CLINICAL RESEARCH PROJECT

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Principal Investigator:
Inhye Ahn, M.D., HB, NHLBI (E)* [redacted]
Accountable Investigator:
Adrian Wiestner, M.D., Ph.D., HB, NHLBI (E)* [redacted]

Medically Responsible Investigator
Adrian Wiestner, M.D., Ph.D., HB, NHLBI (E) [redacted]

Associate Investigators:
Georg Aue, M.D., HB, NHLBI (E)* [redacted]
Mark Roschewski, M.D., NCI (E)* [redacted]
Gerald Marti, M.D, Ph.D, FDA (V) [redacted]
Maryalice Stetler-Stevenson, M.D., CCR (E) [redacted]
Janet Valdez, PA-C, HB, NHLBI (E)* [redacted]
Thomas Hughes, Pharm.D., CC (E) [redacted]
Susan Soto, M.S.N., R.N., Research Nurse, HB, NHLBI (E) [redacted]
Xin Tian, Ph.D., Biostatistician, OBR, NHLBI (E) [redacted]
Clare Sun, M.D., HB, NHLBI (E)* [redacted]
Jennifer Lotter, PA (E)* [redacted]

*Study team members authorized to obtain informed consent

Collaborators:
Ronald Taylor, Ph.D., UVA University of Virginia
Clifton L Dalgard, M.D, CLS Collaborative Health Initiative Research Program, Uniformed Services University

Company Providing Investigational Drug: Novartis

Subjects of Study:	Number	Sex	Age-range
	Up to 46	either	18-99

Project Involves Ionizing Radiation? Yes (research and medically indicated)
Off-Site Project? No

Multi Center Trial? No
DSMB Involvement? Yes

PRECIS

Chemoimmunotherapy has become the standard of care in CLL/SLL. Unfortunately, CLL remains an incurable disease with limited treatment options, and more effective first-line therapies are needed. Ofatumumab has shown great promise in the setting of relapsed/refractory CLL, producing overall response rates of approximately 50% in this difficult-to-treat patient population. Given its proven activity, as well as indications of its superiority to rituximab in terms of CD20 affinity and complement-dependent cytotoxicity, studies examining the efficacy of ofatumumab in combination with purine analog-based therapy in the first-line setting are needed.

Emerging evidence indicates that a risk-adapted approach to choosing initial therapy regimens for patients with CLL may be warranted. Specifically, recent findings by Keating *et al* suggest that the inclusion of cyclophosphamide into the initial chemoimmunotherapy regimen may overcome the adverse prognostic significance of the 11q22 deletion, which is present in a significant percentage of CLL patients who require therapy. Additionally, the presence of the 17p13 deletion remains a markedly adverse prognostic factor that portends poorer overall survival in the face of all existing treatments, making the advancement of more effective chemoimmunotherapy regimens a priority for this group of patients.

Hence, we are proposing a phase II study to evaluate the efficacy of ofatumumab in combination with either fludarabine (FO) or fludarabine and cyclophosphamide (FCO) in previously untreated patients with CLL/SLL. Patients with the aforementioned high-risk genetic features (deletion of chromosomes 11q22 or 17p13) will be given FCO, all others will receive FO.

Prior studies have demonstrated that the eradication of minimal residual disease (MRD) is associated with improved progression-free survival and even overall survival in CLL. Moreover, studies have also shown that the administration of consolidation monoclonal antibody therapy after completion of induction therapy can result in the achievement of MRD negativity, thus improving response durability. Accordingly, all responding patients not achieving MRD-negative status at the completion of induction therapy will undergo an eight month period of consolidation therapy with ofatumumab in an attempt to improve on depth and durability of response.

The Primary objective will be to establish the safety and efficacy of ofatumumab-based induction chemoimmunotherapy followed by consolidation ofatumumab immunotherapy in previously untreated patients with CLL/SLL.

The primary endpoint will be progression-free survival rate 2 years after initiation of induction therapy.

Secondary endpoints will include: toxicity profile, rate of minimal residual disease, rates of overall and complete response, overall survival rate 2 years after initiation of induction chemoimmunotherapy and correlation of biologic endpoints with clinical response using biomarkers.

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1.0 OBJECTIVES

1.1 Primary objective

To establish the safety and efficacy of ofatumumab-based induction chemoimmunotherapy followed by consolidation ofatumumab immunotherapy in previously untreated patients with CLL/SLL.

1.2 Secondary objectives

- To determine the rate of minimal residual disease (MRD) negativity at the completion of induction chemoimmunotherapy
- To determine the rate of MRD negativity at the completion of consolidation immunotherapy
- To determine the rates of overall and complete response to induction chemoimmunotherapy
- To explore the mechanisms of action and predictors of response to chemoimmunotherapy

2.0 BACKGROUND AND SCIENTIFIC JUSTIFICATION

2.1 Chronic lymphocytic leukemia (CLL) and/or small lymphocytic lymphoma (SLL)

The World Health Organization recognizes CLL and SLL as manifestations of the same disease process, distinguishable from each other only by the respective presence or absence of a leukemic phase[1]. According to the updated National Cancer Institute Working Group (NCI-WG) guidelines, CLL is defined as the presence within the peripheral blood of a clonal population of mature-appearing lymphocytes that co-express CD5 and B-cell surface antigens CD19, CD20, and CD23, at a concentration of greater than 5,000 cells per microliter[2]. SLL, meanwhile, is characterized by the involvement of the lymph nodes or other tissues by a monoclonal B-cell population that is morphologically and immunophenotypically identical to that of CLL, but without bone marrow or peripheral blood involvement[2]. The diagnosis of monoclonal B-cell lymphocytosis (MBL) is reserved for those individuals who demonstrate the presence of a clonal lymphocyte population within the peripheral blood that is immunophenotypically consistent with CLL/SLL but does not exceed 5000 cells/μL and is not associated with lymphadenopathy or cytopenias[2]. For the purposes of this study, the term "CLL" will encompass both CLL and SLL, and will exclude MBL.

2.2 Pathophysiology of CLL

CLL is a disease characterized by a variably progressive burden of mature-appearing but immunologically defective B-lymphocytes within the blood, bone marrow, and secondary lymphoid organs. Biologically, CLL can be divided into two main subgroups based on the presence or absence of somatic hypermutations within the immunoglobulin heavy chain gene (IgVH) expressed by the malignant B-cells[3,4]. It was initially hypothesized that mutated CLL (mCLL) originated from the antigen-experienced GC or post-GC lymphocyte while unmutated CLL (uCLL) comprised a distinctly different disease arising from the naïve B-cell[4]. More recently, however, gene expression profiling studies have demonstrated that mCLL and uCLL share a common profile that, when compared to those of the normal B-cell subtypes, bears the most resemblance to that of the memory B-cell[5]. These findings argue against the existence of two separate biologic disease entities and suggest instead that both subtypes of CLL evolve from a common antigen-experienced cell.

The B-cell receptor (BCR) is thought to play a key role in the pathogenesis of CLL. In addition to the somatic hypermutations which implicate antigenic stimulation of the BCR prior to malignant

transformation, recent studies have also identified the existence of "stereotyped" BCR's, or receptors that contain closely homologous or identical sequences within their heavy chain gene complimentarity-determining region 3 (CDR3), in a significant proportion of CLL patients[6,7]. The fact that these specific BCR sequences occur non-randomly in both the mCLL and uCLL populations suggests that BCR stimulation by a common foreign or auto-antigen may somehow be implicated in leukemogenesis. Additionally, both constitutive and antigen-mediated BCR activation in the CLL cell have been shown to induce the expression of genes associated with clinical disease progression[8,9]. Thus, it appears that antigen exposure and activation of BCR signaling pathways may be important in both the development and progression of CLL. The identity of the antigen(s) or the exact nature of the interaction with the BCR, however, remains unknown.

Until recently, disease progression in CLL was thought to result strictly from accumulation, rather than active proliferation, of malignant lymphocytes as a consequence of acquired apoptotic resistance[10,11]. Recent evidence, however, suggests the existence of a significant proliferative component as well, which may to some extent determine disease severity and progression. Messmer *et al* recently demonstrated through heavy water analysis that leukemic cell proliferation rates in CLL patients are at least three orders of magnitude higher than those of normal B-cells in normal individuals, with the higher rates being associated with clinical disease progression[12]. Similarly, Sieklucka *et al* identified higher rates of cell proliferation in patients with advanced stage CLL (Rai III-IV) compared to those with earlier stage[13]. Furthermore, the discovery of reduced telomere length (a marker of replicative history and genomic instability) in CLL cells also argues for a significant proliferative component to CLL and highlights the possible role of genomic instability in disease progression[14].

Lastly, the tumor microenvironment is now recognized as an important contributor to CLL cell survival and proliferation. The importance of the microenvironment was initially suggested by the observation that CLL cells undergo apoptosis readily *in vitro* but not *in vivo*[15]. Since then, multiple cellular and humoral interactions have been identified to both protect CLL cells from apoptosis and promote proliferation, to include CXCR4/SDF1-mediated interaction with bone marrow stromal cells[16] and CD40/CD40L- and IL4-mediated stimulation by activated T-cells within lymphoid proliferative centers[17]. Targeting this protective interaction remains a source of hope for therapeutic advance.

2.3 Epidemiology and clinical course of CLL

CLL is the most common adult leukemia in the western world, comprising approximately 25-30% of all adult leukemias in the United States [18,19]. Caucasians are the most frequently affected ethnic group, with significantly lower rates of incidence seen in the African-American and Asian subpopulations in the United States (approximately 75% and 25% of the Caucasian rate of incidence, respectively)[20] and dramatically lower rates of incidence in east Asian countries compared to those in North America and Europe[21,22]. Incidence among men is nearly two-fold greater than among women, regardless of race [18]. Diagnosis is most commonly made in the sixth to seventh decade of life, with less than 5% of patients diagnosed prior to age 45[18].

To date, there has been little in the way of proven associations between environmental or occupational exposures and CLL. A notable exception is Agent Orange, which in 2003 was deemed by the U.S. Institute of Medicine to be associated with an increased risk of acquiring the disease in veterans exposed during the Vietnam War [23]. Notably, the risk of acquiring CLL was not found to be elevated in atomic bomb survivors, in contrast to the elevated risk of acquiring the other main leukemia subtypes (AML, ALL, and CML)[24]. This finding and others like it have led to the widely held view that CLL is a nonradiogenic malignancy. This remains a somewhat controversial

hypothesis, however, with some arguing that the prolonged clinical latency of CLL, combined with shortcomings in the epidemiologic studies performed to date, have masked the association between radiation and disease development[25].

CLL is well known for its markedly heterogeneous clinical course. Although some patients present with asymptomatic and indolent disease and never require treatment, the majority either progress to a more advanced, treatment-requiring phase after an initial indolent course, or present with symptomatic and aggressive disease at initial diagnosis. Survival is likewise highly variable, ranging from normal lifespan to death within one to two years from diagnosis. The natural history of CLL has demonstrated some change over time, with a lengthening of median survival from approximately 5 years (based on data from the 1960's and 1970's) to approximately 9 years (based on data from the 1980's)[26]. It is possible, however, that the majority of this improvement may be a reflection of the greater number of patients diagnosed in the early asymptomatic stages of the disease rather than a true alteration in its natural history.

CLL can become symptomatic (or life-threatening) as a result of tissue compartment invasion, autoimmune phenomena, or infectious complications, with death being most commonly due to severe infection or bleeding. Progressive tumor burden within the bone marrow can lead directly to severe cytopenias. Likewise, cytopenias can also result from autoimmune phenomena, the most common of which is hemolytic anemia (AIHA), affecting up to 37% of patients [27]. Less common are autoimmune thrombocytopenia and pure red cell aplasia. Although not typically painful, bulky lymphadenopathy and splenomegaly can cause symptoms related to their compressive effect on surrounding anatomical structures. Multiple factors can contribute to an immunocompromised state, leading to infectious complications in 80% of patients with CLL and death from infection in 50-60%. These factors include hypogammaglobulinemia, qualitative immunoglobulin defects, decreased cell-mediated immunity, complement deficiencies, and neutropenia [28].

2.4 Diagnostic and prognostic categories of CLL/SLL

Based on a recent update of the NCI-WG guidelines by the International Workshop on Chronic Lymphocytic Leukemia (IWCLL), the diagnosis of CLL is made if the following criteria are met [2]:

- B-lymphocytosis greater than 5000/μL
- clonality of lymphocytosis confirmed by flow cytometry
- appropriate immunophenotype (CD5/19/20/23+)
- large lymphocytes/prolymphocytes < 55% of blood lymphocytes

The diagnosis of SLL, meanwhile, requires the presence of lymphadenopathy and/or splenomegaly, B-lymphocyte count of less than 5000/μL, and histologic confirmation (by lymph node biopsy when possible)[2].

Two prognostic staging systems, Rai and Binet, have been used in CLL. Introduced in the 1970's, the Rai system distinguishes 5 categories of disease. The more recent Binet system, meanwhile, distinguishes only 3 categories. For the purpose of this study, we will utilize the Rai system when referencing disease stage (see below). Both systems estimate prognosis based on extent of disease at diagnosis as measured by clinical parameters (lymphadenopathy, organomegaly) and peripheral blood counts. Although useful in terms of providing guidance to clinicians regarding appropriate intensity of follow-up and immediacy of therapy, these systems have been criticized for their inability to reliably estimate prognosis in individual patients, especially those diagnosed in early disease stages.

