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Protocol Page

A Pilot study of different doses of Ibrutinib in patients with Chronic Lymphocytic Leukemia
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Study Chair:	Prithviraj Bose
Additional Contact:	Deanna M. Thomas Vicky H. Zoeller Leukemia Protocol Review Group
Department:	Leukemia
Phone:	713-792-7747
Unit:	428
Study Manager:	TBA
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A pilot study of different doses of ibrutinib in patients with chronic lymphocytic leukemia

Prithviraj Bose, M.D.,[1] Varsha V. Gandhi, Ph.D.,[1,2] and Michael J. Keating, M.B., B.S.[1]

Departments of Leukemia[1] and Experimental Therapeutics[2]

MD Anderson Cancer Center, Houston, TX

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1. OBJECTIVES AND ENDPOINTS

1.1 Objectives

1.1.1 Primary: To determine the effects of different daily doses of ibrutinib (420 mg, 280 mg, 140 mg) on free drug and chemokine levels in plasma, BTK occupancy by ibrutinib, BTK mRNA, BTK phospho- and total protein levels, downstream signaling, NF-κB activity and platelet functionality.

1.1.2 Secondary: To observe the effects of different daily doses of ibrutinib (420 mg, 280 mg, 140 mg) on clinical parameters in patients with CLL in terms of efficacy and safety.

1.2 Endpoints

1.2.1 Primary: Ibrutinib plasma and chemokine concentrations, BTK occupancy by ibrutinib, BTK mRNA levels, BTK phospho- and total protein levels, downstream signaling, NF-κB p65 levels and platelet functionality on days 1 (baseline), 8 and 28 of each cycle of treatment.

1.2.2 Secondary: Absolute lymphocyte count (ALC), hemoglobin and platelet levels, CLL-related symptoms, and palpable lymphadenopathy and/or hepatosplenomegaly on day 28 of each cycle of treatment; characterization of safety and tolerability of ibrutinib throughout the study.

2. BACKGROUND

2.1 Chronic lymphocytic leukemia (CLL)

CLL is the most common leukemia in Western countries, typically occurs in elderly patients (median age 67-72) and is characterized by a highly variable clinical course.[1] Patients are generally observed without therapy until they have evidence of progressive or symptomatic/active disease, e.g., disease-related symptoms, progressive marrow failure, massive or progressive or symptomatic splenomegaly or lymphadenopathy, progressive lymphocytosis or autoimmune cytopenias poorly responsive to corticosteroids or other standard therapy (IWCLL guidelines).[2] Chemoimmunotherapy (CIT) with fludarabine, cyclophosphamide and rituximab (FCR) constitutes the current standard of care for younger, fit individuals,[1, 3, 4] and has been shown to lead to very long term remissions ("functional cures") in the subset of patients with mutated *IgVH* and "favorable" cytogenetic characteristics (e.g., normal, del13q, trisomy 12).[5] CIT with bendamustine and rituximab represents an attractive option for patients over the age of 65, and a variety of anti-CD20 monoclonal antibodies in combination with chlorambucil are reasonable choices in patients with comorbidities considered unfit for conventional CIT.[1, 3, 4]

In recent years, the first-in-class, irreversible inhibitor of Bruton's Tyrosine Kinase (BTK), ibrutinib, has surged to the forefront of therapy for patients with disease that has relapsed after or is refractory to CIT,[3] particularly in the setting of del17p/*TP53* mutation.[1] In view of its striking efficacy in this latter poor-prognosis group of patients for whom CIT is relatively ineffective, ibrutinib has additionally received Food and Drug Administration (FDA) approval for use in patients with del17p in any line of therapy.[4] Additionally, the drug has just been approved for previously untreated patients with CLL based on the findings of the RESONATE-2 trial.[6]

2.2 Ibrutinib mechanism of action and preclinical data in CLL

B-cell receptor (BCR) signaling plays a key role in CLL pathogenesis, and BTK has a critical role in BCR signaling in both normal and malignant B-cells.[7, 8] BTK is important for antigen-induced BCR activation in both normal, mature B-cells and in CLL and mediates survival and proliferation via amplification of downstream effector pathways, e.g., phosphatidyl-3-inositol kinase/Akt (PI3K/Akt), mitogen activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) and nuclear factor kappa B (NF-κB).[7, 8] Additionally, autonomous BCR signaling has also been described in CLL, which is not, however, associated with activating mutations in any of the BCR components.[8]

Ibrutinib inactivates BTK through irreversible, covalent bonding to the Cys481 residue in the adenosine triphosphate (ATP)-binding domain of BTK.[9] BTK has been shown to be critical for the development and expansion of CLL and an important target of ibrutinib.[10] Indeed, BTK point mutations (C481S) that convert ibrutinib to a reversible inhibitor, as well as activating mutations in phospholipase C gamma 2 (PLCγ2), located immediately downstream of BTK, have been implicated in the pathogenesis of acquired resistance to ibrutinib in CLL.[11] The success of the more selective BTK inhibitor, acalabrutinib, in heavily pretreated

patients with CLL, further testifies to the relevance of BTK as a major therapeutic target in this disease.[12]

Ibrutinib binding inhibits activity of the enzyme and autophosphorylation of BTK and abrogates activation of downstream survival pathways (PI3K, ERK, NF-κB), inducing modest apoptosis and inhibiting activation-induced proliferation of CLL cells *in vitro.* It effectively blocks survival signals provided externally to CLL cells from the microenvironment.[13] In primary, patient-derived CLL cells (from blood, lymph nodes and bone marrow) as well as in mouse models, ibrutinib inhibits BCR and NF-κB signaling, induces apoptosis and significantly decreases tumor proliferation and growth.[14-16] Additionally, the drug blocks migration of CLL cells in response to tissue homing chemokines (CXCL12, CXCL13, CCL19), possibly through blockade of BCR-induced activation of lymphocyte cytosolic protein 1 (LCP1)[17] and normalization of the imbalance between expression levels of the homing receptors CCR7 and CXCR4 and S1P receptor 1 (S1P1),[18] down-regulates secretion of BCR-dependent chemokines (CCL3, CCL4) by CLL cells, both *in vitro* and *in vivo,* and causes a transient early lymphocytosis in mouse models.[16, 19] This characteristic "redistribution lymphocytosis" is routinely observed in patients receiving ibrutinib and other BCR pathway inhibitors and is believed to be a result of mobilization of CLL cells from their protective tissue microenvironmental niches into the peripheral blood, where they eventually undergo apoptosis from the lack of prosurvival signals.[20] Ibrutinib abolishes the adhesion of CLL cells to fibronectin and VCAM-1 and reduces CLL cell surface expression of key adhesion molecules such as CD49d, CD29 and CD44, potentially explaining this treatment-induced lymphocytosis.[19, 21] Ibrutinib has also been shown recently to block CLL exosome release[22] and translational responses (e.g., of MYC-specific mRNA)[23] to BCR stimulation.

