Official Title: **A Phase II, Multicenter, Single Arm Study to Determine the Efficacy and Safety of Low Dose Fludarabine and Cyclophosphamide Combined with Standard Dose Rituximab as Primary Therapy in Elderly Untreated Patients (≥65 Years Old) with Chronic Lymphocytic Leukemia**

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CLINICAL STUDY PROTOCOL

PROTOCOL NUMBER ML25464

A PHASE II, MULTICENTER, SINGLE ARM STUDY TO DETERMINE THE EFFICACY AND SAFETY OF LOW DOSE FLUDARABINE AND CYCLOPHOSPHAMIDE COMBINED WITH STANDARD DOSE RITUXIMAB AS PRIMARY THERAPY IN ELDERLY UNTREATED PATIENTS (≥65 YEARS OLD) WITH CHRONIC LYMPHOCYTIC LEUKEMIA

PROTOCOL APPROVAL

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A PHASE II, MULTICENTER, SINGLE ARM STUDY TO DETERMINE THE EFFICACY AND SAFETY OF LOW DOSE FLUDARABINE AND CYCLOPHOSPHAMIDE COMBINED WITH STANDARD DOSE RITUXIMAB AS PRIMARY THERAPY IN ELDERLY UNTREATED PATIENTS (≥65 YEARS OLD) WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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LIST OF ABBREVIATIONS

AE	Adverse Event
ALT	Alanine Aminotransferase/GPT
ANC	Absolute Neutrophil Count
AST	Aspartate Aminotransferase/SGOT
ALK PHOSP	Alkaline Phosphatase
CBC	Complete Blood Count
CLL	Chronic Lymphatic Leukemia
CR	Complete Remission
CRF	Case Report/Record Form
CRO	Contract Research Organization
CT	Computerized Tomography
CY	Cyclophosphamide
FACT-CTC	Functional Assessment of Cancer Therapy - Common Terminology Criteria for cancer Adverse Events
FACS	Flow Cytometry (Fluorescence-Activated Cell Sorting)
DAT	Direct Coombs Test
FLU	Fludarabine
G-CSF	Granulocyte-Colony Stimulating Factor
Hb	Hemoglobin
HIV	Human Immune deficiency Virus
IEP	Immuno-ellectrophoresis
I.V.	Intra-Venously
Ig	Immunoglobulin
IRB	Institutional Review Board
IRR	Infusion Related Reaction
LDH	Lactic Dehydrogenase
MHO	Medical Health Organization
ORR	Overall Response Rate
PD	Progressive Disease
PET-CT	Positron Emission Tomography- Computerized Tomography
PFS	Progression Free Survival
P.O.	Per Os/ by mouth/orally
PR	Partial Response
QOL	Quality Of Life
SAE	Serious Adverse Event
TLS	Tumor Lysis Syndrome
TNT	Time to Next Treatment
ULN	Upper Limit of Normal

SYNOPSIS

A PHASE II, MULTICENTER, SINGLE ARM STUDY TO DETERMINE THE EFFICACY AND SAFETY OF LOW DOSE FLUDARABINE AND CYCLOPHOSPHAMIDE COMBINED WITH STANDARD DOSE RITUXIMAB AS PRIMARY THERAPY IN ELDERLY UNTREATED PATIENTS (≥65 YEARS OLD) WITH CHRONIC LYMPHOCYTIC LEUKEMIA

PROTOCOL NUMBER:	ML25464
DATE PROTOCOL FINAL:	30 August 2010
STUDY DRUG:	FLUDARABINE, CYCLOPHOSPHAMIDE, RITUXIMAB
INDICATION:	CHRONIC LYMPHOCYTIC LEUKEMIA
STUDY PHASE:	II

