**CLINICAL RESEARCH PROJECT**

NCT02514083
**Protocol** #: 15-H-0172
**Drug Names:** Ibrutinib and Fludarabine
**IND:** Exempt

**Title:** A Pilot Phase II Study of Ibrutinib and Short-Course Fludarabine in Previously Untreated Patients with Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma (CLL/SLL)

**Short Title:** Ibrutinib and fludarabine in untreated CLL

**Keywords**: Ibrutinib, Fludarabine, BTK Inhibitor, T-cell Modulation, CLL, SLL

**Principal Investigator:**
Inhye Ahn, M.D., NHLBI (E)*

**Accountable and Medically Responsible Investigator**:
Adrian Wiestner, M.D., Ph.D., HB, NHLBI (E)*

**Associate Investigators**:
Raul Braylan, M.D., DLM, CC, NIH (E)
Katherine R. Calvo, M.D., Ph.D, DLM, CC, NIH (E)
Erika Gaglione, BA, HB, NHLBI (C)
Thomas Hughes, Pharm.D., CC (E)
Jennifer Lotter, PA (E)*
Irina Maric, M.D., DLM, CC, NIH (E)
Gerald Marti, M.D., Ph.D., NHLBI (V)*
Pia Nierman, RN., HB, NHLBI (E)
Christopher Pleyer, M.D., HB, NHLBI (E)*
Susan Soto, M.S.N., RN., HB, NHLBI (E)
Maryalice Stetler-Stevenson, M.D., CCR (E)
Clare Sun, M.D., HB, NHLBI (E)*
Jeanine Superata, FNP, HB, NHLBI (E)*
Xin Tian, Ph.D., Biostatistician, OBR, NHLBI (E)
Janet Valdez, PA-C, HB, NHLBI (E)*
Constance Yuan, M.D., CCR (E)
*Indicates who may obtain informed consent.

**Company Providing Investigational Drug:** Pharmacyclics, an AbbVie Company

**Subjects of Study:**

| Number | Sex | Age-range |
|---|---|---|
| 32 | M/F | > 18 |

| | |
|---|---|
| Ionizing Radiation for Research: | Yes |
| Off-Site Project: | No |
| Multi-center trial: | No |
| DSMB Involvement: | Yes |

## PRECIS

Chronic lymphocytic leukemia (CLL) and/or small lymphocytic lymphoma (SLL) are tumors of B cells that often affect elderly patients. While the cause of CLL is still unclear, studies have indicated critical factors required for the tumor cells. First, CLL cells grow and survive because they receive signals through the B-cell receptor (BCR); and second, CLL cells benefit from interactions with other cells, especially T cells.

The stimulation through the BCR can be reduced with ibrutinib, which is an oral drug that selectively inhibits Bruton's tyrosine kinase (BTK). In clinical trials, ibrutinib demonstrated safety and high response rates in patients with high-risk disease. Ibrutinib has gained FDA approval as a treatment for CLL patients with 17p deletion and for those who had at least one prior therapy. However, single-agent ibrutinib has limitations; the drug does not eliminate all the tumor cells, and, with time, the tumor cells may become resistant. Therefore, combination of ibrutinib with other drugs could be beneficial. Here we chose fludarabine because it is a well-tolerated drug that has been used widely to treat CLL. Also, fludarabine can kill both malignant B cells and T cells that support the growth of leukemia cells. With this approach we hope to restore healthier immune system.

This study will investigate the safety and efficacy of ibrutinib combined with fludarabine. This protocol is intended for previously untreated CLL patients. Ibrutinib will be given daily until disease progression or intolerable side effects occur. Fludarabine will be given only in cycles 3 and 4.

## 1.0 OBJECTIVES

### 1.1 Primary objective

- To test the safety and efficacy of ibrutinib-based therapy combined with a short-course fludarabine in previously untreated patients with chronic lymphocytic leukemia (CLL) and/or small lymphocytic leukemia (SLL)

### 1.2 Secondary objectives

- Duration of response
- Best response
- Minimal residual disease status
- To explore the biologic effects on B- and T-cell subsets and function
- To explore the lack of clinical response

## 2.0 BACKGROUND AND SCIENTIFIC JUSTIFICATION

***Chronic Lymphocytic Leukemia (CLL) and/or small lymphocytic lymphoma (SLL)***

The World Health Organization classification of hematopoietic neoplasms describes CLL as a leukemic, lymphocytic lymphoma, being only distinguishable from SLL by its leukemic appearance.[1] In the National Cancer Institute-sponsored Working Group (NCI-WG) guidelines, the diagnosis of CLL requires the presence of at least 5 x $10^9$ clonal B lymphocytes/L (5000/μL) in the peripheral blood.[2] The definition of SLL requires the presence of lymphadenopathy and/or splenomegaly. Clonal B lymphocytes in the peripheral blood should not exceed 5 x $10^9$/L and the diagnosis should be confirmed by histopathologic evaluation of a lymph node biopsy when possible.[3]

CLL/SLL cells coexpress the T-cell antigen CD5 and B-cell surface antigens CD19, CD20 and CD23. The levels of surface immunoglobulin, CD20, and CD79b are characteristically low compared with those found on normal B cells. Each clone of leukemia cells is restricted to express either kappa or lambda immunoglobulin light chains.[3]

For the purposes of this study, the term "CLL" will encompass both CLL and SLL.

### 2.1 Pathophysiology

CLL is characterized by clonal proliferation of auto-reactive B cells mediated by B-cell receptor (BCR) signaling. The BCR is a multimeric complex formed by the assembly of surface immunoglobulin (SIg) homodimer and Igα/Igβ (CD79a/CD79b) heterodimer. Engagement of antigen at BCR phosphorylates immunoreceptor tyrosine-based activation motifs (ITAM) motif and links Ig chains to intracellular Src family tyrosine kinases. Activation of BCR signaling cascade leads to recruitment and activation of the spleen tyrosine kinase (SYK),[4] and downstream activation of Bruton tyrosine kinase (BTK) and phosphoinositide 3-kinase (PI3K).[5] Therefore, inhibition of BCR signaling is an important therapeutic target for CLL.

Another key component of CLL pathogenesis is bidirectional interactions between tumor cells and microenvironment. First, tumor microenvironment has a stimulatory role and is critical for CLL cell survival. CLL cells cultured *in vitro* undergo apoptosis, but can be rescued by co-culturing them with stromal cells or by adding soluble factors.[6,7] In patients, T and CLL cells aggregate to form 'proliferation centers' within bone marrow and LN, and these rallied cells form immune synapses.[8] The crosstalk between T and CLL cells stimulates survival of leukemic cells, and promotes resistance to apoptosis through CD40 ligand.[9,10] Second, T cells in CLL are functionally[8] and phenotypically[11] defective, allowing malignant B cells to escape immune surveillance. These "pseudo-exhausted" T cells receive inhibitory signals from CLL, such as CD200, and PD-L1.[8,12] Taken together, simultaneous targeting of tumor microenvironment and BCR pathway can be a promising therapeutic strategy in CLL.

## 2.2 Epidemiology and clinical course of CLL

CLL is the most common leukemia of adults in Western countries with an annual incidence of 2–4.5 per 100,000 in the general population. Based on 1995-2011 North American Association of Central Cancer Registries (NAACCR), 14,620 men and women (8,140 men and 6,480 women) were estimated to be newly diagnosed with CLL in 2015.[13] CLL affects males more frequently than females, and is a disease of older individuals (median age 73.0 years for Caucasian).[14] The highest rates of incidence are seen in Caucasians followed by African Americans. Lower rates of incidence are seen in Asian and Hispanic populations.

Complications of CLL usually arise from progressive disease. These complications are, but are not limited to, infection, hematologic abnormalities, immunologic phenomena, secondary malignancy, and disease transformation. Up to 70% of patients with CLL will develop infections, which accounts for more than half of deaths in CLL.[15] Hematologic abnormalities include autoimmune hemolytic anemia (37%), pure red cell aplasia (6%), and immune mediated thrombocytopenia (2-4%).[15] Secondary cancers are noted in about 11% and include acute myeloid leukemia and solid tumors. Disease transformation into other lymphomas occurs in approximately 5%, and includes diffuse large B cell lymphoma and Hodgkin's lymphoma.[16]

## 2.3 Diagnostic and prognostic categories of CLL/SLL

Diagnosis of CLL is based on complete blood count (CBC) with differential, flow cytometry of peripheral blood (to determine the immunophenotype of circulating lymphocytes), and examination of peripheral blood smear. According to the 2008 update of the NCI-WG diagnosis and treatment of CLL, following two criteria must be met:[2]

- Absolute B lymphocyte count in peripheral blood $\geq$ 5000/μL (5 x $10^9$/L), with morphologically mature-appearing small lymphocytes
- Clonality of circulating B lymphocytes defined by peripheral blood flow cytometry. A majority of the population should express the following pattern: low levels of surface Ig with either κ or λ (but not both) light chains; expression of B-cell associated antigens (CD19, CD20, and CD23); and expression of the T-cell associated antigen (CD5).

Diagnosis of SLL requires the presence of lymphadenopathy and/or splenomegaly confirmed by histopathologic evaluation of LN through biopsy when possible, while peripheral blood B lymphocytes not exceeding 5 x $10^9$/L.

There are two prognostic staging systems in CLL called Rai and Binet. Rai staging system is based on gradual and progressive increase in leukemic cell burden - starting from blood and bone marrow, progressively involving lymph nodes, and followed by spleen and liver involvement. This will lead to eventual compromise of bone marrow function (Table 1).

Table 1: Rai staging system[17]

| Stage | Risk | Manifestations | Median survival (months) |
|---|---|---|---|
| 0 | Low | Blood and marrow lymphocytosis | 120 |
| I | Intermediate | Lymphocytosis and adenopathy | 108 |
| II | Intermediate | Lymphocytosis and adenopathy | 94 |
| III | High | Lymphocytosis + anemia (Hb < 11g/dL) | 60 |
| IV | High | Lymphocytosis + thrombocytopenia (Plt < 100K/μL) | 60 |

Rai staging system was designed to provide prognostic information, and is of great value in stratifying patients with survival curves corresponding to the Rai low-, intermediate-, and high-risk groups, respectively. However, this risk stratification system has a limitation in underestimating the risk of progression in subsets of early stage patients. For this reason, other prognostic markers, such as immunoglobulin variable heavy chain (IGHV) genes, ξ (zeta)-chain associated protein 70 (ZAP-70), and CD38, have been used to identify patient subsets with aggressive clinical phenotype. IGHV gene is defined as mutated when there is a greater than 2% difference in

nucleotide sequence compared to germline DNA.[18] Unmutated IGHV gene status is associated with shorter survival and the higher risk of relapse after treatment, including hematopoietic stem cell transplantation.[19] ZAP-70 is a tyrosine kinase normally expressed by NK and T cells, but not in normal B cells. A subset of CLL patients express ZAP-70, which is strongly associated with unmutated IGHV status and correlated to poor prognosis.[20]

Genetic abnormalities are another important prognostic marker (Table 2). Development of fluorescence in situ hybridization (FISH) in the late 1980s and early 1990s allowed a more sensitive detection of chromosomal abnormalities. Using the FISH testing in interphase cells, Döhner *et al.* identified that deletion 13q14 was the most common isolated chromosomal abnormality in CLL (64%).[21] Deletion 13q14 is currently believed to be a primary event in CLL, supported by its presence in clonality, and, at many times, as the sole abnormality. The prognosis of patients with deletion 13q14 appears to be better than, if not similar to, that of patients with a normal karyotype.[21] Trisomy 12 is thought to confer poor prognosis, and is associated with an atypical morphology and immunophenotype, high proliferation rate, and advanced clinical stages. Trisomy 12 is typically found as a minority of tumor cells or as a subclone, and may be a secondary abnormality in CLL.[21]

Table 2: Cytogenetic Abnormalities in CLL[21]

| Chromosome | Median OS (months) | Median time to first treatment (months) |
|---|---|---|
| Deletion 17p13 | 32 | 9 |
| Deletion 11q22-23 | 79 | 13 |
| Trisomy 12 | 114 | 49 |
| Normal | 111 | 33 |
| Deletion 13q14 | 133 | 92 |

Deletion 17p is found in 7-10% of newly diagnosed CLL and is a predictor of poor prognosis among patients requiring therapy. Using karyotype banding, Giesler *et al.* reported deletion 17p to be associated with poor survival, and as the only genetic abnormality with an independent prognostic value.[22] Deletions 11q, seen in 17-20% of patients, is associated with certain clinical phenotypes, such as extensive adenopathy, progressive disease and shorter survival in patients under age 55.[21] Ataxia telangiectasia mutated (*ATM*) gene is mapped at chromosome 11q, and its loss or mutation involving both alleles has been associated with poor overall survival in patients, and impaired cellular response to irradiation as well cytotoxic agents in vitro.[23] However, the poor prognosis of 11q deletion may be overcome with the use of fludarabine, cyclophosphamide, and rituximab.[24] The prognostic impact of 12q trisomy is controversial with conflicting evidences. For instance, some studies have demonstrated advanced disease and higher proliferative activity in patients with trisomy 12, while others have demonstrated similar survival to that of normal karyotype.[25] Deletion 13q, present in 45-55% of patients, appears to be associated with a favorable outcome.[21] It is often associated with inactivation of *RB1* gene,[26] and its minimal deleted region is also suspected to harbor non-coding genes with a tumor suppressor role.

Other prognostic markers, such as lymphocyte doubling time (LDT), bone marrow histologic pattern, and β2 microglobulin (B2M), are useful as an adjunct to genetic abnormalities. Actual or projected LDT can provide an estimation of disease kinetics, and its value less than 12 months in untreated patients predicts progressive disease course.[27] The pattern of lymphocyte infiltration in the bone marrow can be correlated to a progressive clinical course (diffuse pattern) versus an indolent one (interstitial and/or nodular pattern).[28] Finally, the B2M levels correlate with disease stage and tumor burden in CLL.[29]

### 2.4 Indications for CLL Treatment

Newly diagnosed patients with asymptomatic early-stage disease (Rai 0, Binet A) should be monitored without therapy unless they have evidence of disease progression.[30] Patients with intermediate to high Rai stages (3 and 4) or Binet stages (B and C) usually benefit from treatment.

According to the International Workshop on Chronic Lymphocytic Leukemia (IWCLL), active disease should meet one of the following criteria.[3]

- Evidence of progressive marrow failure as manifested by the development of, or worsening of, anemia and/or thrombocytopenia
- Massive (i.e., at least 6 cm below the left costal margin) or progressive or symptomatic splenomegaly
- Massive (i.e., at least 10 cm in longest diameter) or progressive or symptomatic lymphadenopathy
- Progressive lymphocytosis with an increase of more than 50% over a 2-month period or 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.
- Autoimmune anemia and/or thrombocytopenia that is poorly responsive to corticosteroids or other standard therapy
- 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 (i.e., ECOG PS 2 or worse; 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.

## 2.5 Current Treatment options for CLL/SLL

### 2.5.1 Watchful Waiting

Immediate treatment is recommended in patients with advanced-stage disease, high tumor burden, and severe disease-related "B" symptoms. Otherwise, a period of observation is recommended. Asymptomatic early-stage disease should be monitored until disease progression.[3] During the observation period, patients are periodically monitored with blood counts and assessed for the evidence of progressive disease through monitoring of symptoms and physical exam.

### 2.5.2 First-line Chemotherapy/Immunotherapy/Chemoimmunotherapy

***Monotherapy:***

Monotherapy with alkylating agents has served as an initial frontline treatment strategy for CLL. Chlorambucil, which has been considered as the gold standard for CLL for several decades, still remains to be an appropriate option for unfit elderly patients.[30] Bendamustine is also approved by the U.S. Food and Drug Administration (FDA) in 2008 for the treatment of CLL. When chlorambucil and bendamustine were compared in previously untreated CLL patients, higher overall response rate (ORR) was achieved with bendamustine (68%) than chlorambucil (48%).[31]

Aside from alkylating agents, purine analogues such as fludarabine, pentostatin and cladribine have been used as monotherapies in CLL. Among those, fludarabine is the best-studied compound with a superior overall response as a monotherapy compared to other alkylating agents.[32]

***Immunotherapy:***

The advent of monoclonal antibodies opened the era of targeted therapy for hematologic malignancies. Rituximab was the first monoclonal antibody targeting CD20, which was initially used as a monotherapy demonstrated improved progression-free survival (PFS) in a consolidation setting (estimated 5-year PFS 73%).[33] The addition of anti-CD20 monoclonal antibodies to chemotherapy (referred as chemoimmunotherapy) has proven to be very efficacious in CLL and will be discussed below.

***Chemoimmunotherapy:***

Alkylating agents, purine analogues and monoclonal antibodies have different mechanisms of action and partially non-overlapping toxicity profiles. When combined together, the regimen led to improved responses in CLL. For instance, the combination of fludarabine, cyclophosphamide, and rituximab (FCR) resulted in an ORR of 90% (complete response (CR) in 44%, partial response (PR) in 46%).[34] Bendamustine combined with rituximab (BR) also has as impressive activities as FCR. In previously untreated CLL, BR led to ORR of 88% (CR in 23%, PR in 65%) and 91% survival after a median observation time of 27 months.[35] Efficacy and safety of BR and FCR were directly compared in German CLL study group, which showed identical ORR of two arms (97.8% in both arms).[36] Depth of response was higher in FCR (CR in 47.4%) than BR (CR in 38.1%), as well as PFS and event-free survival. However, these differences did not translate into survival (OS 94.2% in FCR *vs.* 95.8% in BR at 2 years) due to increase toxicity with FCR.

Several other chemoimmunotherapy regimens have been investigated, which include cladribine plus rituximab, methylprednisolone plus rituximab, fludarabine plus alemtuzumab, and fludarabine, cyclophosphamide plus alemtuzumab. None of these regimens resulted in efficacy comparable to FCR.[37]

Geode *et al.* studied a third generation anti-CD20 monoclonal antibody called obinutuzumab in combination with chlorambucil for previously untreated CLL patients.[38] As expected, obinutuzumab and chlorambucil had a higher efficacy than chlorambucil alone, with median PFS of 27 *vs.* 11 months, respectively. Obinutuzumab gained FDA approval to be used in combination with chlorambucil in 2013.

### 2.5.3 Complications of Chemoimmunotherapy

Major limitations of highly active chemoimmunotherapy in CLL - most notably, the FCR regimen - are the durability of remission and toxicities. Long-term follow up of patients treated with front-line FCR revealed only one third remained in remission while approximately half of them had refractory or relapsing disease.[39] As for toxicities, more than 90% of patients treated with FCR and 78.5% treated with BR experienced moderate to severe adverse events (CTCAE grade 3 to 5).[36] Hematologic toxicities were more frequent in the FCR arm (90.0% *vs.* 66.9%), leading to higher rates of severe neutropenia (81.7%) and severe infections (39.0%) with the FCR arm. Treatment related mortality occurred in 3.9% in the FCR and 2.1% in the BR arm. [36] Monoclonal antibodies also add infusional toxicities as seen in rituximab and obinutuzumab. Toxicities of chemoimmunotherapy can linger or present in a delayed manner. For example, 20% of patients treated with fludarabine and other alkylators were reported to experience persistent cytopenia.[39] Intergroup study E2997 reported 5% incidence of myeloid neoplasia 6 years after fludarabine and cyclophosphamide therapy[31]; the adjusted incidence for myeloid neoplasia rate at 8 years was 8%.

### 2.5.4 Relapsed or Refractory CLL

There is no standard regimen for patients who has relapsed or refractory CLL. Treatment for this population should be individualized based on host and biological factors, including fitness of a patient, comorbidities, disease burden (i.e., bulky or non-bulky), and FISH cytogenetics (i.e., deletion 17p).[32]

Treatment options for relapsed or refractory CLL encompass aforementioned front-line regimens. In a phase II trial of FCR in 284 patients with previously treated CLL (19% were fludarabine-refractory and 35% were rituximab-refractory), ORR was 74 % and median OS was 47 months.[40] Presence of fludarabine-refractory disease, deletion 17p, or more than 3 prior treatments predicted shorter survival in subgroup analysis. Another option is BR, which resulted in ORR of 59% in a multicenter phase II trial of 78 relapsed and/or refractory CLL.[41] However, the duration of response is usually short-lived in this population (median duration of response: 15 months).[41]

Ofatumumab is a second-generation anti-CD20 antibody being used for relapsed/refractory disease. A phase II trial evaluated ofatumumab in 138 heavily pre-treated relapsed and/or refractory CLL patients, and reported ORR of 47-58% with median OS of 13.7-15.4 months.[42] Notably, the activity of ofatumumab appeared to be independent of prior exposures to rituximab. In 2009, the U.S. FDA granted an accelerated approval of ofatumumab for the treatment of CLL patients refractory to both fludarabine and alemtuzumab.

While various treatment options are available for relapsed/refractory CLL patients, they do not offer durable remissions, are certainly not curative, and are frequently toxic. In most cases, the goal of second- or high-lines of treatment is to bridge patients into the next treatment or to reduce tumor burden before hematopoietic stem cell transplant.

### 2.5.5 Stem Cell Transplantation:

Median age of CLL patients at diagnosis is 72.[43] Given the advanced age and relatively benign course of the disease in many patients, few are considered for aggressive treatments such as hematopoietic cell transplantation. Still, stem cell transplant remains as the only curative option for CLL to date, and has demonstrated improvement in transplant-related mortality. This option is currently reserved to those small subsets of patients with good performance status and high-risk features, such as 17p deletion or refractoriness to prior therapies.