Table 1: Rai clinical staging system [29]:

Stage	Risk group	Features at diagnosis	Median survival (mos)
0	Low	Blood and marrow lymphocytosis	120
I	Intermediate	Lymphocytosis and adenopathy	108
II	Intermediate	Lymphocytosis + organomegaly	94
III	High	Lymphocytosis + anemia (Hb <11g/dl)	60
IV	High	Lymphocytosis + thrombocytopenia (Plt<100K/μL)	60

Over the past decade, considerable information has been obtained regarding the prognostic significance of molecular and cytogenetic variation in CLL. Notably, a higher degree of clinical aggressiveness is seen in uCLL compared to mCLL[30]. Additionally, the expression of cell surface CD38 and intracellular zeta-associated protein of 70kDa (ZAP-70) have both been associated with aggressive disease and poor prognosis, with the former demonstrating prognostic significance independent of IGVH mutational status and the latter being strongly associated with uCLL. In a recent study assessing the relative value of CD38, ZAP-70, and IGVH mutational status in predicting time to initial treatment, ZAP-70 positivity demonstrated the strongest predictive value, with highly significant hazard ratios (HR) demonstrated across all disease stages on univariate and multivariate analysis [31]. CD38 positivity, by contrast, demonstrate the least value, with significant HR's attained only in advanced disease stages. IGVH mutational status, meanwhile, improved predictive power only in ZAP-70 negative cases. Based on these results, it appears that ZAP-70 may be the most helpful of the three in determining appropriateness of early therapy in patients without symptomatic indications. Until results of prospective studies utilizing these markers are known, however, their use in aiding treatment decisions outside the context of a clinical trial is not recommended.

Cytogenetic status is also a proven basis for risk stratification in CLL. Due to the low mitotix indices of CLL cells within the peripheral blood and bone marrow, few cases of CLL demonstrate cytogenetic abnormalities by standard metaphase karyotype analysis. Using interphase fluorescence in situ hybridization (FISH), however, cytogenetic abnormalities can be detected in approximately 70 to 80% of patients [32]. The most common of these is a deletion in the long arm of chromosome 13 (del 13q14), which is seen in approximately 50% of patients and is associated with a relatively good prognosis. Deletions in the long arm of chromosome 11 (del 11q22-23) and the short arm of chromosome 17 (del 17p13), by contrast, portend a markedly poorer prognosis. The estimated prevalence and median survival times of the major cytogenetic aberrations in CLL (as estimated by Dohner et al) are as follows [33]:

Karyotype*	Percentage of Cases	Median survival (mos)
del 17p13	7	32
del 11q22-23	17	79
tri 12q	14	114
normal	18	111
del 13q14	36	133

**Karyotypes determined by interphase FISH cytogenetic analysis. Categories defined as follows: patients with 17p deletion; patients with 11q deletion but no 17p deletion; patients with 12q trisomy but no 17p or 13q deletion; patients with normal karyotype; patients with 13q deletion as sole abnormality.*

Patients with the 11q deletion are more likely to have bulky lymphadenopathy[34], while patients with the 17p deletion are more likely to be refractory to fludarabine-based therapy[35]. The poor

prognosis associated with both deletions is thought to be due in large part to chemotherapy resistance mediated by the loss of regulatory genes (*TP53* and *ATM* respectively) crucial to the cell's response to DNA damage. Recent evidence also suggests that genetic factors other than the deletions themselves may contribute to the clinical heterogeneity of the disease. For example, a significant proportion of patients with 11q deletions also have *ATM* mutations in the non-deleted allele, which has been shown to confer an even poorer prognosis than an 11q deletion without such mutation [36]. Even more striking is the recent reporting of the M.D. Anderson and Mayo Clinic data on early stage asymptomatic patients harboring the 17p deletion. This retrospective analysis revealed a somewhat bimodal disease pattern, with approximately half of the patients progressing to the point of requiring treatment within 18 months and the majority of the others retaining stable disease not requiring treatment at follow-up periods of up to 70 months [37].

These findings collectively underscore the pathogenetic complexity of a disease that is unlikely to be optimally prognosticated based on any one molecular or genetic marker. Further studies are needed to better understand the mechanisms underlying the clinical heterogeneity of CLL. Nevertheless, interphase cytogenetic analysis remains at this time a useful and easy (albeit imperfect) means of identifying high-risk patients with CLL.

2.5 Indications for treatment of CLL

The International Workshop on Chronic Lymphocytic Leukemia (IWCLL) recommends initiating treatment in CLL patients with advanced stage disease (Rai III-IV/Binet C), or in patients with intermediate stage disease (Rai I-II/Binet C) so long as there is evidence of active disease as defined by the presence of at least one of the following[2]:

- Weight loss ≥10% within the previous 6 months.
- Extreme fatigue (i.e. ECOG performance status 2 or worse; cannot work or unable to perform usual activities).
- 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.
- Autoimmune anemia and/or thrombocytopenia poorly responsive to corticosteroids or other standard therapy.
- Massive (i.e. >6 cm below the left costal margin) or progressive splenomegaly.
- Massive nodes or clusters (i.e. >10 cm in longest diameter) 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.
- Marked hypogammaglobulinemia or the development of a monoclonal protein in the absence of any of the above criteria for active disease is not sufficient for treatment outside the context of a clinical trial.

Outside the context of a clinical trial, patients with early stage disease (Rai O/Binet A) should not be treated. This recommendation is based on multiple studies which have demonstrated no survival benefit in early stage patients treated with chemotherapy[38-40]. Within the context of a clinical trial, active disease (as described above) should be documented, regardless of clinical stage.

2.6 Treatment options for CLL/SLL

2.6.1 Watchful waiting

This option is generally reserved for early stage patients and intermediate stage patients without evidence of active disease as described in section 2.4.

2.6.2 Chemotherapy/Immunotherapy

For years, chlorambucil was considered standard of care chemotherapy for active CLL. Within the past decade, however, the purine analogs have demonstrated higher response rates and longer progression-free survival (PFS) times than chlorambucil, albeit without an overall survival benefit. In 2000, the CALGB 9011 trial demonstrated that fludarabine yielded a higher response rate (63% versus 37%) and a longer median PFS (25 and 14 months) than chlorambucil in previously untreated patients [41]. Moreover, this study also demonstrated increased toxicity with no appreciable improvement in response with the fludarabine-chlorambucil combination regimen, leading to early closure of this study arm. Similar improvements over chlorambucil were also seen with cladribine, again without improvement in overall survival [42]. Furthermore, fludarabine demonstrated superior response and PFS when compared to anthracycline-based polychemotherapy [43].

Based on these results, subsequent studies have focused primarily on the addition of additional cytotoxic agents and/or immunotherapeutic agents to purine analogs. The US Intergroup E2997 trial demonstrated that the addition of cyclophosphamide to fludarabine resulted in significantly higher rates of OR (74% v 59%), CR (23% v 5%), and PFS (31 months v 19 months) when compared to fludarabine monotherapy, with some increase in hematologic toxicity but no difference in infectious complications [44]. Meanwhile, CALGB 9712 demonstrated an overall response rate of 84% when rituximab was added either concurrently or sequentially to fludarabine [45]. When compared retrospectively with the data from CALGB 9011, the addition of rituximab to fludarabine resulted in a significant increase in OR (84% v 63%), CR (38% v 20%), 2-year PFS (67% v 45%), and 2-year OS (93% v 81%)[46]. A subsequent analysis of IGVH and cytogenetic risk subgoups within CALGB 9712 revealed significantly worse OS in patients with uCLL and those with either 11q or 17p deletion, although none of these risk factors demonstrated an independent association with poorer outcome[47].

The long-term results of adding both cyclophosphamide and rituximab to fludarabine (FCR) have recently been reported by Tam *et al* to be significant for an overall response rate of 95% with 72% CR and a median time to progression of 80 months [48]. Infectious complications occurred in 14% of responders during the first 2 years of remission, with minimal infections thereafter. Although the presence of the 17p deletion was independently associated with poorer response, it did not predict shorter duration of remission in those attaining CR. Furthermore, a recent analysis of all patients with the 11q deletion treated with chemoimmunotherapy at MDACC from 2003 to 2007 (the majority of which received FCR on protocol) revealed significantly better outcomes (3-year RFS of 77%, 3-year OS of 91%) than what has been seen historically in this population[49]. This observation led the authors to hypothesize that FCR may actually negate the adverse prognostic significance of the 11q deletion. In 2008, the superiority of chemoimmunotherapy over chemotherapy was confirmed by the phase III CLL8 and REACH trials (FCR v FC in previously untreated and relapsed/refractory patients, respectively), the results of which were presented at the ASH Annual Meeting. In the CLL8 trial, FCR demonstrated superiority in terms of OR (95% v 88%), CR (52% v 27%), and median PFS (76 months v 62 months), with a trend toward improved overall survival [50]. In the REACH trial, FCR demonstrated significant superiority in terms of OR (70% v 58%), CR (24% v 13%), and median PFS (30.6 months v 20.6 months) [51]. In this study, the benefit of adding rituximab was maintained regardless of IGVH mutational status or presence of the 17p deletion. Furthermore, there was no difference between groups in the rate of grade 3/4 infection (18% FCR v 19% FC). Additionally, a recent study of "FCR-lite" (reduced dosages of both

fludarabine and cyclophosphamide) by Foon *et al* demonstrated an 86% CR rate with low toxicity (8% grade 3-4 neutropenia)[52].

FCR and FR have not yet been compared prospectively, and opinions vary regarding which therapy should be given to patients in the community. Byrd *et al* have proposed implementing a risk-adapted approach based upon validated predictors of poor response to FR [47], which would serve to steer higher-risk patients toward more aggressive and/or alternative therapies. Others currently recommend FCR as front-line therapy in all cases of symptomatic CLL. In a recent US Oncology study evaluating FCR versus P(pentostatin)CR, only 50% of patients were able to complete therapy[53]c, suggesting that the good outcomes achieved by research institutions utilizing more intensive chemoimmunotherapy regimens may not necessarily be reproducible in the community setting. Given such concerns over tolerability and the lack of prospective data comparing FR and FCR, it is our opinion at this time that both regimens remain reasonable options for first-line therapy.

Other agents with demonstrated activity in CLL include bendamustine, flavopiridol, lenalidomide, and alemtuzumab. In a recent phase III study, bendamustine demonstrated superior PFS when compared to chlorambucil[54]. The GCLLSG is currently conducting a study of bendamustine with rituximab (BR) versus FCR in previously untreated patients. Flavopiridol, a cyclin-dependent kinase inhibitor, recently demonstrated an overall response rate of 47% in relapsed patients, with strikingly high response rates in poor risk populations (50% in del 17p; 60% in del 11q)[55]. Lenalidomide has been shown to induce remissions (to include complete responses) in relapsed/refractory disease [56]. Alemtuzumab, a fully human anti-CD52 monoclonal antibody, has demonstrated activity both as a single agent and in combination with chemotherapy, and is known have efficacy in patients with the 17p deletion [57-59]. Lastly, a number of novel agents (BCL-2 inhibitors, CDK inhibitors, PI-3-K inhibitors) are currently being studied in CLL.

In November 2013 the FDA approved the anti CD20 antibody obinutuzumab for use in combination with chlorambucil for the treatment of patients with previously untreated CLL. The study demonstrated an improvement in PFS in a randomized open-label multicenter trial that compared obinutuzumab in combination with chlorambucil with chlorambucil alone in patients with previously untreated CD20-positive CLL. A total of 356 patients were randomly assigned (2:1) to receive obinutuzumab plus chlorambucil (n=238) or chlorambucil alone (n=118). PFS was 23.0 months for patients treated with obinutuzumab plus chlorambucil and 11.1 months for patients treated with chlorambucil alone." [69]

2.6.3 Stem cell transplantation: Despite the aforementioned advancements in therapy for CLL, no currently available agent or regimen is curative, and clinical relapse remains inevitable for all patients who do not die from other causes. Allogeneic hematopoietic stem cell transplantation remains the only potentially curative treatment. However, only a minority of patients are eligible for this option, and transplant-related mortality remains a serious concern.

2.7 Ofatumumab

2.7.1 Mechanism of action: Ofatumumab is a fully human IgG1 anti-CD20 monoclonal antibody. Like its mouse/human chimeric anti-CD20 predecessor, rituximab, it is thought to induce cell death via complement-mediated cytotoxicity (CDC) and antibody-mediated cellular cytotoxicity (ADCC). In vitro, it was found to be considerably more effective than rituximab at inducing CDC in CD20-positive cell lines[60, 61], inducing high levels of cell lysis at low concentrations of CD20 whereas rituximab failed to achieve comparable lysis even in cells with the highest concentration of CD20[61]. Although not completely understood, the possible reasons for this enhanced cytotoxicity

include greater stability of binding (i.e. slower rate of dissociation from CD20) and differences in epitope recognition. With regard to the latter, it has been shown through amino acid substitution and peptide mapping studies that ofatumumab not only binds a different epitope on the large extracellular loop of CD20 than does rituximab, but also binds, unlike rituximab, to an epitope on the small extracellular loop [61]. It is hypothesized that this small loop binding may allow for closer proximity of the antibody to the cell membrane which, in turn, may allow for better recruitment of C1q and more efficient complement-mediated lysis. This hypothesis is supported by the recent findings of Pawluczkowyck *et al*, who demonstrated that ofatumumab-opsonized CLL cells bind significantly more C1q than do rituximab-opsonized cells in the presence of low concentration C1q, and also that ofatumumab induces greater CDC than rituximab even when rituximab-opsonized cells are exposed to higher concentration C1q to achieve comparable C1q binding [62].

2.7.2 Distribution: Steady state volume of distribution is low, reflecting a distribution confined primarily to the systemic circulation.