2.3 Clinical efficacy and safety of ibrutinib monotherapy in CLL in major clinical trials

In a phase 1b-2 multi-center study, 85 patients with relapsed or refractory CLL or small lymphocytic lymphoma (SLL), most of whom were heavily pre-treated and considered to have high-risk disease, received ibrutinib at doses of 420 or 840 mg daily.[24] The overall response rate (ORR) in both dosing cohorts was 71%; and an additional 20% and 15% of patients in the respective groups had a partial response with lymphocytosis (PR-L).[24] At 26 months, the estimated progression-free survival (PFS) rate was 75% and the rate of overall survival (OS) was 83%.[24] In a subsequent phase III study, 391 patients with relapsed or refractory CLL or SLL were randomized 1:1 to ibrutinib or ofatumumab.[25] PFS (median, not reached vs. 8.1 months at 9.4 months' median follow-up), OS (90% vs. 81% at 12 months) and ORR (42.6% vs. 4.1%) were all significantly improved in the ibrutinib group.[25] An additional 20% of ibrutinib-treated patients had a PR-L.[25] Ibrutinib was well-tolerated in these two registration trials, with predominantly grade 1/2 adverse events (AEs), e.g., nausea, diarrhea, fatigue, pyrexia and upper respiratory infection.[24, 25] Ibrutinib was also studied in the frontline setting in older (≥65) patients with CLL/SLL (n=31).[26] The ORR was 71% (13% of

patients achieved complete remission, CR) and another 13% of patients achieved a PR-L after a median follow-up of 22.1 months.[26] The AE profile was very similar. 10% of patients developed grade 3 infections.[26] One patient each developed grade 3 neutropenia and grade 4 thrombocytopenia.[26] In the phase III RESONATE-2 trial that compared ibrutinib to chlorambucil as initial therapy for CLL in this age group (median age 73), median PFS was not reached for ibrutinib versus 18.9 months for chlorambucil, and the risk of progression or death was 84% lower with ibrutinib after median follow-up of 18.5 months.[6] OS was also significantly prolonged with ibrutinib (98% alive at 24 months versus 85% with chlorambucil).[6] ORRs were 86% with ibrutinib and 35% with chlorambucil.[6] AEs occurring in ≥20% of patients on ibrutinib included diarrhea, fatigue, cough and nausea; additionally, peripheral edema, dry eye, arthralgia, neutropenia (including grade 3) and vomiting occurred in ≥10%.[6] The results of 3-year (median) follow-up of 132 patients receiving ibrutinib as a single agent have recently been reported.[27] Longer treatment was associated with improvement in response quality over time and durable remissions.[27] Toxicity with longer follow-up diminished with respect to occurrence of grade ≥3 cytopenias, fatigue, and infections.[27] Progression was uncommon, occurring primarily in some patients with relapsed del17p and/or del11q disease.[27] Infections in CLL patients have been shown to decline over time during ibrutinib therapy.[28] Recently, reasons for discontinuation of ibrutinib by CLL patients and outcomes in this setting have been reported.[29, 30] In the MD Anderson Cancer Center experience, 33 of 127 patients discontinued ibrutinib, 14 due to disease progression and 14 due to AEs or sudden death.[29] In the Ohio State University experience, 76 of 308 patients discontinued ibrutinib, 31 because of disease progression and 37 because of AEs or sudden cardiac death.[30] In both studies, outcomes after discontinuation of ibrutinib were poor.[29, 30]

The redistribution lymphocytosis characteristic of treatment with ibrutinib has been extensively investigated.[31] While an efflux of tumor cells from the tissue compartments into the blood has been conclusively shown,[32] mathematical models have estimated the fraction of the tissue CLL cells redistributed into the blood during ibrutinib therapy to be 23.3% ± 17% of the total tissue disease burden, arguing that the reduction of tissue disease burden is due more to CLL cell death and less to egress from nodal compartments.[33] The duration of the lymphocytosis may be shorter in patients with trisomy 12.[34] Lymphocytosis may take longer to peak in patients whose initial ALC doubles by day 28, compared with those with a lower rate of increase.[35] While the lymphocytosis resolves within 8 months in the majority of patients, it may last for over a year in some patients.[36] Importantly, this persistent lymphocytosis does not represent disease progression or clonal evolution, and these patients do not have inferior PFS.[36] These observations have called attention to the current IWCLL response criteria, which would consider lymphocytosis to represent progressive disease (PD), and highlighted the need to refine clinical endpoints in the era of novel, targeted agents.[37]

While ibrutinib is mostly used as a single agent at the present time, the safety and feasibility of combining it with anti-CD20 monoclonal antibodies[38, 39] and with CIT[40, 41] has been demonstrated. Such combinations have produced high response rates and blunt the redistribution lymphocytosis associated with ibrutinib monotherapy; however, no clear advantage over ibrutinib monotherapy has yet been shown. Trials to evaluate ibrutinib in high risk individuals with CLL who do not meet current criteria for initiation of treatment are planned.[42, 43]

2.4 Targets of ibrutinib beyond BTK and off-target toxicity of ibrutinib

BTK belongs to the TEC family kinases (TFKs), and other members of this family (TEC, ITK, BMX, RLK/TXK) are also targeted by ibrutinib, albeit at higher concentrations (e.g., 78 nM for TEC).[44] Other kinases targeted by ibrutinib include the HER family kinases EGFR, HER2/ErbB2 and HER4/ErbB4, and JAK3.[8, 44] Ibrutinib inhibits yet other kinases at low nanomolar concentrations, e.g., BRK, CSK, FRG, HCK, BLK; indeed, the IC_{50} for BLK is the same as for BTK, i.e., 0.5 nM.[9, 44] While some of these off-target effects point to potential uses of the drug beyond B-cell malignancies (e.g., $HER2^{+}$ breast cancer, EGFR-mutant lung cancer, asthma, rheumatoid arthritis), others could be responsible for some of it's toxicities, e.g., atrial fibrillation (AF), bleeding, and possibly, long-term deleterious effects on bone homeostasis.[44]

Much attention has been directed towards ibrutinib's irreversible inhibition of interleukin-2-inducible kinase (ITK) in T-cells, resulting in subversion of Th2 immunity and potentiation of Th1-based immune responses.[45] This action of ibrutinib may underlie the synergism between the drug and immune checkpoint inhibitors (anti-PD1/PDL1 monoclonal antibodies) in mouse models of a number of tumor types.[46] Conversely, ibrutinib has been reported to antagonize rituximab-dependent NK-cell mediated cytotoxicity[47, 48] and impair the phagocytosis of rituximab-coated patient-derived CLL cells,[49] but these effects have not been demonstrated in murine xenograft models[48] or observed in clinical trials,[38, 39] possibly due to promotion by ibrutinib of both positive and negative interactions with anti-CD20 monoclonal antibodies.[50] Interestingly, inhibition of ITK and resultant modulation of cellular immunity have been invoked as potentially contributing to the development of panniculitis, an unusual, recently recognized AE in ibrutinib-treated patients.[51]