Background and rationale Chronic lymphocytic leukemia (CLL) is characteristically diagnosed in the older population with a median age at diagnosis ranging between 65 and 72 years. Because the prevalence of co morbidity increases with age, concomitant disease is often present in this age group. Additional factors which may also adversely affect treatment outcome at standard doses in elderly patients include: altered pharmacokinetics and poorer host tissue tolerance ; changes in the bone marrow hematopoietic reserve and microenvironment leading to increased treatment related myelotoxicity and higher rates of treatment related complications such as infections. As a result, elderly patients are often excluded from clinical trials or even from routine treatment on the basis of their age and / or comorbidity. Furthermore when these patients are treated, the drug doses are often markedly reduced in an attempt to avoid causing toxicity and complications. The challenge in treating elderly CLL patients relates to finding the correct balance between drug efficacy and toxicity and because of this optimal treatment for this group of patients is often hard to achieve.

Study Objectives

The aim of this study is to evaluate the safety and efficacy (response rate) of a combination of low dose Fludarabine (FLU) and Cyclophosphamide (CY) combined with standard dose Rituximab given as primary treatment for elderly patients with chronic lymphocytic leukemia.

Primary end point: -Response rate

Secondary end points:

- Safety - Special issues: incidence of neutropenic fever, hospitalizations (no^{o}- days),
- Thrombocytopenia ≥grade 3, neutropenia ≥grade 3, Infection rate.
- Progression Free Survival (PFS).
- Time to Next Treatment (TNT).
- QOL

STUDY DESIGN:

This is a multicenter, phase II, single arm study designed to evaluate the efficacy and safety of the combination of low dose Fludarabine (FLU) and Cyclophosphamide (CY) combined with standard dose Rituximab in elderly previously untreated CLL patients ($\geq$ 65 years of age). Potential study subjects will sign an informed consent prior to undergoing any study related procedure. Number of patients to be enrolled 40.

Treatment plan: Flu 12.5mg/m^2/d will be given intravenously (I.V.) for days 1-3 every 28 days, for a total of 6 cycles.

CY will be given I.V. at the dose of 150 mg/ m^2/d on days 1-3 every 28 days, for a total of 6 cycles.

Rituximab given before chemotherapy cycle1: 375 mg/m^2 I.V. on day 0, cycles 2-6: 500 mg/m^2 I.V. on day 1 every 28 days.

Dose reduction or delays are recommended in case of grade $\geq$3 (NCI-CTC) adverse events.

This study consists of 3 periods for each study subject: Pre-treatment, Treatment and Follow up.

Pre-treatment period: patients will undergo screening for protocol eligibility within 28 days (4 weeks) of recruitment.

Subjects who meet all the inclusion criteria and have signed an approved informed consent will be enrolled.

Treatment period FLU 12.5mg/m^2/d I.V. will be given on days 1-3 every 28 days, for a total of 6 cycles. CY will be given (I.V.) given before chemotherapy cycle 1: 375 mg/m^2 intravenously on day 0, cycles 2-6: 500 mg/m^2 I.V. on day 1 every 28 days.

Premedication: antihistamines (e.g. 2 mg clemastin or promethazine 25 mg i.v.) and paracetamol (500-1000 mg orally) 30 min before first infusion of Rituximab, and thereafter only if clinically indicated. Prednisone/prednisolone 100 mg I.V. only if clinically indicated.

Dose reduction or delay in case of drug induced hematological toxicity grade≥ 3 level.

During the treatment period, all patients will attend periodic monthly study center visits in order to assess the safety and efficacy of the treatment.

Complete blood count will be performed on day 1 and 14 of each of the first two cycles, and on day 1 in the other 4 cycles, or more frequently at the investigators discretion.

Bone marrow aspiration and biopsy and CT scan will be performed within 3-to 6 months after the completion of the treatment period.

Follow up period: All patients will be assessed for progression and survival every 3 months via clinic visits, for a total of 30 months.

Complete blood count and chemistry will be performed every 3 months, or more frequently at the investigators discretion.

In the event of suspected progression or relapse BMB and or CT scan will be performed at the treating physicians' discretion.