### 2.5.6 Targeted Tyrosine Kinase Inhibitors

Orally bioavailable kinase inhibitors are currently being developed.[44] Idelalisib (CAL-101, GS-1101) is a selective inhibitor of the p110δ isoform of PI3Kδ. Firocari *et al.* reported a phase 1 study that enrolled 54 heavily pretreated relapsed/refractory CLL patients, and treated them with continuous once- or twice-daily doses ranging from 50 to 350 mg per dose.[45] After a median of 9 months drug exposure, ORR was 39%, and nodal PR was 50% (defined as 50% reduction of lymph node size from baseline). Median PFS was 17 months, which increased to 29 months for those receiving 150 mg twice per day or greater. Dose-limiting toxicities were not observed, and potentially treatment-related adverse events (fatigue, rash, diarrhea, respiratory tract infections, and reversible increases in hepatic transaminases) resulted in discontinuation of treatment in only 7% of patients. Because PI3Kδ influences clonal expansion and differentiation of suppressor T cells, some of these events, particularly diarrhea and/or colitis, may represent on-target toxicities of idelalisib. Idelalisib was FDA approved in 2014 for the treatment of patients with relapsed CLL, in combination with rituximab, for whom rituximab alone would be considered appropriate therapy due to other co-morbidities.

Given promising response rates of kinase inhibitors, investigators have started using these kinase inhibitors in combinations with chemotherapy, monoclonal antibodies, and other kinase inhibitors. The majority of these studies remain early in accrual and maturity. Idelalisib has been studied in combination with rituximab versus placebo and rituximab.[46] Sharman *et al.* demonstrated that the median PFS was not reached in idelalisib arm as compared to 5.5 months in the placebo arm (hazard ratio for progression or death in the idelalisib group, 0.15; P<0.001). Patients receiving idelalisib versus placebo had improved rates of ORR (81% *vs.* 13%; odds ratio, 29.92; P<0.001) and OS at 12 months (92% *vs.* 80%; hazard ratio for death, 0.28; P=0.02). Serious adverse events occurred in 40% of the patients receiving idelalisib and rituximab and in 35% of those receiving placebo and rituximab.

Ibrutinib also gained FDA approval in 2014 as a treatment for CLL who had at least one prior therapy or who has deletion 17p. Reported clinical experiences as a single-agent and in combinations with ibrutinib will be discussed in detail in section **2.6.2.**

### 2.5.7 Other Options

The intrinsic apoptotic pathway is often dysregulated in relapsed CLL/SLL due to a deficiency in pro-apoptotic proteins such as *TP53* and overexpression of anti-apoptotic proteins such as Bcl-2. ABT-199 is a selective, potent, orally bioavailable, small molecule Bcl-2 inhibitor that can trigger apoptosis. Seymour et al looked at 56 patients were enrolled with a median time on study of 10.0 months. ABT-199 showed activity in patients with relapsed/refractory CLL with a response rate of 84% including 20% CR and CR with incomplete marrow recovery (CRi). The most common adverse events (all grades involving 25% or greater patients) were diarrhea (46%), neutropenia (43%), fatigue (34%), upper respiratory tract infection (29%), and cough (25%). Grade 3 or 4 adverse events occurring in 4 or more patients were neutropenia (41%), TLS (11%), thrombocytopenia (10%), hyperglycemia (10%), anemia (7%), and febrile neutropenia

(7%). This study is continuing enrollment using a revised dosing schedule designed to reduce the identified risk of tumor lysis syndrome. A phase 2 monotherapy study in patients with relapsed CLL patients with deletion 17p has commenced as well as combination studies with either rituximab or obinutuzumab in patients with relapsed CLL. [47]

## 2.6 Ibrutinib

### 2.6.1 Mechanism of Action, Distribution, Metabolism and Clearance[48]

Ibrutinib (PCI-32765) is a covalent BTK inhibitor, which blocks BCR signaling and induces apoptosis of malignant B cells.[49] *In vitro*, ibrutinib forms a potent irreversible bond with cysteine-481 residue at the active site of BTK (IC50 = 0.39 nM), and results in sustained inhibition of BTK catalytic activity and enhanced selectivity over other kinases that do not contain a cysteine at this position. When added directly to human whole blood, ibrutinib inhibits signal transduction from the BCR (IC50 = 80 nM) as assayed by anti-IgM stimulation followed by CD69 expression.[50] In human B cells, ibrutinib blocked the transcriptional up-regulation of B-cell activation genes within 6 hours. 1-hour pulse exposure of ibrutinib followed by washout was sufficient to prevent up-regulation of these genes, indicating that ibrutinib acts as an irreversible inhibitor of the BCR pathway.[51]

The plasma protein bindings of ibrutinib and PCI-45227 are 97.3% and 91.0%, respectively. The apparent steady-state volume of distribution (Vdss/F) is approximately 10,000L (based on non-compartmental and population PK analyses), suggesting extensive distribution to peripheral tissues and/or binding to macromolecules in circulation. The blood-to-plasma ratio around Tmax is approximately 0.7.

Following oral administration of ibrutinib at doses ranging of 420, 560, and 840 mg/day, exposure to ibrutinib increased as doses increased with substantial intersubject variability. The mean half-life (t1/2) of ibrutinib across 3 clinical studies ranged from 4 to 9 hours, with a median time to maximum plasma concentration (Tmax) of 2 hours. Taking into account the approximate doubling in mean systemic exposure when dosed with food and the favorable safety profile, ibrutinib can be dosed with or without food. Ibrutinib is extensively metabolized primarily by cytochrome P450 (CYP) 3A4. The on-target effects of metabolite PCI-45227 are not considered clinically relevant. Steady-state exposure of ibrutinib and PCI-45227 was less than 2-fold of first dose exposure. Less than 1% of ibrutinib is excreted renally. Ibrutinib exposure is not altered in patients with creatinine clearance (CrCl) >30 mL/min. Patients with severe renal impairment or patients on dialysis have not been studied. Following single dose administration, the AUC of ibrutinib increased 2.7-, 8.2- and 9.8-fold in subjects with mild (Child-Pugh class A), moderate (Child-Pugh class B), and severe (Child-Pugh class C) hepatic impairment compared to subjects with normal liver function. A higher proportion of Grade 3 or higher adverse reactions were reported in patients with B-cell malignancies (CLL, MCL and WM) with mild hepatic impairment based on NCI organ dysfunction working group (NCI-ODWG) criteria for hepatic dysfunction compared to patients with normal hepatic function.

Excretion is predominantly via feces (approximately 80% recovered within 2 days), and less via urine (~8%). The apparent clearance in subjects with B-cell malignancies is high: in the order of 1000 L/h. The half-life of ibrutinib is 4-6 hours. Overall, these pharmacokinetic (PK) characteristics resulted in minimal accumulation of both parent compounds and metabolite PCI-45227 on repeated daily dosing of ibrutinib. Steady-state exposure of ibrutinib and PCI-45227 was less than 2-fold of first dose exposure.

### 2.6.2 Clinical Experience in CLL

Once-daily administration of ibrutinib causes sustained inactivation of BTK resulting inhibition of BCR signaling and tumor microenvironment interactions.[52,53] _ENREF_53 The phase 1b/2 multicenter ibrutinib trial reported by Byrd *et al.* involved 85 relapsed CLL patients where 51 received 420 mg, and 34 received 840 mg.[54,55] ORRs were 71% in both dosing groups, with an additional 15-20% had a partial response with lymphocytosis (PR-L). At 26 months, the estimated PFS was 75%, and OS was 83%. A single-agent

ibrutinib was overall well tolerated, while commonly observed side effects included thrombocytopenia, diarrhea, bruising, neutropenia, anemia, upper respiratory tract infection, fatigue, musculoskeletal pain, rash, fever, constipation, peripheral edema, arthralgia, nausea, stomatitis, sinusitis and dizziness. When ibrutinib was compared to ofatumumab in 391 previously treated CLL in a randomized phase 3 study, ibrutinib achieved significantly higher ORR (42.6% vs. 4.1%, $p<.001$) and showed survival benefit (12-month OS: 90% vs. 91%, hazard ratio 0.43, $p=.005$).[55]

Responses to ibrutinib appear to be independent of clinical and genomic risk factors. In the elderly CLL population of age 65 years or older, ibrutinib was a well-tolerated initial therapy, and led to ORR of 71% (13% had CR; 3% had nodal PR, 55% ad PR).[56] In the high-risk CLL population with *TP53* mutation, a ibrutinib was an active single-agent treatment as an initial therapy as well as for relapsed or refractory CLL, and led to ORR of 97% (55% had PR; 42% had PR-L).[57]

In July 2014, ibrutinib received a full approval in the US for CLL patients who have received at least one prior therapy, and for the patients with 17p deletion. Response to ibrutinib appears to be independent of clinical and genomic risk factors, and it currently stands as the sole FDA-approved agent with the treatment indication for CLL with 17p deletion. Long-term data regarding responses and toxicity are still maturing for ibrutinib. Notably, a 3-year post-initiation data of 132 previously untreated and relapsed/refractory CLL patients on ibrutinib did not show new safety signal.[58] The observed median duration of response was not reached for all treated patients on study, while it was 25 months for the subset with deletion 17p. 30-month PFS was 96% among patients who received ibrutinib as a frontline.[59]

Ibrutinib has been investigated in combinations with other agents including chemoimmunotherapies in CLL, and early experiences were reported to date. Barrientos *et al.* reported on 30 patients with relapsed/refractory CLL/SLL that received up to 6 cycles of BR with a continuous fixed ibrutinib dose of 420 mg/day.[60] With the median treatment duration of 16 months, the ORR was 93% (28/30 patients, including 5 CRs and 3 nodal PRs), and one additional patient achieved a PR-L. The estimated 12 month PFS was 90%, and the responses appeared to be independent of high-risk features. The most frequently reported adverse events were diarrhea (70%), nausea (66.7%), fatigue (46.7%), neutropenia (40%) and upper respiratory tract infection (36.7%). Adverse events in grade 3 or higher in severity included neutropenia (40%), maculopapular rash and fatigue (10% each), and thrombocytopenia, febrile neutropenia, and cellulitis (6.7% each).

Ibrutinib in combination with rituximab was investigated in two different phase II trials. The first study reported 40 high-risk CLL patients who were treated with daily ibrutinib and 6 cycles of rituximab.[61] At the median follow up of 14 months, 32 of 40 patients continue on therapy without disease progression. Based on 2008 IWCLL guideline, ORR was 95% (87% in PR; 8% in CR), and one patient in CR achieved minimal residual disease negativity by flow cytometry. Notably, the response rate of patients with 17p deletion or *TP53* mutation was 90%, with the majority of them achieving PR. Treatment generally was well tolerated, with infectious complications being the most common adverse event. 8 patients came off study; 3 patients died from unrelated infectious complications (2 cases of sepsis, 1 case of pneumonia), 1 died from unrelated respiratory and cardiovascular failure, 2 came off study due to possible ibrutinib-related toxicity (one subdural hematoma, one grade 3 mucositis), 1 had progressive disease, and 1 proceeded to stem cell transplantation. The second study randomly assigned 93 relapsed CLL patients to either ibrutinib alone or ibrutinib and rituximab, and reported more rapid abrogation of lymphocytosis in the combination arm.[62]

Byrd *et al.* reported on ibrutinib in combination with ofatumumab for treatment of relapsed CLL/SLL. For the 27 enrolled CLL/SLL patients, ORR defined by IWCLL criteria was 100%, PFS was 100% at the median follow-up of 9.8 months.[63] 89% of CLL/SLL/prolymphocytic leukemia (PLL) patients remain on study and only one patient has discontinued treatment by proceeding to stem cell transplant. The majority of adverse events were grades 1 or 2, and no grade 3 or 4 infusion reactions, neutropenia, or thrombocytopenia have been observed.

Limitations of ibrutinib have been identified as clinical experiences are accumulating. First, deep responses with a single-agent ibrutinib are rare. When ibrutinib was combined with other agents, CR rate increased to 8% (ibrutinib/rituximab)[61] and 16% (ibrutinib plus BR),[64] which still fall short of conventional approaches.[34,35] Second, acquired mutations in BTK and in phospholipase C-gamma 2 (PLCγ2), a direct downstream target of BTK, were identified in six CLL patients who developed secondary resistance to ibrutinib.[65,66] Among them, five showed a cysteine-to-serine mutation in BTK (C481S), which disrupts the covalent binding between the drug and the kinase, and thereby dramatically decreases the potency of ibrutinib. Two patients, including one with C481S mutation, had gain-of-function mutations in PLCγ2 leading to autonomous BCR signaling.[65]

## 2.7 Summary of Clinical Safety

A brief summary of safety data from monotherapy and combination therapy studies is provided in below. For more comprehensive safety information please refer to the current version of the IB. Additional safety information may be available for approved indications in regional prescribing labels where the study is conducted.

***For monotherapy studies:***
Pooled safety data for a total of 1318 subjects treated with ibrutinib monotherapy in 13 studies that have completed primary analysis or final analysis as of the 31 May 2016 cutoff date for the current IB update in B-cell malignancies are summarized below.

Most frequently reported treatment-emergent adverse events (TEAEs) in subjects receiving ibrutinib as monotherapy (N=1318):

| Most frequently reported TEAEs ≥ 15% [a] | Most frequently reported Grade 3 or 4 TEAEs ≥ 3% [b] | Most frequently reported Serious TEAEs ≥ 2% [c] |
|---|---|---|
| Diarrhea | Neutropenia | Pneumonia |
| Fatigue | Pneumonia | Atrial fibrillation |
| Nausea | Thrombocytopenia | Febrile neutropenia |
| Cough | Anemia | Pyrexia |
| Anemia | Hypertension | |
| Pyrexia | Diarrhea | |
| Neutropenia | Atrial fibrillation | |
| Upper respiratory tract infection | | |
| Thrombocytopenia | | |
| Edema peripheral | | |

[a] *Source is Table 6 of IB (v11),* [b] *Source is Table 8 of IB (v11),* [c] *Source is Table 9 of IB (v11).*

Rarely, in less than 1% of cases, pneumonia was attributed to the infection of Pneumocystis jirovecii. Most cases of Pneumocystis pneumonia (PCP) were reported as a single case from different monotherapy and combination studies.

***For combination therapy studies:***
Pooled safety data for a total of 423 subjects treated with various therapies in combination with ibrutinib from 4 studies conducted in B-cell malignancies, which included 1 randomized-control study, are summarized below. Therapies used in combination with ibrutinib in these studies, included BR (bendamustine and rituximab), FCR (fludarabine, cyclophosphamide, and rituximab), ofatumumab, and R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone).

Most frequently reported TEAEs in subjects receiving ibrutinib in combination therapy (N=423):

| Most frequently reported TEAEs ≥ 20% [a] | Most frequently reported Grade 3 or 4 TEAEs ≥ 3% [b] | Most frequently reported Serious TEAEs-≥ 2% [c] |
|---|---|---|
| Neutropenia | Neutropenia | Febrile neutropenia |
| Diarrhea | Thrombocytopenia | Pneumonia |
| Nausea | Febrile neutropenia | Atrial fibrillation |
| Thrombocytopenia | Pneumonia | Pyrexia |
| Fatigue | Hypertension | Cellulitis |
| Anemia | Neutrophil count decreased | |
| Pyrexia | Anemia | |
| | Fatigue | |
| | Diarrhea | |

[a] *Source is Table 10 of IB (v11),* [b] *Source is Table 12 of IB (v11),* [c] *Source is Table 13 of IB (v11).*

Rarely, in less than 1% of cases, pneumonia was attributed to the infection of *Pneumocystis jirovecii*. Most cases of Pneumocystis pneumonia (PCP) were reported as a single case from different monotherapy and combination studies.

### 2.7.1 Clinical and scientific justification for protocol design

Combinations of chemoimmunotherapies as front-line CLL treatment have significantly improved patient outcomes. Nevertheless, approximately 10% of CLL patients do not respond to fludarabine-based therapies and approximately half eventually progresses with relapsed or refractory diseases. Chemoimmunotherapies also leads to short- and long-term toxicities. These limitations call for a novel treatment approach that is both efficacious and safe.

BCR signaling, a pathway critical for malignant B-cell activation, is pharmacologically targetable. Inhibition of BTK with ibrutinib was proven to be effective and well tolerated in CLL,[52] leading to FDA-approval of ibrutinib for CLL patients who had at least one prior treatment or CLL with deletion 17p. The use of ibrutinib upfront is justified in patients with deletion 17p, and the proposed combination of ibrutinib and fludarabine may be particularly desirable in this patient population.

Along with the accumulation of clinical experiences, a single-agent ibrutinib is identified to have several limitations. First, deep responses are infrequent. CR was seen in only 2% of relapsed or refractory CLL[54] and in 13% of treatment-naïve CLL.[56] When ibrutinib was combined with other agents, CR rate increased to 8% (ibrutinib/rituximab)[67] and 16% (ibrutinib/bendamustine/rituximab),[64] which still fall short of conventional approaches.[34,35] Moreover, acquired mutations in BTK and phospholipase C-gamma 2 (PLCγ2), a direct downstream target of BTK, were identified in 6 CLL patients who developed secondary resistance to ibrutinib.[65,66] Among them, five showed a cysteine-to-serine mutation in BTK (C481S), which disrupts the covalent binding between the drug and the kinase, thereby dramatically decreasing the potency of ibrutinib.[65] We hypothesize that the addition of a second agent could augment the depth of response and prevent drug resistance. This constitutes one rationale for the trial design.

Notably, T-cells in CLL are capable of stimulating tumor cells and promoting resistance to apoptosis through CD40 ligand and other pathways.[9,10] *In vivo*, T-cells and CLL cells were shown to aggregate and form 'proliferation centers' within bone marrow and lymphoid tissues, and these rallied cells were necessary for CLL cell proliferation.[68] Using fludarabine as the second agent provides a mean to target these T-cells.

In this context, immunotherapy is an attractive option for long-term disease control. Allogeneic stem cell transplantation set the paradigm of a curative approach using immune reconstitution; however, it is only available for a minority of patients. The clinical successes with chimeric antigen-receptor (CAR) T cells provide a strategy for more general application of T-cell immunotherapy in CLL. However, T-cells in CLL

are functionally[69] and phenotypically[11] defective, or "pseudo-exhausted", due to inhibitory signals from leukemic B cells - such as CD200, PD-L1 and B7-H3[8] - and these signals appear to reduce the efficacy of CAR T cells. Fludarabine has been used successfully as a preparative agent before immunotherapies and can promote reconstitution of the T-cell compartment through homeostatic proliferation.[70]

Thus, we view CLL as a clonal proliferation of auto-reactive B-cells that escape immune surveillance, and are stimulated through BCR signaling and T-cell interaction. We propose to combine ibrutinib with a short-course fludarabine to achieve two goals: first, eliminate or greatly decrease T-cell help towards CLL cells; and second, replace the dysfunctional T-cell population. Fludarabine, a purine analog cytotoxic to both B and T cells,[32,72,73] is a biologically plausible addition to ibrutinib with a large body of experience in CLL treatment.[32,34,71] In addition, fludarabine is frequently used as a part of conditioning regimen during hematopoietic stem cell transplant[72,73] and adoptive cell transfer[74,75] with an aim to deplete lymphocytes and reconstitute immune microenvironment. Preclinical and clinical evidences support the immune modulatory role of fludarabine and subsequent recovery of T cells in CLL. Notably, reconstitution of CD4+ T, CD8+ T, and NK cells were demonstrated in CLL after treatment with fludarabine.[70] Regulatory T cells decreased after fludarabine exposure.[76]

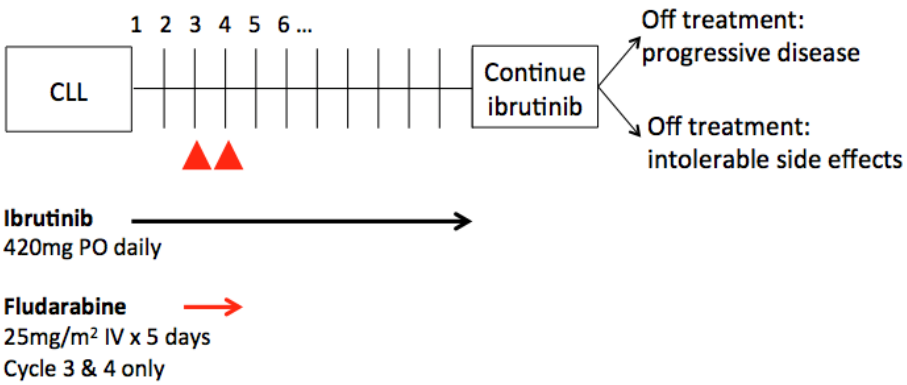
We hypothesize that recovering T-cell populations after a short-course fludarabine will have an improved functional capacity, and can exert anti-leukemic effects by the recovery of immune surveillance function. We consider the combination of ibrutinib and fludarabine as a possible platform to which immune therapeutic approaches, such as vaccines, CAR T-cells, or checkpoint inhibitors, may be added in subsequent steps. In this study, we will investigate the effect of the combination therapy on the depth of response and on the composition of the T-cell compartment. We will compare the correlative data to our experience with single-agent ibrutinib in over 70 patients, and the ongoing study using fludarabine, cyclophosphamide, and ofatumumab in previously untreated CLL patients.

The following specific issues have been considered in the study design:

- Studying untreated CLL/SLL is justifiable as the side effect profile of ibrutinib is favorable and fludarabine is the current standard front-line agent.
- The combination of ibrutinib and chemotherapy has been well tolerated[64] and there are ongoing clinical trials combining ibrutinib with chemoimmunotherapies, including a phase III study of BR plus ibrutinib (NCT01611090), a phase II study of FCR plus ibrutinib (NCT02251548), and a phase III study with an arm for ibrutinib plus rituximab (NCT02048813).

## 3.0 STUDY DESIGN

This is a phase II single center trial. It is a prospective, single-arm, open-label study of ibrutinib and a short-course fludarabine in previously untreated subjects with CLL or SLL. Each cycle is 28 days. Patients will initiate ibrutinib first for cycle 1 and 2, followed by the addition of short-course fludarabine for cycle 3 and 4. Ibrutinib will be given continuously until disease progression or intolerable side effects.