AF and atrial flutter have occurred in 6-9% of patients treated with ibrutinib (IMBRUVICA® package insert, revised 01/2015). This effect has been attributed to the binding of ibrutinib to BTK and TEC in the heart and subsequent inhibition of cardioprotective PI3K/Akt signaling.[52] Other targets of ibrutinib, viz., HER2/ErbB2, HER4/ErbB4 and BMX play important roles in cardiac physiology, and their inhibition could represent additional mechanisms through which ibrutinib could cause AF and cardiac dysfunction.[44]

Bleeding is a relatively common AE of ibrutinib and grade ≥3 bleeding events have occurred in up to 6% of patients (IMBRUVICA® package insert, revised 01/2015). Bleeding events of any grade, including bruising and petechiae, have occurred in approximately half of patients (IMBRUVICA® package insert, revised 01/2015). The low frequency of subarachnoid hemorrhages in several patients receiving concomitant warfarin in the phase 1b-2 trial of ibrutinib prompted the exclusion of concurrent therapy with oral vitamin K antagonists in all subsequent studies of ibrutinib.[3] Additionally, ibrutinib is typically held for 3 to 7 days before and after surgical procedures.[3] Both BTK and TEC play important roles in collagen-induced platelet adhesion mediated through glycoprotein (GP) VI, and TEC is able to compensate for loss of function of BTK in this setting.[53, 54] However, BTK is essential for von Willebrand factor (vWF)-induced (GPIb-dependent) platelet aggregation and thrombus formation.[55] Ibrutinib inhibits platelet signaling and function downstream of the collagen receptor, GPVI and firm platelet adhesion to vWF, leading to decreased platelet aggregation that correlates with bleeding events.[56, 57] The risk of bleeding in CLL patients treated with ibrutinib does appear to decrease with continued therapy.[58]

Mice lacking BTK and TEC show severe osteopetrosis caused by a defect in bone resorption.[59] Receptor activator of NF-κB (RANK) and immunoreceptor tyrosine-based activation motif (ITAM)-harboring adaptors signal through BTK, TEC, B-cell linker (BLNK) and PLCγ to activate an essential calcium signal in osteoclasts, and inhibition of TEC reduces osteoclastic bone resorption in models of osteoporosis and inflammation-induced bone destruction.[59] While this could argue for a therapeutic role of ibrutinib in diseases of enhanced osteoclastic bone resorption such as osteoporosis or rheumatoid arthritis, these findings raise concern over the long-term effects of ibrutinib treatment on bone homeostasis, particularly since systematic studies of bone density have not been performed in patients receiving ibrutinib.[44]

3. RATIONALE FOR STUDYING DIFFERENT DOSES OF IBRUTINIB IN PATIENTS WITH CLL

Dose of ibrutinib verus clinical response

In a phase I study of ibrutinib in 56 patients with relapsed or refractory B-cell malignancies, BTK occupancy ≥95% was achieved 4 hours post-dose in all patients receiving 2.5 mg/kg/day.[60] Additionally, complete or near-complete BTK occupancy was observed in patients with area under the curve (AUC) exceeding 160 ng x h/ml.[60] BTK occupancy was measured using a highly specific fluorescent affinity probe, whose binding to the BTK active site had previously been shown to tightly correlate with blockade of BCR signaling and in vivo efficacy.[9] Dose escalation proceeded up to three dose levels above the level at which full BTK occupancy was observed, i.e., 12.5 mg/kg/day, and all dosing cohorts from 2.5 through 12.5 mg/kg/day had >95% BTK occupancy and similar response rates.[60] In the context of clinical observations in patients with CLL that show that patients whose ibrutinib

dose is reduced to 280 mg/d or 140 mg/d because of AEs continue to respond (Michael J. Keating, MD, unpublished observations), these data provide a compelling argument that doses of ibrutinib lower than the approved 420 mg/d will be effective in CLL.

Decrease in off-target effects

Lower doses should result in lower concentrations of free drug in plasma and reduced binding to off-target kinases such as TEC, likely leading to a decreased incidence of AEs such as AF and bleeding manifestations. Acalabrutinib, a new and more selective irreversible BTK inhibitor, does not inhibit other kinases such as EGFR, TEC and ITK at pharmacologically active concentrations.[12] In a phase I/II trial in 61 heavily pretreated patients (median 3 prior therapies, 31% with del17p, 75% with unmutated IgVH), acalabrutinib produced an ORR of 95% (85% PRs, 10% PR-Ls, 100% ORR in patients with del17p) without any major hemorrhage or AF.[12]

Decrease of total BTK in CLL cells during ibrutinib therapy

Prior investigations in murine models demonstrated that BTK protein total levels increased after B-cell receptor pathway stimulation.[61] Similar observations were recently made in an adoptive transfer CLL murine model.[62] The mechanism involved the CXCR4-CXCL12 signaling pathway. As a corollary, inhibition of BTK by ibrutinib in murine model resulted in a decline in total BTK along with internalization of CXCR4 receptors.

Data from our laboratory further extends these observations. We found that total BTK protein was decreased in primary circulating CLL cells obtained from patients undergoing ibrutinib therapy (Gandhi, unpublished data). Collectively, these observations suggest that total BTK protein levels decline with ibrutinib treatment. Finally, BTK-dependent NF-κB activation, resulting from both BCR[63, 64] and toll-like receptor (TLR)[65] activation, is inhibited by ibrutinib, and NF-κB regulates the level of BTK protein through control of transcription,[66] providing yet another mechanism by which ibrutinib therapy may lead to reduced levels of BTK protein. Because ibrutinib is an irreversible inhibitor and one molecule of drug is needed for stochiometrically inhibiting the enzyme, a lower level of protein would mean a lower concentration of ibrutinib required for BTK inhibition. In the presence of higher levels of circulating ibrutinib than are required to establish full BTK occupancy, these phenomena would be expected to increase the likelihood of off-target binding of the drug, potentially leading to AEs.

Impact on drug cost

Finally, the cost of ibrutinib therapy (average wholesale US price $11,002 per month), especially in the likely future scenario of an expanded frontline indication, is prohibitive. Costs of indefinite therapy with tyrosine kinase inhibitors (TKIs) have already been noted to be unsustainable in the context of chronic myeloid leukemia.[67] In the current paradigm,

therapy with ibrutinib is continued indefinitely in the absence of disease progression or intolerable toxicity, particularly since the drug does not induce a minimal residual disease (MRD)-negative state on its own.[42] In a recent analysis, the average lifetime cost of treatment of CLL per patient was estimated to be over $700,000 for patients beginning therapy in the 2017-2025 period.[68] In this analysis, the incremental cost per life-year gained was $204,000 and that per quality-adjusted life-year gained was $262,000.[68] Another recent analysis compared the costs of CLL treatment before the approval of ibrutinib to the current cost using ibrutinib as salvage therapy and to the potential future cost, assuming approval of ibrutinib for first-line use.[69] Estimated 10-year pharmaceutical costs per newly diagnosed patient and per treated patient, respectively, were: $45,659 and $157,446 for the historical scenario, $77,948 and $268,788 for the current scenario for ibrutinib, and $164,141 and $566,002 for the potential future scenario.[69] Total out-of-pocket cost per treated patient with newly diagnosed CLL under Medicare Part D increased from $325 under the historical scenario to $8,800 under the current scenario and to $35,564 under the future scenario.[69]

4. DRUG INFORMATION

4.1 Product description

Ibrutinib is a white to off-white solid with the empirical formula C25H24N6O2 and a molecular weight 440.50. Ibrutinib is freely soluble in dimethyl sulfoxide, soluble in methanol and practically insoluble in water. The chemical name for ibrutinib is 1-[(3R)-3-[4-amino-3-(4-phenoxyphenyl)-1Hpyrazolo[3,4-d]pyrimidin-1-yl]-1-piperidinyl]-2-propen-1-one.