Duration of study

The duration of the treatment period is approximately 6 months. This time period is required to complete the therapy, to determine the safety profile of the drug combination and the response rate to therapy. The duration of the follow up period will be 30 months. The occurrence of PD will determine the duration of progression-free survival of each patient.

Recruitment period will be 24 months.

Inclusion criteria:

- Diagnosis of B-CLL is established by the presence of: An absolute lymphocyte count> $5X10^9$/L, typical morphology in the peripheral blood smear as seen by light microscopy, CLL cells must show CD5/CD19/CD23 positivity with light chain restriction as recorded by flow cytometry. Low surface immunoglobulin/CD 79b expression is also expected to be evident on these cells while FMC7 should be negative-are both additional typical features on flow cytometry (optional).
- Previously untreated B-CLL patients $\geq$ 65 years old.
- Patients requiring treatment (active Binet stage A and B, all stage C disease).
- Written informed consent

Exclusion criteria:

- Calculated or measured creatinine clearance < 30 ml/min
- Hepatic enzymes or bilirubin > 2X ULN, unless thought to be due to CLL
- Suspected or documented CNS involvement by CLL
- Known HIV positivity
- Active or uncontrolled infections, including opportunistic infection, active hepatitis B / C
- Various concomitant diseases requiring chronic steroid administration
- Active Coombs positive hemolytic anemia
- Prior treatment for CLL
- CLL with transformation (Richter syndrome).
- Presence of active second neoplasia (excluding non-melanoma skin cancer, or in situ cervix or breast carcinoma) < 2 years prior to the study.
- Use of other investigational agents or participation in any other clinical trials < 30 days prior to the study.
- Low compliance.
- Performance status: ECOG performance status $\geq$4.
- Patients who mentally or physically are unable to comply with all aspects of the study.

Assessments:

Efficacy

The following evaluations will be done to record the efficacy of the treatment regimen:

- Assessment of disease response (revised International Response Criteria: updated NCI-IWG 2008).[46].
- Date of documentation of disease progression.
- Bone marrow biopsy examination
- Peripheral blood immunophenotyping by FACS.
- Changes in ANC, Hb levels and platelets counts from baseline blood counts and those done during the study period.
- Chest, abdominal and pelvic computerized tomography
- Physical examination for lymphadenopathy and hepato-splenomegaly

Safety

The following evaluations will be recorded to assess the safety of the regimen:

- Complete physical examination including vital signs.
- Clinical laboratory evaluations (hematology, biochemistry)
- Concomitant medication and procedures
- Adverse events
- Health outcomes assessment (any extra medical appointment)

Efficacy analysis:

Primary efficacy will be determined by objective overall response rate. Exact tests for assessing these will be used to compare objective overall response rates. Kaplan Meier procedure will be used to characterize the duration of response for PFS and TNT. Summary statistics (standard deviation, mean, median, minimum and maximum) will be provided for the relevant variables

Safety Analysis:

Data from all subjects who receive any study drug will be included in the safety analyses. The severity of the toxicities will be graded according to the NCI CTC v3 or v2- for cytopenia, whenever possible. In the by-subject analysis, a subject experiencing the same event more than once will be counted only once.

Adverse events will be defined and summarized by the worst NCI CTC grade. Adverse events leading to death or to discontinuation of treatment, events classified as NCI CTC Grade 3 or Grade 4, study-drug-related events, and serious adverse events will be summarized separately. Laboratory data will be graded according to NCI CTC severity grade.