2.7.3 Metabolism and clearance: Like other IgG molecules, ofatumumab is cleared from the circulation by both a target-independent route and a target-dependent route dependent on the availability of B-lymphocytes. Accordingly, half life is considerably shorter after the first infusion (approximately one day) than after subsequent infusions (over ten days).

2.7.4 Prior clinical experience in relapsed/refractory CLL

The safety and efficacy of ofatumumab in relapsed/refractory CLL was first demonstrated in a multinational phase I/II dose-escalation study (Hx-CD20-402) [63]. A total of 33 patients were treated in three cohorts, with cohorts A and B containing three patients each and cohort C containing 27 patients. Each cohort was treated with four weekly doses of ofatumumab, with the following dosing schedule:

Cohort	Week 1	Weeks 2, 3 and 4
A	100mg	500mg
B	300mg	1000mg
C	500mg	2000mg

A total of 246 adverse events (AE's) were reported, with 61% deemed related to treatment and approximately one-third occurring on the day of first infusion. Grade 3 or 4 AE's comprised 8% of all events. A total of 9 patients reported 10 severe adverse events (SAE's) with 5 deemed related to treatment: herpes zoster, interstitial lung disease, and cytolytic hepatitis each occurred in 1 patient, and severe neutropenia occurred in 2 patients. Overall response rate was 44%, with 1 responder in cohort A and the remaining responders all in cohort C. All responses were partial except for 1 patient in cohort C who achieved a nodular partial response (nPR). Median PFS was 106 days, with a median time to next treatment of 1 year.

A second multinational study (Hx-CD20-406) evaluated ofatumumab in patients either refractory to both fludarabine and alemtuzumab (or "double-refractory" (DR)) or refractory to fludarabine and with bulky lymphadenopathy (BFR) [64]. Patients were given 8 weekly infusions followed by 4 monthly infusions. The first dose was 300mg and all subsequent doses were 2000mg. There were no unexpected safety concerns identified in the interim analysis. Grade 3-4 neutropenia occurred in 12% of patients, with 28% experiencing major infection (requiring hospitalization for longer than 48 hours). Overall response rates were 51% and 44% for the DR and BFR groups, respectively. Median overall survival was 14 months for DR patients and 15 months for BFR patients, with response being significantly correlated with longer survival times in both groups. In October 2009, based on the

results of this interim analysis, ofatumumab received accelerated approval from the FDA for treatment of double-refractory patients.

2.8 Clinical and scientific justification for protocol design

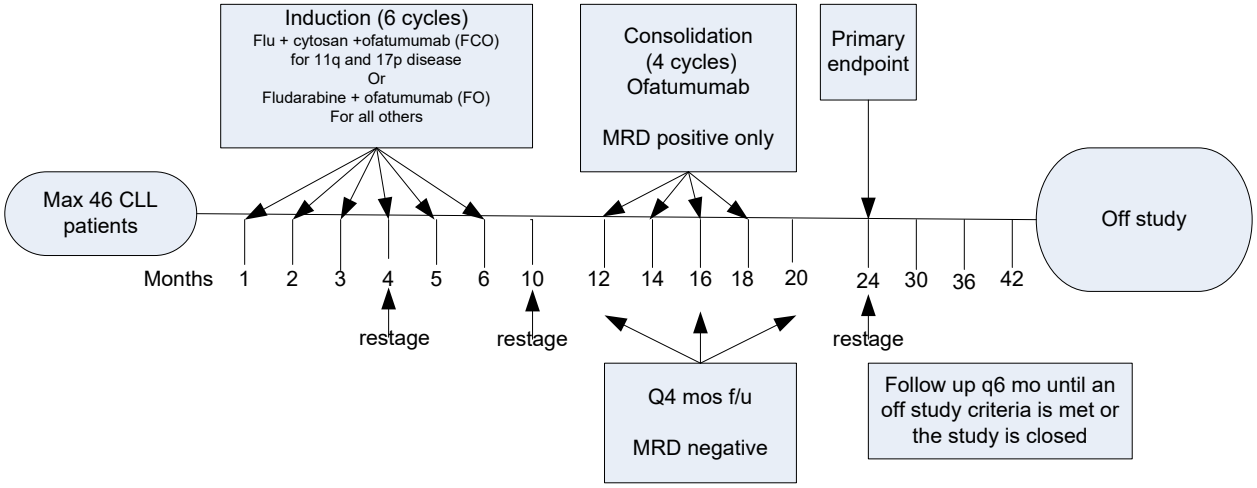
Chemoimmunotherapy is now considered standard of care in previously untreated CLL. As demonstrated by CALGB 9712 and the CLL8 and REACH trials, the addition of anti-CD20 immunotherapy extends progression-free survival in previously untreated patients, with no increase in infectious or hematologic complications. Despite this improvement, however, CLL remains a disease incurable by existing non-transplant regimens. Moreover, many patients with adverse disease features either fail to respond to therapy or enjoy very short progression-free intervals. For these reasons, more effective first-line chemoimmunotherapeutic regimens are needed. It stands to reason that the addition of a highly potent anti-CD20 monoclonal antibody that has demonstrated both safety and impressive single-agent activity in relapsed/refractory disease (as well as clear superiority to rituximab in vitro) may improve upon the both the depth and durability of response currently achievable by existing rituximab-based regimens. Furthermore, there is no indication from any study to date of any unexpected or unexpectedly severe toxicity related to ofatumumab, nor is there mechanistically any reason to suspect greater toxicity from ofatumumab in combination with chemotherapy than from rituximab in such combination. Hence, we are proposing a phase II study of ofatumumab-based chemoimmunotherapy in previously untreated patients with CLL.

FR and FCR are two of the most commonly used first-line regimens, yet they have not been prospectively compared. Although FCR has demonstrated the highest response rate to date, there are concerns regarding tolerability and feasibility of administration within the community, and consequently there is currently no consensus regarding the utility of adding cyclophosphamide to FR. In light of the adverse prognostic significance of factors such as the 11q/17p deletions, however, one can argue that it is reasonable to adopt a risk-adapted approach that favors more aggressive therapy for patients with a statistically greater chance of early relapse, while reserving the less aggressive therapy for those patients without adverse risk features. This approach is supported both by the aforementioned MDACC analysis which strongly suggests that FCR may abrogate the adverse prognostic significance of the 11q deletion, and by the recent analysis of CALGB 9712 which demonstrated poorer overall survival among 11q- and 17p- patients treated with FR. Consequently, we are proposing a risk-adapted strategy whereby the addition of cyclophosphamide to fludarabine-based chemoimmunotherapy is reserved only for those patients with high-risk interphase cytogenetics.

In CLL, the eradication of minimal residual disease (MRD) has been associated with longer PFS and, in some studies, OS [65-67]. Furthermore, the administration of consolidation therapy, such as rituximab and alemtuzumab, after completion of induction therapy has been shown to result in achievement of MRD-negative status in a substantial proportion of initially MRD-positive patients, which has translated into improved PFS [67,68]. Whereas a recent GCLLSG study of alemtuzumab consolidation after induction FC was stopped early due to an unacceptable incidence of severe infection, consolidation rituximab has not been shown to increase toxicity. Given this demonstrated ability of rituximab to safely achieve MRD negativity in a consolidation setting, we hypothesize that a more efficient anti-CD20 monoclonal antibody (such as ofatumumab) would eradicate MRD in a greater proportion of patients and therefore lead to even more durable remissions. In line with this hypothesis, we are proposing the administration of consolidation ofatumumab to all patients not achieving MRD negativity after completion of induction chemoimmunotherapy.

3.0 STUDY DESIGN

We propose a phase II study of risk-adapted fludarabine/ofatumumab-based induction chemoimmunotherapy followed by consolidation ofatumumab in previously untreated patients with CLL. All responders not achieving MRD negativity by four-color flow cytometric analysis of the peripheral blood two months after completion of induction therapy will be given consolidation ofatumumab every two months for a total of four doses.



4. ELIGIBILITY ASSESSMENT AND ENROLLMENT

4.1 Inclusion criteria

4.1.1 Histologically confirmed CLL or SLL as defined by the following:

- B-lymphocytosis greater than 5000 cells/μL (may be less than 5000 cells/μL if lymphadenopathy is present with histologic confirmation of lymph node involvement by SLL)
- Immunophenotypic profile consistent with CLL as demonstrated by flow cytometry
- Appropriate immunophonotype (CD5/19/23+)
- Clonality of lymphocytosis confirmed by flow cytometry
- Large lymphocytes <55% of blood lymphocytes

4.1.2 Active disease as defined by at least one of the following:

- 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 months0

4.1.3 Measurable disease (defined as two dimensional disease on imaging or quantifiable leukemic disease)

4.1.4 Ages 18 and over

4.2 Exclusion criteria

4.2.1 Prior monoclonal antibody therapy with agents having anti-CLL activity

4.2.2 Prior cytotoxic chemotherapy with agents having anti-CLL activity (Fludarabine, Cyclophosphamide, Bendamustine, Chlorambucil).

4.2.3 Transformed CLL

4.2.4 Active autoimmune hemolytic anemia or thrombocytopenia

4.2.5 Any medical condition that requires the chronic use of corticosteroids

4.2.6. Active or latent Hepatitis B infection

4.2.7 HIV infection

4.2.8 Severe chronic obstructive pulmonary disease, severe cardiac disease, or other uncontrolled medical condition that would, in the opinion of the principal investigator, place the subject at an unreasonable risk of life-threatening adverse events due to chemoimmunotherapy

4.2.9 ECOG performance status 3 or worse

4.2.10 Creatinine ≥2 mg/dL or creatinine clearance ≤30 mL/min

4.2.11 Bilirubin ≥2 mg/dL or active hepatic or biliary disease (with exception of patients with Gilbert's syndrome, asymptomatic gallstones, or stable chronic liver disease per investigator assessment)

4.2.12 Female patients: Current pregnancy or unwilling to take oral contraceptives 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.

4.2.13 Psychiatric illness/social situations that would limit the patient's ability to tolerate and/or comply with study requirements.

4.2.14 Unable to understand the investigational nature of the study or give informed consent.

5.0 TREATMENT PLAN

5.1 Drug administration

Study drug administration will take place in the Clinical Center Day hospital. However, at the PI's discretion, select subjects may be admitted to the inpatient unit for the first few days of an induction cycle. Subjects will have a peripheral line placed. Per PI discretion, a central line may be placed in select patients.

All subjects will be given six cycles of fludarabine and ofatumumab (FO) EXCEPT:

- Subjects under the age of 70 who possess either the 11q or the 17p deletion by interphase FISH analysis will be given an induction regimen consisting of six cycles of fludarabine, cyclophosphamide, and ofatumumab **(FCO).**

- Subjects ages 70 and older who possess the 11q or 17p deletion will be given six cycles of reduced dosages of both fludarabine and cyclophosphamide **(FCO-lite)** due to concerns over the tolerability of FCO in the elderly population(see section 2.6.2)

5.1.1 Ofatumumab infusion:

All ofatumumab doses during induction and consolidation therapy will be 1000 mg Day 1, except during the first cycle of induction therapy, subjects will receive a 300 mg dose will be given on Day 1 and a 1000 mg dose will be given on Day 8.

All doses will be prepared in 1,000mL of 0.9% sodium chloride injection, USP.
Rates of infusion will comply with the recommendations listed in the product label as follows:

Interval After Start of Infusion (min)	Dose 1 (300mg) (mL/hour)	Dose 2 (1000mg) (mL/hour)	Subsequent Doses (mL/hour)
0-30	12	12	25
31-60	25	25	50
61-90	50	50	100
91-120	100	100	200
>120	200	200	400

Infusions will be interrupted for infusion reactions of any severity and medical management will be instituted as follows:

- For grade 4 reactions, the infusion will not be resumed.
- For grade 1, 2, or 3 reactions, if the reaction resolves or remains less than or equal to grade 2, the infusion will be resumed with the following modifications according to the initial grade of infusion reaction:
    - Grade 1 or 2: Infuse at one-half of the previous rate.
    - Grade 3: Infuse at a rate of 12 mL/hour.
- After resuming the infusion, the infusion rate may be increased according to the table above, based on patient tolerance.

All subjects will be pre-medicated with acetaminophen, an antihistamine and a corticosteroid (see section 5.4).

5.1.2 Fludarabine

Fludarabine will be given after completion of ofatumumab infusion as an IV infusion over 30 minutes administered once daily on days 2-6 (FO) or 2-4 (FCO) of the first induction cycle and Days1-5 (FO) or 1-3 (FCO) of each subsequent induction cycle. Dosing will be based on actual weight for all patients. An allowance of 20 minutes to 60 minutes will be tolerated for fludarabine infusions to account for variations in administration.

- Subjects receiving FO will be dosed at 25mg/m^2/d for 5 days regardless of age (total dose of 125 mg/m2 fludarabine each cycle)

- Subjects receiving FCO who are under the age of 70 will be dosed at 25 mg/m^2/d for 3 days (total dose of 75 mg/m2 fludarabine each cycle)

- Subjects receiving FCO who are age 70 or older will be dosed at 20 mg/m^2/d for 3 days (total dose 60 mg/m2 fludarabine each cycle)

Dose reduction in the event of renal impairment: A 20% dose reduction in fludarabine will be implemented for subjects with a creatinine clearance of 30-59 mL/min. using the Cockroft Gault equation with actual body weight. Fludarabine will be held for subjects with a creatinine clearance of less than or equal to 30mL/min at the discretion of the PI.

The actual treatment dose of fludarabine will remain unchanged from cycle to cycle unless there is evidence of marrow toxicity, or the subject has greater than 10% change in their body weight.