## 4.0 ELIGIBILITY ASSESSMENT AND ENROLLMENT

### 4.1 Inclusion Criteria

1. Men and women with histologically confirmed disease as defined by the following:
   - CLL: clonal B-lymphocytosis ≥ 5,000 cells/μL .
   - SLL: lymphadenopathy with the tissue morphology of CLL but that are not leukemic, < 5,000 cells/μL.
   - Immunophenotypic profile or immunohistochemistry read by an expert pathologist as consistent with CLL. This will include CD5, CD19, and CD20 expression by the CLL cells typically also with CD23 expression, but CD23 negative cases may be included if there is an absence of t (11;14)
2. Active disease as defined by at least one of the following (IWCLL consensus criteria):
   - Weight loss ≥10% within the previous 6 months
   - Extreme fatigue
   - Fevers of greater than 100.5ºF for ≥2 weeks without evidence of infection
   - Night sweats for more than one month without evidence of infection
   - Evidence of progressive marrow failure as manifested by the development of, or worsening of, anemia and/or thrombocytopenia
   - Massive or progressive splenomegaly
   - Massive nodes or clusters or progressive lymphadenopathy
   - Progressive lymphocytosis with an increase of >50% over a 2-month period, or an anticipated doubling time of less than 6 months
3. Treatment naïve CLL/SLL patients
   - Treatment-naïve CLL indicates no prior anti-CLL therapy. Anti-CLL therapy includes chemotherapies, monoclonal antibodies, and targeted agents with known or reasonably expected anti-leukemic activity.
4. Eastern Cooperative Oncology Group (ECOG) performance status of ≤ 2
5. ANC > 750/uL, platelets > 50,000/uL
6. Agreement to use acceptable methods of contraception during the study and for 90 days after the last dose of study drug if sexually active and able to bear or beget children
   - Female subjects who are of non-reproductive potential (i.e., post-menopausal by history - no menses for ≥1 year; OR history of hysterectomy; OR history of bilateral tubal ligation; OR history of bilateral oophorectomy). Female subjects of childbearing potential must have a negative serum pregnancy test upon study entry.
   - Male and female subjects who agree to use both a highly effective method of birth control (e.g., implants, injectables, combined oral contraceptives, some intrauterine devices, complete abstinence, or sterilized partner) and a barrier method (e.g. condoms, vaginal ring, sponge, etc.) during the period of therapy and for 90 days after the last dose of study drug
   - Complete abstinence is a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatments. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the subject.
7. Willing and able to participate in all required evaluations and procedures in this study protocol including swallowing capsules without difficulty
8. Ability to understand the purpose and risks of the study and provide signed and dated informed consent and authorization to use protected health information (in accordance with national and local subject privacy regulations)

### 4.2 Exclusion Criteria

- Transformed CLL, including Hodgkin and non-Hodgkin lymphoma
- Active autoimmune hemolytic anemia or thrombocytopenia

- Known bleeding disorders
- Impaired hepatic function: Total bilirubin ≥1.5 x upper limit of normal unless due to Gilbert's disease, AST/ ALT ≥ 2.5 x institutional upper limit of normal unless due to infiltration of liver, Child-Pugh class B or C
- Impaired renal function: estimated GFR < 30ml/min/1.73m$^2$ based on CKD-EPI
- Life-threatening illness, medical condition or organ system dysfunction which, in the investigator's opinion, could compromise the subject's safety, interfere with the absorption or metabolism of ibrutinib and fludarabine, or put the study outcomes at undue risk
- Concomitant immunomodulatory therapy, chemotherapy, radiotherapy or experimental therapy
- Active Hepatitis B or Hepatitis C infection
- HIV infection
- Female patients who are currently in pregnancy, or unwilling to use acceptable methods of contraception or refrain from pregnancy if of childbearing potential or currently breastfeeding. Male patients who are unwilling to follow the contraception requirements described in this protocol.
- Psychiatric illness/social situations that would limit the patient's ability to tolerate and/or comply with study requirements.
- Unable to understand the investigational nature of the study or give informed consent.
- Individuals < 18 years old
- Known hypersensitivity to any component of ibrutinib or fludarabine
- Requires concomitant anticoagulation with Coumadin (warfarin) or other vitamin K antagonists.
- Prior malignancy, except for adequately treated basal cell or squamous cell skin cancer, in situ cervical cancer, or other cancer from which the subject has been disease-free for ≥ 2 years or which will not limit survival to < 2 years.
- Malabsorption syndrome, disease significantly affecting gastrointestinal function, or resection of the stomach or small bowel or ulcerative colitis, symptomatic inflammatory bowel disease, or partial or complete bowel obstruction.
- History of stroke or intracranial hemorrhage within 6 months before the first dose of study drug
- Major surgery within 4 weeks of first dose of study drug.
- Currently active, clinically significant cardiovascular disease such as uncontrolled or symptomatic arrhythmias, Class 3 or 4 congestive heart failure as defined by New York Heart Association Functional Classification, or a history of myocardial infarction, or unstable angina, or acute coronary syndrome within 6 months of screening.
- Subjects who received a strong cytochrome P450 (CYP) 3A inhibitor within 7 days prior to the first dose of ibrutinib or subjects who require continuous treatment with a strong CYP3A inhibitor.

## 5.0 TREATMENT PLAN

### 5.1 Drug administration

Ibrutinib is an orally administered product. Study drug ibrutinib administration will take place in the outpatient facility of the NIH clinical center or at the patient's home. The drug dose is 420 mg PO daily. There is one specific dietary restriction to avoid grapefruit juice and Seville oranges due to CYP450 3A4 inhibition. Each dose of ibrutinib is to be taken with 8 ounces (approximately 240mL) of water. The capsule should be swallowed intact and subjects should not attempt to open the capsule or dissolve them in water. Ibrutinib will be given daily until disease progression or intolerable side effects.

Fludarabine is given as an intravenous infusion on cycle 3 and 4. Fludarabine administration will take place in the Clinical Center Day hospital. At the PI's discretion, select subjects may be admitted to the inpatient unit for the first few days of fludarabine infusion. Subjects will have a peripheral line placed. Per PI discretion, a central line may be placed in select patients. Fludarabine dose is 25 mg/m$^2$/day on days 1-5 of cycles 3 and 4. Dosing will be based on actual weight for all patients. No dose adjustment is required based on age. In the event of renal impairment, dose reduction will be as follows;

- Creatinine clearance of 30-70 mL/min/1.73m$^2$ using the CKD-EPI equation with actual body weight: Fludarabine will be dose reduced by 20%.
- Creatinine clearance of ≤ 30 mL/min/1.73m$^2$ using the CKD-EPI equation with actual body weight: Fludarabine will be held at the discretion of the PI.

**5.2 Therapy schedule:** All cycles will be 28 (+/- 5) days long.

- Cycle 1 and 2: ibrutinib 420mg PO daily on day 1-28
- Cycle 3 and 4: ibrutinib 420mg PO daily on day 1-28, fludarabine 25mg/m2/day on day 1-5
- Cycle 5 and subsequent cycles up to C27: ibrutinib 420mg PO daily on day 1-28

**5.3 Prophylactic Medications:**

- Allopurinol 300mg PO once daily (or alternative agent in case the subject cannot tolerate allopurinol) during cycle 3 and 4 on days 1-7 for all subjects
- Sulfamethoxazole/trimethoprim DS PO once daily 3 times per week (or alternative agent in case of sulfa allergy) from cycle 1 to 9 and beyond at the discretion of the PI
- Acyclovir 800 mg PO twice daily or valacyclovir 500mg PO once daily at the discretion of the PI (not mandatory)

**5.4 Holding and dose adjustments of study drug administration in the case of treatment related toxicity**

**5.4.1 Cytopenia:**

| Drug | Cycle | Condition at the start of cycle | Action* |
|---|---|---|---|
| Ibrutinib | 1 and onwards | ANC <500/μL **OR** PLT <50,000/μL | Check CBC with differential weekly.<br>Hold ibrutinib if cytopenia persists for ≥ 7 days **OR** if PLT<50,000/μL with bleeding.<br>Resume ibrutinib when ANC ≥750/μL **AND** PLT ≥50,000/μL.<br>• First occurrence: Resume ibrutinib at 420 mg once daily (original dose).<br>• Second occurrence: Resume ibrutinib at 280 mg once daily. If cytopenia does not recur after 2 cycles of ibrutinib 280 mg once daily, investigator may increase ibrutinib to 420 mg once daily. |
| Fludarabine | 3 and 4 | HGB <11 g/dL with indications of autoimmune hemolysis | Omit fludarabine. |
| | | ANC 750 - <1,000/μL **OR** PLT 50,000 - <75,000/μL | Defer fludarabine and check CBC with differential weekly.<br>Resume fludarabine at original dose when ANC ≥1,000/μL **AND** PLT ≥75,000/μL.<br>If count recovery does not occur in 8 weeks, omit fludarabine. |
| | | ANC <500/μL, **OR** PLT <50,000/μL | Defer fludarabine, and check CBC with differential weekly.<br>Resume fludarabine at 20% reduced dose when ANC ≥1,000/μL **AND** PLT ≥75,000/μL.<br>If count recovery does not occur in 8 weeks, omit fludarabine. |

ANC - absolute neutrophil count; PLT – platelet

* Dose delay or reduction is not required if cytopenia is considered related to CLL.

### 5.4.2 Atrial fibrillation:

- For new-onset atrial fibrillation, ibrutinib will be held until cardiac evaluation has been completed. The decision to restart ibrutinib will be made after the PI and consulting physician determine the subject is sufficiently stable following diagnostic testing and institution of treatment if required.
- Anticoagulation with atrial fibrillation: If anticoagulation is deemed necessary by consulting physician and/or PI, patients will be allowed to start treatment with anti-platelet or anti-coagulant therapies except warfarin. Co-administration with warfarin has been associated with grade 3 or higher bleeding events such as (subdural hematoma, gastrointestinal bleeding, hematuria, and post procedural hemorrhage). Other forms of anti-platelet, anti-coagulant, direct thrombin inhibitors, or factor Xa inhibitors may be used concomitantly with ibrutinib, however the risk of increased bruising or bleeding is unknown. Patients will be counseled and monitored closely for signs of bleeding and/or bruising and will be instructed to contact the research team and primary care physician in the case of any complications.

### 5.4.3 Non-hematologic toxicities:

- After a grade 3 or higher non-hematological toxicity (despite optimal supportive care) resolves, ibrutinib will be restarted as follows;

| Occurrence | Action |
|---|---|
| 1st | Hold ibrutinib until recovery to Grade ≤ 1 or baseline; restart at the original dose level (420mg daily). |
| 2nd | Hold ibrutinib until recovery to Grade ≤ 1 or baseline; restart at one dose level lower (280mg daily). |
| 3rd | Hold ibrutinib until recovery to Grade ≤ 1 or baseline; restart at one dose level lower (140mg daily). |
| 4th | Discontinue ibrutinib. |

- A high number of circulating malignant cells (>400,000/μL) may confer increased risk; these subjects should be closely monitored. Administer supportive care such as hydration and/or leukapheresis as indicated. Ibrutinib may be temporarily held, and PI should be contacted.
- In the event that ibrutinib is withheld for a toxicity of 28 consecutive days the study drug should be discontinued. However, if clinical benefit outweighs the risk of restarting treatment then patient may restart study drug after > 28 consecutive days off study drug per PI discretion.
- For fludarabine, after a grade 3 non-hematological toxicity (despite optimal supportive care), when the toxicity resolves to grade 2 or lower and clinical benefit outweighs the risk, treatment can be restarted with 20% dose reduction on the subsequent cycle. Patients experiencing ≤ Grade 2 side effects may continue treatment. Supportive measures as appropriate should be taken to minimize side effects. An interruption of study drug administration is permissible. In select cases where a subject has uncomfortable grade 2 toxicities, dose may be reduced by one dose level per PI discretion.

Dose changes must be recorded in the Dose Administration eCRF.

### 5.4.4 Dose modification for hepatic impaired subjects:

- Ibrutinib is metabolized in the liver and therefore subjects with clinically significant hepatic impairment at the time of screening (Child- Pugh class B or C) are excluded from study participation.
- For subjects with existing chronic mild hepatic impairment (Child-Pugh class A) at enrollment, the starting dose has to be adjusted to a level of 280 mg daily (two capsules).
- For subjects who develop mild liver impairment while on study (Child-Pugh class A), the recommended dose reduction for ibrutinib is to a level of 280 mg daily (two capsules).

- For subjects who develop moderate liver impairment while on study (Child-Pugh class B), the recommended dose reduction is to a level of 140 mg daily (one capsule).
- Subjects who develop severe hepatic impairment (Child-Pugh class C) must hold study drug until resolved to moderate impairment (Child-Pugh class B) or better.
- Subjects who develop acute hepatic toxicity with liver enzymes Grade 3 or higher while on study should be managed per standard dose modification guidelines in Section 5.4.3.

## 5.5 Holding of Study Drug Administration

### 5.5.1 Ibrutinib and Surgical Procedures:

Consider the benefit-risk of withholding ibrutinib for at least 3 to 7 days pre and post-surgery depending upon the type of surgery and the risk of bleeding.

### 5.5.2 Medically Necessary Conditions to Hold Study Drugs:

- If medically necessary reasons to hold ibrutinib occur during treatment (i.e. concurrent infection, impending workups for diagnostic and/or therapeutic purposes), ibrutinib can be held based on the study team's discretion.
- If medically necessary reasons to hold fludarabine occur during treatment (i.e. concurrent infection, emergence of autoimmune cytopenia, impending workups for diagnostic and/or therapeutic purposes), fludarabine can be delayed up to 2 cycles while continuing ibrutinib until the medical reasons to hold fludarabine resolve or return to baseline. If fludarabine is delayed beyond 2 cycles, fludarabine will be omitted and the patient will continue treatment with ibrutinib only.
- Supportive medications including allopurinol, and acyclovir (if indicated) intended to start at cycle 3 will be delayed until fludarabine starts or not given if fludarabine is omitted. Weekly labs intended for cycles 3 and 4 will be delayed until fludarabine starts or not performed if fludarabine is omitted.

### 5.5.3 Permanent discontinuation of study drug administration in case of treatment related adverse events:

- Serious or life threatening cardiac arrhythmias (grade 3 or 4)
- Severe infection (requiring vasopressor support > 24h, or intubation)
- Any grade 4 toxicity excluding readily reversible metabolic or laboratory abnormalities or hematologic toxicities
- Progressive disease as defined in section 7.3
- Pregnancy or unwillingness to use acceptable method of contraception

Patients who will not be able to receive further study drug administration will be followed for safety and after resolution of the event will continue to be followed at regular intervals and will undergo reassessment of disease as outlined in section 6 and 7.

## 5.6 Supportive Care (non-investigational)

**5.6.1** **Blood Products**: Patients will be transfused packed red blood cells and platelets as clinically indicated. All required blood products will be irradiated prior to transfusion.

**5.6.2** **Growth Factors**: Filgrastim or peg-filgrastim may be used to accelerate count if ANC < 500/uL at the PI's discretion, for example, for febrile neutropenia.

**5.6.3** Anti-infective agents will be used as indicated for treatment of intercurrent infections.

**5.6.4** **PCP prophylaxis**: Sulfamethoxazole/trimethoprim DS PO once daily 3 times per week (or alternative agent in case of sulfa allergy) from cycle 1 to 9 and beyond, at the discretion of the PI. Patients who may be at an increased risk of PCP infection include those who are taking long-term

high-dose steroids or immune suppressants, and those who had prior PCP infection. Patients allergic to sulfa drugs who are deemed appropriate for prophylaxis may be given an alternative agent at the discretion of the PI.

**5.6.5** **Anti-viral prophylaxis**: Acyclovir 800 mg PO twice daily or valacyclovir 500mg PO once daily may be used the discretion of the PI (not obligatory).

## 5.7 Permitted and Non Permitted Concomitant Medications (Investigators Brochure)

Patients may continue most medications they were prescribed prior to study enrollment for co-morbid conditions. No formal drug-drug interaction studies have been conducted therefore we ask patients to report all medications and over the counter drugs they are taking so we can monitor for any drug-drug interactions. Treatment for autoimmune cytopenias is permitted only during cycles with ibrutinib alone, but not during cycle 3 and 4 when fludarabine is added. Treatment for autoimmune cytopenias should not exceed 14 days at doses that do not exceed 100mg per day of prednisone or equivalent.

### 5.7.1 Guideline for Use of CYP Inhibiting Drugs

Ibrutinib is metabolized primarily by CYP3A. Avoid co-administration with strong CYP3A or moderate CYP3A inhibitors and consider alternative agents with less CYP3A inhibition.

- If a strong CYP3A inhibitor (eg, ketoconazole, indinavir, nelfinavir, ritonavir, saquinavir, clarithromycin, telithromycin, itraconazole, nefazadone, posaconazole, or cobicistat) must be used, reduce ibrutinib dose to 140 mg or withhold treatment temporarily (for 7 days or less). Subjects should be monitored for signs of ibrutinib toxicity.
- If a moderate CYP3A inhibitor (eg, voriconazole, erythromycin, amprenavir, aprepitant, atazanavir, ciprofloxacin, crizotinib, darunavir/ritonavir, diltiazem, fluconazole, fosamprenavir, imatinib, verapamil, amiodarone, or dronedarone) must be used, reduce ibrutinib to 140 mg (for 840 mg/day dose, reduce to 280 mg) for the duration of the inhibitor use. Avoid grapefruit and Seville oranges during ibrutinib treatment, as these contain moderate inhibitors of CYP3A.
- No dose adjustment is required in combination with mild inhibitors.
- Avoid concomitant use of strong CYP3A inducers (eg, carbamazepine, rifampin, phenytoin, and St. John's Wort). Consider alternative agents with less CYP3A induction.
- A list of common CYP3A inhibitors and inducers is provided in Appendix 3 of the IB. A comprehensive list of inhibitors, inducers, and substrates may be found at http://medicine.iupui.edu/clinpharm/ddis/main-table/. This website is continually revised and should be checked frequently for updates.
- For the most comprehensive effect of CYP3A inhibitors or inducers on ibrutinib exposure, please refer to the current version of the IB.

### 5.7.2 Concomitant Use of QT Prolonging Agents

Any medications known to cause QT prolongation should be used with caution; periodic ECG and electrolyte monitoring should be considered.

### 5.7.3 Concomitant Use of Antiplatelet Agents and Anticoagulants

Use ibrutinib with caution in subjects requiring anticoagulants or medications that inhibit platelet function. In an in vitro platelet function study, inhibitory effects of ibrutinib on collagen induced platelet aggregation were observed. Supplements such as fish oil and vitamin E preparations should be avoided during treatment with ibrutinib. Bleeding events of any grade, including bruising and petechiae, occurred in subjects treated with ibrutinib. Subjects with congenital bleeding diathesis have not been studied. Ibrutinib should be held at least 3 to 7 days pre- and post-surgery depending upon the type of surgery and the risk of bleeding. For guidance on ibrutinib and the use of anticoagulants during procedures/surgeries see Section 5.5.

Subjects requiring the initiation of therapeutic anticoagulation therapy (eg, atrial fibrillation) should be observed closely for signs and symptoms of bleeding and the risks and benefits of continuing ibrutinib treatment should be considered.

### 5.8 Special instructions for patients

**5.8.1 Immunizations:** Live vaccines are contraindicated in this patient population. Subjects who would like to receive other routine non-attenuated vaccinations will be allowed to do so. Patients will be advised not to receive live viral vaccines. The ability to generate an immune response to any vaccine following administration of ibrutinib has not been studied.

**5.8.2 Birth control:** Subjects with reproductive potential who are sexually active must use acceptable methods of contraception during the study and for 90 days after the last dose of ibrutinib. Examples of acceptable methods of contraception include condoms, implants, injectables, combined oral contraceptives, intrauterine devices, true sexual abstinence, or sterilized partner. Note that that periodic abstinence, e.g., calendar, ovulation, symptothermal, post-ovulation methods or withdrawal, are not acceptable methods of contraception.

### 5.9 Prohibited Concomitant Medications

- Any chemotherapy, anticancer immunotherapy, experimental therapy, or radiotherapy are prohibited while the subject is receiving ibrutinib treatment.
- For patients who were newly diagnosed with a second malignancy, the study drug can be interrupted while the patient undergoes further evaluation and treatment with definitive, time-limited anti-cancer therapy (i.e. localized breast cancer requiring surgery and adjuvant chemoradiation). If the second malignancy requires long-term therapy (> 6 months) or a definite therapy is not available (i.e. metastatic solid tumor), the patient will be taken off study.
- Corticosteroids for the treatment of the underlying disease is prohibited. Corticosteroids for the treatment of non-cancer related reasons for longer than 14 consecutive days at doses >20mg/day of prednisone or equivalent, or at doses >100mg of prednisone or its equivalent at any time point is prohibited.

## 6.0 CLINICAL MONITORING

Samples will be ordered and tracked through the CRIS screens. Should a CRIS screen not be available, the NIH form 2803-1 will be completed and will accompany the specimen and be filed in the medical record.

Results from other NIH protocols may be used as a part of the study evaluation. Selected results performed from outside institutions are accepted, which are viral studies, flow cytometry, CT of neck/chest/abdomen/pelvis, and FISH.