4.2 Storage and handling

Bottles should be stored at room temperature: 20°C to 25°C (68°F to 77°F). Excursions are permitted between 15°C and 30°C (59°F to 86°F). Ibrutinib capsules should be retained in their original package until dispensing.

4.3 How supplied

Ibrutinib capsules for oral administration are supplied as white opaque capsules that contain 140 mg ibrutinib as the active ingredient. Each capsule also contains the following inactive ingredients: croscarmellose sodium, magnesium stearate, microcrystalline cellulose, and sodium lauryl sulfate. The capsule shell contains gelatin, titanium dioxide and black ink. Each white opaque capsule is marked with “ibr 140 mg” in black ink. The capsules are available in white HDPE bottles with a child-resistant closure:

- 90 capsules per bottle: NDC 57962-140-09
- 120 capsules per bottle: NDC 57962-140-12

4.4 Route of administration and pharmacokinetics (PK)

Oral.

Absorption:

Ibrutinib is absorbed after oral administration with a median T_{max} of 1 to 2 hours.[60] Ibrutinib exposure increases with doses up to 840 mg. The steady-state AUC (mean ± standard deviation) observed in patients at 560 mg is 953 ± 705 ng·h/mL and in patients at 420 mg is 680 ± 517 ng·h/mL. Administration with food increased ibrutinib C_{max} and AUC by approximately 2 to 4- and 2-fold, respectively, compared with administration of ibrutinib after overnight fasting. However, when administered once daily in uncontrolled food-intake conditions (>/=30 min before or 2 h after), exposures were slightly (approximately 30 %) lower than in fed condition (30 min after); thus, no food restrictions are warranted.[70]

Distribution:

Reversible binding of ibrutinib to human plasma protein *in vitro* was 97.3% with no concentration dependence in the range of 50 to 1000 ng/mL. The volume of distribution at steady state ($V_{d,ss}$) was 683 L, and the apparent volume of distribution at steady state ($V_{d,ss}/F$) was approximately 10000 L.[71]

Metabolism:

Metabolism is the main route of elimination for ibrutinib. It is metabolized to several metabolites, primarily by cytochrome P450, CYP3A,[72] and to a minor extent by CYP2D6. The active metabolite, PCI-45227, is a dihydrodiol metabolite with inhibitory activity towards BTK approximately 15 times lower than that of ibrutinib. The range of the mean metabolite to parent ratio for PCI-45227 at steady-state is 1 to 2.8.

Elimination:

Intravenous clearance was 62 and 76 L/h in fasted and fed conditions, respectively. In line with the high first-pass effect, the apparent oral clearance is approximately 2000 and 1000 L/h in fasted and fed conditions, respectively.[71] The half-life of ibrutinib is 4 to 6 hours and the drug does not accumulate after repeated oral dosing.[60] Ibrutinib, mainly in the form of metabolites, is eliminated primarily via feces.[73] After a single oral dose (140 mg) of radiolabeled [14C]-ibrutinib in healthy subjects, approximately 90% of radioactivity was excreted within 168 hours, with the majority (80%) excreted in the feces and less than 10% accounted for in urine.[73] Unchanged ibrutinib accounted for approximately 1% of the radiolabeled excretion product in feces and none in urine, with the remainder of the dose being metabolites.[73]

Renal Impairment:

Ibrutinib is not significantly cleared renally; urinary excretion of metabolites is < 10% of the dose. Creatinine clearance > 25 mL/min had no influence on the exposure to ibrutinib.

There are no data in patients with severe renal impairment (CrCL < 25 mL/min) or in patients on dialysis.

Hepatic Impairment:

Ibrutinib is metabolized in the liver. In a hepatic impairment trial, a single dose of 140 mg of ibrutinib was administered in non-cancer subjects. Ibrutinib AUC increased 2.7-, 8.2- and 9.8-fold, respectively, in subjects with mild (n=6), moderate (n=10) and severe (n=8) hepatic impairment relative to subjects with normal liver function. Ibrutinib C_{max} increased 5.2-, 8.8- and 7.0-fold, respectively, in subjects with mild, moderate and severe hepatic impairment relative to subjects with normal liver function. For patients with mild liver impairment (Child-Pugh class A), the recommended dose is 140 mg daily (one capsule).

4.5 Availability

Commercially available supplies of ibrutinib will be used in this study.

4.6 Agent destruction and return

Not applicable.

4.7 Contraindications

None.

4.8 Drug interactions

Ibrutinib is primarily metabolized by cytochrome P450 enzyme 3A.

CYP3A Inhibitors:

In healthy volunteers, co-administration of ketoconazole, a strong CYP3A inhibitor, increased C_{max} and AUC of ibrutinib by 29- and 24-fold, respectively.[72] The highest ibrutinib dose evaluated in clinical trials was 12.5 mg/kg (actual doses of 840 – 1400 mg) given for 28 days with single dose AUC values of 1445 ± 869 ng · hr/mL, which is approximately 50% greater than steady state exposures seen at the highest approved dose (560 mg, in mantle cell lymphoma (MCL)). Concomitant administration of ibrutinib with strong or moderate inhibitors of CYP3A should be avoided. For strong CYP3A inhibitors used short-term (e.g., antifungals and antibiotics for 7 days or less, e.g., ketoconazole, itraconazole, voriconazole, posaconazole, clarithromycin, telithromycin), interruption of ibrutinib therapy during the duration of inhibitor use should be considered. Chronic use of strong CYP3A inhibitors should be avoided. If a moderate CYP3A inhibitor must be

used, the dose of ibrutinib should be reduced to 140 mg daily (from the approved dose of 420 mg daily). Patients taking concomitant strong or moderate CYP3A4 inhibitors should be monitored more closely for signs of toxicity. Grapefruit and Seville oranges should be avoided during ibrutinib treatment, as these contain moderate inhibitors of CYP3A.