Starting with cycle #3 subjects receiving FO, the fludarabine day 3 through 5 may be given by their home Oncologists. In subjects receiving FCO, the fludarabine day 3 may be given by their home Oncologists.

5.1.3 Cyclophosphamide (approximately 30% of the study population)

Subjects with high-risk cytogenetics (as defined in section 2.4) will also receive cyclophosphamide after the completion of ofatumumab infusion. Cyclophosphamide will be administered as an IV infusion over 30 minutes once daily on days 2-4 of the first induction cycle and on days 1-3 of each subsequent induction cycle. Dosing will be based on actual weight for all patients. An allowance of 20 minutes to 60 minutes will be tolerated for cyclophosphamide infusions to account for variations in administration.

Subjects receiving FCO who are under the age of 70 will be dosed at 250 mg/m^2/d for 3 days (total dose of 750 mg/m2 cyclophosphamide each cycle).

Subjects receiving FCO who are ages 70 or older will be dosed at 150 mg/m^2/d for 3 days (total dose of 450 mg/m2 cyclophosphamide each cycle).

Pre and post hydration will not routinely be given, rather will be per research team discretion.

The actual treatment dose of cyclophosphamide will remain unchanged from cycle to cycle unless there is evidence of marrow toxicity, or the subject has greater than 10% change in their body weight.

Starting with cycle #3 subjects receiving FCO, the cyclophosphamide on day 3 may be given by their home Oncologists.

5.2 Induction therapy schedule: Typically cycles will be 28 (+/-4) days long. However, cycles may be 28 (+8) days long in cases of travel hardship.

Cycle 1: At the PI's discretion, select subjects may be admitted to the inpatient unit for the first few days of an induction cycle.

FO: ofatumumab (300mg) on D1, ofatumumab (1000mg) on D8, fludarabine on D2-D6

FCO: ofatumumab (300mg) on D1, ofatumumab (1000mg) on D8, fludarabine D2-D4 and cyclophosphamide on D2-D4

Cycles 2 through 6:

FO: ofatumumab (1000mg) on D1, fludarabine on D1-D5

FCO: ofatumumab (1000mg) on D1, fludarabine D1-D3 and cyclophosphamide on D1-D3

5.3 Consolidation therapy

Those patients demonstrating MRD positivity at the time of post-cycle 6 restaging will be given consolidation ofatumumab (1000mg) every 2 months starting 2 months after post-cycle 6 restaging and continuing for a total of 4 doses.

Those patients who achieve MRD negativity at the time of post-cycle 6 restaging will not receive consolidation therapy but will instead return to the clinic for follow-up and MRD assessment at 12, 16, and 20 months (+/- 2 weeks) after start of induction therapy

5.4 Premedication (induction cycles) All subjects will be pre-medicated prior to each induction cycle as follows:

- *dexamethasone* 16mg PO given 60 minutes prior to ofatumumab infusion on D1 and D8 of induction cycle 1; if no severe infusion reaction occurs, then subsequent doses may be reduced or deferred at the discretion of the PI
- *acetaminophen* 650mg PO given 30-60 minutes prior to ofatumumab infusion
- *diphenhydramine* 50mg IV/PO given 30-60 minutes prior to ofatumumab infusion
- *allopurinol* 300mg PO once daily given during induction cycle 1 on days 0-14
- *additional antiemetic:* For subjects receiving FCO, a single dose of a 5-HT3 antagonist anti-emetic (oral or intravenous) will be given as a pre-medication 30 to 60 minutes.

prior to chemotherapy. Options include ondansetron 24 mg po x 1 dose or 0.15 mg/kg IVPB x 1 dose, or granisetron 2 mg po x 1 dose or 10 mcg/kg IV x 1 dose, however other anti-emetic agents are allowed based on the discretion of the prescribing physician

A window of 25 minutes to 3 hours prior to study drug administration will be allowed for all premedications in induction cycles to allow for variations in administration.

5.5 Premedication (consolidation cycles) All subjects will be pre-medicated prior to the consolidation cycles as follows:

- *dexamethasone* 16mg PO given 60 minutes prior to ofatumumab infusion on D1 of consolidation cycle 1; if no severe infusion reaction occurs, then subsequent doses may be reduced or deferred at the discretion of the PI
- *acetaminophen* 650mg PO given 30-60 minutes prior to ofatumumab infusion
- *diphenhydramine* 50mg IV/PO given 30-60 minutes prior to ofatumumab infusion

A window of 25 minutes to 3 hours prior to study drug administration will be allowed for all premedications in induction cycles to allow for variations in administration.

5.6 Dose adjustments for hematologic toxicities:

5.6.1 Neutropenia: ANC must be greater than 1000 prior to the start of each induction cycle unless deemed by the PI to be due to disease. Best clinical judgment (i.e. consideration of baseline ANC, baseline disease burden, etc.) will be used to make this determination.

- For ANC less than 1000 prior to next cycle not due to disease, therapy will be held until neutrophil recovery >1000 and may be given with a 25% dose reduction in chemotherapy. Best clinical judgment (i.e. ANC nadir, pre-treatment ANC, and clinical information) will be used to make this determination.

- For ANC nadir <500 deemed not due to disease, fludarabine and cyclophosphamide will be dose reduced by 25%. Dose reductions may also be taken at the PI's discretion for ANC nadir between 500-1000. Best clinical judgment (i.e. ANC nadir, pre-treatment ANC, and clinical information) will be used to make this determination.

- Subsequent dose reductions will be an additional 25%, making the total dose reduction 50% from the original dose.

- If more than 2 dose reductions are required, induction cycle study drug administration will be discontinued. If the subject has completed > 3 cycles of induction therapy, the subject will remain on study. If the subject has completed < 3 cycles of therapy, the subject will go off study.

5.6.2 Thrombocytopenia: Platelet count must be greater than 75,000 prior to the start of each induction cycle unless deemed by the PI to be due to disease. Best clinical judgment (i.e. baseline platelet count, baseline disease burden, etc.) will be used to make this determination.

- If platelet count is less than 75,000 not due to disease, study drug administration will be held until platelet recovery and then given with a 25% dose reduction in chemotherapy.

- Subsequent dose reductions will be an additional 25%, making the total dose reduction 50% from the original dose.

- If more than 2 dose reductions are required, induction cycle study drug administration will be discontinued. If the subject has completed > 3 cycles of induction therapy, the subject will remain on study. If the subject has completed < 3 cycles of therapy, the subject will go off study.

5.7 Dose adjustments for non-hematologic toxicity:

For non-hematologic toxicities > grade 2 deemed by the PI to be related to chemotherapy, subsequent doses of chemotherapy will be reduced by 25%. If more than 2 dose reductions are required, study treatment will be discontinued and the patient will go off study per section 9.7. Readily reversible electrolyte and metabolic abnormalities or infections controlled by appropriate therapy are exempt.

5.8 Supportive care (non-investigational)

5.8.1 PCP prophylaxis: PCP prophylaxis will be given to all patients in the form of TMP/SMX one double-strength tablet twice daily for two days out of every week, or once daily for three days out of every week, throughout the duration of induction therapy. Antibiotic prophylaxis may be given past this point at clinician discretion. Patients allergic to sulfa drugs will be given an accepted form of alternative prophylaxis.

5.8.2 Viral prophylaxis: Herpes zoster prophylaxis will be offered to all patients in the form of valacyclovir 500mg tablet once daily or acyclovir 800mg twice daily for up to 2 years after initiating induction therapy. Patients will retain the option to decline viral prophylaxis.

5.8.3 Growth factors: Filgrastim or pegfilgrastim may be used to accelerate count recovery, especially in the event of febrile neutropenia, at the PI's discretion. Erythroid growth factors will not be used.

5.8.4 Blood products: Patients will be transfused packed red blood cells and platelets as clinically indicated. Due to the risk of GVHD in lymphopenic patients, all required blood products will be irradiated prior to transfusion. If the patient will also be followed by an off-site hematologist and/or primary care physician, personal communication will be made to the provider(s) to ensure that the patient will receive only irradiated blood products should off-site transfusion be necessary. We will discuss with the subject's referring home health care provider, the absolute need to receive only irradiated blood products should off-site transfusion be necessary. If patient's need to receive a blood product elsewhere (i.e. through an ER), we will inform the patient that he/she must verbalize the need to receive irradiated products.

5.9 Permitted concomitant medications

Patients may continue any 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. Medications prescribed for the intent to "treat" the malignancies in this protocol must be discontinued prior to study enrollment as previously stated. During protocol participation, no lymphoid malignancy directed treatments may be initiated.

5.10 Special instructions for patients

5.10.1 Immunizations: The safety of and ability to generate a primary or anamnestic response to immunization with live attenuated or inactivated vaccines during ofatumumab treatment has not been studied. The response to vaccination could be impaired when B cells are depleted. It is recommended that the investigator review the patient's immunization history as part of the initial screening procedure for a patient being considered for treatment with ofatumumab. Vaccination of the patient, in compliance with local area vaccination guidelines for the patient population being treated, is recommended prior to administration of ofatumumab. In particular, prior to administration of ofatumumab, hepatitis B vaccination, in patients with risk factors for hepatitis B infection or in areas with a high prevalence of hepatitis B, as per local area treatment guidelines should be considered. Administration of live attenuated vaccines should be avoided after treatment with ofatumumab and until B cell counts are normalized.

5.10.2 Birth control: Women of childbearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to and for the duration of study participation.

5.11 Hepatitis Evaluation

Subjects who are HBsAg negative, anti-HBc positive and HBV DNA negative may be included in the study but must undergo HBV DNA monitoring. Consult with a physician experienced in care & management of subjects with hepatitis B to manage/treat subjects who are anti-HBc positive. Initiate anti-viral therapy if required. If a subject's HBV DNA becomes positive during the study, notify the Novartis medical monitor. For subjects who have not completed planned ofatumumab therapy, discuss with the medical monitor the risks and benefits of continuing or discontinuing ofatumumab before appropriate treatment decisions are made for that individual subject.

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. Bone marrow aspirate and lymph node biopsies will be read by a pathologist and used for diagnostic purposes.

6.1 Pre-study evaluations: Baseline assessments will include the following and can be accepted from any prior NIH approved protocol (within the previous 2 months unless otherwise noted):

- Complete medical history
- Physical exam
- Concomitant medication review
- ECOG performance evaluation
- Complete blood count with differential
- Chem 20 panel
- Reticulocyte count (preferably on the day of screening, but acceptable within 2 months)
- Coagulation panel (PT,PTT)

- Serum free light chains, quantitative immunoglobulins (preferably on the day of screening, but acceptable within 2 months)
- Isohemagglutinin titer and blood type (preferably on the day of screening, but acceptable within 2 months)
- Beta-2 microglobulin
- Direct antiglobulin test (DAT) (preferably on the day of screening, but acceptable within 2 months)
- Viral serologies for hepatitis B and C, HIV 1/2 , HSV within 12 months. For individuals with a positive hepatitis B core antibody, HBV DNA PCR will be performed to screen for subclinical infection.
- For females of childbearing potential, 2 negative pregnancy tests sensitive to 50 m IU/ml.
- HLA typing of sibling(s) if patient is a suitable candidate for stem cell transplantation
- Bone marrow aspirate and biopsy performed at NIH is strongly desired within 12 months preceding start of the first induction cycle
- Interphase FISH cytogenetics for deletion 13q14 trisomy 12, deletion 11q22-23, and deletion 17p13 within 12 months preceding start of the first induction cycle
- Lymph node biopsy if clinically indicated, or optional for research purposes
- Lymphocyte phenotyping (T, B, NK)
- Peripheral blood flow cytometry panel for CLL if not done in the past 12 months
- Complete staging CT of the neck, chest, abdomen and pelvis if not already obtained within the preceding 3 months. IV and PO contrast will be used unless the patient has a contrast allergy (for research purposes)
- PET scan as clinically indicated
- EKG if not already obtained within the preceding 3 months
- Lymphapheresis optional for research purposes

6.2 Induction and post-induction evaluations

6.2.1 Pre-induction cycle evaluation: Prior to each induction cycle and at the end of the final (6^{th}) induction cycle, the following assessments will be completed:

- Interval History and Physical exam
- Concomitant medication review
- ECOG performance status evaluation
- Complete blood count with differential (within 5 days prior to start of each cycle)
- Chem 20 panel (within 5 days prior to start of each cycle)
- C-reactive protein (within 5 days prior to start of each cycle)
- DAT (within 5 days prior to start of each cycle)
- Haptoglobin (within 5 days prior to start of each cycle)
- Repeat pregnancy test (women of child bearing potential)
- Repeat EKG (if clinically indicated)

6.2.2 Monitoring following induction cycles

Between induction cycles (at least weekly [+/- 4 days]), subjects will be assessed with a CBC (with differential) either at the NIH or through their home MD. If subjects are followed at home, laboratory results must be faxed to the NIH 3SE nurses' station (301-402-4269).

- Repeat lymph node biopsy (day 9 induction cycle 1) when accessible lymph nodes are present is strongly desired for research purposes. This biopsy will be performed at NIH.