### 6.1 Study Evaluations: Screening and Baseline (see schedule of events Appendix A)

**Screening:**
Screening evaluation will include the following (also see schedule of events).

- Complete medical history: relevant past medical history includes medical diagnoses and/or conditions; 1) that are currently ongoing, OR 2) occurred within 1 year of study enrollment, OR 3) occurred before 1 year of study enrollment and were potentially associated with major organ function or with long-term sequelae (i.e. hypertension, hyperlipidemia, diabetes, surgery of major organ system, any malignancy).
- ECOG performance evaluation
- Physical exam will be performed, and subjects will be monitored clinically for atrial fibrillation
- Concomitant medication review

- Complete blood count (CBC) with differential
- Acute Care Panel (includes Na, K, Cl, CO2, Creatinine, Glucose, and Urea Nitrogen)
- Mineral Panel (Phosphorus, Magnesium, Albumin, and Calcium)
- Total Protein, CK, Uric Acid, and LDH
- Hepatic Panel (includes Alkaline Phosphatase, ALT, AST, Total Bilirubin, and Direct Bilirubin)
- Reticulocyte count
- Coagulation panel (includes PT, PTT)
- Beta-2 microglobulin (B2M)
- Haptoglobin
- Direct antiglobulin test (DAT)
- Iron Studies (includes ferritin, transferrin, iron)
- Folate, Vitamin B12
- Viral serologies for hepatitis B and C, HIV 1/2. For individuals with a positive hepatitis B core antibody, HBV DNA PCR will be performed to screen for subclinical infection within 12 months preceding the start of study drug.
- For females of childbearing potential, one negative pregnancy tests sensitive to 50 mIU within 2 weeks prior to starting study drug
- Flow cytometry panel for CLL (may be done on blood and/or bone marrow and/or lymph node) or immunohistochemistry of lymph node or bone marrow demonstrating CLL/SLL within 3 months prior to starting study drug
- CT of the neck, chest, abdomen and pelvis within 3 months prior to starting study drug. IV and PO contrast will be used unless the patient has a contrast allergy or impaired renal function.
- EKG within 3 months prior to starting study drug

**Baseline (day -28 to day 0):**
Baseline evaluation will include the following (also see schedule of events).

- Complete medical history: relevant past medical history includes medical diagnoses and/or conditions; 1) that are currently ongoing, OR 2) occurred within 1 year of study enrollment, OR 3) occurred before 1 year of study enrollment and were potentially associated with major organ function or with long-term sequelae (i.e. hypertension, hyperlipidemia, diabetes, surgery of major organ system, any malignancy).
- ECOG performance evaluation
- Physical exam will be performed, and subjects will be monitored clinically for atrial fibrillation
- Concomitant medication review
- HLA typing (if clinically indicated)
- CBC with differential
- Acute Care Panel (Na, K, Cl, CO2, Creatinine, Glucose, and Urea Nitrogen)
- Mineral Panel (Phosphorus, Magnesium, Albumin, and Calcium)
- Total Protein, CK, Uric Acid, and LDH
- Hepatic Panel (Alkaline Phosphatase, ALT, AST, Total Bilirubin, and Direct Bilirubin)
- Reticulocyte count
- Coagulation panel (PT, PTT)
- Serum protein electrophoresis with immunofixation (SPEP)
- Serum free light chains, quantitative immunoglobulins
- C-reactive protein (CRP)
- Beta-2 microglobulin (B2M)
- Haptoglobin
- Direct antiglobulin test (DAT)
- For females of childbearing potential, pregnancy test
- Lymphocyte phenotyping (T, B, NK)
- IGHV mutation analysis (as this test does not change with time, any prior report is acceptable)

- Flow cytometry panel (optional)
- Bone marrow aspirate and biopsy within 3 months of starting treatment
- Peripheral blood interphase FISH within 3 months prior to starting study drug. FISH panel contains probes for *ATM* (11q22.3), *D12Z3* (12 cen), *D13S319* (13q14.3), *LAMP1* (13q34) and *TP53* (17p13.1).
- Lymph node biopsy for research if the patient is willing and has an accessible lymph node (optional)
- Lymphapheresis for research (optional)
- Research blood (up to 80ml)

## 6.2 On therapy evaluations (see schedule of events Appendix A)

**From cycle 1 to 6 (+/- 5-day window):**
Prior to each cycle, the following assessments will be completed. If the assessments have been already performed within 5 days of the study drug initiation as a part of screening or baseline evaluations, the study team can decide whether these assessments may or may not be repeated.

- Interval History
- ECOG performance status evaluation
- Physical exam
- Concomitant medication review
- CBC with differential
- Acute Care Panel (Na, K, Cl, CO2, Creatinine, Glucose, and Urea Nitrogen)
- Mineral Panel (Phosphorus, Magnesium, Albumin, and Calcium)
- Total Protein, Uric Acid, and LDH
- Hepatic Panel (Alk Phosphatase, ALT, AST, Total Bilirubin, and Direct Bilirubin)
- Reticulocyte count
- Beta-2 microglobulin (B2M)
- Direct antiglobulin test (DAT)
- For females of childbearing potential, repeat pregnancy test
- Research blood (up to 80ml)

**Additional blood draws, procedures, or imaging include (+/- 5-day window, except for CT scan and bone marrow biopsy):**

***Cycle 3&4***

- Prior to Cycle 3 (within day -14 to 0): Lymphocyte phenotyping (T, B, NK), CT of neck/chest/ abdomen/pelvis
- Cycle 3&4 Day 5 (+/- 5 days): Research blood (up to 80ml, optional)
- Cycle 3&4 Day 14, and 22 (+/- 5 days): CBC with differential (may be performed outside of NIH), research blood (up to 80ml) (if the blood was drawn at the NIH)

***Cycle 5***

- Cycle 5 Day 1 (+/-5-day window): Lymphocyte phenotyping (T, B, NK)

***Others***

- Up to 3 lymph node biopsies may be obtained for research at any time during therapy (optional, with at least 4 weeks between biopsies).

**After cycle 6 (+/- 14-day window):**
Following assessments will be done every 3 months while on therapy until disease progression or intolerable side effects.

- Interval History
- ECOG performance status evaluation

- Physical exam
- Concomitant medication review
- CBC with differential
- Acute Care Panel (Na, K, Cl, CO2, Creatinine, Glucose, and Urea Nitrogen)
- Mineral Panel (Phosphorus, Magnesium, Albumin, and Calcium)
- Total Protein, Uric Acid, and LDH
- Hepatic Panel (Alk Phosphatase, ALT, AST, Total Bilirubin, and Direct Bilirubin)
- Reticulocyte count
- Beta-2 microglobulin (B2M)
- Direct antiglobulin test (DAT)
- For females of childbearing potential, repeat pregnancy test
- Research blood (up to 80ml)

### 6.3 Response assessment (see schedule of events Appendix A) (+/- 30-day window)

Response assessment will be performed after 6 cycles, and annually (at the anniversary date of the first dose of study drug).

- Interval History
- ECOG performance status evaluation
- Physical exam
- Concomitant medication review
- CBC with differential
- Acute Care Panel (Na, K, Cl, CO2, Creatinine, Glucose, and Urea Nitrogen)
- Mineral Panel (Phosphorus, Magnesium, Albumin, and Calcium)
- Total Protein, Uric Acid, and LDH
- Hepatic Panel (Alk Phosphatase, ALT, AST, Total Bilirubin, and Direct Bilirubin)
- Reticulocyte count
- Coagulation panel (PT, PTT)
- Serum free light chains, quantitative immunoglobulins
- Beta-2 microglobulin (B2M)
- Haptoglobin
- Direct antiglobulin test (DAT)
- For females of childbearing potential, pregnancy test
- Lymphocyte phenotyping (T, B, NK)
- Flow cytometry panel (optional)
- CT of the neck, chest, abdomen and pelvis. IV and PO contrast will be used unless the patient has a contrast allergy or impaired renal function.
- Bone marrow biopsy after 6 cycles (mandatory). May also obtain annually starting at the anniversary date of the first dose of the study drug (optional). After 1 year on study, bone marrow biopsy and aspirate may be performed 3 months ± 15-days after the patient met CT criteria and hematologic criteria for complete response (optional).
- EKG if medically indicated
- Lymphapheresis for research (optional)
- Lymph node biopsy for research if the patient is willing and has an accessible lymph node. Up to 3 lymph node biopsies may be obtained for research at any time during therapy (optional, with at least 4 weeks between biopsies).
- Research blood (up to 80ml)

### 6.4 At progression evaluation (see schedule of events Appendix A)

All response assessments as listed in 6.3 may be performed at progression. Additional optional studies may be performed which includes the following:

- Serum protein electrophoresis with immunofixation (SPEP)
- C-reactive protein (CRP)
- PET/CT of the neck, chest, abdomen and pelvis. IV and PO contrast will be used unless the patient has a contrast allergy or impaired renal function
- Peripheral blood interphase FISH with FISH panel containing probes for *ATM* (11q22.3), *D12Z3* (12 cen), *D13S319* (13q14.3), *LAMP1* (13q34) and *TP53* (17p13.1)
- IGHV mutational analysis

## 7.0 CRITERIA FOR RESPONSE

Responses for spleen and lymphadenopathy will be assessed using CT scan scans. In the absence of a CT scan assessment by physical exam may be substituted. Response assessments will be made by IWCLL 2018 guidelines.[77] Response includes complete response, partial response, and partial response with lymphocytosis (Table 4).

Table 4. Criteria of response

| Response | CR | PR[F] | PRL[F] | PD[G] |
|---|---|---|---|---|
| ***Group A*** | | | | |
| Lymphadenopathy[A] | None >1.5cm | Decrease ≥ 50% | Decrease ≥ 50% | Increase ≥ 50% or any new lesion > 1.5cm |
| Splenomegaly/ Hepatomegaly[B] | None | Decrease ≥ 50% | Decrease ≥ 50% | Increase ≥ 50% or any new splenomegaly |
| Blood lymphocytes[C] | < 4000/μL | Decrease ≥ 50% from baseline | Increase or <50% decrease over baseline | Increase ≥ 50% over baseline or > 5000/μL |
| Bone marrow[D] | Normocellular, <30% lymphocytes, no B-lymphoid nodules | Not applicable | Not applicable | Not applicable |
| ***Group B[E]*** | | | | |
| Platelet count | >100,000/μL | > 100,000/μL or increase ≥ 50% over baseline | > 100,000/μL or increase ≥ 50% over baseline | Decrease ≥ 50% from baseline secondary to CLL |
| Hemoglobin | > 11.0g/dL | > 11.0 g/dL or increase ≥ 50% over baseline | > 11.0 g/dL or increase ≥ 50% over baseline | Decrease ≥ 50% from baseline secondary to CLL |
| Neutrophils | > 1500/μL | > 1500/μL or increase ≥ 50% over baseline | Not applicable | Not applicable |

CR = complete response; CRi = CR with incomplete blood count recovery; CT = computed tomography; PD = progressive disease; PR = partial response; PRL = partial response with lymphocytosis

***Footnotes***

[A] Sum of the product (SPDs) of up to 6 lymph nodes as evaluated by CT scans. If CT is unavailable, the absence of enlarged lymph nodes (> 1.5cm) documented by physical exam is acceptable.

[B] Splenomegaly assessed by CT scan. In the absence of a CT scan assessment by physical exam may be substituted. New splenomegaly or hepatomegaly is assessed by physical exam.

[C] Patients with treatment-related lymphocytosis remain on study unless associated with other signs of progressive disease. Treatment-related lymphocytosis may occur at the start of treatment or at any time when resuming treatment after a dose interruption.
[D] Complete response requires confirmation with bone marrow biopsy. In the absence of a confirmatory bone marrow biopsy the response can be classified as CRu. In case of a hypocellular marrow the response can be classified as CRi.
[E] PR or PRL, at least one Group B criterion has to be met. CR, all criteria in A and B must be met. In subjects meeting Group A criteria for CR but not Group B, the response can be classified as CRi.
[F] PR and PRL criteria refer to changes from baseline.
[G] PD criteria refer to changes from baseline in subjects who never responded and to changes from best response (nadir) in subjects who responded.

**Complete Response (CR)**
• Is defined as meeting all criteria in A and B.
• Meet all criteria in A and B but bone marrow confirmation is pending: unconfirmed complete response (CRu).
• Meet all criteria in A but bone marrow is hypocellular, or criteria in B are not met: complete response with incomplete blood count recovery (CRi).

**Partial Response (PR)**
• Is defined by 2 criteria in Group A if abnormal before therapy and at least 1 Group B criterion.
• In subjects with just 1 involved Group A site (e.g., lymphadenopathy in a subject with SLL), the response is PR when the criterion for that site is met.
• If criteria for PR, except for a decrease in the number of blood lymphocytes by 50% or more from the value before therapy, are met, then assessment will be PRL.
• SLL subjects need to have both ALC >50% and ALC >5k to be PRL.

**Progressive Disease (PD)**
Progressive disease is defined by:
• Progressive lymphocytosis ≥50% from nadir, confirmed due to expansion of CLL cells by flow cytometry (≥5,000 B lymphocytes/μL).
• or an increase ≥50% in the sum of the products of at least two lymph nodes with at least one lymph node >1.5 cm in greatest diameter,
• or the appearance of a new pathologic lymph node >1.5 cm in greatest diameter,
• or new onset splenomegaly or hepatomegaly on physical exam, or other new CLL organ infiltrates,
• or an increase ≥50% in splenomegaly or hepatomegaly when occurring in the absence of a confounding process (e.g., infection), and that is maintained or continues to progress over a period of at least 3 months.

Other criteria for PD include:
• Transformation to a more aggressive histology (e.g., Richter syndrome). Whenever possible, this diagnosis should be established by lymph node biopsy.
• The progression or development of cytopenia (excluding autoimmune cytopenia), as documented by a decrease of hemoglobin levels by >2 g/dL, or by a decrease of platelet counts by >50%, that persists for at least 3 months defines disease progression if a marrow biopsy supports a disease related etiology.

A rise of the lymphocyte count at the beginning of therapy or resumption of therapy after a period of drug hold in absence of other indications of progressive disease will not be considered as evidence of progressive disease because this type of agent does typically lead to a mobilization of tumor cells into the peripheral blood.

## 8.0 ANCILLARY LABORATORY RESEARCH STUDIES

### 8.1 Collection of samples

**Blood samples:** A volume not to exceed 550 ml of peripheral blood will be requested during the initial 8-week period. Subsequent research blood draws will typically consist of < 100 ml of peripheral blood at F/U visits (not to exceed 550 mL in any 8-week period).

**Lymphapheresis:** A 4-5 liter lymphapheresis procedure for research may be obtained prior to initiating therapy in consenting subjects.

**BM biopsies and aspirate:** A bone marrow biopsy cylinder and up to 10cc of bone marrow aspirate may be obtained for research at the time of bone marrow biopsy as indicated in sections 6.1 and 6.2.

**Lymph node biopsies:** An excisional or core lymph node biopsies may be obtained pre-treatment and after treatment. During the course of participating on this study, an additional 10 cc of blood (NIH visits only) and 5 cc of bone marrow aspirate each time a patient has a bone marrow examination may be requested. These samples will be stored with the subject's permission for other exploratory laboratory research studies reviewed and approved by the IRB and listed in Appendix B. Research samples will be coded and stored in the secure laboratory of Dr. Wiestner.

### 8.2 Intended use

These samples will be used for the following intentions.

1. To explore the biologic effects on B- and T-cell subsets and function, we will;
    - Enumerate T (CD4, CD8), B, and NK cells in circulation
    - Characterize T-cell subsets, including T-regulatory cells, Th-1, Th-2, Th-17
    - Characterize T-cell activation state and differentiation
    - Assess expression of checkpoint inhibitory molecules such PD-L1, PD-1, and CTLA-4
    - Genomic profiling of the B- and T-cell repertoire by CDR3 analysis
    - Measure pertinent cytokine and chemokine levels

2. To explore mechanisms of clinical response, we will;
    - Assess shifts in clonal composition on treatment assessed using interphase FISH and gene sequencing for somatic mutations

These specimens will not be used for diagnostic purposes.

Leftover and additional samples will be used for the following descriptive or exploratory ancillary research studies which may be done and if done, may be correlated with the presence or absence of response. Samples may also be sent to Pharmacyclics, an AbbVie Company, once an MTA has been established

### 8.3 Storage, tracking and disposition of samples and data

**Storage:** All samples will be stored in the laboratory of Dr. Wiestner. Samples collected will be de-identified prior to storage in the laboratory of the principal investigator following current NHLBI DIR BSI Policy. Efforts to ensure protection of patient information include:

- Each sample is assigned a unique number.
- Vials holding patient samples are labeled with the sequential laboratory accession ID number that does not contain any personal identifier information.
- An electronic database is used to store patient information related to the coded samples
- The laboratory is located in a controlled access building and laboratory doors are kept locked. Visitors to the laboratory are required to be accompanied by laboratory staff at all times.
- Hard copy records or electronic copies of documents containing patient information are kept in the locked laboratory or other controlled access locations.

**Tracking:** Samples will be ordered and tracked through the CRIS Research Screens. Should a CRIS screen not be available, the NIH form 2803-1 will be completed and will accompany the specimen and be filed in the medical record. Samples will not be sent outside NIH without IRB notification and an executed MTA or CTA.

De-identified human serum and/or plasma and/or tumor samples and clinical data from patients with B-cell malignancies may be sent to Dr. Dan Landau, Weill Cornell Medical College.

**End of study procedures**: Samples from consenting subjects will be stored until they are no longer of scientific value or if a subject withdraws consent for their continued use, at which time they will be destroyed.

**Loss or destruction of samples**: Should we become aware that a major breech in our plan for tracking and storage of samples has occurred, the IRB will be notified.

## 9.0 BIOSTATISTICAL CONSIDERATIONS

### 9.1 Primary endpoint:

Primary efficacy endpoint is the rate of complete response at 24 weeks or after 6 cycles. Response to treatment will be determined according to IWCLL 2008 criteria incorporating 2012 and 2013 clarifications pertaining to patients treated with kinase inhibitors.

Primary safety endpoint is the rate of treatment discontinuation within the first 24 weeks or 6 cycles due to intolerable side effects.

### 9.2 Secondary endpoints:

- Progression-free survival (PFS)
- Overall survival (OS)
- Best response during treatment
- Duration of response
- Minimal residual disease (MRD) state defined by flow cytometry
- Clonal dynamics on treatment assessed using interphase FISH and gene sequencing for somatic mutations
- Shifts in cellular and humoral immunity on treatment (e.g. immunoglobulin levels, myeloid-derived stromal cells, B-cell repertoire, T-cell subsets including regulatory T cells, T helper 1, T helper 2, T helper 17)
- Expression of checkpoint inhibitory molecules such as PD-L1, PD-1, and CTLA-4

### 9.3 Sample size

The study will enroll 27 evaluable subjects for response assessment for primary efficacy and safety endpoints. Up to 5 additional subjects may be enrolled to account for early discontinuation before completing 6 cycles due to non-treatment related reasons. Those subjects with non-treatment related early discontinuation will not be evaluable for the primary efficacy and safety endpoints.

As for the primary efficacy endpoint, CR with ibrutinib alone has been reported to be 13% in previously untreated CLL population,[56] and 0-2% in relapsed/refractory CLL.[54,55] When ibrutinib and rituximab were combined in high-risk CLL patients, CR rate was 8%.[61] Based on the reported data, a CR rate exceeding 20% would generate an interest for further studies. We will use a Simon's minimax two-stage design to test the null hypothesis ($H_0$) that the CR rate in the study is $\leq$5% against the alternative hypothesis ($H_1$) that it is >5% with type I error of 0.05. If the true CR rate is 20%, the study using the following two-stage design has 80% power. In the first stage, we will enroll 13 subjects and assess CR rate after 6 cycles. If there is no CR in these 13 subjects, we will stop the study; if 1 or more CRs are observed, we will enter an additional 14 subjects for a total of 27 evaluable subjects. The null hypothesis will be rejected if 4 or more CRs are observed in 27 subjects. Early discontinuation within the first 24 weeks or 6 cycles due to toxicity related to study drug are counted as non-response towards the primary efficacy endpoint.

As for the primary safety endpoint, treatment discontinuation rate has been reported to be 26% with the treatment with FCR,[78] in contrast to 4% with the single-agent ibrutinib.[55] Based on reported data, a treatment discontinuation rate <10% at 24 weeks would favor the improved safety and tolerability of the proposed regimen compared to conventional chemoimmunotherapy. We will use a Simon's minimax two-stage design to test the null hypothesis ($H_0$) that the treatment discontinuation rate is ≥30% against the alternative hypothesis ($H_1$) that it is <30%. In the first stage, up to 13 subjects will be enrolled: if 3 or more subjects discontinue treatment within 6 cycles due to intolerable side effects, further enrollment will be put on hold and we will request a review by the DSMB. If 2 or fewer subjects discontinue treatment within 6 cycles among the first 13 subjects, an additional 14 subjects will be enrolled for a total of 27 subjects. The null hypothesis will be rejected if 4 or fewer subjects discontinue treatment due to intolerable side effects among 27 subjects. The above design for the primary safety endpoint has a power of 81% and a type I error of 0.05 if the true treatment discontinuation rate is 10%.

### 9.4 Statistical Methods

The planned analyses will include descriptive statistics on the proportions of overall responses. The response probabilities will be estimated using the sample proportions and their inferences including confidence intervals and hypotheses testing will be evaluated using Binomial distributions, and, if appropriate, normal approximations. The time to responses, overall survival and the PFS time will be analyzed using appropriate nonparametric tools in survival analysis such as Kaplan-Meier estimates taking consideration of random censoring.

In addition, methods based on survival analysis, cumulative incidence rates and other competing risk models will be used to evaluate the treatment effects. Graphical tools will be used to display the appropriate estimates (i.e. estimated proportions and Kaplan-Meier curves) and their corresponding 95% confidence intervals. Methods based on multiple regression, analysis of variance, logistic regression and nonparametric regression will also be employed if deemed appropriate.