CYP3A Inducers:
Administration of ibrutinib with rifampin, a strong CYP3A inducer, decreased ibrutinib C_{max} and AUC by approximately 13- and 10-fold, respectively.[72] Concomitant use of strong CYP3A inducers (e.g., carbamazepine, rifampin, phenytoin and St. John's Wort) should be avoided, and alternative agents with less CYP3A induction considered.

4.9 Warnings and precautions

Hemorrhage:
Fatal bleeding events have occurred in patients treated with ibrutinib. Grade ≥3 bleeding events (subdural hematoma, gastrointestinal bleeding, hematuria and post procedural hemorrhage) have occurred in up to 6% of patients. Bleeding events of any grade, including bruising and petechiae, occurred in approximately half of patients treated with ibrutinib. The mechanism for the bleeding events is not well understood. Ibrutinib may increase the risk of hemorrhage in patients receiving antiplatelet or anticoagulant therapies. Based on the risk-benefit ratio, ibrutinib is suggested to be withheld for at least 3 to 7 days pre- and post-surgery depending upon the type of surgery and the risk of bleeding.

Infections:
Fatal and non-fatal infections have occurred with ibrutinib therapy. Grade ≥3 infections occurred in 14% to 26% of patients. Cases of progressive multifocal leukoencephalopathy (PML) have occurred in patients treated with ibrutinib. Patients should be monitored for fever and infections and evaluated promptly.

Cytopenias:
Treatment-emergent Grade 3 or 4 cytopenias including neutropenia (range, 19 to 29%), thrombocytopenia (range, 5 to 17%), and anemia (range, 0 to 9%) occurred in patients treated with ibrutinib. Complete blood counts (CBCs) should be monitored monthly.

Atrial Fibrillation:
Atrial fibrillation and atrial flutter (range, 6 to 9%) have occurred in patients treated with ibrutinib, particularly in patients with cardiac risk factors, acute infections, and a previous history of atrial fibrillation. Patients should be clinically monitored periodically for atrial fibrillation. Patients who develop arrhythmic symptoms (e.g., palpitations, lightheadedness) or new onset dyspnea should have an electrocardiogram (ECG) performed. If atrial fibrillation persists, the risks and benefits of treatment and dose modification should be considered.

Second Primary Malignancies:
Other malignancies (range, 5 to 14%) including non-skin carcinomas (range, 1 to 3%) have occurred in patients treated with ibrutinib. The most frequent second primary malignancy was non-melanoma skin cancer (4 to 11 %).

Tumor Lysis Syndrome (TLS):
TLS has been reported with ibrutinib therapy. Patients should be monitored closely and appropriate precautions taken in patients at risk for TLS (e.g. high tumor burden).

Embryo-Fetal Toxicity:
Based on findings in animals, ibrutinib can cause fetal harm when administered to a pregnant woman. Ibrutinib caused malformations in rats at exposures 14 times those reported in patients with MCL and 20 times those reported in patients with CLL or Waldenstrom's macroglobulinemia (WM), receiving ibrutinib doses of 560 mg per day and 420 mg per day, respectively. Reduced fetal weights were observed at lower exposures. Women should avoid becoming pregnant while taking ibrutinib. If this drug is used during pregnancy or if the patient becomes pregnant while taking this drug, the patient should be apprised of the potential hazard to a fetus.

5 ELIGIBILITY CRITERIA

5.1 Inclusion criteria (all criteria must be met):

5.1.1 Patients with a diagnosis of CLL (any stage) with ALC ≥20 x 10^9/l, requiring therapy.

5.1.2 Able to receive ibrutinib through commercial supply, i.e., insured patients meeting FDA-approved indications.

5.1.3 Age ≥18 years.

5.1.4 Eastern Cooperative Oncology Group (ECOG) performance status of 0-2.

5.1.5 Adequate end organ function, defined as the following: total bilirubin ≤1.5 x upper limit of normal (ULN, unless due to Gilbert syndrome, in which case it should be ≤3.0 x ULN), ALT and AST ≤2.5 x ULN, CrCL ≥25 ml/min.

5.1.6 Able to understand and sign the IRB-approved informed consent document for this trial.

5.1.7 Women of childbearing potential (WOCBP) must practice 2 effective methods of birth control during the course of the study. Male patients who are partners of WOCBP should also practice an effective method of contraception. Effective methods of birth control include diaphragm or condoms with spermicidal foam or jelly, birth control pills (BCPs), injections or patches, intra-uterine devices (IUDs) and surgical sterilization.

- Postmenopausal women must be amenorrheic for ≥12 months to be considered of non-childbearing potential.
- Women and men must continue birth control for the duration of the trial and ≥3 months after the last dose of study drug.
- All WOCBP MUST have a negative pregnancy test prior to beginning ibrutinib on study.

5.1.8 Patients should have discontinued any and all other therapy for CLL ≥48 hours prior to start of study therapy and recovered from any toxicity due to these therapies to grade ≤1.

5.2 Exclusion criteria:

5.2.1 Previous treatment with ibrutinib.

5.2.2 Current therapy with warfarin or other anticoagulants at therapeutic doses, e.g., low molecular weight heparin, fondaparinux, dabigatran, rivaroxaban, apixaban or edoxaban that are unable to be discontinued.

5.2.3 Active gastrointestinal conditions that are expected to impair absorption of orally administered medications.

5.2.4 Active, uncontrolled infection.

5.2.5 Inability to understand a written informed consent document.

5.2.6 Pregnancy or lactation.

5.2.7 Patients with leukemic involvement of the central nervous system.

5.2.8 Patients who currently have or have a history of the following within 6 months preceding study entry are not eligible:

- Unstable angina (UA) or myocardial infarction (MI).
- Clinically significant atrial or ventricular arrhythmias (e.g., AF, atrial flutter, ventricular tachycardia, ventricular fibrillation, or *torsades de pointes*).
- New York Heart Association (NYHA) class III or IV heart failure.

5.2.9 Patients on strong CYP3A inducers or inhibitors that are unable to be discontinued. The list of drugs that interact with cytochrome P450 enzymes can be found online at: http://medicine.iupui.edu/clinpharm/DDIs/ClinicalTable.aspx

6 TREATMENT PLAN

6.1 General

All patients should be registered with the Data Management Office PDMS/CORE system.

6.2 Treatment plan

Patients will receive ibrutinib orally daily for 3 cycles. Cycles will be 28 days long. Ibrutinib will be administered continuously, at approximately the same time each day, without regard to food. In the first cycle, the daily dose of ibrutinib will be 420 mg (3 capsules), in the second cycle, 280 mg (2 capsules) and in the third cycle, 140 mg (1 capsule). Patients will swallow the ibrutinib capsules whole with water without opening, breaking or chewing them.