6.2.3 Post-cycle 3 (induction) restaging

Subjects will be restaged prior to induction cycle 4 using the following assessments in addition to those delineated in section 6.2.1:

- Peripheral blood flow cytometry panel for MRD
- CT of the neck, chest, abdomen, and pelvis. IV and PO contrast will be given unless the patient has a contrast allergy (for research purposes)
- PT and PTT (done for safety pre bone marrow biopsy)
- Bone marrow aspirate and biopsy for research (optional)
- Reticulocyte count
- Serum free light chains, quantitative immunoglobulins
- Repeat EKG (if clinically indicated)

6.2.4 Post-cycle 6 (induction) monitoring and restaging

Subjects will be restaged 3 months (+/- 2 weeks) after completion of induction cycle 6, using the following assessments in addition to those delineated in section 6.2.1:

- Peripheral blood flow cytometry panel for MRD
- CT of the neck, chest, abdomen, and pelvis. IV and PO contrast will be given unless the patient has a contrast allergy (standard of care)
- PT and PTT (done for safety pre bone marrow biopsy)
- Bone marrow aspirate and biopsy for response assessments
- Reticulocyte count
- Serum free light chains, quantitative immunoglobulins
- Lymphocyte phenotyping (T, B, NK)
- Repeat EKG (if clinically indicated)
- Complete blood count with differential may be done monthly depending on blood counts post induction

6.3 Consolidation and post consolidation evaluations (Only those patients that have completed ≥3 cycles of induction therapy and are MRD positive)

6.3.1 Pre-cycle evaluation (consolidation)

Prior to the start of each consolidation cycle, MRD positive subjects will return to the NIH every 2 months (+/- 2 weeks) for consolidation and the following assessments:

- Interval history and physical exam
- Concomitant medication review
- ECOG performance status evaluation
- Complete blood count with differential (within 5 days prior to start of cycle)
- Chem 20 panel (within 5 days prior to start of cycle)
- C-reactive protein (within 5 days prior to start of cycle)
- Lymphocyte phenotyping (T, B, NK)

- DAT (within 5 days prior to start of cycle)
- Haptoglobin (within 5 days prior to start of cycle)
- Pregnancy test
- Peripheral blood flow cytometry panel for MRD (optional) prior to all consolidation cycles.
- Repeat EKG (if clinically indicated)

6.3.2 Interim cycle evaluations (consolidation)

Subjects will be followed with a CBC (with differential) one month after each dose of consolidation ofatumumab, either at the NIH or through their home MD. If subjects are to be seen at home, laboratory results must be faxed to the NIH 3SE nurses' station (301-402-4269)

6.3.3 Post-cycle 4 (consolidation) evaluation

Subjects will be evaluated 2 months (+/- 2 weeks) after the last dose of consolidation ofatumumab is given, using the following assessments in addition to those delineated in section 6.2.1:

- Peripheral blood flow cytometry panel for MRD (optional)
- Serum free light chains, quantitative immunoglobulins

6.4 Primary endpoint evaluation (2 years after start of induction)

All patients (both MRD positive and MRD negative) will be restaged two years (24 months +/- 1 week) after the start of induction therapy using the following assessments:

- Interval history and physical exam
- Concomitant medication review
- ECOG performance status evaluation
- Complete blood count with differential
- Chem 20 panel
- C-reactive protein
- Reticulocyte count
- Serum free light chains, quantitative immunoglobulins
- Lymphocyte phenotyping (T, B, NK)
- DAT
- haptoglobin
- Peripheral blood flow cytometry panel for MRD
- CT of the neck, chest, abdomen, and pelvis. IV and PO contrast will be given unless the patient has a contrast allergy(standard of care)
- bone marrow biopsy (optional)
- Repeat EKG (if clinically indicated)

6.5 Off study medication monitoring:

6.5.1 MRD negative subjects through primary endpoint (2 years)

Subjects who are MRD negative will return to NIH at 12, 16, and 20 months (+/- 2 weeks) after start of induction therapy

- Interval history and physical exam
- Concomitant medication review
- ECOG performance status evaluation
- Complete blood count with differential
- Chem 20 panel
- C-reactive protein
- Lymphocyte phenotyping (T, B, NK)
- DAT
- Haptoglobin
- Serum free light chains and quantitative immunoglobulins
- Peripheral blood flow cytometry panel for MRD (optional)
- Repeat EKG (if clinically indicated)

6.5.2 All subjects (MRD negative and positive after the primary endpoint visit)

All subjects will continue to be followed at the NIH every 6 months (+/- 2 weeks) (yearly if subject has travel hardships) after the primary endpoint visit with the following assessments so long as they remain on study.

- Interval history and physical exam
- Concomitant medication review
- ECOG performance status evaluation
- Complete blood count with differential
- Chem 20 panel
- C-reactive protein
- Lymphocyte phenotyping (T, B, NK)
- DAT
- Haptoglobin
- Serum free light chains and quantitative immunoglobulins
- B2 Microglobulin
- Peripheral blood flow cytometry panel for MRD (at discretion of PI)
- Repeat EKG (if clinically indicated)
- Lymphapheresis (optional)

6.5.3 All subjects other laboratory testing

- Vitamin D levels may be drawn at up to 3 time points for clinical and research purposes.
- Specialized platelet testing may be drawn at any time as long as it is maintained within blood draw guidelines (Section 8.1). This testing may include von Willebrand Factor, von Willebrand Antigen, and Factor VIII. Specialized platelet function assays may be performed which may include ADP, epinephrine, and collagen. In addition, VCAM and thrombomodulin may also be assessed at this time.

7.0 CRITERIA FOR RESPONSE

Each subject will be assigned a response category at each restaging and follow-up visit. Responses will be categorized using the updated NCI-WG guidelines, which are as follows [2]:

7.1 Criteria for complete response (CR): CR requires all of the following:

- Peripheral blood lymphocytes < 4000/μL
- Absence of significant lymphadenopathy by physical examination and appropriate radiographic techniques (CT or MRI). All lymph nodes must have regressed to <=1.5cm in greatest diameter
- Absence of hepatomegaly or splenomegaly by physical examination, or appropriate radiographic techniques. Spleen, if enlarged before therapy must have regressed in size and must not be palpable by physical exam.
- Absence of constitutional symptoms
- Normal CBC, defined as:
  - Polymorphonuclear cells ≥ 1500/uL
  - Platelets > 100 000/uL (untransfused)
  - Hemoglobin > 11 g/dL (untransfused)
- Bone marrow biopsy demonstrates normal cellularity for age, with less than 30% of nucleated cells being lymphocytes. Lymphoid nodules should be absent
- Subjects who fulfill all the criteria of complete response but who have a persistent anemia or thrombocytopenia apparently unrelated to CLL will be deemed a CR with incomplete bone marrow recovery (CRi)

7.2 Criteria for partial response (PR): PR requires demonstration of at least one of the elements of a normal CBC as defined above, in addition to at least one of the following:

- ≥ 50% decrease in peripheral blood lymphocyte count from the pretreatment baseline value
- ≥ 50% reduction in the sum of the products of the greatest diameters (SPD) of lymph nodes of up to 6 nodes or nodal masses. No new sites of disease or increase in size of nodes.
- ≥ 50% reduction in pathologic enlargement of the liver and/or spleen by 50% as determined by measurement below the respective costal margin or CT scan

7.3 Criteria for minimal response (MR): MR must exhibit all of the following:

- ≥ 25% but ≤ 50% decrease in peripheral blood lymphocyte count from the pretreatment baseline value
- ≥ 25% but ≤ 50% reduction in the sum of the products of the greatest diameters (SPD) of lymph nodes of the 4 largest dominant nodes or nodal masses. No new sites of disease or increase in size of nodes.
- ≥ 25% but ≤ 50% reduction in pathologic enlargement of the liver and/or spleen by 50% as determined by measurement below the respective costal margin or CT scan

7.4 Criteria for progressive disease (PD): PD or treatment failure is characterized by at least one of the following:

- ≥50 % increase in the SPD of at least 2 lymph nodes (at least one node must be ≥ 2 cm); appearance of any new lymph nodes on physical examination or imaging

- ≥50% increase in the size of the liver and/or spleen as determined by measurement below the respective costal margin or CT scan or appearance of palpable hepatomegaly or splenomegaly, which was not previously present

- ≥50% increase in the absolute number of circulating lymphocytes to at least 5000/ul

- Transformation to a more aggressive histology
- Occurrence of any cytopenia attributable to CLL. After treatment: the progression of any cytopenia (unrelated to autoimmune cytopenia), as documented by a decrease of Hb levels by more than 20 g/L (2 g/dL) or to less than 100 g/L (10 g/dL), or by a decrease of platelet counts by more than 50% or to less than 100,000/µl which occurs at least 3 months after treatment, defines disease progression, if the marrow biopsy demonstrates an infiltrate of clonal CLL cells.

7.5. **Criteria for stable disease** (SD): Defined as not achieving CR, PR, or MR, but not fulfilling the criteria for PD.

7.6 **Progression-free survival (PFS):** Defined as the length of time from the initiation of therapy until criteria for progressive disease (PD) **or death** are reached.

7.7 **Overall survival (OS):** Defined as the length of time from the initiation of therapy until death.

7.8 **Minimal residual disease (MRD):** Defined as the presence of peripheral blood lymphocytes demonstrating the CLL phenotype, using a limit of detection of 0.01% of leukocytes.

8.0 ANCILLARY LABORATORY RESEARCH STUDIES

8.1 Collection of samples

Blood samples: Depending on the ALC, 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 restaging and MRD time points.

Bone marrow biopsy: An additional 5 cc of bone marrow aspirate (baseline, restaging visits, primary endpoint, off study evaluations) may be collected for exploratory ancillary laboratory research studies.

Lymph node biopsy: Samples will be obtained from the diagnostic procedures subjects undergo at study entry and at the scheduled follow-up evaluations. During cycle 1, a dedicated research lymph node core biopsy may be obtained in subjects who have superficial lymph nodes (axillary, cervical or inguinal areas). More invasive procedures, e.g. biopsy of abdominal lymph nodes, will NOT be used to obtain this research biopsy.

MRD assessment: For research purposes, MRD (peripheral blood flow cytometry) may also be assessed at 3, 12, 16, and 20 months from start of induction cycle one (in addition to required MRD assessments at 10 and 24 months from start of induction cycle one).

8.2 Intended use

These specimens will not be read by a pathologist or used for diagnostic purposes. Rather, the 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.

- MRD using 4-color flow cytometry

- Effects of ofatumumab alone and in combination with chemotherapy on tumor cell biology using flow cytometry, gene expression profiling and protein studies. These studies aim to define predictors of response and investigate possible indicators of synergy between chemotherapy and immunotherapy

- Ofatumumab serum levels and elimination half-life and/or complement activation and CD20 levels. Coded samples will be sent to Ronald P.Taylor, PhD, Professor of Biochemistry 434-924-2664 University of Virginia for these analyses. No transfer of material will be accomplished until a Material Transfer Agreement is fully executed through the NHLBI Office of Technology Transfer and Development (OTTAD)

- Biomarker development

- Additional studies which are approved by the NHLBI IRB and listed in the Appendix of the protocol may be done on stored samples

8.3 Storage, tracking and disposition of samples

Storage: All samples will be stored in the laboratory of Dr. Wiestner. Research samples will be stored using BSI in accordance with NHLBI DIR Biospecimen 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.

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: To determine the progression-free survival rate 2 years after initiation of induction therapy.

9.2 Secondary endpoints:

- The toxicity profile ofatumumab-based induction chemoimmunotherapy followed by consolidation ofatumumab immunotherapy using the CTCAE criteria.
- Incidence of MRD negativity at the completion of induction chemoimmunotherapy.
- Incidence of MRD negativity at the completion of consolidation immunotherapy in those patients failing to achieve MRD negativity two months after completion of induction chemoimmunotherapy.
- Complete and overall response rates to induction chemoimmunotherapy.
- Overall survival rate 2 years after initiation of induction chemoimmunotherapy.
- Correlate biologic endpoints with clinical response by measuring biomarkers.

9.3 Sample size

We plan to evaluate progression-free survival (PFS) as our primary endpoint. We use the concurrent fludarabine/rituximab regimen as studied by Byrd *et al* as our comparator regimen. This regimen achieved PFS in 67% of patients at two years. Using 67% as our alternative PFS and 40% as our null hypothesis, we plan to enroll a total of 38 evaluable patients to achieve 90% power at α level of 0.05. The sample size calculation was based on a two-sided binomial test, and we reject the null hypothesis if the number of PFS patients is no more than 8 or greater than 21. Based on our experience and that of the German CLL8 study group, our best estimate of the patient population to be risk stratified is as follows: *By Ig-mutated/ZAP-70* (estimated 1/3 with Ig-mutated/ZAP-70 negative disease and 2/3 with Ig-unmutated/ZAP-70 positive Disease), *by cytogenetics* (estimated 40-50% 13q del, 10-20% no abnormality, 10% trisomy 12, 25% 11q del: and 5% 17p del) and *By treatment regimen* (estimated 70% will receive FO and 30% will receive FCO).

Subjects will be considered evaluable for the primary endpoint providing they have completed at least 3 induction cycles. Based on an assumed dropout rate of 15-20% and the goal of having 38 evaluable subjects, an additional 8 subjects may be enrolled.

9.4 Statistical methods

The planned analyses will include descriptive statistics on the proportions of responses (i.e. % subjects with partial or complete response). 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 and the PFS time will be analyzed using appropriate nonparametric tools in survival analysis such as Kaplan-Meier estimates taking consideration of random censoring. We may also use

the two tailed, one-sample log-rank test to test if the actual PFS rate at 2 years is greater than 40%, assuming the PFS time is exponentially distributed under the null hypothesis.

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 Stopping rules for treatment-related SAEs

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. Severe infection requiring vasopressor >24h or intubation
2. Any Grade IV toxicity **excluding**

    - readily reversible metabolic or laboratory abnormalities
    - hematologic toxicities

3. Persistent neutropenia <500 or thrombocytopenia <20,000 persisting for greater than four weeks after completion of an induction or consolidation cycle, unless deemed to be secondary to disease.