STUDY DURATION: five years **TOTAL SAMPLE SIZE**: 40

2. Background Information

Chronic lymphocytic leukemia (CLL) is characteristically diagnosed in older people with a median age at diagnosis ranging between 65 and 72 years. Because the prevalence of co morbidity increases with age, concomitant disease is often evident in this age group. Goede et al [29] reported that increasing numbers of more severe concomitant comorbid illnesses are associated with a shortened survival in patients with CLL. Most recently Thurmes and colleagues [1] have shown that these comorbid conditions are in fact less important than both the age of the patient and the disease stage, in predicting prognosis in untreated patients.
Additional factors which may also adversely affect treatment outcome in elderly patients include: altered pharmacokinetics and poorer host tissue tolerance ; changes in bone marrow hematopoietic reserve and microenvironment which may lead to increased treatment related myelotoxicity and higher rates complications such as infections [21,26]. As a result, elderly patients are often excluded from clinical trials or even from routine treatment at standard doses of drugs, on the basis of their age and / or comorbidity and when treated the drug doses are often markedly reduced when given.
The basic challenge in treating elderly CLL patients appears to be related to finding the correct balance between treatment efficacy and drug toxicity and because of this, optimal treatment for older patients is hard to achieve and remains an open question and topic of debate.

Purine analogues drugs which inhibit DNA synthesis are very effective in CLL and fludarabine, cladribine and pentostatin have all been used effectively in the treatment of CLL [14]. Fludarabine is the most extensively studied purine analogue in CLL and is available in tablet form as well as for intravenous use. When used in combination with cyclophosphamide, fludarabine has been shown to be more effective than when used alone. [13,14,27,28]. In this respect the U.K. MRC LRF CLL4 trial reported in 2007 [11] compared therapy with chlorambucil alone vs fludarabine vs fludarabine (F) alone +

cyclophosphamide (FC) in 777 newly diagnosed CLL patients, and showed that FC achieved superior response rates and better progression-free survival for all CLL patients . Because of this, FC was proposed as the gold standard treatment of all CLL patients irrespective of age [11]. Similar results were published by the German CLL Study Group and the North American Intergroup [14].

However although Fludarabine - based regimes are most efficacious, they also appear to be more toxic, inducing more prolonged myelosuppression with more infections.

Pollizzotto et al [15] investigated the influence of increasing age on the ability to deliver fludarabine based regimens as well as the toxicity obtained thereafter, in patients with indolent lymphoproliferative disorders. They reported a gradual increase in toxicity with advancing age especially in patients age ≥ 70 years but still concluded that regimens were generally well tolerated and could be delivered safely to older patients who had a good performance status (PS). They noted a modestly increased degree of myelosuppression but no increase in the incidence of severe infectious complications or treatment-related mortality.

Table 1

PR	CR	OR	Patients age	Oral Cyclophosphamide dose	Oral Fludarabine dose	Disease status	Journal
NHL-59% CLL-100%	NHL-35% CLL-0%	NHL-94% CLL-100%	61-85 Median 73.5	150mg/m^2 X 4 days Every 28 days , 4 courses	25mg/m^2 (total 40 mg) X 4 days	Mostly relapsed refractory CLPD-28 Patients CLL-9 p	Hematology 2004 (16)
All-44% SLL-4/8	All-40% SLL-3/8	All-84% SLL-7/8	66-85 Med 74	150mg/m^2 X 4 days	25mg/m^2 (total 40 mg) X 4 days	Untreated 25 patients SLL-8	BJH 2007 (17)

				Every 28 days ; 4 courses			
40%	54%	94%	30% above age 70	150mg/m^2 X 5 days Every 28 dX6	24mg/m^2 X5 days	Untreated (196)	CLL4 (11)

2.1 Rituximab

Since the introduction of Rituximab (Rituxan™, IDEC) (FDA Approval Letter Nov. 26, 1997) in the USA and shortly thereafter in Europe in 1998 (MabThera™ (Hoffmann-LaRoche AG) for use in the treatment of CLL and indolent non-Hodgkins' lymphoma (NHL), it has been used extensively in the treatment of indolent and aggressive lymphoma, both as a single agent or more often in combination with chemotherapy. Today Rituximab is already being used to treat a variety of autoimmune disorders and by now has also been accepted as the model monoclonal antibody for the treatment of cancer. Rituximab is a chimeric mouse variable heavy and light chains (IDEC 2B8) with a human IgG_1 constant kappa antibody (C2B8) that reacts specifically with the CD20 antigen found on the surface of malignant and normal B cells and established B-cell lines [31,33]. The antigen is expressed at lower levels on chronic lymphocytic leukemia (CLL) and plasma cells [31]. CD20 is not shed from the surface of CD20+ cells after antibody binding and it is not internalized or downregulated [34]. Compared with its murine counterpart, Rituximab has a longer half-life in humans, interacts with human effector cells [34, 35], and is less immunogenic [36].