### 9.5 Study Stopping Rules

The study will be monitored to ensure that the occurrence of a specified set of treatment related serious adverse events (TRSAEs) that occur during the treatment period does not substantially exceed an anticipated rate. The following specified TRSAEs determined to be probably or definitely related to therapy will be considered for early stopping of the study

1. Death
2. Severe infection requiring vasopressor >24h or intubation
3. Any grade 4 toxicity **excluding**
    - readily reversible metabolic or laboratory abnormalities
    - hematologic toxicities

We anticipate the rate of these specified TRSAEs within the 6 months period (completion of primary endpoint) to be 15% or less. Following Geller et al. (2003, "Design of Early Trials in Stem Cell Transplantation: A Hybrid Frequentist-Bayesian Approach"),[79] our stopping rule is determined by a Bayesian approach. The stopping boundary for an experiment is reached if the Bayesian posterior probability that the true probability of developing one or more of the specified TRSAE's exceeds this benchmark rate of 15% is at least 90%. We take our prior distribution to be a beta distribution so that our prior clinical opinion is worth 20% of the weight we will place on the new data. This gives prior parameters alpha = 0.9, beta = 5.1. Hence when we make decisions about stopping the study, the data from the study will dominate over the prior opinion. We begin monitoring the TRSAEs when 3 subjects are evaluable for TRSAEs within the 6 months.

| Number of subjects in the experiment | Stop if the number of subjects who have developed any of the specified TRSAE's reaches: |
|---|---|
| ≤ 6 | 3 |
| ≤ 11 | 4 |
| ≤ 16 | 5 |
| ≤ 21 | 6 |
| ≤ 26 | 7 |
| ≤ 32 | 8 |

We investigated the performance of the above stopping rule by a simulation study. In each simulation run, we generated a study with 32 independent Bernoulli trials, each had a probability p for having TRSAE and q=1-p for not having TRSAE and compared the TRSAE outcomes with the above stopping boundary to determine whether the study was stopped. We repeated the simulation 100,000 times and computed the proportion of stopped studies (i.e. "number of stopped studies"/100,000) which were stopped using the above stopping rule. The following table summarizes the proportions of stopped studies under a number of scenarios for *p*:

| Probability of TRSAE = *p* | 0.1 | 0.15 | 0.20 | 0.25 | 0.30 |
|---|---|---|---|---|---|
| Proportion of Stopped Studies | 4.4% | 17.9% | 41.3% | 66.1% | 84.2% |
| Average number of subjects | 31.1 | 28.9 | 25.1 | 20.6 | 16.3 |
| Average number TRSAEs | 3.1 | 4.3 | 5.0 | 5.2 | 4.9 |

These results suggest that our stopping rule has a low probability stopping a study when the proportion of TRSAE is below the benchmark value of 15%, and the probability of stopping a study is high when the true proportion of TRSAE exceeds this benchmark value. Based on these results, we believe that our Bayesian stopping rule has satisfactory statistical properties.

### 9.6 Stopping rule for Mortality:

In addition, we have a stopping rule for TRM (death that are probably or definitely related to the protocol regimen and are not due to disease progression). We anticipate the TRM rate within one year to be 5% or less. Using the same Bayesian approach, the stopping boundary is reached if the Bayesian posterior probability that the true probability of developing TRM's exceeds 5% is at least 95%. We take our prior distribution to be a beta distribution with parameters $(\alpha, \beta) = (0.3, 5.7)$. This indicates that the relative weight we place on our prior opinion is approximately 20% of the weight we will place on the results of the new study. The following table summarizes the threshold numbers for stopping the study based on above Bayesian approach.

| Number of subjects in the experiment | Stop if the number of subjects who have TRMs reaches: |
|---|---|
| ≤ 9 | 2 |
| ≤ 21 | 3 |
| ≤ 32 | 4 |

We investigated the performance of the above stopping rule by a simulation study. In each simulation run, we generated a study with 32 independent Bernoulli trials, each had a probability *p* for having TRM and *q*=1-*p* for not having TRM and compared the TRM outcomes with the above stopping boundary to determine whether the study was stopped. We repeated the simulation 100,000 times and computed the proportion of stopped studies (i.e. "number of stopped studies"/100,000) which were stopped using the above stopping rule. The following table summarizes the proportions of stopped studies under a number of scenarios for *p*:

| Probability of TRM = $p$ | 0.03 | 0.05 | 0.07 | 0.10 | 0.15 | 0.20 |
|---|---|---|---|---|---|---|
| Proportion of Stopped Studies | 4.7% | 14.2% | 27.7% | 49.6% | 78.5% | 93.0% |
| Average number of subjects | 31.1 | 29.4 | 27.1 | 23.3 | 17.1 | 12.4 |
| Average number TRMs | 0.9 | 1.5 | 1.9 | 2.3 | 2.6 | 2.5 |

These results suggest that our stopping rule has a low probability stopping a study when the proportion of TRM is below the benchmark value of 5%, and the probability of stopping a study is high when the true proportion of TRM exceeds this benchmark value. Based on these results, we believe that our Bayesian stopping rule has satisfactory statistical properties.

### 9.7 Off Study Criteria (for subject participation)

**Patient choice:** Subjects may be removed from the study at their request. The risks of withdrawing will be discussed, as will alternative treatment options. Subjects who opt to withdraw from the protocol will be strongly encouraged to continue to have labs (CBC, chemistries) monitored for two months after study withdrawal for their safety.

**PI Decision:** Study drug administration will be discontinued for adverse events as detailed in section 5.5. These subjects will be followed until resolution of the event and continue to be followed on protocol. Other off study criteria include:

- Initiation of non-protocol treatment.
    - For patients who were newly diagnosed with a second malignancy, the study drug can be interrupted while the patient undergoes further evaluation and treatment with definitive, time-limited systemic therapy (i.e. breast cancer requiring surgery and adjuvant chemoradiation). If the new second malignancy requires long-term therapy (>6 months) or a definite therapy is not available (i.e. metastatic solid tumor), the patient will be taken off study.
- Significant progression of disease or a concomitant condition that would make the subject ineligible for further protocol participation. In selected cases, PI can decide if ibrutinib may be continued after progression until contingency plan for the next line of therapy is in place, and the benefit of treatment continuation outweighs potential risks.
- Pregnancy or begins breast feeding
- Subject becomes significantly noncompliant with study drug administration, study procedures, or study requirements, which might increase risk or substantially compromise the interpretation of study results.

Completion of the study: Subjects will be followed indefinitely until an off study criterion is met or the study is closed to further follow up care.

Once protocol participation is complete, the subject will be referred back to his or her referring physician, consented to the Hematology Branch evaluation and treatment protocol (94-H-0010) for consideration for standard therapy or evaluated for eligibility for another branch protocol, depending on what is considered to be in the best interest of the subject.

## 10.0 DATA AND SAFETY MONITORING

**Principal Investigator:** Accrual, efficacy and safety data will be monitored by the principal investigator Inhye Ahn, M.D., and by the accountable and medically responsible investigator, Adrian Wiestner, M.D., Ph.D.

**NHLBI's IRB:** Prior to implementation of this study, the protocol and the proposed patient consent and assent forms will be reviewed and approved by the properly constituted Institutional Review Board (IRB) operating according to 45 CFR 46. Quality assurance and control monitoring will be consistent with the NHLBI Division of Intramural Research Clinical Research Quality Assurance and Quality Control Policy.

**NHLBI DSMB:** The NHLBI Data Safety and Monitoring Board will review the protocol, progress report, accrual, efficacy and safety data at six- or twelve-month intervals as scheduled. All AEs and SAEs observed during the clinical trial and for which there is a relationship with the use of the ibrutinib and fludarabine, or the conduct of the study will be reported to the DSMB at the regularly scheduled DSMB meeting. The DSMB may recommend early termination of the study for considerations of safety and efficacy. We request review by the NHLBI DSMB since this is the first time ibrutinib and fludarabine are being used in combination in a clinical trial.

**Pharmacyclics, an AbbVie Company:** Pharmacyclics, an AbbVie Company will approve all amendments to the protocol or informed consent prior to submission to the IRB and conduct continuing annual review so long as the protocol is open to accrual or sample and/or data analysis continues. Accrual and safety data will also be monitored and reviewed annually by the IRB. An annual progress report, any amendments to the protocol, and any change in the status of the protocol will be forwarded to medsciences@pcyc.com.

### 10.1 Assessment of Safety

**Definitions**

*Adverse Event (AE):* Any untoward medical occurrence in a human subject, including any abnormal sign (e.g., abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research.

An abnormal laboratory value will be considered an AE if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization
- Is judged by the Investigator to be of significant clinical impact
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

Serious Adverse Event (SAE):

- results in death;
- is life-threatening (places the subject at immediate risk of death from the event as it occurred);
- results in in-patient hospitalization or prolongation of existing hospitalization;
- results in a persistent or significant incapacity;
- results in a congenital anomaly/birth defect; or
- based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition.

*Suspected adverse reaction:* Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A

suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

*Serious event:* An event is serious if it meets the definition of a serious adverse event (above) or if it requires immediate corrective action by a PI and/or IRB to protect the safety, welfare or rights of subjects.

*Unexpected adverse reaction:* An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application. "Unexpected", also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

*Unanticipated Problem (UP)*: Any incident, experience, or outcome that meets <u>all</u> of the following criteria:

1. **unexpected** in terms of nature, severity, or frequency in relation to
   a. the research risks that are described in the IRB-approved research protocol and informed consent document; Investigator's Brochure or other study documents; and
   b. the characteristics of the subject population being studied; and
2. **related or possibly related** to participation in the research; and
3. places subjects or others at a **greater risk of harm** (including physical, psychological, economic, or social harm) than was previously known or recognized.

*Unanticipated Problem that is not an Adverse Event*: An unanticipated problem that does not fit the definition of an adverse event, but which may, in the opinion of the investigator, involves risk to the subject, affect others in the research study, or significantly impact the integrity of research data. For example, report occurrences of breaches of confidentiality, accidental destruction of study records, or unaccounted-for study drug.

*Asymptomatic Treatment-related Lymphocytosis:* This event should also not be considered an AE. Subjects with treatment-related lymphocytosis should remain on study treatment and continue with all study-related procedures.

*Protocol Deviation (PD):* Any change, divergence, or departure from the IRB approved research protocol.

*Non-compliance:* The failure to comply with applicable NIH HRPP policies, IRB requirements, or regulatory requirements for the protection of human research. Noncompliance may be further characterized as:

**1. Serious non-compliance:** Non-compliance that:
   a. Increases risks, or causes harm, to participants
   b. Decreases potential benefits to participants
   c. Compromises the integrity of the NIH HRPP
   d. Invalidates the study data

**2. Continuing non-compliance:** Non-compliance that is recurring. An example may be a pattern of non-compliance that suggests a likelihood that, absent an intervention, non-compliance will continue. Continuing noncompliance could also include a failure to respond to IRB requests to resolve previous allegations of non-compliance.

**3. Minor (non-serious) non-compliance:** Non-compliance that, is neither serious nor continuing.

Safety assessments will consist of monitoring and recording AEs and SAEs; measurements of protocol-specified hematology, clinical chemistry, and other laboratory variables; measurement of protocol-specified vital signs; and other protocol-specified tests that are deemed critical to the safety evaluation of the study drug.

### 10.1.1 Severity

Definitions found in the Common Terminology Criteria for Adverse Events version 4.03 (CTCAE v4.03) will be used for grading the ***severity*** (intensity) of **non-hematologic** AEs:

Grade 1 (Mild AE) – experiences which are usually transient, requiring no special treatment, and not interfering with the patient's daily activities
Grade 2 (Moderate AE) – experiences which introduce some level of inconvenience or concern to the patient, and which may interfere with daily activities, but are usually ameliorated by simple therapeutic measures
Grade 3 (Severe AE) – experiences which are unacceptable or intolerable, significantly interrupt the patient's usual daily activity, and require systemic drug therapy or other treatment
Grade 4 (Life-threatening or disabling AE) – experiences which cause the patient to be in imminent danger of death
Grade 5 (Death related to AE) – experiences which result in patient death

**Grading Scale for Hematologic Toxicity in CLL [Hallek 2008]**

| Grade[1] | Decrease in platelets[2] or Hb[3] (nadir) from pretreatment value | Absolute neutrophil count/μL[4] (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 |

1. 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 reported as Grade 5.
2. 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.
3. Hemoglobin (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.
4. If the 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 granulocyte colony-stimulating factor (G-CSF) is not relevant to the grading of toxicity, but should be documented.

### 10.1.2 Pregnancy

Before study enrollment, subjects must agree to take appropriate measures to avoid pregnancy. However, should a pregnancy occur in a female study subject, consent to provide follow-up information regarding the outcome of the pregnancy and the health of the infant until 30 days old will be requested.

A female subject must immediately inform the Investigator if she becomes pregnant from the time of consent to 90 days after the last dose of study drug. A male subject must immediately inform the Investigator if his partner becomes pregnant from the time of consent to 90 days after the last dose of study drug. Any female subjects receiving study drug(s) who become pregnant must immediately discontinue study drug. The Investigator should counsel the subject, discussing any risks of continuing the pregnancy and any possible effects on the fetus.

Although pregnancy itself is not regarded as an adverse event, the outcome will need to be documented. Any pregnancy occurring in a subject or subject's partner from the time of consent to 90 days after the last dose of study drug must be reported. Any occurrence of pregnancy must be reported to Pharmacyclics Drug Safety, or designee, per SAE reporting timelines. All pregnancies will be followed for outcome, which is defined as elective termination of the pregnancy, miscarriage, or delivery of the fetus. Pregnancies with an outcome of live birth, the newborn infant will be followed until 30 days old and this must be reported to Pharmacyclics Drug Safety, or designee, per SAE reporting timelines. Any congenital anomaly/birth defect noted in the infant must be reported as a serious adverse event.

### 10.1.3 AE and SAE causality assessments

The following general guideline will be followed:

| | |
|---|---|
| Unrelated: | Another cause of the adverse event is more plausible; a temporal sequence cannot be established with the onset of the adverse event and administration of the investigational product; or, a causal relationship is considered biologically implausible. |
| Unlikely to be Related: | A temporal sequence with the onset of the adverse event and administration of the investigational product is improbable, but not impossible. Concurrent or underlying disease, or the use of other drugs or procedures provide plausible explanations for the adverse event. |
| Possibly Related: | There is a clinically plausible time sequence between onset of the adverse event and administration of the investigational product, but the adverse event could also be attributed to concurrent or underlying disease, or the use of other drugs or procedures. Possibly related should be used when the investigational product is one of several biologically plausible adverse event causes. |
| Probably Related: | There is a clinically plausible time sequence between onset of the adverse event and administration of the investigational product, and the adverse event is unlikely to be attributed to concurrent or underlying disease, or the use of other drugs or procedures. Clinical response to withdrawal of the investigational product may indicate the adverse event is probably related. Re-challenge with the investigational drug is not required. |
| Definitely Related: | The adverse event is clearly related to use of the investigational product. |

## 10.2 Documenting and Reporting of Adverse and Serious Adverse Events

Investigators will assess the occurrence of AEs and SAEs at all patient evaluation time points during the study. All AEs and SAEs whether volunteered by the patient, discovered by study personnel during questioning, or detected through physical examination, clinically significant laboratory test, or other means will be recorded in the patient's medical record.

## 10.3 NHLBI-IRB and CD reporting

### 10.3.1 Serious Events

Reports to the IRB and CD:

The PI must report serious UPs, and serious PDs, to the IRB and CD as soon as possible but not more than 7 days after the PI first learns of the event.

Reports to the IRB Chair and CD:
The PI must report all SAEs that do not meet the definition of UP to the IRB chair and CD not more than 14 days after the PI first learns of the event.

Reports to Pharmacyclics LLC, an AbbVie Company:
All serious adverse events and AE of special interest (initial and follow-up information) will be reported on FDA Medwatch (Form 3500A) or Suspect Adverse Event Report (CIOMS Form 1) IRB Reporting Form and sent via email (AEintakeCT@pcyc.com) or fax ((408) 215-3500) to Pharmacyclics Drug Safety, or designee, within 24 hours of the event. Pharmacyclics may request follow-up and other additional information from the Sponsor Investigator.

### 10.3.2 Non-serious Events

Reports to the IRB and CD:
The PI must report all UPs that are not serious to the IRB and CD, and PDs that are not serious to the IRB, not more than 14 days after the PI first learns of the event.

### 10.3.3 Deaths

The PI must report all deaths (that are not UPs) to the CD, and Pharmacyclics as soon as possible, but not more than 7 days after the PI first learns of the event.

## 10.4 Reports at the time of continuing IRB review:

At continuing review, the PI will provide to the IRB a summary of:

- All UPs
- All PDs (except for those granted a waiver of reporting)
- All AEs (except for those granted a waiver of reporting or any AEs occurred before the initiation of the study drugs)
- If, while preparing the continuing review, the PI identifies a greater frequency or level of severity of expected adverse events than was previously identified in the protocol or investigational brochure (IB), these should be reported separately as a UP. If such an observation occurs before the time of continuing IRB review, it should be reported to the IRB and CD as a UP in the time frames noted above, and summarized at the time of continuing review.

*Exclusions to data reporting*:
The following events will be captured only in the source documents and will not be reported to the IRB at the time of continuing review:

- Laboratory values that do not meet the definition of AE listed in Section 9.2.2.
- All grade 1 events listed as expected in the investigator's brochure.
- Study drug non-compliance will not be reported to the IRB unless patients are less than 80% compliant with study medications.

### 10.4.1 Reporting Period for AEs

The AE reporting period for this study begins when the patient takes the first dose of study drug and ends with the safety follow-up visit. If an SAE is present at the safety follow-up visit or within 30 days of the last dose of study drug (whichever is later), it should be followed to resolution or until the Investigator assesses the subject as stable, a new anticancer therapy is initiated, or the subject is lost to follow-up or

withdraws consent. Resolution/stable means the subject has returned to baseline state of health or the Investigator does not expect any further improvement or worsening of the event.

*Reporting for other malignancies*
Occurrences of any new malignant tumors including solid tumors, skin malignancies and hematologic malignancies will be reported throughout study participation, including duration of study treatment and during any protocol specified follow-up periods including post-progression follow-up for overall survival.

### 10.4.2 Reporting Period and Reporting Requirements for SAEs

**NHLBI IRB:** Please see section 10.3.1 above.

**Pharmacyclics LLC, an AbbVie Company,** will be notified for Drug Safety, or designee of any serious, unexpected, fatal or life-threatening adverse event or adverse drug reaction as soon as possible, but no later than 24 hours in the case of death or life-threatening serious adverse events after the Investigator's receipt of the information or becoming aware of the occurrence. All other SAEs will be reported no later than 24 hours after receipt of the information or becoming aware of the occurrence. Pharmacyclics LLC may request follow-up and other additional relevant clinical records or information from the investigator.

| Drug Safety Contact Information | |
|---|---|
| Email: | AEintakeCT@pcyc.com |
| US Fax: | 1-408-215-3500 |

The following may not be reported to the IRB immediately unless they meet the criteria of an SAE:

- A standard procedure for protocol therapy administration.
- The administration of blood or platelet transfusion.
- A procedure for protocol/disease-related investigations (e.g., surgery, scans, endoscopy, sampling for laboratory tests, bone marrow sampling, pharmacokinetic or biomarker blood sampling).
- Prolonged hospitalization for technical, practical, or social reasons in the absence of an adverse event
- A procedure planned before entry into the study

### 10.4.3 Adverse Events of Special Interest (AESI)

Specific adverse events, or groups of adverse events, will be followed as part of standard safety monitoring activities. These events (regardless of seriousness) will be reported to Pharmacyclics Drug Safety per SAE reporting timelines.

***Major Hemorrhage***
Major hemorrhage is defined as any of the following:

- Any treatment-emergent hemorrhagic adverse events of Grade 3 or higher*.
- Any treatment-emergent serious adverse events of bleeding of any grade
- Any treatment-emergent central nervous system hemorrhage/hematoma of any grade

*All hemorrhagic events requiring transfusion of red blood cells should be reported as grade 3 or higher AE per CTCAE v4.
Events meeting the definition of major hemorrhage will be captured as an event of special interest according to above.

## 10.5 Data management

The principal investigator will be responsible for overseeing entry of data into an in-house password protected electronic system and ensuring data accuracy, consistency and timeliness. The principal investigator, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts to ensure that data is verifiable and evaluable. Data will be abstracted from Clinical Center progress notes as well

as from progress notes forwarded from the subjects' home physician. Laboratory data from NIH will be imported electronically from CRIS into an in-house clinical trial database. Laboratory values from referring home physicians will be entered into the system.

We will maintain the confidentiality of identifiable private information collected in this Clinical Trial and protect the privacy of the individual human subjects. Primary data containing individually identifiable information obtained during the conduct of the protocol will be kept in secure network drives or in approved alternative sites that comply with NIH information security standards. Neither individual personal identifiers nor the key linking coded data to individuals will be released to Pharmacyclics, an AbbVie Company without prior IRB approval and an executed CDA or MTA. Identifiable data will not be sent outside NIH without prior IRB approval or appropriate conditions for disclosure outlined in the executed CDA or MTA.

**End of study procedures**: Data will be stored in locked cabinets and in a password protected database until it is no longer of scientific value. Upon completion of the data analysis, the Investigator will send to Pharmacyclics, an AbbVie Company a copy of the de-identified data set and final Clinical Study Report as requested.

**Loss or destruction of data**: Should we become aware that a major breech in our plan to protect patient confidentiality and trial data has occurred, the IRB will be notified.

### 10.6 Protocol Amendments

Per the IST Agreement, any amendments to the Protocol or Informed Consent Form protocol must be sent to Pharmacyclics, an AbbVie Company for review and approval prior to submission to the IRB. Written verification of IRB approval will be obtained before any amendment is implemented.

**Publication Policy**: Given the research mandate of the NIH, patient data including the results of testing and responses to treatment will be entered into an NIH-authorized and controlled research database. Any future research use will occur only after appropriate human subject protection institutional approval such as prospective NIH IRB review and approval or an exemption from the NIH Office of Human Subjects Research Protections (OHSRP).

Per the IST Agreement, the Investigator is required to submit to Pharmacyclics, an AbbVie Company a copy of a planned publication (abstract, poster, oral presentation or manuscript) prior to the submission thereof for publication or disclosure. Pharmacyclics, an AbbVie Company may provide scientific comments and suggestions understanding that the Investigator has sole editorial responsibility, and retains the authority to make the final determination on whether or not to incorporate Pharmacyclics, an AbbVie Company comments or requests for additional information.