We anticipate the rate of these specified TRSAEs within the 2-year period to be 25% or less. Following Geller et al. (2003, "Design of Early Trials in Stem Cell Transplantation: A Hybrid Frequentist-Bayesian Approach"), 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 25% is at least 95%. We take our prior distribution to be a beta distribution with parameters $(\alpha, \beta) = (1.25, 3.75)$. The parameters are chosen so that the mean $\alpha / (\alpha + \beta) = 0.25$ as the expected proportion of specified TRSAE's and the sum $\alpha + \beta = 5$ as the "worth" we place on our prior clinical opinion. This indicates that the relative weight we place on our prior opinion is approximately 10% of the weight we will place on the results of the new study. Hence when we make decisions about stopping the study, the data from the study will dominate over the prior opinion. 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 developed any of the specified TRSAE's reaches or exceeds:
≤ 3	3
≤ 5	4
≤ 8	5
≤ 11	6
≤ 14	7
≤ 17	8
≤ 20	9

Number of subjects in the experiment	Stop if the number of subjects who have developed any of the specified TRSAE's reaches or exceeds:
≤ 23	10
≤ 26	11
≤ 29	12
≤ 33	13
≤ 36	14
≤ 39	15
≤ 42	16
≤ 46	17

We investigated the performance of the above stopping rule by a simulation study. In each simulation run, we generated a study with 46 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	0.2	0.25	0.30	0.35	0.40	0.45
Proportion of Stopped Studies	4.13%	13.3%	31.8%	56.6%	78.7%	92.3%
Average number of subjects	44.7	42.2	37.7	31.3	24.4	18.4
Average number TRSAEs	8.9	10.5	11.3	11.0	9.8	8.3

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 25%, 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). 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 $(\alpha, \beta) = (0.25, 4.75)$. This indicates that the relative weight we place on our prior opinion is approximately 10% 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 or exceeds:
≤ 5	2
≤ 15	3

Number of subjects in the experiment	Stop if the number of subjects who have TRMs reaches or exceeds:
≤ 5	2
≤ 27	4
≤ 39	5
≤ 46	6

We investigated the performance of the above stopping rule by a simulation study. In each simulation run, we generated a study with 46 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	0.04	0.05	0.07	0.10	0.15	0.20
Proportion of Stopped Studies	4.9%	9%	20.9%	44.9%	79.4%	94.7%
Average number of subjects	44.6	43.5	40.5	34.3	23.8	16.3
Average number TRMs	1.8	2.2	2.8	3.4	3.6	3.3

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: Should any of the following adverse events occur, study drug administration will be discontinued. The subject will be followed until resolution of the event. Labs will be monitored for 6 months after discontinuation of study drug administration, or until the subject initiates alternative CLL therapy, at which time the subject's participation on this study will be considered complete and he or she will go off study. Off study events include:

- Grade IV neutropenia that persists for longer than three weeks after completion of a cycle
- Grade IV thrombocytopenia refractory to platelet transfusions
- Serious or life threatening cardiac arrhythmias (grade 3 or 4)
- Severe infection (requiring vasopressor support >24h or intubation) considered definitely related to the study medication
- Any Grade IV toxicity considered related to the study medication excluding readily reversible metabolic or laboratory abnormalities or hematologic toxicities
- More than 2 chemotherapy dose reductions in response to hematologic or non-

hematologic toxicities
- Irreversible renal insufficiency precluding administration of fludarabine for more than one cycle
- Significant progression of disease or a concomitant condition that would make the subject ineligible for further protocol participation
- Pregnancy or unwillingness to refrain from double barrier method of contraception
- Initiation of non-protocol therapy for CLL

Completion of the study: Subjects will be followed indefinitely per section 6.5.2 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

10.1 Safety monitoring

Event Characterization and Reporting to the IRB, Clinical Director (CD) , and Sponsor

Approved by HSRAC on September 30, 2013
Date effective: October 28, 2013

Adverse events, Protocol deviations, Unanticipated problems (UP), serious adverse events, Sponsor and serious, are defined as described in NIH HRPP SOP 16 ("Reporting Requirements for Unanticipated Problems, Adverse Events and Protocol Deviations."). All adverse events occurring during the study, including those observed by or reported to the research team, will be recorded. Serious unanticipated problems, and serious protocol deviations, will be reported to the IRB and Clinical Director as soon as possible but not more than 7 days after the PI first learns of the event. Not serious unanticipated problems will be reported to the IRB and Clinical Director as soon as possible but not more than 14 days after the PI first learns of the event. Not serious protocol deviations will be reported to the IRB as soon as possible but not more than 14 days after the PI first learns of the event. In accordance with NHLBI policy, SAEs that do not meet the criteria of Unanticipated Problem (UP) must be reported to the IRB Chair and Clinical Director within 14 days of learning of the event using the SAE form in PTMS.

Deaths will be reported to the Clinical Director within 7 days after the PI first learns of the event.

Unanticipated Problems and Protocol Deviations

An unanticipated problem is any incident, experience, or outcome that is:

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. (An AE with a serious outcome will be considered increased risk.)

Protocol Deviation
Any change, divergence, or departure from the study design or procedures of an IRB-approved research protocol.

Principal Investigator: Accrual, efficacy and safety data will be monitored by the Principal Investigator: Inhye Ahn, M.D.

***NHLBI IRB*:** Accrual and safety data will be monitored reviewed annually by the Institutional Review Board (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 Title 45 CFR 46. This committee will also approve all amendments to the protocol or informed consent, and conduct continuing annual review so long as the protocol is open to accrual or follow up of subjects.

***NHLBI DSMB*:** The NHLBI Data Safety and Monitoring Board will review the protocol at six or twelve month intervals. A progress report will be forwarded to the DSMB at these times. The DSMB may recommend early termination of the study for considerations of safety and efficacy.

FDA: An annual progress report, any amendments to the protocol, and any change in the status of the protocol will be forwarded to FDA to:

Melanie Pierce, Senior Regulatory Health Project Manager
Patricia Keegan, M.D., Division Director
Division of Biologic Oncology Products
Office of Oncology Drug Products
Center for Drug Evaluation and Research, Food and Drug Administration
5901-B Ammendale Road, Beltsville, MD 20705-1266
Telephone: 301-796-1273

Novartis: An annual progress report, any amendments to the protocol, and any change in the status of the protocol will be forwarded to:

Christina Kovacs, Clinical Research Scientist
CDMA Oncology
Email Christina.kovacs@Novartis.com
Tel 866-778-7791

We request a waiver from continuing to record and report the following types of previously identified protocol deviations that have been resolved through previous amendments to the protocol:

- Out of window administration of chemoimmunotherapy and premedications. The protocol was amended to increase flexibility to accommodate for timing associated with these standard procedures.

10.2 Adverse events

Adverse event monitoring plan used to evaluate the safety of this protocol regimen will include:

Clinical findings: any unlabeled unfavorable and unintended signs, symptoms or diseases which either occur during the study (having been absent at baseline), or if present at baseline appear to worsen, will be reported. All tumor lysis reactions and all ofatumumab infusion related reactions will be reported.

Abnormal laboratory findings: any unlabeled change from baseline laboratory assessments (done prior to first dose of study medication) that result in a progression to a grade 3 or 4 laboratory toxicity. In view of the underlying disease, many of these subjects may be dependent on red cells, platelets and/or growth factors. The PI will make a decision as to the baseline hematologic reserve of the patient and designate the appropriate laboratory measure which will be used as the baseline.

The AEs will be attributed (unrelated, unlikely, possibly, probably or definitely) to study medication and/or disease using the following rules and graded by severity utilizing CTCAE version 3.0.

Attribution of adverse events:

Criteria for Determining Category of Relationship of Clinical Adverse Events to Treatment		
1	**Not related**	This category applies to those adverse events which, after careful consideration, are clearly and incontrovertibly due to extraneous causes (disease, environment, etc.)
2	**Unlikely** **(must have two)**	In general, this category can be considered applicable to those adverse events which, after careful medical consideration at the time they are evaluated, are judged to be unrelated to the test drug. An adverse event may be considered unlikely if or when: 1. It does not follow a reasonable temporal sequence from administration of the test drug. 2. It could readily have been produced by the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject. 3. It does not follow a known pattern of response to the test drug. 4. It does not reappear or worsen when the drug is re-administered.
3	**Possibly** **(must have two)**	This category applies to those adverse events for which, after careful medical consideration at the time they are evaluated, a connection with the test drug administration appears unlikely but cannot be ruled out with certainty. An adverse event may be considered possibly related if or when: 1. It follows a reasonable temporal sequence from administration of the test drug. 2. It could not readily have been produced by the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject. 3. It follows a known pattern of response to the test drug.
	Probably **(must have three)**	This category applies to those adverse events for which, after careful medical consideration at the time they are evaluated, are felt with a high degree of certainty to be related to the test drug. An adverse event may be considered probably related if or when: 1. It follows a reasonable temporal sequence from administration of the test drug. 2. It could not be reasonably explained by the known characteristics of the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject. 3. It disappears or decreases on cessation or reduction in dose. There are important exceptions when an adverse event does not disappear upon discontinuation of the drug, yet drug-relatedness clearly exists (e.g., bone marrow depression, fixed drug eruptions, tardive dyskinesia). 4. It follows a known pattern of response to the test drug.
5	**Definitely** **(must have all)**	This category applies to those adverse events which, the Investigator feels are incontrovertibly related to test drug. An adverse event may be assigned an attribution of definitely related if or when: 1. It follows a reasonable temporal sequence from administration of the test drug. 2. It could not be reasonably explained by the known characteristics of the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject.

Criteria for Determining Category of Relationship of Clinical Adverse Events to Treatment		
		3. It disappears or decreases on cessation or reduction in dose with re-exposure to drug. (Note: this is not to be construed as requiring re-exposure of the subject, however, a category of definitely related can only be used when a recurrence is observed.) 4. It follows a known pattern of response to the test drug.

10.3 Serious adverse events

Any serious adverse events as defined in the "NIH Guidelines for Adverse Event Reporting" will be reported to the IRB immediately. This includes any untoward medical occurrence that:

- results in death
- is life threatening
- requires (or prolongs) hospitalization
- causes persistent or significant disability/incapacity
- results in congenital anomalies or birth defects or
- other conditions which in the judgment of the investigators represent significant hazards.

Serious adverse events will be attributed as definitely (clearly related to the research), probably (likely related to the research), possibly (may be related to the research), unlikely (doubtfully related to the research) and unrelated (clear not related to the research).

Treatment related SAEs (TRSAEs) As detailed in section 9.7, a subset of TRSAEs considered to be probably or definitely related to study medication will be monitored and considered for early stopping of the study according to statistically determined criteria. John Tisdale M.D. (NIH/NIDDK) will serve as the independent monitor who reviews the attribution of TRSAEs.
Hospitalizations for administrative issues (e.g., to receive a transfusion) or upgrading to ICU for routine monitoring will not be reported as an SAE.

After the 2-year follow-up/restaging visit, only those AEs and SAEs that are deemed definitely related to a procedure or test that was administered as part of this protocol will be reported.

10.4 Reporting of serious adverse events

Principal Investigator*:* All serious adverse events will be reported to the Principal Investigator of this study.

IRB*:* Serious adverse events will be reported to the IRB immediately with a written report to follow within 7 days of a death or 15 days of any other serious adverse event using the Serious Adverse Event Form.

DSMB*:* Reports of serious adverse events that are **unexpected and thought to be related** to the experimental drug will be forwarded immediately to the Data and Safety Monitoring Board (DSMB). A summary of all SAEs will be included for review annually by the DSMB. If the unexpected serious adverse event is thought to be due to the experimental component of the protocol, accession to the protocol will be stopped until a full discussion with the IRB has been held.

FDA: IND # 107803. A summary of all SAEs will be submitted to the FDA with the annual progress report. SAEs that are unexpected and thought to be related to the experimental drug will be forwarded within 15 days of the study team learning of the event using an NHLBI SAE report form and a Medwatch form to:

Melanie Pierce, Senior Regulatory Health Project Manager
Patricia Keegan, M.D., Division Director
Division of Biologic Oncology Products
Office of Oncology Drug Products
Center for Drug Evaluation and Research, Food and Drug Administration
5901-B Ammendale Road, Beltsville, MD 20705-1266
Telephone: 301-796-1273

Novartis: All unexpected and possibly, probably or definitely related SAEs occurring during the study or within 30 days of the last administration of ofatumumab will be reported to Novartis within 24 hours of the research team learning of the event. A copy of the SAE report (NHLBI SAE report form) will be forwarded as soon as possible, but no later than seven (7) days in the case of death or life-threatening serious adverse events or within fifteen (15) days after the occurrence of all other forms of serious adverse events. If the SAE is unexpected and determined possibly, probably or definitely related to study drug the SAE report (NHLBI SAE report form and Medwatch form) will be forwarded to Novartis and FDA. Follow-up reports regarding the patient's subsequent course will be submitted until the SAE has resolved or until the patient's condition stabilizes (in the case of persistent impairment) or the patient dies. The SAE report will contain a full written summary detailing relevant aspects of the adverse events in question. Where applicable, information from relevant hospital case records and autopsy reports will be included. The investigator will always provide an assessment of causality at the time of the initial report as described in 'Assessment of Causality'.