6.3 Dose modifications

Ibrutinib therapy should be interrupted for any grade ≥3 non-hematologic toxicity, grade ≥3 neutropenia with infection or fever, or grade 4 hematologic toxicity, as assessed by the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE, version 4.03). Ibrutinib may be resumed at the starting dose for that particular cycle once the toxicity has resolved to grade ≤1 or baseline. If the toxicity recurs, the dose should be reduced by 1 capsule (140 mg) daily if this occurs within the first or second cycles. A second reduction of dose by 1 capsule (140 mg) daily may be made for patients in their first cycle of treatment. For patients already taking 1 capsule (140 mg) daily at the time of occurrence of the toxicity (i.e., patients in their third cycle of therapy or second cycle patients after a first dose reduction), therapy with ibrutinib should be discontinued.

6.4 Missed doses

If a dose of ibrutinib is not taken at the scheduled time, it can be taken as soon as possible on the same day with a return to the normal schedule the following day. Extra capsules of ibrutinib should not be taken on the following day(s)to make up for the missed dose(s).

6.5 Duration of therapy

The duration of treatment with ibrutinib will be three 28-day cycles. Beyond this point, patients may continue to take ibrutinib at their physician-recommended dose, or switch to an alternative therapy.

6.6 Therapies prohibited during the study

Any other therapy for the treatment of CLL, including corticosteroids at doses greater than 10 mg/day of prednisone, therapeutic doses of warfarin or other anti-coagulants, strong CYP3A inhibitors or inducers.

6.7 Permitted therapies

Including but not limited to hematopoietic growth factors, antimicrobials for prophylaxis or treatment of infections, allopurinol and/or rasburicase for prophylaxis or treatment of TLS.

7 PRETREATMENT EVALUATION

7.1 A complete history and physical examination including blood pressure, performance status and concomitant medications within 2 weeks prior to start of study treatment.

7.2 CBC and differential, albumin, alkaline phosphatase, total, direct and indirect bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, LDH, phosphorus, potassium, total protein, SGPT (ALT), SGOT (AST), sodium and urinalysis within 1 week prior to start of study treatment.

7.3 Serum beta-2-microglobulin and immunoglobulin levels (IgG, IgA, IgM) within 1 week prior to start of study treatment.

7.4 Pregnancy test (blood or urine) for female patients of childbearing potential within 7 days (1 week) prior to initiation of study treatment.

7.5 Baseline electrocardiogram (ECG) within 1 week prior to start of study treatment.
7.6 Baseline bone marrow evaluation and CT scans for assessment of baseline lymphadenopathy and organomegaly are NOT required for study enrollment, but may be performed at any time as needed as standard of care tests.

8 EVALUATION DURING STUDY

8.1 Physical examination along with evaluation of performance status, recording of AEs and concomitant medications on day 28 (±2 days) of each cycle.
8.2 CBC and differential on day 28 (±2 days) of each cycle.
8.3 Albumin, alkaline phosphatase, total, direct and indirect bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, LDH, phosphorus, potassium, total protein, SGPT (ALT), SGOT (AST), and sodium on day 28 (±2 days) of each cycle.
8.4 Serum beta-2-microglobulin and immunoglobulin levels (IgG, IgA, IgM) on day 28 (±2 days) of each cycle.
8.5 ECG on day 28 (±2 days) of each cycle.
8.6 Plasma concentrations of ibrutinib by high performance liquid chromatography with tandem mass spectrometric detection as previously described,[60] just before dosing and at 4 and 24 hours post-dosing on days 1, 8, and 28 (but before the first dose of the next cycle) of each cycle. Exception: there will be no 4-hour blood draw on day 28. Chemokines will also be measured, using enzyme-linked immunosorbent assay (ELISA).
8.7 BTK occupancy level by fluorescent affinity probe detection as previously described,[9, 60] just before dosing and at 4 and 24 hours post-dosing on days 1, 8, and 28 (but before the first dose of the next cycle) of each cycle. Exception: there will be no 4-hour blood draw on day 28. See section 8.11.
8.8 Measurement of BTK mRNA and BTK and phospho-BTK protein and downstream signaling, as previously described,[74, 75] just before dosing and at 4 and 24 hours post-dosing on days 1, 8, and 28 (but before the first dose of the next cycle) of each cycle. Exception: there will be no 4-hour blood draw on day 28.
8.9 Quantification of NF-κB activation by ELISA as previously described,[76] just before dosing and at 4 and 24 hours post-dosing on days 1, 8, and 28 (but before the first dose of the next cycle) of each cycle. Exception: there will be no 4-hour blood draw on day 28.
8.10 Measurement of platelet functionality as previously described,[77] just before dosing and at 24 hours post-dosing on days 1 and 28 (but before the first dose of the next cycle) of each cycle.
8.11 Peripheral blood samples for plasma ibrutinib and cytokine levels, BTK occupancy, total and phospho-BTK mRNA and protein levels as well as levels of other downstream mediators of BCR signaling, and NF-κB activity (appendix C) will be sent for processing to the laboratory of Dr. Varsha V. Gandhi, Ph.D. at:

1901 East Road
Unit # 1950
Houston, TX 77054
Room # 3SCR4.4121
Phone: 713-792-2989
Fax: 713-745-1710
Email: vgandhi@mdanderson.org

For the BTK occupancy assay, peripheral blood mononuclear cells from blood collected from patients will be isolated in Dr. Gandhi's laboratory. The frozen cell pellets then will be transported on dry ice to Pharmacyclics (at the address indicated below). All patient samples are de-identified and neither the hospital number nor the names are included. Laboratories within Pharmacyclics will analyze these samples for the BTK occupancy assay.

Pharmacyclics LLC
999 East Arques Avenue
Sunnyvale, CA 94087, USA.

Study Calendar

	Pre-Study	Wk 1	Wk 2	Wk 3	Wk 4	Wk 5	Wk 6	Wk 7	Wk 8	Wk 9	Wk 10	Wk 11	Wk 12
Ibrutinib		A	A	A	A	A	A	A	A	A	A	A	A
Informed consent	X												
Demographics	X												
Medical history	X												
Concurrent meds	X	X----------X											
Physical exam	X				X^f				X^f				X^f
Vital signs	X				X^f				X^f				X^f
Height	X												
Weight	X				X^f				X^f				X^f
Performance status	X				X^f				X^f				X^f

CBC w/diff, plts	X				X[f]				X[f]				X[f]
Serum chemistry[a]	X				X[f]				X[f]				X[f]
Serum beta-2-microglobulin	X				X[f]				X[f]				X[f]
IgG, IgA and IgM levels	X				X[f]				X[f]				X[f]
ECG	X				X[f]				X[f]				X[f]
Adverse event evaluation		X----------X											
ß-HCG	X[b]												
Urinalysis	X												
Plasma ibrutinib and chemokine levels		X[c]	X[d]		X[e]	X[c]	X[d]		X[e]	X[c]	X[d]		X[e]
BTK occupancy levels		X[c]	X[d]		X[e]	X[c]	X[d]		X[e]	X[c]	X[d]		X[e]
BTK mRNA, protein and downstream signaling		X[c]	X[d]		X[e]	X[c]	X[d]		X[e]	X[c]	X[d]		X[e]
NF-κB activation assay		X[c]	X[d]		X[e]	X[c]	X[d]		X[e]	X[c]	X[d]		X[e]
Platelet functionality assay		X[g]			X[g]	X[g]			X[g]	X[g]			X[g]