### 10.7 Protocol Monitoring

As per ICH-GCP 5.18 and FDA 21 CFR 312.5 clinical protocols are required to be adequately monitored by the study sponsor. The monitoring of this study will be conducted by Clinical Research Associates (CRAs)/Monitors working under an agreement with NHLBI to monitor aspects of the study in accordance with the appropriate regulations and the approved protocol. The objectives of a monitoring visit will be: 1) to verify the existence of signed informed consent form (ICF) and documentation of the ICF process for each monitored subject; 2) to verify the prompt and accurate recording of all monitored data points, and prompt reporting of all SAEs; 3) to compare abstracted information with individual subjects' records and source documents (subject's charts, laboratory analyses and test results, physicians' progress notes, nurses' notes, and any other relevant original subject information); and 4) to help ensure investigators are in compliance with the protocol. The monitors also will inspect the clinical site regulatory files to ensure that regulatory requirements (Office for Human Research Protections-OHRP), FDA and applicable guidelines (ICH-GCP) are being followed. During

the monitoring visits, the investigator (and/or designee) and other study personnel will be available to discuss the study progress and monitoring visit.

The investigator (and/or designee) will make study documents (e.g., consent forms and pertinent hospital or clinical records readily available for inspection by the local IRB, the FDA, the site monitors, and the NHLBI staff for confirmation of the study data.

## 11.0 HUMAN SUBJECT PROTECTION

The investigator(s) accept their responsibilities for protecting the rights and welfare of human research subjects and will permit, with reasonable advance notice and at reasonable times, the designated research monitors to monitor the conduct of the research, as well as to audit source documents to the extent necessary to verify compliance with FDA Good Clinical Practice and the approved protocol.

### 11.1 Rationale for Subject Selection

#### 11.1.1 Predicted distribution by gender, age and race:

CLL is a rare neoplasm that comprises a substantial proportion of all leukemia in middle-aged persons and is the most common type among elderly persons in western populations. Epidemiologic studies suggest that distribution by gender will be 66% males and 33% females.[13] This trend appears to be lost with age. CLL is more common in Caucasian and African-American but rare in Hispanics and very rare in the Asian population. This study will be open to all patients who fit the inclusion criteria and provide informed consent to protocol participation. We would predict that distribution should be comparable to that seen on the NHLBI Hematology Branch screening protocol as follows:

- by gender: 33% females; 66% males
- by age: ages 23-79, median 60
- by race: 2% Asian, 11% Black, 8% Hispanic, 79% White

#### 11.1.2 Special Populations:

**Justification for inclusion of previously untreated CLL:** Ibrutinib is FDA-approved for the treatment of relapsed/refractory CLL and is therefore available outside of clinical trials. Furthermore, a substantial proportion of relapsed/refractory CLL is refractory to fludarabine-based regimens, which is typically given as a first-line drug. Thus, the combination of ibrutinib and short-course fludarabine is likely more effective in previously untreated CLL than in relapsed/refractory CLL.

**Justification for exclusion of children**: CLL is uncommon in patients less than 45 years of age and is virtually unknown in patients less than 20 years of age. At the time of diagnosis, more than 95% of patients are 45 years old and above.[14] CLL may also be, biologically, a different disease in children. Ibrutinib has not been studied in human subjects under 18 years of age. For these reasons, individuals < 18 years old have been excluded from protocol participation.

**Justification for Exclusion of pregnant women**: There are no clinical studies that were done on pregnant women, and it is unknown whether ibrutinib or its metabolites are excreted in human milk. In addition, it is highly unlikely that a woman of pre-menopausal age will present with CLL or SLL at the Clinical Center. CLL/SLL is a malignancy of B cells that predominantly affects the elderly population. Diagnosis is typically made in adults over the age of 50 and more than half of the people with CLL/SLL are over the age of 70.

**Justification for Exclusion of patients with impaired hepatic or renal function:** No specific clinical studies have been conducted to date in patients with impaired hepatic or renal function. To minimize risks, patients enrolled must have AST and ALT levels < 3.0 × the institutional upper limit of normal (ULN) and a creatinine level ≤ 2.0 × the institutional ULN.

**Justification for Exclusion of Cognitively Impaired Subjects:** Cognitively impaired and institutionalized persons will not participate in this study. Subjects must be able to provide informed consent, and understand and comply with the treatment plan and follow-up.

**Inclusion of NIH Staff (At NIH CC only):** NIH staff may voluntarily participate in this protocol. Recruitment, enrollment and compensation of NIH staff will be consistent with the Guidelines for the Inclusion of Staff in NIH Intramural Research Studies (April 2016) (HRPP SOP 14F, Appendix A; also Appendix C of this protocol) and NIH Policy Manual Chapter 2300-630-3 "Leave Policy for NIH Employees Participating in NIH Medical Research Studies" (HRPP SOP 14F, Appendix B).

**Recruitment**: The study will be listed on the clinicaltrials.gov, Clinical Center research studies, and the National Heart, Lung and Blood Institute patient recruitment websites. If recruitment goals are not met, a recruitment plan will be developed by the Clinical Center Office of Patient Recruitment.

**Payment for participation: $0 –** Subjects will not be compensated for their participation in this study. There is no payment for the blood samples obtained for research.

**Reimbursement for protocol participation travel, food, and lodging** will be consistent with NIH guidelines.

**Competition with other Branch protocols:** This trial is the only active treatment protocol for treatment-naïve CLL/SLL patients without deletion 17p. There will be little to no competition with existing branch protocols.

## 11.2 Risks and discomforts

### 11.2.1 Risks related to Ibrutinib

***Bleeding-related events***

There have been reports of hemorrhagic events in subjects treated with ibrutinib both with and without thrombocytopenia. These include primarily minor hemorrhagic events such as contusion, epistaxis, and petechiae; and major hemorrhagic events, some fatal, including gastrointestinal bleeding, intracranial hemorrhage and hematuria. Use of ibrutinib in subjects requiring other anticoagulants or medications that inhibit platelet function may increase the risk of bleeding. Subjects with congenital bleeding diathesis have not been studied. See Section 6.2.4 for guidance on concomitant use of anticoagulants, antiplatelet therapy and/or supplements. See Section 5.5 for guidance on ibrutinib management with surgeries or procedures.

***Cardiac Arrhythmias***

Atrial fibrillation and atrial flutter, and cases of ventricular tachyarrhythmia including some fatal events, have been reported in subjects treated with ibrutinib, particularly in subjects with cardiac risk factors, hypertension, acute infections, and a previous history of cardiac arrhythmia. Subjects who develop arrhythmic symptoms (eg, palpitations, lightheadedness) or new onset of dyspnea should be evaluated clinically, and if indicated, have an ECG performed. For cardiac arrhythmia which persists, consider the risks and benefits of ibrutinib treatment and follow the protocol dose modification guidelines (see Section 5.4).

***Cytopenias***
Treatment-emergent Grade 3 or 4 cytopenias (neutropenia, thrombocytopenia, and anemia) were reported in subjects treated with ibrutinib. Subjects should be monitored for fever, weakness, or easy bruising and/or bleeding.

***Diarrhea***
Diarrhea is the most frequently reported non-hematologic AE with ibrutinib monotherapy and combination therapy. Other frequently reported gastrointestinal events include nausea, vomiting, and constipation. These events are rarely severe. Should symptoms be severe or prolonged follow the protocol dose modification guidelines (see Section 5.4).

***Infections***
Infections (including sepsis, bacterial, viral, or fungal infections) were observed in subjects treated with ibrutinib therapy. Some of these reported infections have been associated with hospitalization and death. Consider prophylaxis according to standard of care in subjects who are at increased risk for opportunistic infections. Although causality has not been established, cases of progressive multifocal leukoencephalopathy (PML) and hepatitis B reactivation and pneumocystis pneumonia have occurred in patients treated with ibrutinib. Subjects should be monitored for signs and symptoms (fever, chills, weakness, confusion, vomiting and jaundice) and appropriate therapy should be instituted as indicated.

***Tumor Lysis Syndrome (TLS)***
There have been reports of tumor lysis syndrome (TLS) events in subjects treated with single-agent ibrutinib or in combination with chemotherapy. Subjects at risk of tumor lysis syndrome are those with comorbidities and/or risk factors such as high tumor burden prior to treatment, increased uric acid (hyperuricemia), elevated lactate dehydrogenase (LDH), bulky disease at baseline, and pre-existing kidney abnormalities.

***Liver Failure***
Rare cases of liver failure have been reported in patients treated with ibrutinib.

***Non-Melanoma Skin Cancer***
Non-melanoma skin cancers have occurred in patients treated with ibrutinib. Monitor patients for the appearance of non-melanoma skin cancer.

***Rash***
Rash has been commonly reported in subjects treated with either single agent ibrutinib or in combination with chemotherapy. Most rashes were mild to moderate in severity. Isolated cases of severe cutaneous adverse reactions (SCARs) including Stevens-Johnson syndrome (SJS) have been reported in subjects treated with ibrutinib. Subjects should be closely monitored for signs and symptoms suggestive of SCAR including SJS. Subjects receiving ibrutinib should be observed closely for rashes and treated symptomatically, including interruption of the suspected agent as appropriate. In addition, hypersensitivity-related events including erythema, urticaria, and angioedema have been reported.

***Interstitial lung disease***
Cases of interstitial lung disease (ILD) have been reported in subjects treated with ibrutinib. Subjects should be monitored and evaluated for symptoms (eg, dyspnea, cough or pyrexia) and treated symptomatically, including interruption of the suspected agent as appropriate. Should symptoms develop follow the protocol dose modification guidelines.

***Leukostasis***
There were isolated cases of leukostasis reported in subjects treated with ibrutinib. A high number of circulating lymphocytes (>400,000/μL) may confer increased risk.

***Lymphocytosis***
Upon initiation of treatment, a reversible increase in lymphocyte counts (i.e., ≥50% increase from baseline and an absolute count 5000/mc μL), often associated with reduction of lymphadenopathy, has been observed in most subjects with CLL/SLL treated with ibrutinib as a single agent. This effect has also been observed in some subjects with MCL treated with ibrutinib. This observed lymphocytosis is a pharmacodynamic effect and should not be considered progressive disease in the absence of other clinical findings. In both disease types, lymphocytosis typically occurs during the first few weeks of ibrutinib therapy (median time 1.1 weeks) and typically resolves within a median of 8.0 weeks in subjects with MCL and 14 weeks in subjects with CLL/SLL. This pharmacodynamic effect was less prominent or not observed in other indications.

A large increase in the number of circulating lymphocytes (eg, >400,000/μL) has been observed in some subjects. Lymphocytosis was not commonly observed in subjects with Waldenström's macroglobulinemia treated with ibrutinib. Lymphocytosis appeared to occur in lower incidence and at lesser magnitude in subjects with CLL/SLL receiving ibrutinib in combination with chemoimmunotherapy.

***Hypertension***
Hypertension has been commonly reported in subjects treated with ibrutinib. Monitor subjects for new onset of hypertension or hypertension that is not adequately controlled after starting ibrutinib. Adjust existing anti-hypertensive medications and/or initiate anti-hypertensive treatment as appropriate.

**Potential for Drug-Drug Interactions:** Ibrutinib is primarily metabolized by CYP3A.

**Agents That May Increase ibrutinib Plasma Concentrations (CYP3A Inhibitors):**
Concomitant use of ibrutinib and drugs that strongly or moderately inhibit CYP3A can increase ibrutinib exposure and strong CYP3A inhibitors should be avoided.

***Strong CYP3A inhibitors***
Co-administration of ketoconazole, a strong CYP3A inhibitor, in 18 healthy subjects, increased exposure (Cmax and AUC0-last) of ibrutinib by 29- and 24-fold, respectively. In a dedicated drug-drug interaction study in patients with B-cell malignancies, co-administration of voriconazole increased Cmax and AUC by 6.7-fold and 5.7-fold, respectively. In clinical studies, the maximal observed ibrutinib exposure (AUC) was ≤ 2-fold in 37 patients treated with mild and/or moderate CYP3A inhibitors when compared with the ibrutinib exposure in 76 patients not treated concomitantly with CYP3A inhibitors. Clinical safety data in 66 patients treated with moderate (n=47) or strong CYP3A inhibitors (n=19) did not reveal meaningful increases in toxicities. Voriconazole and posaconazole can be used concomitantly with ibrutinib as per the dosing guidance described in the specific clinical study protocol. All other strong inhibitors of CYP3A (eg, ketoconazole, indinavir, nelfinavir, ritonavir, saquinavir, clarithromycin, telithromycin, itraconazole, nefazodone and cobicistat) should be avoided, and an alternative with less CYP3A inhibitory potential should be considered. If the benefit outweighs the risk and a strong CYP3A inhibitor must be used, see the recommended dose modifications described in the specific clinical study protocol.

***Moderate and mild CYP3A inhibitors***
In patients with B-cell malignancies, co-administration of the CYP3A inhibitor erythromycin increased Cmax and AUC by 3.4-fold and 3.0-fold, respectively. If a moderate CYP3A inhibitor (eg, fluconazole, erythromycin, amprenavir, aprepitant, atazanavir, ciprofloxacin, crizotinib, diltiazem, fosamprenavir, imatinib, verapamil, amiodarone, dronedarone) is indicated in patients with B-cell malignancies, reduce the ibrutinib dose to 280 mg for the duration of the inhibitor use. or as per recommended dose modifications described in the specific clinical study protocol. No ibrutinib dose modifications for moderate inhibitors are required in patients with cGVHD dosed with ibrutinib 420 mg.

No dose adjustment is required in combination with mild inhibitors. Monitor patient closely for toxicity and follow dose modification guidance as needed. Avoid grapefruit and Seville oranges during ibrutinib treatment as these contain moderate inhibitors of CYP3A.

***Agents That May Decrease ibrutinib Plasma Concentrations (CYP3A Inducers)***
Administration of ibrutinib with strong inducers of CYP3A decreases ibrutinib plasma concentrations by up to 90%. Avoid concomitant use of strong CYP3A inducers (eg, carbamazepine, rifampin, phenytoin and St. John's Wort). Consider alternative agents with less CYP3A induction.

***Drugs that may have their plasma concentrations altered by ibrutinib***
In vitro studies indicated that ibrutinib is a weak reversible inhibitor toward CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4/5 and does not display time--dependent CYP inhibition. The dihydrodiol metabolite of ibrutinib is a weak inhibitor toward CYP2B6, CYP2C8, CYP2C9, and CYP2D6. Both ibrutinib and the dihydrodiol metabolite are at most weak inducers of CYP isoenzymes in vitro. Therefore, it is unlikely that ibrutinib has any clinically relevant drug-drug interactions with drugs that may be metabolized by the CYP enzymes.
In vitro studies indicated that ibrutinib is not a substrate of P-gp, nor other major transporters, except OCT2. The dihydrodiol metabolite and other metabolites are P--gp substrates. Ibrutinib is a mild inhibitor of P--gp and breast cancer resistance protein (BCRP). Ibrutinib is not expected to have systemic drug-drug interactions with P--gp substrates. However, it cannot be excluded that ibrutinib could inhibit intestinal P--gp and BCRP after a therapeutic dose. There are no clinical data available. To minimize the potential for an interaction in the GI tract, narrow therapeutic range P--gp or BCRP substrates such as digoxin or methotrexate should be taken at least 6 hours before or after ibrutinib. Ibrutinib may also inhibit BCRP systemically and increase the exposure of drugs that undergo BCRP -mediated hepatic efflux, such as rosuvastatin.

**Contraindications:**
Ibrutinib is contraindicated in subjects with clinically significant hypersensitivity to any of the compound's structural components. Ibrutinib has not been used in subjects with biliary obstruction, acute hepatitis, severe liver failure, or severely impaired renal function; hence, avoid use of ibrutinib in patients with these conditions.

**11.2.2 Risks related to blood draws:** No major risks are involved with blood draws. Minor complications including bleeding, pain, and hematoma formation at the site of blood draws, vasovagal reactions or infections may rarely occur.

**11.2.3 Risks related to CT Scan:** CT (computed tomography), sometimes called CAT scan, uses special x-ray equipment to obtain image data from different angles around the body and then uses computer processing of the information to show a cross-section of body tissues and organs. Oral and/or intravenous contrast agents will be used and are usually well tolerated. However, some subjects will experience allergic reactions to intravenous contrast. To lower the risk of allergic reactions, low allergenic contrast agents are administered at NIH clinical center. In addition, subjects will be advised that approximately 2-7% of patients who receive contrast agents will experience a temporary reduction in kidney function lasting up to 2 weeks following infusion and that in rare instances, permanent renal damage can result from the use of the IV contrasting agent. Therefore, in subjects with impaired kidney function, we will not use intravenous contrast.

The amount of radiation subjects will receive from the research scans in this study is 1.3 rem of radiation annually, which is below the guideline of 5 rem (or 0.5 rem in children) per year allowed for research subjects by the NIH Radiation Safety Committee. All female subjects will receive pregnancy testing prior to radiation exposure.

**11.2.4 Related to pregnancy and nursing mothers**: There are no clinical studies in pregnant women, and it is unknown whether ibrutinib or its metabolites are excreted in human milk. Men and women of child-bearing potential must use highly effective contraceptive (eg, condoms, implants, injectables, combined oral contraceptives, some intrauterine devices [IUDs], sexual abstinence, or sterilized partner) protection while on study and for 90 days after the last dose of study drug. If a female subject or the partner of a male subject becomes pregnant, the sponsor must be notified. Male subjects should refrain from sperm donation.

**11.2.5 Related to central line placement** (only when indicated): A catheter may be placed in a large vein of the neck, chest, or arm using local anesthetic. Patients will sign a separate consent for the placement procedure. Only trained experienced staff will place the line in order to minimize these procedure related risks. The risks from the procedure are low; they include bleeding, bruising, or infection at the site of insertion. Some patient may experience a vasovagal reaction (lightheadedness, or, rarely, fainting due to temporary lowering of blood pressure). Very rarely (less than 1% of the time), the line placement may nick the lung causing it to collapse during line insertion. If the lung collapses, a tube may have to be inserted into the chest and remain in place until the lung re-expands. Because of this risk, patients will have a chest x-ray following the procedure to make sure the line is in the correct place and that the lung is not collapsed. Once placed, the line will remain in place until drug administration is complete.

**11.2.6 Risks related to bone marrow biopsy:** The anesthetic can cause some temporary stinging and burning. A pulling sensation and discomfort may be felt as the marrow is withdrawn. Although rare, there is a potential for bleeding at the site and local infection. Bleeding can be stopped by applying local pressure, and infection can be treated with antibiotics.

**11.2.7 Risks related to transfusions:** Some risks with the transfusion with blood and /or blood products include fever or allergic reactions. These risks are uncommon and are usually mild, but on rare occasions may be severe or life threatening. Extremely rare risks include infections with viruses, such as hepatitis or HIV or serious incompatibility reactions.

### 11.3 Risks in Relation to Benefit

The benefits to the adult patient could be a reduction or a disappearance of the CLL/SLL resulting in an improved quality of life, a decreased susceptibility to infections, and foremost a significant improvement in survival time. Potentially, treatment with other therapies could also be avoided or postponed. Therefore, this research involves greater than minimal risk to subjects with the prospect of direct benefit (45 CFR 46.102).

### 11.4 Informed Consent processes and procedures

The investigational nature and research objectives of this trial, the procedure and its attendant risks and discomforts will be carefully explained to the subject and a signed informed consent document will be obtained prior to entry onto this study.

At any time during participation in the protocol, should new information become available relating to risks, adverse events, or toxicities, this information will be provided orally or in writing to all enrolled or prospective patient participants. Documentation will be provided to the IRB and if necessary the informed consent amended to reflect relevant information.

If there is an unexpected enrollment of a research participant for which there is no translated extant IRB approved consent document, the principal investigator and or those authorized to obtain informed consent will use the Short Form Oral Consent Process as described in MAS Policy M77-2, 45 CFR 46.117 (b) (2), and 21

CFR50.27 (b) (a)0. The summary that will be used is the English version of the extant IRB approved consent document.

We request prospective IRB approval of the use of the short form for up to a maximum of **5** research participants in a given language and will notify the IRB at the time of continuing review of the frequency of the use of the Short Form. Should we reach the threshold of 5, we will notify the IRB of the need for an additional use of the Short Form and that we will have that consent document translated into the given inherent language.

**Informed consent process for NIH employees :** If the individual requesting to participate is a co-worker, the consent from the employee (co-worker) will not be obtained by the employee's direct supervisor but by another research staff member approved for obtaining informed consent and who is also not a co-worker. Neither participation nor refusal to participate as a subject in this protocol will have an effect, either beneficial or adverse, on the participant's employment or position at NIH. However, all subjects will be made aware that there are limits to these protections. The PI, through the consenting staff member will make the "NIH Information Sheet on Staff Research Participation" available to the NIH staff who are considering enrolling in research. (HRPP SOP 14F, Appendix A; also in Appendix C of the protocol). In addition, all NIH staff that choose to participate in this study can access HRPP SOP 14F, Appendix B (NIH Policy Manual Chapter 2300-630-3 "Leave Policy for NIH Employees Participating in NIH Medical Research Studies").

### 11.5 Conflict of Interest

Pharmacyclics, an AbbVie Company is providing ibrutinib for this study to NIH without charge. No NIH ivestigator involved in this study receives any payment or other benefits from Pharmacyclics, an AbbVie Company. The Principal Investigator assures that each associate investigator listed on the protocol title page received a copy of the NIH's Guide to preventing conflict of interest. No members of the research team reported a potential conflict of interest.

### 11.6 Technical Transfer Agreements

The protocol has the following associated CRADA: Between NHLBI, and Pharmacyclics, an AbbVie Company.