You should report SAEs to Novartis by fax to:

- **U.S. Drug Safety & Epidemiology at Fax #: 877-778-9739**
  - (Should the designated SAE Fax# be non-functional please send SAEs to the designated SAE mailbox: clinicalsafetyop.phuseh@novartis.com)
- **A Novartis SAE Coversheet must be attached to all SAE submissions**
- **SAE Submissions must reference your Novartis Study Code**

For medical emergencies, the contact is:

Gaetano Bonifacio, MD
Medical Director
US CD MA Hematology
Novartis Pharmaceuticals Corporation
One Health Plaza, 345/4th
East Hanover, NJ 07936-1080
Mobile +1 610 427 3613
gaetano.bonifacio@novartis.com

10.5 Reporting of pregnancy

Subjects who become pregnant during the study should discontinue the study drug administration immediately. The investigator, or his/her designee, will collect pregnancy information on any subject who becomes pregnant while participating in this study. The investigator, or his/her designee, will submit pregnancy information to Novartis within two weeks of learning of a subject's pregnancy. Information on the status of the mother and child will be forwarded to Novartis. Generally, follow-up will be no longer than 6 to 8 weeks following the estimated delivery date. Any premature termination of the pregnancy will be reported.

While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy for medical reasons will be recorded and reported to Novartis as an AE or SAE.

A spontaneous abortion is always considered to be an SAE and will be reported to Novartis. Furthermore, any SAE occurring as a result of a post-study pregnancy and is considered reasonably related to the investigational product by the investigator, will be reported to Novartis. While the investigator is not obligated to actively seek this information in former study participants, he/she may learn of an SAE through spontaneous reporting.

10.6 Data management

Storage of data:

The PI 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.

All human subjects personally identifiable information (PII) as defined in accordance to the Health Insurance Portability and Accountability, eligibility and consent verification will be recorded in DIR's Clinical Data System (CDS) or the Laboratory of Cardiac Energetics (LCE) database. Primary data obtained during the conduct of the protocol will be kept in secure network drives or in approved alternative site that complies with NIH security standards. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant, e.g., study-specific identifying number (SSPIN) generated by CDS or other unique code, or minimum PII required for subject identification.

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.

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.

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 (OHSR).

11.0 HUMAN SUBJECT PROTECTION

11.1 Rationale for subject selection

Predicted distribution by gender, age and race: CLL/SLL 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. 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

Recruitment: The study will be listed on the ClinicalTrials.gov, Clinical Center research studies, the leukemia and Lymphoma Foundation, Physician's Desk Query, 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.

Compensation for participation: $0.

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

For travel from home: Travel from home for the first NIH visit will not be reimbursable. If the patient consents to protocol participation travel home following the first visit will be reimbursable. Subjects will be reimbursed 100% of government rate for travel once the subject has been determined eligible to participate and signs consent.

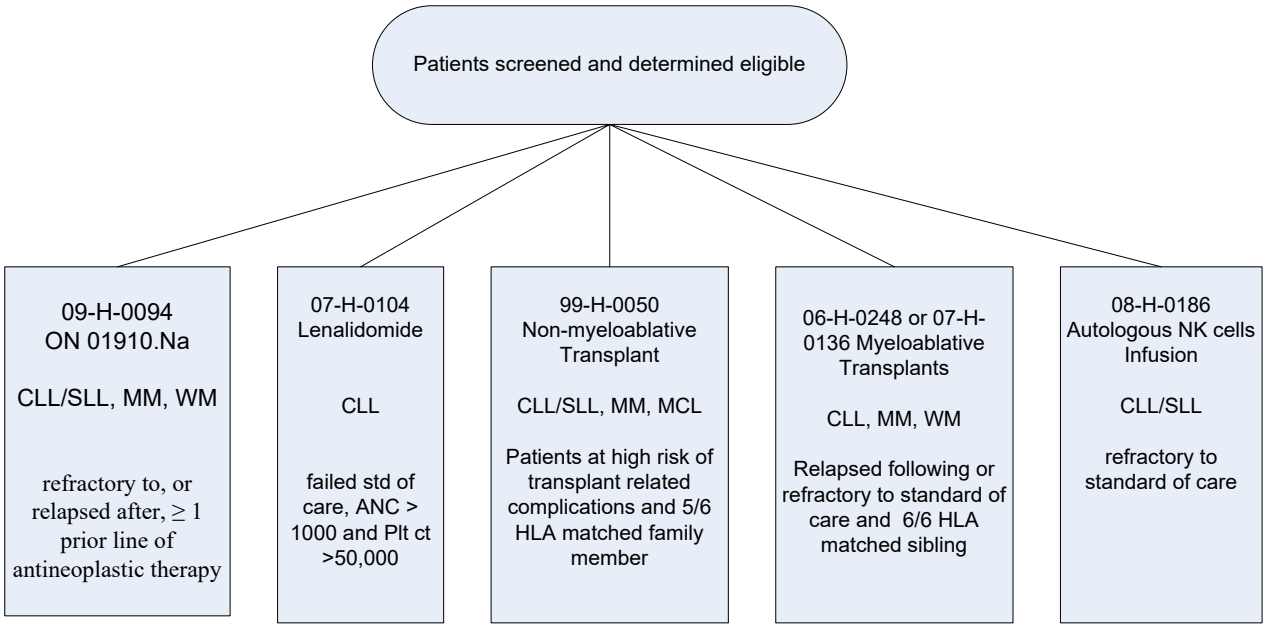
Local travel (car/taxi/shuttle/train/bus): Subjects will be reimbursed for local train/bus and/or shuttle costs. Car mileage will be reimbursed $0.41/mile when the distance from home is greater than 50 miles. Reimbursement for mileage less than 50 miles from home is not provided. Subjects will not be reimbursed for rental car cost beyond the car mileage rate. Taxi will be paid only when medically necessary and authorized by the PI

Meals: Subjects will not be reimbursed for meals.

Lodging: Subjects will be reimbursed for hotel/motel lodging at a rate of $60/night for a maximum of 7 days after which the reimbursement is $30/night. If space is available, the children's inn ($64/night) or the family lodge ($65/night) will be paid directly to the facility.

Guardian coverage: Subjects will be reimbursed for guardian travel (100% of government rate) and lodging ($15.00/night) provided the services of a guardian are medically indicated and pre-approved.

Competition with other Branch protocols:



11.2 Participation of children: CLL is very 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 50 years and above. CLL may also be biologically a different disease in children. For these reasons, individuals < 18 yrs old have been excluded from protocol participation.

11.3 Risks and discomforts

11.3.1 Risks related to ofatumumab

The safety of monotherapy with ofatumumab was evaluated in 181 patients with relapsed or refractory CLL. 90 percent of the patients received at least 8 infusions.

Infusion reactions. Intravenous administration of ofatumumab has been associated with infusion reactions. These reactions may result in temporary interruption or withdrawal of treatment or death. Pre-medications prior to administration attenuate infusion reactions but these may still occur, predominantly during or following the first intravenous infusion. Infusion reactions following IV administration of ofatumumab may include, but are not limited to, anaphylactic reaction, bronchospasm, cardiac events (e.g. myocardial ischaemia /infarction, bradycardia), chills/rigors, cough, cytokine release syndrome, diarrhea, dyspnoea, fatigue, flushing, hypertension, hypotension, nausea, pain, pruritus, pulmonaryoedema, pyrexia, rash and urticaria. Even with pre-medication, severe reactions, including cytokine release syndrome, have been reported following ofatumumab use.

In cases of severe infusion reaction, the infusion of ofatumumab must be interrupted immediately and symptomatic treatment instituted. Infusion reactions occur more frequently on the first day of infusion and tend to decrease with subsequent infusions. Subjects with a history of decreased pulmonary function may be at a greater risk for pulmonary complications from severe reactions and should be monitored closely during infusion of ofatumumab. Pre-medication consisting of acetaminophen, oral or intravenous antihistamine, and oral and/or intravenous glucocorticoids has been administered in clinical studies in oncology and rheumatoid arthritis populations. This pre-

medication attenuates infusion reactions and should be administered prior to intravenous administration of ofatumumab.

Cytopenias. Prolonged (≥1 week) severe neutropenia and thrombocytopenia can occur with ofatumumab. Complete blood counts (CBC) and platelet counts will be monitored at regular intervals during therapy, and in patients who develop Grade 3 or 4 cytopenias this monitoring will be increased in frequency. Among the 181 patients, a total of 108 patients (70%) experienced bacterial, viral or fungal infections. A total of 45 patients (29%) experienced ≥ Grade 3 infections, of which 19 (12%) were fatal. Of 108 patients with normal neutrophil counts at baseline, 45(42%) developed ≥ grade 3 neutropenia. 19 (18%) develop grade 4 neutropenia. Some patients experienced new onset Grade 4 neutropenia > 2 weeks in duration.

Tumor Lysis Syndrome. In patients with CLL, tumor lysis syndrome (TLS) may occur with use of ofatumumab. Management of TLS includes correction of electrolyte abnormalities, monitoring of renal function, maintenance of fluid balance and supportive care.

Progressive multifocal leukoencephalopathy (PML). Progressive multifocal leukoencephalopathy (PML) and death have been reported in oncology patients receiving cytotoxic pharmacotherapy, including ofatumumab. A diagnosis of PML should be considered in any ofatumumab patient who reports the new onset of or changes in pre-existing neurologic signs and symptoms. If a diagnosis of PML is suspected, ofatumumab should be suspended and the patient referred to a neurologist. Patients with confirmed PML or for who PML cannot be ruled out ofatumumab should be discontinued.

Hepatitis B reactivation. Immuno-compromised patients receiving cytotoxic chemotherapy and/or CD20-directed cytolytic antibodies including ofatumumab are at risk of hepatitis B infection and reactivation resulting in fulminant hepatitis, hepatic failure, and death. Cases have been reported in patients without prior history of infection and in patients with evidence of prior infection (hepatitis B surface antigen (HBsAg) negative; anti-hepatitis B core antibody (anti-HBc) positive). All patients should be screened for HBV infection by measuring HBsAg and anti-HBc before initiation of ofatumumab treatment. Patients who are anti-HBc positive and HBsAg negative (evidence of prior HBV infection), and whose HBV DNA test is negative, can be enrolled but should be closely monitored for clinical and laboratory signs of active HBV infection or reactivation during treatment with ofatumumab. For such patients, a consult should be obtained from a physician experienced in the care and management of patients exposed to HBV infection. Because HBV reactivation has been reported up to 12 months following completion of ofatumumab therapy, anti-HBc positive patients should be monitored for signs of infection or reactivation for 6-12 months following the last infusion of ofatumumab. In patients who present with evidence of new HBV infection or reactivation, ofatumumab and any concomitant chemotherapy should be interrupted immediately and appropriate treatment should be instituted (e.g., antiviral therapy). A consult regarding HBV antiviral therapy should be obtained from a physician experienced in the care and management of hepatitis B. Insufficient data exist regarding the safety of resuming ofatumumab in patients who develop HBV reactivation. Resumption of ofatumumab in patients whose HBV reactivation resolves should be discussed with physicians with expertise in managing hepatitis B.

Intestinal obstruction. Bowel obstruction has been reported in oncology patients receiving anti-CD20 monoclonal antibody therapy, including ofatumumab. Patients who present with severe abdominal pain, especially early in the course of ofatumumab therapy, should be evaluated and appropriate treatment instituted.

Cardiovascular. Patients with a history of cardiac disease should be monitored closely.

Ofatumumab should be discontinued in patients who experience serious or life-threatening cardiac arrhythmias.

Adverse Reactions Seen in CLL. Events denoted with an asterisk under CLL are likely attributable to ofatumumab in the setting of an infusion reaction and typically occur after the start of infusion and within 24 hours after the completion of the infusion.

Infections and Infestations: Hepatitis B (infection and reactivation)

Gastrointestinal Disorders: nausea*, vomiting*, abdominal pain*, small intestinal obstruction, diarrhoea*

Respiratory, Thoracic and Mediastinal Disorders: pharyngolaryngeal pain*, dyspnoea*, cough*, bronchospasm*, chest discomfort*, nasal congestion*, hypoxia*

Skin and Subcutaneous Tissue Disorders: rash*, pruritus*, urticaria*, flushing*

General Disorders and Administration Site Conditions: fatigue*, chills*, rigors*, hyperhidrosis*, cytokine release syndrome*, pyrexia*, infusion-related reaction
Cardiac Disorders: tachycardia*, bradycardia*

Vascular Disorders: hypertension*, hypotension*, pulmonary edema*
Musculoskeletal and Connective Tissue Disorders: back pain*

Metabolism and Nutrition Disorders: tumor lysis syndrome

Immune System Disorders: hypersensitivity*, anaphylactic reaction*, anaphylactic shock*

Blood and Lymphatic Disorders: neutropenia

Other Potential Adverse Reactions in Hematologic Malignancies:
Infections: bacterial, viral, and fungal infections.

Blood and lymphatic system disorders: leukopenia, (e.g. neutropenia and lymphopenia), thrombocytopenia and anemia.

Skin and subcutaneous disorders: Toxic epidermal necrolysis/Stevens Johnson Syndrome. Various cutaneous reactions can occur in patients with malignancies, particularly in individuals with non-Hodgkin non-Hodgkintients with malignancimal necrolysis or SJS have been reported with rituximab; however, in patients with hematologic malignancies the occurrence of these events may be confounded due to the occurrence of various paraneoplastic syndromes involving the skin (e.g., paraneoplastic pemphigus).