A: Ibrutinib, orally daily, 420 mg/d in cycle 1 (weeks 1-4), 280 mg/d in cycle 2 (weeks 5-8), 140 mg/d in cycle 3 (weeks 9-12).

a: Albumin, alkaline phosphatase, total, direct and indirect bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, LDH, phosphorus, potassium, total protein, SGOT [AST], SGPT [ALT], sodium.

b: Serum or urine pregnancy test (women of childbearing potential).

c: On day 1 of each cycle, samples at 0 (cycle 1 only), 4, and 24 hours (see appendix C). Please note that the day 1 time zero collection in cycles 2 and 3 coincides with the day 28 24-hour collection of the previous cycles; therefore, no separate day 1 time zero collection is required in cycles 2 and 3.

d. On day 8 of each cycle, samples at 4 and 24 hours (see appendix C).

e. On day 28 of each cycle, samples at 24 hours (see appendix C).

f. On day 28 (±2 days) of each cycle.

g. Pre-treatment (cycle 1 day 1) and 24 hours post-dosing on days 1 and 28 of each cycle.

9 ASSESSMENT OF RESPONSE

There will be no formal response assessment due to the short duration of ibrutinib treatment in this study. For reference, IWCLL criteria[2] for response, stable and progressive disease are provided in Appendix A. As noted above, lymphocytosis alone does not represent disease progression in the context of ibrutinib therapy.[37]

10 CRITERIA FOR REMOVAL FROM THE STUDY

10.1 Patients with clinically significant progressive disease.
10.2 Poor compliance with study treatments or protocol-mandated study procedures.
10.3 Unacceptable severe (grade 3-4) toxicity despite dose optimization and optimal management of toxicity.
10.4 Patient request.
10.5 Lack of appreciable benefit in the presence of better therapeutic options in the opinion of the investigator.

11 STATISTICAL CONSIDERATIONS

Ibrutinib is an inhibitor of Bruton's tyrosine kinase (BTK). The FDA has approved it for relapsed patients with CLL and frontline patients with del17p. Its standard dose is 420 mg/day (three 140-mg capsules). The primary objective of this pilot study is to determine the effects of different daily doses of ibrutinib (420 mg, 280 mg, 140 mg) on free drug levels in plasma, BTK occupancy by ibrutinib, BTK mRNA, BTK phospho- and total protein levels and NF-κB activity. In total, 12 patients will be enrolled in this study. All patients will receive ibrutinib orally daily for 3 cycles. Each cycle will be 28 days long. In the first cycle, the daily dose of ibrutinib will be 420 mg (3 capsules), in the second cycle, 280 mg (2 capsules) and in the third cycle, 140 mg (1 capsule). That is, each patient will go through the three cycles with decreasing dose levels as described above. The half-life of ibrutinib is 4 to 6 hours and the drug does not accumulate after repeated oral dosing. Therefore, the influence of a previous cycle on the subsequent cycle is minimal.

Among the above pharmacokinetic and pharmacodynamic (PK/PD) parameters, a key factor is BTK occupancy. A dose level with BTK occupancy by ibrutinib ≥95% (alternative hypothesis, H1) is regarded as sufficient, and that with an occupancy rate <85% (null hypothesis, H0) is insufficient. Using a t-test to test H0 vs. H1, when the standard deviation of BTK occupancy rate (used as a continuous variable) is 0.10, a sample size of 10 to 12 patients can provide 80-88% power, at 2-sided significance level 0.05. This t-test will be conducted for each dose level. The data set described below shows that the standard deviation of BTK occupancy rate is about 5% when its mean is higher than 85%. As such, a sample size of 8 can provide 95% power at significance level 0.01. Therefore, even considering multiple testing adjusted by the Bonferroni method, and sample size reduction due to various reasons, this trial will still have sufficient power. An ideal dose level is sufficiently high to provide a high (≥95%) BTK occupancy level by ibrutinib, but not higher, so that there is not much "left over drug" in plasma to cause toxicities, i.e, a low free drug level in plasma is desirable.

Previous clinical experience has shown that lower than standard doses of Ibrutinib have similar efficacy and lower toxicities. In a phase I study of ibrutinib in a different population of patients (with relapsed or refractory B-cell malignancies), BTK occupancy ≥95% was achieved 4 hours post-dose in all patients receiving 2.5 mg/kg/day,[60] which equals 175 mg/day for patients weighing 70 kg. In total, 56 patients were enrolled onto this study with 7 dose levels, and 6 to 9 patients at each dose level, to provide data on means (SDs) of the concentration of ibrutinib over time, areas under the curves (AUCs) of the receiver operating characteristics (ROCs), BTK occupancy (in %) for different dose levels over time, and other PK/PD parameters. For the 7 dose levels, the mean AUCs over 0-24 hours range roughly from 100 to 1500 ng x h/mL, with their SDs ranging roughly from 20 to 400 ng x h/mL, and higher dose levels had larger mean AUCs and larger SDs. For the highest dose level, the mean (SD) concentrations of ibrutinib were roughly 20 (5), 100 (35), 135 (45), 55 (15) ng/mL at 1, 2, 3, and 4 hours after taking the drug. These data show that the sample sizes of 6 to 9 patients at each dose level are sufficient to provide reasonably accurate estimates for these PK/PD parameters. This pilot study will enroll up to 12 patients, but have each patient receive all the three proposed dose levels, one at a time, as described above.

We will also observe the effects of different daily doses of ibrutinib (420 mg, 280 mg, 140 mg) on clinical parameters in patients with CLL in terms of efficacy and safety. We will measure the absolute lymphocyte count (ALC), hemoglobin and platelet levels, CLL-related symptoms, and palpable lymphadenopathy and/or hepatosplenomegaly on day 28 of each cycle of treatment. We will characterize the safety and tolerability of ibrutinib throughout the study. Descriptive statistics will be used. Continuous variables e.g., clinical characteristics such as CBC parameters, and laboratory correlates such as plasma drug levels, BTK occupancy, mRNA and protein levels, and NF-κB activity levels, will be summarized using the mean (SD) and/or median (range). Frequency tables will be used to summarize categorical variables.

12 REPORTING REQUIREMENTS

Please see Appendix B.

13 CONFIDENTIALITY PLAN

All data will be entered in PDMS/CORE.

Appendix A. IWCLL categories of response in CLL.