Reduced FC doses

There are only a few published reports (including relatively small numbers of CLL/SLL patients which describe the results of an FC regimen in elderly CLL patients-(≥ 70 years). These are listed in Table1.

2.2 Use of Rituximab and Combination of Rituximab and Fludarabine in CLL

Although initial responses to single agent treatment with Rituximab were low and partial responses and not complete remissions, interest continued in the use of this agent in CLL [37,39,40]. The original studies employed 375 mg/m^2 of Rituximab once a week for four weeks. Subsequent studies suggested using this dose three times a week for four weeks for a total of 12 doses instead of 4 doses which resulted in better overall responses [41]. Dose limiting toxicities of Rituximab have not been demonstrated perhaps due to the cost of the drug. In this respect it should be noted that in the initial studies using Rituximab to treat follicular NHL, responses were much higher than in CLL. This was attributed to the lower level of CD20 expression on CLL lymphocytes and the presence of circulating CD20 antigen. These differences were partially overcome when either the dose of Rituximab was increased or the frequency of the standard dose of 375 mg/m2 was increased [41, 44].

After establishing the safety and efficacy of standard dose Rituximab 375 mg/m2 three times a week for four weeks for a total of twelve doses, Byrd et al went on, to compare sequential and concurrent therapy with RF [43].

The concurrent group was therefore treated with 11 doses of Rituximab while the sequential cohort only received four doses. This was a randomized phase II trial of concurrent versus sequential Fludarabine and Rituximab with 51 and 53 patients per arm, respectively. The overall response rate in the concurrent arm was 90%, including CR in 47%while in the sequential arm the overall response rate was 77%, with CR in 28%. The disease-free and overall median survival has yet to be reached yet, but will be in excess of 23 months [44]. Three major side effects were observed: infusion related toxicity, myelosuppression, and infections. Infusion related toxicity were greatest in concurrent treated patients (20%) and almost not existent in the sequentially treated patients. In a phase II study, the German CLL Study Group (GCLLSG) [44] reported the treatment of 20 untreated and 11 relapsed CLL patients. Treatment consisted of Fludarabine administered at standard doses

(25mg/m2/d; days 1-5, 29-33, 57-61, 85-89) and Rituximab (375mg/m2/d) given on days 57, 85. The overall response rate (CR and PR) was 87% with a CR/CRu of 33%. The median duration of response was 75 weeks. Severe anemia (grade III/IV) was observed in 10% of patients, neutropenia in 42%, and thrombocytopenia in 9%, respectively. The most frequent non-hematologic side effects were infections (52%) and infusion-related symptoms. The majority of infections were respiratory and herpes virus infections.

A retrospective comparative analysis of the CALGB 9712 [47] (Rituximab plus Fludarabine) [48] and the CALGB 9011 (Fludarabine only) studies demonstrated that the addition of Rituximab to Fludarabine resulted in a significantly better 2 years progression free survival and overall survival compared to patients treated with Fludarabine alone (67% *Vs* 45% and 93% *Vs* 81%, respectively) and in both treatment approaches the infectious toxicity was similar [45].

The MD Anderson Cancer Center group reported a single arm study that utilized the combination of Rituximab, Fludarabine and Cyclophosphamide (FCR) in untreated and in relapsed and refractory CLL patients (224 and 177 patients, respectively) [24, 25]. In the untreated patients the overall response rate was 95% with a CR rate of 70%, a nodular PR rate of 10% and a PR rate of 15%. Although, during the follow-up period the time to treatment was not reached, a calculated analysis showed that 69% of patients were projected to relapse at 4 years. The major toxicities were myelosupression, predominantly of grade 3 and 4, neutropenia (in 52% of all courses) and infections.