Cases of PRES (posterior reversible encephalopathy syndrome) have been reported with rituximab and with the administration of many other medications in a wide variety of clinical settings (e.g., transplantation, oncology and rheumatology). PRES can clearly occur in the setting of hypertension (e.g., eclampsia) and during immunosuppressive therapy. The pathogenesis of PRES is not clear though it is considered to be related to changes in the blood-brain barrier and cerebral vascular function. The onset is generally abrupt and characterized by headache followed by altered consciousness. Loss of vision, seizures and localized neurologic findings (e.g.,

hemiparesis) may occur. The diagnosis is made clinically with significant support from MRI scanning. Once recognized and treated, the condition is often reversible though occasionally hemorrhage may occur.

11.3.2 Risks related to fludarabine

The most common side effects include: general body discomfort, loss of appetite, muscle pain, nausea, tiredness, vomiting, weakness.

Severe side effects include: severe allergic reactions (rash; hives; itching; difficulty breathing; tightness in the chest; swelling of the mouth, face, lips, or tongue); abnormal thinking; black, tarry, or bloody stools; blood in the urine; changes in strength or walk; chest pain; confusion; coughing or vomiting blood; difficult or painful urination; fainting; hearing loss; irregular heartbeat; lower back or side pain; mental or mood changes (eg, agitation, confusion); numbness or tingling in the hands or feet; red, swollen, blistered, or peeling skin; seizures; severe or persistent tiredness or weakness; signs of infection (eg, fever, chills, cough, or sore throat); shortness of breath; skin changes; sores on the mouth or lips; swelling of the fingers, hands, or feet; unusual bruising or bleeding; vision changes or blindness; yellowing of the eyes or skin. Patients will be advised to contact the research team immediately in the event of a severe side effect.

11.3.3 Risks related to cyclophosphamide

The most common side effects include: appetite loss; absence of menstrual periods; color change in skin; diarrhea; general unwell feeling; hair loss; nausea; skin rash; stomach discomfort or pain; texture change in nails; vomiting; weakness.

Severe allergic reactions (rash; hives; itching; difficulty breathing; tightness in the chest; swelling of the mouth, face, lips, or tongue); blood in urine; bloody or black tarry stools; chest pain; chills; fever; hallucinations; increased or decreased urination; infection; lower back or abdominal pain; pain, swelling, or redness at the injection site; painful urination; persistent cough; pneumonia; red, swollen, or blistered skin; seizures; severe stomach pain; shortness of breath; sore throat; sores in the mouth; swelling of the hands or feet; unusual bruising or bleeding; unusual or severe tiredness or weakness; yellowing of the skin or eyes. Patients will be advised to seek medical attention right away if any of these SEVERE side effects occur when using cyclophosphamide.

11.3.4 ***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.

11.3.5 ***Risks related to bone marrow aspirate and biopsy:***

Aspirates are obtained from the posterior iliac crest. After preparation of the skin with alcohol and betadine, the epidermis, and periosteal layers are infiltrated with 1 % xylocaine. Two to eight individual aspirates are obtained through a 16 gauge Illinois or Jamshidi needle, yielding 0.5-2x 10^9 nucleated cells. Generally, a maximum of 7 aspirates are obtained from each site; the needle is advanced about 0.5 cm between each aspiration. A maximum of 20 cc total of bone marrow is collected. Bone marrow is aspirated into a heparinized syringe or into a syringe containing anticoagulated culture medium, depending on the use for which it is intended. A research core biopsy may also be obtained with prior consent. No major risks are involved with bone marrow aspirate and biopsy, however, there is a small risk of infections, pain, bleeding, and hematoma formation at the site of the aspiration.

11.3.6 ***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. Subjects will undergo a total of four CT scans during the course of the study. Two of these (prior to induction chemoimmunotherapy and after completion of induction) are medically indicated. The other two scans (prior to start of induction cycle 4 and two years after start of induction therapy) are not required for medical care and are for research purposes only. The amount of radiation subjects will receive from the research scans in this study is 3.2 rem per year 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. 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.

11.3.7 ***Risks related to lymph node biopsy*****:** lymph nodes are part of the immune system. They are found in the neck, behind the ears, in the armpits, and in the groin. A lymph node biopsy removes lymph node tissue to be looked at under a microscope for signs of cancer. There are several ways to do a lymph node biopsy.

- A **fine or core needle aspiration biopsy** puts a thin needle into the lymph node and removes cells to look at. Subjects will feel only a quick sting from the local anesthesia used to numb the skin and may feel some pressure from the biopsy needle. After a core needle biopsy, the site may be tender for 2 to 3 days.

- An **open biopsy** makes a cut in the skin and removes the lymph node. General anesthesia may be required. For 1 to 2 days subjects may feel tired and have a mild sore throat from the tube that was used to help them breathe during the biopsy. Throat lozenges and gargling with warm salt water will be recommended. After the open biopsy, the biopsy site may feel tender, firm, swollen, and/or bruised. Subjects may be advised not do any heavy lifting or other activities that stretch or pull the muscles around the area. Patients will be instructed on wound care and timing of suture removal.

11.3.8 Risks related to pregnancy and nursing mothers:

Pregnancy

There are limited data from the use of ofatumumab in pregnant women. The effect on human pregnancy is unknown. Precautions should be undertaken to avoid pregnancy and adequate contraception should be used while using ofatumumab and for at least 6 months after the last ofatumumab treatment. Ofatumumab should not be administered to pregnant women unless the possible benefit to the mother outweighs the possible risk to the fetus. Animal studies do not indicate direct or indirect harmful effects with respect to maternal toxicity, pregnancy or embryonal/fetal development.

Lactation

The safe use of ofatumumab in humans during lactation has not been established. It is not

known whether ofatumumab is secreted in human milk; however human IgG is secreted in human milk. Published data suggest that neonatal and infant consumption of breast milk does not result in substantial absorption of these maternal antibodies into circulation. Because the effects of local gastrointestinal and limited systemic exposure to ofatumumab are unknown, caution should be exercised when ofatumumab is administered to a nursing woman.

Fertility

There are no data on the effects of ofatumumab on human fertility.

11.3.9 Risks 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 patients 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 a vein causing one lung 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.

11.3.10 Risks related to lymphapheresis (optional research procedure)

The possible risks from lymphapheresis include bleeding from the site where the blood is extracted and returned, lightheadedness, infection, low blood pressure, and muscle cramping.

11.4 Risks in relation to benefit

For adult subjects: The benefits to the adult patient could be a reduction or a disappearance of the chronic lymphocytic leukemia, 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 and significant benefit (45 CFR 46.102).

11.5 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.

Non English speaking subjects

We anticipate the possible enrollment of non-English speaking research participants into this study. The IRB approved full consent document will be translated into the subject's language in accordance

with the Clinical MAS Policy M77-2. 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, 45CFR 46.117(b)(2) and 21CFR50.27(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 five 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 five, 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.

11.6 Conflict of interest

The Principal Investigator assured that each associate investigator listed on the protocol title page received a copy of the NIH's Guide to preventing conflict of interest. Investigators added subsequent to the initial circulation were provided a copy of the document when they were added. Copies of the Conflict of Interest Statement were forwarded to the Clinical Director. No initial or subsequent members of the research team reported a potential conflict of interest.

11.7 Technical transfer agreements

This protocol has no associated patents or CRADAs.

The protocol has the following associated CTAs:

- between NHLBI and Novartis

The protocol has the following associated MTAs:

- between NHLBI and Ronald P.Taylor, PhD, Professor of Biochemistry 434-924-2664 University of Virginia --in place
- between NHLBI and Uniformed Services University for sequencing and analysis of de-identified RNA and DNA samples.

12.0 PHARMACEUTICALS

12.1 Ofatumumab (Arzerra™)

Description: ofatumumab is an IgG1κ human monoclonal antibody that was generated via transgenic mouse and hybridoma technology and is produced in a recombinant murine cell line (NS0) using standard mammalian cell cultivation and purification technologies.

Mechanism of Action: ofatumumab binds specifically to both the small and large extracellular loops of the CD20 molecule. The CD20 molecule is expressed on normal B lymphocytes (pre-B- to mature B-lymphocyte) and on B-cell CLL. The CD20 molecule is not shed from the cell surface and is not internalized following antibody binding. The Fab domain of ofatumumab binds to the CD20 molecule and the Fc domain mediates immune effector functions to result in B-cell lysis *in vitro*. Data suggest that possible mechanisms of cell lysis include complement-dependent cytotoxicity and antibody-dependent, cell-mediated cytotoxicity.

Pharmacokinetics: pharmacokinetic data were obtained from 146 patients with refractory CLL who received a 300-mg initial dose followed by 7 weekly and 4 monthly infusions of 2,000 mg. The C_{max} and $AUC_{(0-\infty)}$ after the 8th infusion in study 1 were approximately 40% and 60% higher than after the 4th infusion in study 2. The mean volume of distribution at steady-state (V_{ss}) values ranged from 1.7 to 5.1 L. Ofatumumab is eliminated through both a target-independent route and a B cell-mediated route. Ofatumumab exhibited dose-dependent clearance in the dose range of 100 to 2,000 mg. Due to the depletion of B cells, the clearance of ofatumumab decreased substantially after subsequent infusions compared to the first infusion. The mean clearance between the 4th and 12th infusions was approximately 0.01 L/hr and exhibited large inter-subject variability with CV% greater than 50%. The mean t1/2 between the 4th and 12th infusions was approximately 14 days (range: 2.3 to 61.5 days).

Indication: ofatumumab (Arzerra™) is indicated for the treatment of patients with chronic lymphocytic leukemia (CLL) refractory to fludarabine and alemtuzumab. It was approved by the FDA October 27, 2009 as a fast track product only for people suffering from chronic lymphocytic leukemia (CLL) who do not respond to current available treatments.

Chemical Name: HuMax-CD20, CAS number 679818-59-8.

Molecular Weight : approximately 149 kDa.

Supply: ofatumumab is a sterile, colorless, preservative-free liquid concentrate (20 mg/mL) for dilution and intravenous administration provided in single-use glass vials with a latex-free rubber stopper and an aluminum overseal. Each vial contains 100 mg ofatumumab in 5 mL of solution. Store diluted solution between 2° to 8°C (36° to 46°F).

Novartis will provide the commercial supply of ofatumumab, presented as 20 mg/ml concentration (5 ml fill = 100 mg per vial). 3154 vials will be provided for the life of the study.

The formulation supplies are 5mLs of solution at a concentration of 20mg/mL (total 100 mg ofatumumab) in a 10mL vial. It uses citric acid monohydrate as the inert buffering agent. The drug substance is produced in batches of 2000 liters.

The formulation supplies for this study will also be 50mLs of solution at a concentration of 20mg/mL (1000mg ofatumumab) in a 60mL vial. It uses acetate as the inert buffering agent. The drug substance is produced in batches of 20,000 liters.

Preparation of solution:

300-mg dose: withdraw and discard 15 mL from a 1,000-mL polyolefin bag of 0.9% Sodium Chloride Injection, USP. Withdraw 5 mL from each of 3 vials of ofatumumab and add to the bag. Mix diluted solution by gentle inversion.

1,000 mg dose: withdraw and discard 50 mL from a 1,000 mL bag of 0.9% Sodium Chloride Injection, USP. Withdraw 5 mL from each of 10 vials of ofatumumab and add to the bag. Mix diluted solution by gentle inversion.

Administration:

- Dilute and administer an intravenous infusion.
- Do not administer as an intravenous push or bolus.
- Do not mix ofatumumab with, or administer as an infusion with, other medicinal products.
- Administer with an infusion pump, an in-line filter supplied with product and polyvinyl chloride (PVC) administration sets.
- Do not shake product.
- Inspect parenteral drug products visually for particulate matter and discoloration prior to administration. Ofatumumab should be a colorless solution and may contain a small amount of visible translucent-to-white, amorphous, ofatumumab particles. The solution should not be used if discolored or cloudy, or if foreign particulate matter is present.
- Flush the intravenous line with 0.9% Sodium Chloride Injection, USP before and after each dose.
- Start infusion within 12 hours of preparation.
- Discard prepared solution after 24 hours.

Storage: store ofatumumab refrigerated between 2° to 8°C (36° to 46°F). Do not freeze. Vials should be protected from light.

Shipping: the NIH Pharmaceutical Development Services will be responsible for receiving, storing, dispensing and accounting for drug product. The shipping address for Novartis supplied investigational agent is

National Institutes of Health
[redacted]

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 NIH Pharmaceutical Development Services 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 Novartis. Disposition of all unused boxes of study drug will be carried out according to instructions provided by the sponsor and/or Novartis at the end of the study after drug accountability is performed by the study monitor.

12.2 CYCLOPHOSPHAMIDE (Cytoxan, Neosar)

Supply: commercially available.
Product description: cyclophosphamide is available as a lyophilized powder for injection in multiple vial sizes.
Preparation: cyclophosphamide powder for injection should be reconstituted with sterile water for injection to yield a concentration of 20 mg/mL as described in the product labeling. Once reconstituted, the prescribed dose will be further diluted in 250 mL of 0.9% sodium chloride injection or 5% dextrose in water for intravenous administration over 60 minutes.
Storage and stability: vials of cyclophosphamide are stored at room temperature. Once

reconstituted as directed, solutions of cyclophosphamide are stable for 24 hours at room temperature, or 6 days when refrigerated at 2-8° C.
Route of administration: the prescribed dose of cyclophosphamide will be diluted in an additional 100 mL of 0.9% sodium chloride injection or 5% dextrose in water for intravenous administration over 30 minutes.

12.3 FLUDARABINE PHOSPHATE (Fludara)

Supply: commercially available.
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, USP 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 over 30 minutes.
Storage and Stability: fludarabine vials should be stored under refrigeration between 2-8 °C (36- 46 °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 over 30 minutes.

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APPENDIX A 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 serumchemokines 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 inocculation 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 microdisection 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