## 12.0 PHARMACEUTICALS

### 12.1 Ibrutinib[48]

**Product description:** Ibrutinib is commercially available. Note for more detailed and comprehensive background information please refer to the ibrutinib Package Insert. Chemical name of ibrutinib is PCI-32765-00, which is 1-[(3R)-3-[4-amino-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4 d]pyrimidin-1-yl]-1-piperidinyl]-2-propen-1-one. PCI-32765-00 is a white to off-white crystalline solid, and given as an oral formulation containing micronized PCI-32765-00.

**Formulation, Packaging, and Storage:** Ibrutinib is provided as a gray, hard gelatin capsule containing 140 mg of ibrutinib. The capsules are packaged in opaque high-density polyethylene (HDPE) plastic bottles with labels bearing the appropriate label text as required by governing regulatory agencies. All study drug will be dispensed in child-resistant packaging. The drug product is manufactured for Pharmacyclics LLC, an AbbVie Company, by a contract manufacturer. All formulation excipients are compendial and are commonly used in oral formulations. Each bottle contains 92 capsules. The recommended storage condition for ibrutinib is room temperature (15 to 25°C).

**Dosage and Administration:** The investigator must ensure that patients receive ibrutinib only from personnel who fully understand the procedures for administering the drug.

At any given monthly visit, only enough ibrutinib for 1 cycle should be dispensed. After cycle 6 when follow ups are done every 3 months, only enough ibrutinib until the next follow up visit should be dispensed. In cases where there are patient scheduling conflicts additional doses of ibrutinib may be given to accommodate visits within the window specified in the protocol. Drug dispensed will be recorded in ID MRS for drug accountability. Missed or held doses will be recorded at every clinic visit. If the patient is taken off from the study, unused ibrutinib capsules must be returned to ID MRS for local destruction according to institution's standard operating procedures. Returned capsules must not be redispensed.

Ibrutinib 420 mg (3 capsules at 140-mg per capsule) is intended to be administered orally once daily with a glass of water (avoid GRAPEFRUIT JUICE and SEVILLE ORANGES). The capsules should be swallowed intact and patients should not attempt to open capsules or dissolve them in water.

If a dose is missed, it can be taken up to 6 hours after the scheduled time with a return to the normal schedule the following day. If it has been greater than 6 hours, the dose should not be taken, and the patient should take the next dose at the scheduled time the next day. The missed dose will not be made up.

**Overdose:** Any dose of study drug in excess of that specified in this protocol is considered to be an overdose. Signs and symptoms of an overdose that meet any Serious Adverse Event criterion must be reported as a Serious Adverse Event in the appropriate time frame and documented as clinical sequelae to an overdose.

There is no specific experience in the management of ibrutinib overdose in patients. No maximum tolerated dose (MTD) was reached in the Phase 1 study in which subjects received up to 12.5 mg/kg/day (1400 mg/day). Healthy subjects were exposed up to single dose of 1680 mg. One healthy subject experienced reversible Grade 4 hepatic enzyme increases (AST and ALT) after a dose of 1680 mg. Subjects who ingested more than the recommended dosage should be closely monitored and given appropriate supportive treatment.

Refer to Section 10.4.1 for further information regarding AE reporting.

**Supply:** The drug product ibrutinib is manufactured by Pharmatek Laboratories, Inc (San Diego, CA) or Aptuit (Kansas City, MO).

**Shipping:**

National Institutes of Health
PHARM DEV SVC, Room 1C230
10 Center Drive, MSC 1196, Building 10
Bethesda, Maryland 20892-1196
Shipping Designee Name: Hope Decederfelt, RPh
Shipping Designee Phone No: (301) 402-8153
Shipping Designee FAX No: (301) 402-3268
Shipping Designee e-mail: HDecederfe@cc.nih.gov

### 12.2 Fludarabine:

**Product description:** fludarabine phosphate is commercially available as both a lyophilized powder for injection in vials containing 50 mg of fludarabine phosphate with mannitol 50 mg and sodium hydroxide for pH adjustment and a solution for injection in 2 mL vials containing 50 mg of fludarabine phosphate (25 mg/mL of fludarabine) with 25 mg/mL mannitol and sodium hydroxide for pH adjustment.

**Preparation:** fludarabine lyophilized powder for injection should be reconstituted with 2 mL of sterile water for injection, up to a concentration of 25 mg/mL. The prescribed dose of fludarabine should be diluted in 100 mL of either 0.9% sodium chloride or 5% dextrose in water for intravenous administration.

**Storage and Stability:** fludarabine vials should be stored under refrigeration between 2-8 $^{o}$C (36- 46 $^{o}$F). Reconstituted fludarabine phosphate is chemically and physically stable for 24 hours at room temperature or for 48 hours if refrigerated. The manufacturer recommends use of either the reconstituted powder for injection or the solution for injection (once diluted for administration) within 8 hours because neither product contains an antimicrobial preservative.

**Administration:** the prescribed dose of fludarabine should be diluted in 100 mL of either 0.9% sodium chloride or 5% dextrose in water for intravenous administration.

**Supply:** commercially available.

### 12.3 Off-Label Use of Drugs

Ibrutinib and fludarabine will be used in this study beyond what is indicated in their Package Inserts. Ibrutinib and fludarabine are approved by the FDA for the treatment of patients with CLL regardless of prior treatment status; however, this protocol will be investigating ibrutinib and fludarabine on subjects who are treatment naive. The use of this drug meets the requirements for an exemption from the IND regulations, 21 CFR 312, specifically:

1. The investigational drug is lawfully marketed in the United States.
2. The investigation is not intended to be reported to the FDA as a well-controlled study in support of a new indication for use of the drug product.
3. The investigation is not intended to support a significant change in advertising to an existing lawfully marketed prescription drug product.
4. The investigation does not involve a route of administration or dosage level or use in a patient population or other factor that significantly increases the risks (or decreases the acceptability of the risks) associated with the use of the drug product.
5. The investigation will be conducted in compliance with the requirements for institutional review set forth in FDA regulations 21 CFR 56, and requirements for informed consent as set forth in FDA regulations 21 CFR 50.
6. The investigation will be conducted in compliance with FDA regulations 21 CFR 312.7: Promotion and charging for investigational drugs.

### 12.4 Accountability Procedures

Drug accountability records will be maintained for all clinical supplies. All empty and partially used vials and clinical trial supplies will be destroyed locally according to the institution's standard operating procedures for drug destruction. The pharmacy will maintain detailed documentation of the number and identification of vials which are destroyed, and copies of these documents will be provided to the sponsor and Pharmacyclics, an AbbVie Company. Disposition of all unused boxes of study drug will be carried out according to instructions provided by the sponsor and/or Pharmacyclics, an AbbVie Company at the end of the study after drug accountability is performed by the study monitor.

## 13.0 REFERENCES


1. Müller-Hermelink HK, Montserrat E, Catovsky D, Harris NL. Chronic lymphocytic leukemia/small lymphocytic lymphoma. In: Jaffe ES, Harris NL, Stein H, Vardiman JW, editors. World Health Organization Classification of Tumours: Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues. Lyon, France: IARC Press; 2001. pp. 127–130.
2. Cheson BD, Bennett JM, Grever M, et al. National Cancer Institute-sponsored Working Group guidelines for chronic lymphocytic leukemia: revised guidelines for diagnosis and treatment. Blood 1996;87:4990-7.
3. 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;111:5446-56.
4. Friedberg JW, Sharman J, Sweetenham J, et al. Inhibition of Syk with fostamatinib disodium has significant clinical activity in non-Hodgkin lymphoma and chronic lymphocytic leukemia. Blood;115:2578-85.
5. Staudt LM. Oncogenic activation of NF-kappaB. Cold Spring Harb Perspect Biol;2:a000109.
6. Lagneaux L, Delforge A, Bron D, De Bruyn C, Stryckmans P. Chronic lymphocytic leukemic B cells but not normal B cells are rescued from apoptosis by contact with normal bone marrow stromal cells. Blood 1998;91:2387-96.
7. Burger JA, Quiroga MP, Hartmann E, et al. High-level expression of the T-cell chemokines CCL3 and CCL4 by chronic lymphocytic leukemia B cells in nurselike cell cocultures and after BCR stimulation. Blood 2009;113:3050-8.
8. Ramsay AG, Clear AJ, Fatah R, Gribben JG. Multiple inhibitory ligands induce impaired T-cell immunologic synapse function in chronic lymphocytic leukemia that can be blocked with lenalidomide: establishing a reversible immune evasion mechanism in human cancer. Blood 2012;120:1412-21.
9. Luqman M, Klabunde S, Lin K, et al. The antileukemia activity of a human anti-CD40 antagonist antibody, HCD122, on human chronic lymphocytic leukemia cells. Blood 2008;112:711-20.
10. Kater AP, Evers LM, Remmerswaal EB, et al. CD40 stimulation of B-cell chronic lymphocytic leukaemia cells enhances the anti-apoptotic profile, but also Bid expression and cells remain susceptible to autologous cytotoxic T-lymphocyte attack. Br J Haematol 2004;127:404-15.
11. Van den Hove LE, Vandenberghe P, Van Gool SW, et al. Peripheral blood lymphocyte subset shifts in patients with untreated hematological tumors: evidence for systemic activation of the T cell compartment. Leukemia research 1998;22:175-84.
12. Riches JC, Davies JK, McClanahan F, et al. T cells from CLL patients exhibit features of T-cell exhaustion but retain capacity for cytokine production. Blood 2013;121:1612-21.
13. Cancer Facts and Figures 2015. American Cancer Society Data accessed January 6, 2015.
14. Chomarat P, Banchereau J. Interleukin-4 and interleukin-13: their similarities and discrepancies. Int Rev Immunol 1998;17:1-52.
15. Mauro FR, Foa R, Cerretti R, et al. Autoimmune hemolytic anemia in chronic lymphocytic leukemia: clinical, therapeutic, and prognostic features. Blood 2000;95:2786-92.
16. Rossi D, Gaidano G. Richter syndrome: molecular insights and clinical perspectives. Hematol Oncol 2009;27:1-10.
17. Rai KR, Sawitsky A, Cronkite EP, Chanana AD, Levy RN, Pasternack BS. Clinical staging of chronic lymphocytic leukemia. Blood 1975;46:219-34.
18. Damle RN, Wasil T, Fais F, et al. Ig V gene mutation status and CD38 expression as novel prognostic indicators in chronic lymphocytic leukemia. Blood 1999;94:1840-7.
19. Wiestner A, Rosenwald A, Barry TS, et al. ZAP-70 expression identifies a chronic lymphocytic leukemia subtype with unmutated immunoglobulin genes, inferior clinical outcome, and distinct gene expression profile. Blood 2003;101:4944-51.
20. Rassenti LZ, Jain S, Keating MJ, et al. Relative value of ZAP-70, CD38, and immunoglobulin mutation status in predicting aggressive disease in chronic lymphocytic leukemia. Blood 2008;112:1923-30.
21. Dohner H, Stilgenbauer S, Benner A, et al. Genomic aberrations and survival in chronic lymphocytic leukemia. N Engl J Med 2000;343:1910-6.
22. Geisler CH, Philip P, Christensen BE, et al. In B-cell chronic lymphocytic leukaemia chromosome 17 abnormalities and not trisomy 12 are the single most important cytogenetic abnormalities for the prognosis: a

cytogenetic and immunophenotypic study of 480 unselected newly diagnosed patients. Leukemia research 1997;21:1011-23.
23. Austen B, Skowronska A, Baker C, et al. Mutation status of the residual ATM allele is an important determinant of the cellular response to chemotherapy and survival in patients with chronic lymphocytic leukemia containing an 11q deletion. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2007;25:5448-57.
24. Tsimberidou AM, Tam C, Abruzzo LV, et al. Chemoimmunotherapy may overcome the adverse prognostic significance of 11q deletion in previously untreated patients with chronic lymphocytic leukemia. Cancer 2009;115:373-80.
25. Garcia-Marco JA, Price CM, Catovsky D. Interphase cytogenetics in chronic lymphocytic leukemia. Cancer Genet Cytogenet 1997;94:52-8.
26. Corcoran MM, Rasool O, Liu Y, et al. Detailed molecular delineation of 13q14.3 loss in B-cell chronic lymphocytic leukemia. Blood 1998;91:1382-90.
27. Molica S, Alberti A. Prognostic value of the lymphocyte doubling time in chronic lymphocytic leukemia. Cancer 1987;60:2712-6.
28. Lipshutz MD, Mir R, Rai KR, Sawitsky A. Bone marrow biopsy and clinical staging in chronic lymphocytic leukemia. Cancer 1980;46:1422-7.
29. Moreno A, Villar ML, Camara C, et al. Interleukin-6 dimers produced by endothelial cells inhibit apoptosis of B-chronic lymphocytic leukemia cells. Blood 2001;97:242-9.
30. Chemotherapeutic options in chronic lymphocytic leukemia: a meta-analysis of the randomized trials. CLL Trialists' Collaborative Group. J Natl Cancer Inst 1999;91:861-8.
31. Knauf WU, Lissichkov T, Aldaoud A, et al. Phase III randomized study of bendamustine compared with chlorambucil in previously untreated patients with chronic lymphocytic leukemia. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2009;27:4378-84.
32. Rai KR, Peterson BL, Appelbaum FR, et al. Fludarabine compared with chlorambucil as primary therapy for chronic lymphocytic leukemia. N Engl J Med 2000;343:1750-7.
33. Del Poeta G, Del Principe MI, Buccisano F, et al. Consolidation and maintenance immunotherapy with rituximab improve clinical outcome in patients with B-cell chronic lymphocytic leukemia. Cancer 2008;112:119-28.
34. Hallek M, Fischer K, Fingerle-Rowson G, et al. Addition of rituximab to fludarabine and cyclophosphamide in patients with chronic lymphocytic leukaemia: a randomised, open-label, phase 3 trial. Lancet 2010;376:1164-74.
35. Fischer K, Cramer P, Busch R, et al. Bendamustine in Combination With Rituximab for Previously Untreated Patients With Chronic Lymphocytic Leukemia: A Multicenter Phase II Trial of the German Chronic Lymphocytic Leukemia Study Group. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2012;30:3209-16.
36. Fink A-M, Busch R, Lange E, et al. Chemoimmunotherapy With Fludarabine (F), Cyclophosphamide (C), and Rituximab (R) (FCR) Versus Bendamustine and Rituximab (BR) In Previously Untreated and Physically Fit Patients (pts) With Advanced Chronic Lymphocytic Leukemia (CLL): Results Of a Planned Interim Analysis Of The CLL10 Trial, An International, Randomized Study Of The German CLL Study Group (GCLLSG). Blood 2013;122:526.
37. Hallek M. Therapy of chronic lymphocytic leukaemia. Best Pract Res Clin Haematol 2010;23:85-96.
38. Goede V, Fischer K, Busch R, et al. Obinutuzumab plus chlorambucil in patients with CLL and coexisting conditions. N Engl J Med 2014;370:1101-10.
39. Tam CS, O'Brien S, Wierda W, et al. Long-term results of the fludarabine, cyclophosphamide, and rituximab regimen as initial therapy of chronic lymphocytic leukemia. Blood 2008;112:975-80.
40. Badoux XC, Keating MJ, Wang X, et al. Fludarabine, cyclophosphamide, and rituximab chemoimmunotherapy is highly effective treatment for relapsed patients with CLL. Blood 2011;117:3016-24.
41. Fischer K, Cramer P, Busch R, et al. Bendamustine combined with rituximab in patients with relapsed and/or refractory chronic lymphocytic leukemia: a multicenter phase II trial of the German Chronic Lymphocytic Leukemia Study Group. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2011;29:3559-66.

42. Wierda WG, Kipps TJ, Mayer J, et al. Ofatumumab as single-agent CD20 immunotherapy in fludarabine-refractory chronic lymphocytic leukemia. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2010;28:1749-55.
43. Eichhorst B, Dreyling M, Robak T, Montserrat E, Hallek M. Chronic lymphocytic leukemia: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Annals of oncology : official journal of the European Society for Medical Oncology / ESMO 2011;22 Suppl 6:vi50-4.
44. Brown JR, Wiestner A. Chronic lymphocytic leukemia: moving forward-rapidly. Seminars in hematology 2014;51:157.
45. Fiorcari S, Brown WS, McIntyre BW, et al. The PI3-kinase delta inhibitor idelalisib (GS-1101) targets integrin-mediated adhesion of chronic lymphocytic leukemia (CLL) cell to endothelial and marrow stromal cells. PLoS One 2013;8:e83830.
46. Fuhrman RR, Sharman JP, Coutre SE, et al. A Phase 3, Randomized, Double-Blind, Placebo-Controlled Study Evaluating the Efficacy and Safety of Idelalisib and Rituximab for Previously Treated Patients with Chronic Lymphocytic Leukemia (CLL). Blood 2013;122:LBA-6.
47. Davids MS, Pagel JM, Kahl BS, et al. Bcl-2 Inhibitor ABT-199 (GDC-0199) Monotherapy Shows Anti-Tumor Activity Including Complete Remissions In High-Risk Relapsed/Refractory (R/R) Chronic Lymphocytic Leukemia (CLL) and Small Lymphocytic Lymphoma (SLL). Blood 2013;122:872.
48. San Miguel JF, Schlag R, Khuageva NK, et al. Bortezomib plus melphalan and prednisone for initial treatment of multiple myeloma. N Engl J Med 2008;359:906-17.
49. Buggy JJ, Elias L. Bruton Tyrosine Kinase (BTK) and Its Role in B-cell Malignancy. Int Rev Immunol 2012;31:119-32.
50. Herman SE, Gordon AL, Hertlein E, et al. Bruton tyrosine kinase represents a promising therapeutic target for treatment of chronic lymphocytic leukemia and is effectively targeted by PCI-32765. Blood 2011;117:6287-96.
51. Honigberg LA, Smith AM, Sirisawad M, et al. The Bruton tyrosine kinase inhibitor PCI-32765 blocks B-cell activation and is efficacious in models of autoimmune disease and B-cell malignancy. Proc Natl Acad Sci U S A 2010;107:13075-80.
52. Advani RH, Buggy JJ, Sharman JP, et al. Bruton tyrosine kinase inhibitor ibrutinib (PCI-32765) has significant activity in patients with relapsed/refractory B-cell malignancies. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2013;31:88-94.
53. Herman SE, Mustafa RZ, Gyamfi JA, et al. Ibrutinib inhibits BCR and NF-kappaB signaling and reduces tumor proliferation in tissue-resident cells of patients with CLL. Blood 2014;123:3286-95.
54. Byrd JC, Furman RR, Coutre SE, et al. Targeting BTK with ibrutinib in relapsed chronic lymphocytic leukemia. N Engl J Med 2013;369:32-42.
55. Byrd JC, Brown JR, O'Brien S, et al. Ibrutinib versus ofatumumab in previously treated chronic lymphoid leukemia. N Engl J Med 2014;371:213-23.
56. O'Brien S, Furman RR, Coutre SE, et al. Ibrutinib as initial therapy for elderly patients with chronic lymphocytic leukaemia or small lymphocytic lymphoma: an open-label, multicentre, phase 1b/2 trial. The Lancet Oncology 2014;15:48-58.
57. Farooqui MZ, Valdez J, Martyr S, et al. Ibrutinib for previously untreated and relapsed or refractory chronic lymphocytic leukaemia with TP53 aberrations: a phase 2, single-arm trial. The Lancet Oncology 2015;16:169-76.
58. Kumar SK, Dingli D, Lacy MQ, et al. Autologous stem cell transplantation in patients of 70 years and older with multiple myeloma: Results from a matched pair analysis. Am J Hematol 2008;83:614-7.
59. Byrd JC, Furman RR, Coutre SE, et al. Three-year follow-up of treatment-naive and previously treated patients with CLL and SLL receiving single-agent ibrutinib. Blood 2015;125:2497-506.
60. Barrientos JC, Barr PM, Flinn I, et al. Ibrutinib In Combination With Bendamustine and Rituximab Is Active and Tolerable In Patients With Relapsed/Refractory CLL/SLL: Final Results Of a Phase 1b Study. Blood 2013;122:525.
61. Burger JA, Keating MJ, Wierda WG, et al. Safety and activity of ibrutinib plus rituximab for patients with high-risk chronic lymphocytic leukaemia: a single-arm, phase 2 study. The lancet oncology 2014;15:1090-9.