Parameter	CR*	PR*	PD*
Group A			
Lymphadenopathy†	None > 1.5 cm	Decrease ≥ 50%	Increase ≥ 50%
Hepatomegaly	None	Decrease ≥ 50%	Increase ≥ 50%
Splenomegaly	None	Decrease ≥ 50%	Increase ≥ 50%
Blood lymphocytes	< 4000/μL	Decrease ≥ 50% from baseline	Increase ≥ 50% over baseline
Marrow‡	Normocellular, < 30% lymphocytes, no B-lymphoid nodules. Hypocellular marrow defines CRi (5.1.6).	50% reduction in marrow infiltrate, or B-lymphoid nodules	
Group B			
Platelet count	> 100 000/μL	> 100 000/μL or increase ≥ 50% over baseline	Decrease of ≥ 50% from baseline secondary to CLL
Hemoglobin	> 11.0 g/dL	> 11 g/dL or increase ≥ 50% over baseline	Decrease of > 2 g/dL from baseline secondary to CLL
Neutrophils‡	> 1500/μL	> 1500/μL or > 50% improvement over baseline	

Group A criteria define the tumor load, group B criteria define the function of the hematopoietic system (or marrow).

*CR (complete remission): all of the criteria have to be met, and patients have to lack disease-related constitutional symptoms; PR (partial remission): at least two of the criteria of group A plus one of the criteria of group B have to be met; SD is absence of progressive disease (PD) and failure to achieve at least a PR; PD: at least one of the above criteria of group A or group B has to be met.

†Sum of the products of multiple lymph nodes (as evaluated by CT scans in clinical trials, or by physical examination in general practice).

‡These parameters are irrelevant for some response categories.

Appendix B. Reporting requirements.

These guidelines will be followed for the recording and reporting of adverse and serious adverse events.

1. Baseline events will be recorded in the medical history section of the case report form and will include the terminology event name, grade and start date of the event.
    a. Baseline events are any medical condition, symptom, or clinically significant lab abnormality present before the informed consent is signed.
        i. Hematologic laboratory abnormalities will not be recorded as baseline events for patients with acute leukemia, myelodysplastic syndrome, chronic lymphocytic leukemia, or chronic myeloid leukemia in blast phase.
        ii. If exact start date is unknown, month and year or year may be used as the start date of the baseline event.
2. The maximum grade of the adverse event will be captured per course of protocol defined visit date.
3. These adverse events will be recorded in the case report form:
    a. Any grade adverse event that is possibly, probably or definitely related to the study drugs(s).
    b. All serious adverse events regardless of attribution to the study drug(s).
    c. Any grade adverse event regardless of attribution to the study drug(s) that results in any dose modification.
4. Hematologic adverse events will not be recorded or reported for studies in patients with acute leukemia, myelodysplastic syndrome, chronic lymphocytic leukemia, or chronic myeloid leukemia in blast phase except for:
    a. Prolonged myelosuppression as defined by the NCI-CTCAE criteria specific for leukemia, e.g., marrow hypocellularity on day 42 or later (6 weeks) from start of therapy without evidence of leukemia (<5% blasts), or that results in dose modifications, interruptions or meets the protocol definition of dose limiting toxicity (DLT) or serious adverse event (SAE).
5. Serious adverse events will be reported according to institutional policy.
6. Protocol specific language regarding the recording and reporting of adverse and serious adverse events will be followed in the event of discordance between the protocol and Leukemic-specific adverse event recording and reporting guidelines.

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

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

- Death
- A life-threatening adverse drug experience – any adverse experience that places the patient, in the view of the initial reporter, at immediate risk of death from the adverse experience as it occurred. It does not include an adverse experience that, had it occurred in a more severe form, might have caused death.

- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
- A congenital anomaly/birth defect.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse (21 CFR 312.32).

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

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

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

- Unless otherwise noted, the SAE log will be utilized for safety reporting to the MDACC IRB.

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

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

Reporting to FDA:

- Serious adverse events will be forwarded to FDA by the MDACC IRB according to 21 CFR 312.32.

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

Investigator Communication with Supporting Companies: N/A

Reporting of External SAEs

The MDACC institutional policy for reporting of external SAEs will be followed.

APPENDIX C. Pharmacodynamic Investigations

The objectives of the pharmacodynamic investigations during this clinical trial are as follows:

To determine the effects of different daily doses of ibrutinib (420 mg, 280 mg, 140 mg) on free drug levels in plasma, BTK occupancy by ibrutinib, BTK mRNA, BTK phospho- and total protein levels, B-cell receptor down-stream signaling targets, NF-κB activity and platelet functionality such as Syk protein activation.

All patients will be investigated as this clinical trial is designed with pharmacodynamics as the primary investigation. Patients will sign consent forms to enter these investigations. Once a patient has consented to participate in the investigation, please contact Ms. Yuling Chen (713-404-2550) or Dr. Lisa Chen's office (713-793-6962) in the Department of Experimental Therapeutics. Please collect blood samples as indicated below in green- or yellow-top tubes at the following times.

Pre-Dose (Pretreatment sample) **(two green top tubes, one yellow top tube)**
Cycle 1,2,3, Day 1 - 4 hr **(one green top tube)** ***and 24 hr*** **(two green top tubes, one yellow top tube)**
Cycle 1,2,3, Day 8 - 4 hr **(one green top tube)** ***and 24 hr*** **(two green top tubes)**
Cycle 1,2,3, Day 28 - 24 hr **(two green top tubes, one yellow top tube)**

Each blood sample (~10 ml) will be collected in a Vacutainer green top tube. The samples will be mixed and immediately put on ice-bath. Blood tubes will be picked up by Dr. Gandhi's laboratory personnel and further processed to isolate plasma, platelets and CLL lymphocytes. Plasma will be used for ibrutinib pharmacology and for cytokine/chemokine assays; while platelets and lymphocytes will be used for protein and mRNA assays.

Plasma pharmacology: Ibrutinib levels will be quantitated by high performance liquid chromatography with tandem mass spectrometric detection at 4 and 24 hours post-dosing on days 1, 8, and 28 (but before the first dose of the next cycle) of each cycle. Exception: there will be no 4-hour blood draw on day 28.

Chemokine profiling: Plasma will be used to quantitate changes in CCL3, CCL4, and SDF1 levels.

BTK occupancy assay: This will be done using fluorescent affinity probe detection in pretreatment and at 4 and 24 hours post-dosing on days 1, 8, and 28 (but before the first dose of the next cycle). Exception: there will be no 4-hour blood draw on day 28.

Measurement of platelet functionality: Syk protein activation will be evaluated in platelets isolated 24 hours post-treatment on days 1 and 28 of each cycle to determine if changes occur with different doses of ibrutinib during therapy.

Molecular profiling: The lymphocytes obtained in real-time during pre- and post-therapy will be saved and later sent for molecular profiling to determine a spectrum of total and phospho-proteins that have significance in CLL as well as in the B-cell receptor pathway.

BTK downstream pathway analysis: The leukemic lymphocytes will be washed, pelleted and saved to determine the decrease in target proteins BTK, pBTK (Tyr^{223}) and downstream ERK, pERK(Thr^{202}/Tyr^{204}), AKT, pAKT (ser^{473}) and NK-kB. Additional studies may be done to determine other downstream pathway molecules.

RT-PCR assays: To determine if changes in protein levels (identified during immunoblot assays) are due to changes in transcript levels, we will do RT-PCR assays on these samples.

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