62. Blade J, Rosinol L, Sureda A, et al. High-dose therapy intensification compared with continued standard chemotherapy in multiple myeloma patients responding to the initial chemotherapy: long-term results from a prospective randomized trial from the Spanish cooperative group PETHEMA. Blood 2005;106:3755-9.
63. Byrd JC. A phase Ib/II study evaluating activity and tolerability of BTK inhibitor PCI-32765 and ofatumumab in patients with chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) and related diseases. ASCO 2012.
64. Fermand JP, Ravaud P, Chevret S, et al. High-dose therapy and autologous peripheral blood stem cell transplantation in multiple myeloma: up-front or rescue treatment? Results of a multicenter sequential randomized clinical trial. Blood 1998;92:3131-6.
65. Woyach JA, Furman RR, Liu TM, et al. Resistance mechanisms for the Bruton's tyrosine kinase inhibitor ibrutinib. N Engl J Med 2014;370:2286-94.
66. Furman RR, Cheng S, Lu P, et al. Ibrutinib resistance in chronic lymphocytic leukemia. N Engl J Med 2014;370:2352-4.
67. Attal M, Harousseau JL, Stoppa AM, et al. A prospective, randomized trial of autologous bone marrow transplantation and chemotherapy in multiple myeloma. Intergroupe Francais du Myelome. N Engl J Med 1996;335:91-7.
68. Bagnara D, Kaufman MS, Calissano C, et al. A novel adoptive transfer model of chronic lymphocytic leukemia suggests a key role for T lymphocytes in the disease. Blood 2011;117:5463-72.
69. Ramsay AG, Evans R, Kiaii S, Svensson L, Hogg N, Gribben JG. Chronic lymphocytic leukemia cells induce defective LFA-1-directed T-cell motility by altering Rho GTPase signaling that is reversible with lenalidomide. Blood 2013;121:2704-14.
70. Ysebaert L, Gross E, Kuhlein E, et al. Immune recovery after fludarabine-cyclophosphamide-rituximab treatment in B-chronic lymphocytic leukemia: implication for maintenance immunotherapy. Leukemia 2010;24:1310-6.
71. Flinn IW, Neuberg DS, Grever MR, et al. Phase III trial of fludarabine plus cyclophosphamide compared with fludarabine for patients with previously untreated chronic lymphocytic leukemia: US Intergroup Trial E2997. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2007;25:793-8.
72. Kornblit B, Maloney DG, Storb R, et al. Fludarabine and 2-Gy TBI is superior to 2 Gy TBI as conditioning for HLA-matched related hematopoietic cell transplantation: a phase III randomized trial. Biol Blood Marrow Transplant 2013;19:1340-7.
73. Khouri IF, Wei W, Korbling M, et al. BFR (bendamustine, fludarabine, and rituximab) allogeneic conditioning for chronic lymphocytic leukemia/lymphoma: reduced myelosuppression and GVHD. Blood 2014;124:2306-12.
74. Kochenderfer JN, Dudley ME, Kassim SH, et al. Chemotherapy-refractory diffuse large B-cell lymphoma and indolent B-cell malignancies can be effectively treated with autologous T cells expressing an anti-CD19 chimeric antigen receptor. J Clin Oncol 2015;33:540-9.
75. Lee DW, Kochenderfer JN, Stetler-Stevenson M, et al. T cells expressing CD19 chimeric antigen receptors for acute lymphoblastic leukaemia in children and young adults: a phase 1 dose-escalation trial. Lancet 2015;385:517-28.
76. Beyer M, Kochanek M, Darabi K, et al. Reduced frequencies and suppressive function of CD4+CD25hi regulatory T cells in patients with chronic lymphocytic leukemia after therapy with fludarabine. Blood 2005;106:2018-25.
77. Hallek M, Cheson BD, Catovsky D, et al. iwCLL guidelines for diagnosis, indications for treatment, response assessment, and supportive management of CLL. Blood 2018;131:2745-60.
78. Keating MJ, O'Brien S, Albitar M, et al. Early results of a chemoimmunotherapy regimen of fludarabine, cyclophosphamide, and rituximab as initial therapy for chronic lymphocytic leukemia. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2005;23:4079-88.
79. Sonneveld P, Schmidt-Wolf IG, van der Holt B, et al. Bortezomib induction and maintenance treatment in patients with newly diagnosed multiple myeloma: results of the randomized phase III HOVON-65/ GMMG-HD4 trial. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2012;30:2946-55.

## APPENDIX A: Schedule of Events

| Study Day/NAME: | Screening | Baseline | C1 & 2 | C3 &4 | | | | | | C5 &6 | Follow up every 3 months | Response Assessment After cycle 6 | Response Assessment Annually | At progression |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Days (Plus/Minus) | N/A | ±5 | ±5 | ±5 | | | | | | ±5 | ±14 | ±30 | ±30 | N/A |
| Day of the cycle | N/A | N/A | D1 | D1 | D 2 | D 3 | D 4 | D5 | D14 D21 | D1 | N/A | N/A | N/A | N/A |
| NIH visit | X | X | X | X | X | X | X | X | home | X | X | X | X | X |
| **Clinical** | | | | | | | | | | | | | | |
| Consent | | X | | | | | | | | | | | | |
| History, ECOG PS | X | X | X | X | | | | | | X | X | X | X | X |
| Physical Exam | X | X | X | X | | | | | | X | X | X | X | X |
| Medication Review | X | X | X | X | | | | | | X | X | X | X | X |
| **Specific Labs/Other Tests** | | | | | | | | | | | | | | |
| HLA Typing (as clinically indicated) | | X | | | | | | | | | | | | |
| CBC with Differential | X | X | X | X | | | | | X | X | X | X | X | X |
| Acute Care & Mineral Panel | X | X | X | X | | | | | | X | X | X | X | X |
| Tot. protein, Uric Acid, LDH | X | X | X | X | | | | | | X | X | X | X | X |
| Hepatic Panel | X | X | X | X | | | | | | X | X | X | X | X |
| Reticulocyte Count | X | X | X | X | | | | | | X | X | X | X | X |
| PT, PTT | X | X | | | | | | | | | | X | X | X |
| SPEP with IFE | | X | | | | | | | | | | | | [R] |
| SFL with qIG | | X | | | | | | | | | | X | X | X |
| CRP | | X | | | | | | | | | | | | [R] |
| β2 Microglobulin | X | X | X | X | | | | | | X | X | X | X | X |
| Haptoglobin | X | X | | | | | | | | | | X | X | X |
| DAT | X | X | X | X | | | | | | X | X | X | X | X |
| Iron studies (ferritin, transferrin, iron) | X | | | | | | | | | | | | | |
| Folate, Vit B12 | X | | | | | | | | | | | | | |
| Viral Studies (HIV, Hepatitis) | X | | | | | | | | | | | | | |
| Pregnancy test | X | X | X | X | | | | | | X | X | X | X | X |
| TBNK# | | X | | X | | | | | | X | | X | X | X |
| IGHV mutation analysis | | X | | | | | | | | | | | | [R] |
| Flow CLL (w/in 3 mo of starting) | X | | | | | | | | | | | X | X | X |
| CT Neck, CAP (w/in 3 mo of starting) | X | | | X | | | | | | | | X | X | X |
| Bone Marrow( w/in 3mo of starting) | | X | | | | | | | | | | X | [R] ** | [R] |
| CLL/FISH (w/in 3 mo of starting) | | X | | | | | | | | | | | | [R] |
| EKG (w/in 3 mo of starting, then if medically indicated) | X | | | | | | | | | | | | | |
| **Research Samples** | | | | | | | | | | | | | | |
| Lymphapheresis | | [R] | | | | | | | | | | [R] | [R] | [R] |
| Lymph node biopsy* | | [R] | [R] | [R] | | | | | | | | [R] | [R] | [R] |
| Research blood | | X | X | X | | | | [R] | | [R] | X | X | X | X |

**Footnotes for Schedule of Events*:***

[A] If C1D1 labs were collected within 5 days for screening or baseline studies, the study team may decide not to repeat the labs.
* Up to 3 lymph node biopsy can be performed in consented patients.
** Bone marrow biopsy may be done to confirm complete response (optional).
# TBNK is performed baseline, before C3 and at response assessments (after cycle 6 and annually thereafter).
[R] Optional, drawn at discretion of clinical team
X Mandated

**APPENDIX B**:
NHLBI HEMATOLOGY BRANCH LABORATORY RESEARCH STUDIES -2/5/2013

| | DESCRIPTION OF LABORATORY STUDY BY BRANCH SECTION | Does this test pose a greater than minimal risk to pediatric subjects per 45 CFR 46.404? | Does this test pose a greater than minimal risk to healthy pediatric donors per 45 CFR 46.404? |
|---|---|---|---|
| | | | |
| **A** | **Stem Cell Allotransplantation Section (Dr. A. John Barrett)** | | |
| **A.1** | Measurement of lymphocyte function and immune responses directed toward allogeneic tissues, malignant cells, and infectious agents. Assay of a variety of antigens, including standard proliferation, cytotoxicity, and intracellular cytokine detection including GVHD predictive markers. Measurement of antigen-specific responses including employment of tetramers, ELISPOT technique, gene amplification-based assays, and flow cytometry. Selection of cells using immunomagnetic beads or flow cytometry. Culture, expansion, and selection of cells. Surface marker analysis of PB MC using flow cytometry. Cytokine/chemokine analysis of plasma/serum samples using ELISA and/or Luminex techniques. | No | No |
| **A.2** | Generation of cell lines for the study of immune cell interactions with other cells. Transformation of B-lymphocytes using Epstein-Barr virus. Derivation of malignant cell lines from patient leukemic or solid tumor samples. | No | No |
| **A.3** | Infection of cells and cell lines with recombinant genes to ascertain the effects of expressed molecules on immune responses and on growth and development. Transfection of cell lines with specific molecules to study antigen-specific responses. | No | No |
| **A.4** | Assays of peripheral blood and bone marrow progenitor cells including primitive and late erythroid progenitor-derived colonies, myelomonocytic colonies, and primitive multi- potential progenitor-derived colonies. | No | No |
| **A.5** | Injection of human cells into experimental animals to study the immune system and the growth of normal and malignant cells under varying conditions. | No | No |
| **A.6** | Testing of selection methods, cell isolation, and cell expansion leading to the development of new cell-based therapies requiring scale-up for clinical application. | No | No |
| **A.7** | Identification of individual T cell clones by their T cell receptor sequence. | No | No |
| **A.8** | Measurement of tumor and tissue specific antigens in cells of subjects and donors by mRNA,protein, or peptide expression in cells or fluids. | No | No |
| **A.9** | Laser capture micro dissection of cells from biopsies for GVHD to determine clonotypes. | No | No |
| **A.10** | DNA and RNA typing of genes that control immune responses in lymphocytes. | No | No |

| | | | |
|---|---|---|---|
| **A.11** | Microassay studies utilizing cellular DNA, cDNA, and RNA for neoplasia and host-tumor interactions. | No | No |
| | | | |
| **B** | **Molecular Hematopoiesis Section (Dr. Cynthia Dunbar)** | | |
| **B.1** | Flow cytometric analysis of cell surface and cytoplasmic proteins, including cell adhesion molecules, putative retroviral receptors, and markers of differentiation, using bone marrow and mobilized peripheral blood cells. | No | No |
| **B.2** | Hematopoietic progenitor-derived colony ascertainment in vitro (as described above), and engraftment of immunodeficient mice for detection of human stem cell number and function. | No | No |
| **B.3** | Testing ability of hematopoietic progenitor cells to be transduced with retroviral, lentiviral, and novel gene transfer vectors in vitro. | No | No |
| **B.4** | Reprogramming of adult mature cells, including skin fibroblasts and blood cells, into induced pluripotent stem cells in vitro. | No | No |
| | | | |
| **C** | **Cell Biology Section (Dr. Neal Young)** | | |
| **C.1** | Studies of blood and bone marrow hematopoietic progenitor numbers, including early and late erythroid progenitors, myelomonocytic progenitors, and multi-potential progenitor cells. In addition, bone marrow may be placed in long-term bone marrow culture to assess the function of stroma and stem cells and to assay more primitive progenitors, as well as organelle culture. Whole or selected bone marrow populations are cultured short-term for CD34 cell expansion. | No | No |
| **C.2** | Assays of apoptosis in hematopoietic cells and their progeny, using flow cytometric methods such as annexin and caspase-3 staining, propidium iodide uptake, and mitochondrial permeability tests. | No | No |
| **C.3** | Separation and functional study of cell populations characteristic of paroxysmal nocturnal hemoglobinuria, identified by absence of glycosylphosphatidylinositol anchored proteins. | No | No |
| **C.4** | Studies of mutation rates in hematopoietic cells and in buccal mucosa cells, using conventional hypoxanthine phosphoribosyltransferase activity functional assays, sequencing of mitochondrial DNA after specific gene amplification, and measurement of GPI-anchored deficient cells in blood and bone marrow. | No | No |
| **C.5** | Assays of immune function of T-cells, including intracellular cytokine staining, ELISPOT, semiquantitative gene amplification for gamma-interferon, tumor necrosis factor, interleukin-2, and other cytokines, and functional assessment in co-culture using specific neutralizing monoclonal antibodies. In addition, peripheral blood lymphocytes are subjected to spectratyping for CDR3 size distribution as well as nucleotide sequence of CDR3 peaks obtained. | No | No |
| **C.6** | Studies of engraftment of human normal and diseased bone marrow and peripheral blood in immunodeficient mice in order to determine the presence of hematopoietic repopulating stem cells as well as functional differences among selected populations. | No | No |

| | | | |
|---|---|---|---|
| **C.7** | Flow cytometric analysis of blood and bone marrow for lymphocyte phenotype, especially for evidence of activation of lymphocytes, for markers of apoptosis, and for antigens associated with primitive and mature hematopoietic cell populations. | No | No |
| **C.8** | Flow cytometric analysis of blood and bone marrow for hematopoietic stem cell progenitors and CD34 positive cells. | No | No |
| **C.9** | Studies of chromosomal instability in myelopdysplastic syndromes including BM cell and CD34 cell response to PAS crosslinking and examination of the cytotoxic effect of lymphocytes to the abnormal clone of cells. | No | No |
| **C.10** | Surface Enhanced Laser/Desorption Ionization (SELDI) time-of-flight mass spectrometry (Ciphergen) (proteomics methodology). | No | No |
| **C.11** | Mitochondrial DNA (mtDNA) sequence heterogeneity. | No | No |
| **C.12** | Measurement of EBV viral load. | No | No |
| **C.13** | Measurement of EBV LMP-1 via RT-PCR for LMP-1 RNA or flow cytometry for LMP-1. | No | No |
| **C.14** | Outgrowth assay of EBV transformed B cells. | No | No |
| **C.15** | Quantification of serum chemokines and cytokines (e.g. SDF-1, IL-10, IL-6, CXCR4, CXCL12). | No | No |
| **C.16** | Quantification of EBV cytotoxic T cells (tetramerstaining). | No | No |
| **C.17** | Telomere length measurement by Southern blot, Q-PCR, flow-fish, in situ hybridization and STELA | No | No |
| **C.18** | Telomere repair complex gene mutations by nucleotide sequencing of some or all of the following: *DKC1* ,*TERC*, *TERT*, *SBDS*, *NOp10* , *NHP2.* | No | No |
| **C.19** | Analysis of inflammatory markers and/or bacterial, viral, fungal or protozoal elements in plasma or serum using molecular, colorimetric, enzymatic, flow cytometric or other assays in subjects receiving immunosuppressive therapy, chemotherapy and/or bone marrow transplantation. | No | No |
| **C.20** | Confocal microscopic imaging of bone marrow. | No | No |
| **C.21** | Characterization of intracellular signaling proteins by cell permeabilization and flow cytometry, and quantitative immunoblots. | No | No |
| **C.22** | Assays for chromosomal aneuploidy by florescence in situ hybridization (FISH) and other molecular techniques. | No | No |
| **C.23** | Conversion of human dermal fibroblasts into hematopoietic progenitors using Oct4 transfection. | No | No |
| | | | |
| **D** | **Virus Discovery Section (Dr. Neal Young) THESE ASSAYS WILL NOT BE PERFORMED ON SAMPLES FROM HEALTHY PEDIATRIC DONORS** | | |
| **D.1** | Assays of serum, blood cells, and bone marrow cells for B19 parvovirus and possible B19 variants using gene amplification, cell culture, and hematopoietic colony inhibition assays. | No | N/A |

| | | | |
|---|---|---|---|
| **D.2** | Assays of blood, bone marrow, liver, and other tissues for potentially novel viruses, using a variety of techniques including RNA and DNA assays, differential display, gene amplification with conserved and random primers, cell culture assays, immunohistochemical methods, and inoculation of mice, rabbits, and monkeys, as well as antibody measurements. | No | N/A |
| **D.3** | Assays of blood, bone marrow, and liver for known viruses, including herpesviruses such as cytomegalovirus, human herpesviruses 6, 7, and 8, enteric viruses such as A-6, circiviruses, and parvoviruses, using assays as in (2). | No | N/A |
| **D.4** | Spectra-typing of blood cells to determine response to known or putative viral infections. | No | N/A |
| **D.5** | HLA typing or subtyping to determine risk factors/determinants for hepatitis-AA studies. | No | N/A |
| **D.6** | Cytotoxic lymphocyte assays with intracellular cytokine measurement for determining anti-viral response and lymphocyte cloning to obtain clones with specific antiviral activity. | No | N/A |
| | | | |
| **E** | **Solid Tumor Section (Dr. Richard Childs)** | | |
| **E.1** | Cr51 cytotoxicity assay to evaluating killing of patient tumor cells by patient NK cell clones and T-cells. | No | No |
| **E.2** | ELISA for IL-12 maturity of DC's made from subjects monocytes. | No | No |
| **E.3** | ELISA for IFN ã to evaluate specificity of CTL clones. | No | No |
| **E.4** | H thymidine uptake to evaluate proliferation potential of antigen specific T-cells. | No | No |
| **E.5** | PCR of STR to assess chimerism status of cellular subsets grown in-vitro or retrieved from subjects post-transplant. | No | No |
| **E.6** | Flow sorting of PBL and/or tissue samples to evaluate chimerism of different subsets. | No | No |
| **E.7** | Surface marker analysis of peripheral blood mononuclear cells using flow cytometry. | No | No |
| **E.8** | cDNA expression arrays to evaluate T-cells expression/gene patterns in subjects with GVHD and a GVT effect. | No | No |
| **E.9** | Geno typing of tumor or tissue samples by high density cDNA arrays. | No | No |
| **E.10** | VHL mutation analysis on kidney cancer tissue. | No | No |
| **E.11** | Transduction of dendritic and tissue cells with tumor antigens using plasmids, viral vectors and hybrid fusions. | No | No |
| **E.12** | Lasar capture microdissection of cells from tumor biopsies and tissue samples to determine origin (donor vs patient). | No | No |
| **E.13** | Quantification of polyoma virus BK exposure by serology and PCR in stem cell transplant donors and recipients from blood and urine samples. | No | No |
| **E.14** | Quantification of polyoma virus BK specific T cells in stem cell transplant donors and recipients from peripheral blood samples. | No | No |

| | | | |
|---|---|---|---|
| **E.15** | Determination of origin of neovasculature endothelial cells in tumor and tissue samples obtained from subjects post-transplant. | No | No |
| **E.16** | Quantification of lymphocyte subsets CD34 progenitors and endovasculator progenitors in G-CSF mobilized peripheral cell allografts. | No | No |
| **E.17** | Testing for polyoma virus BK latency in CD34 progenitors, B cells and T cells in the G-CSF mobilized peripheral cell allografts. | No | No |
| **E.18** | Determination of etiology of membraneous nephropathy using serum from subjects. | No | No |
| **E.19** | Serum Proteomic patterns analysis to diagnose complications related to allogeneic transplantation. | No | No |
| **E.20** | Determine cell origin (donor vs patient) of tissue samples using IHC, IF, sorting, and FISH. | No | No |
| | | | |
| **F** | **Lymphoid Malignancies Section (Dr. Adrian Wiestner)** | | |
| **F.1** | Culture of cells from research subjects to investigate molecular disease mechanisms, model host tumor interactions, and to test effect of drugs on cell survival and cellular functions. | No | No |
| **F.2** | Generation of stable cell lines for the study of hematologic malignancies. | No | No |
| **F.3** | Modifications of cells using standard expression systems or biologic molecules, e.g. interfering RNA, to investigate the effects of candidate genes on cellular functions. | | |
| **F.4** | Identification and monitoring of B or T cell populations as identified by flow cytometry and by their B cell or T cell receptor expression. | No | No |
| **F.5** | Measurement of gene expression in cells or tissues. Techniques frequently used include gene expression profiling on microarrays, quantitative RT-PCR, Western blotting, flow cytometry and ELISA assays. | No | No |
| **F.6** | Analysis of chromosomal abnormalities or mutations in malignant cells and non-malignant cells including FISH technology and DNA sequencing. | No | No |
| **F.7** | Assays of immune function of B-cells and T-cells, including intracellular cytokine staining, ELISPOT, quantitative RT-PCR for cytokines or other immune regulatory genes. | No | No |
| **F.8** | Analysis of antibody specificities in serum and antigen specificity of the B-cell receptor on cells. Techniques may include expression of antibodies in phage display systems, generation of antibodies in cell culture systems and use of such antibodies to screen for cognate antigens. | No | No |
| **F.9** | Transplantation of human cells into mice (xenograft model) to study disease biology and to investigate the effect of experimental therapy. | No | No |
| **F.10** | Measurements of drug concentrations, biologic molecules and disease markers in blood, serum, and plasma. | No | No |

**APPENDIX C: NIH INFORMATION SHEET ON STAFF RESEARCH PARTICIPATION (APRIL 2016)**

As an NIH employee, contractor, Special Volunteer, Guest Researcher, or trainee, you may participate in intramural research studies unless it is prohibited by your Institute or Center (IC), or if you are excluded by the criteria of the protocol in which you want to enroll. The inclusion of NIH staff in a particular protocol must also be approved by the IRB. You may be motivated by altruism, a commitment to research in your own or related fields, or want access to clinical trials of potential direct therapeutic benefit. When deciding, you should make an informed decision about participation. This information sheet offers some points to consider for NIH staff who are considering research participation at NIH.

First, similar to any individual who is considering research participation, you should seek adequate information about the study purpose, what is required of you in terms of procedures, interventions and time, and the potential risks and benefits of participation. For more information, see the NIH Clinical Center's public website "Are Clinical Studies for You?" at http://www.cc.nih.gov/participate/studies.shtml. When you are thinking about participation in a research study that is being conducted by your supervisor, or others with whom you work closely in your laboratory, branch, or unit, you should consider some additional factors:

**A. Possible bias:** Are you confident that you can be unbiased about reporting answers, side effects, or other information that could influence the study outcome or risk to you?

**B. Confidentiality:** Has the principal investigator (PI) spoken about what information will be collected from you as part of the study? Has the PI discussed what information will be available to those within, and outside, the study team? If applicable, are you comfortable sharing your medical history (including, for example, mental health history or STDs) and your social history (e.g. substance use) with study investigators who may be your coworkers, or with the possibility of them discovering something about your health during the study (e.g. pregnancy status or a new diagnosis)? Although every effort will be made to protect your information and keep it private and confidential, your information may, depending on the nature of the protocol, become available in medical records or to authorized users outside of the study team. Discuss any concerns with the PI.

**C. Pressure:** Do you perceive any pressure or expectations from your supervisor or colleagues regarding participation? Could that pressure influence your decision or make it difficult for you to choose whether or not to participate? Remember that it is your choice whether or not to participate and that your decision to participate or not should not have an effect, either beneficial or adverse, on your position at NIH.