In the relapsed and refractory patients the overall response was 73% with 25% CR, 16% nodular PR and 32% PR and a third of the complete responders achieved molecular remission. The median time to progression in responding patients was 28 months with an overall median survival time of 42 months. About 46% of the patients completed all six intended courses and myelosupression was the major cause for discontinuing treatment (66% and 18% grade 3-4 neutropenia and thrombocytopenia, respectively) with 16 % of the patients having major infection.

Recently, two pivotal phase III studies (CLL8 and REACH) [49,50] evaluated 6 cycles of rituximab (375mg/m^2 on cycle-1 and 500mg/m^2 on cycle 2-6) plus fludarabine (25mg/m^2 d1-3) and cyclophosphamide (250mg/m^2 d1-3) chemotherapy compared with 6 cycles of fludarabine and cyclophosphamide alone. The primary endpoint of both studies was progression-free survival. Secondary endpoints for both studies included overall survival, event-free survival, duration of response, response rate and complete response. CLL8 involved 817 patients with previously untreated (first-line) CLL and good physical fitness. With a median observation time of 37.7 months, statistically significant differences were observed in OS between the two treatment arms. The OS rate was 84.1% in the FCR arm versus 79 % in the FC arm (p=0.01). In both arms, the median OS has still not been reached. Patients who received rituximab together with chemotherapy had a median progression free survival (PFS) of 51.8 months compared to 32.3 months for those who received chemotherapy alone. (hazard ratio 0.56; $p < 0.001$). The overall response rate in patients receiving rituximab plus chemotherapy as first-line therapy was 95.1% vs. 88.4% in those receiving chemotherapy alone and with more complete remissions in the FCR arm (44.1 vs 21.8%; $p<0.001$). Severe (grade 3 or greater) adverse events that occurred more often in the rituximab arm included haematologic toxicity (56% vs. 39%), neutropenia (34% vs. 21%) and leukocytopenia (24% vs. 12%).

The REACH study[49] involved 552 patients with previously treated CLL (second line therapy). Patients who received rituximab plus chemotherapy had a median PFS of 30.6 months compared to 20.6 months for those who received chemotherapy alone (hazard ratio 0.65; p=0.0001). The overall response rate in the Rituximab-containing arm was 70% vs. 58% in those receiving chemotherapy alone without Rituximab. Grade 3 or greater events such as neutropenia (42% vs. 40%), febrile neutropenia (12% vs. 12%) and neoplasms (7% vs. 3%) occurred more often in the rituximab plus chemotherapy arm. Based on the CLL8 and REACH results the FDA approved the use of rituximab as first- and second-line treatment of CLL. In Europe, Rituximab is also approved for treatment of first and relapsing CLL patients.

In the light of the above literature, the combined use of Rituximab and Fludarabine and cyclophosphamide (FCR) is currently regarded as the gold standard and backbone treatment for physically fit patients with CLL.

2.3 Fludarabine Cyclophosphamide Rituximab (FCR) regimens for elderly patients with CLL

Due to major concerns of myleotoxicity and infection complications, this chemo-immunotherapy combination regimen cannot be applied safely to all elderly CLL patients. To address this topic efforts are being made to establish novel FCR-based protocol that will be useful for the average elderly CLL patient. In this regard, Foon and colleagues [50] reported a phase II single arm study of reduced dosages of Fludarabine and Cyclophosphamide (FCR-lite). Fifty untreated CLL patients were treated with 6 cycles of Fludarabine (20mg/m^2) and Cyclophosphamide (150mg/mg^2) combined with high–dose Rituximab (500mg/m^2) given every other week during induction and later as maintenance every 3 months for two years. The OR and CR rates were 100% and 79%, respectively. Median duration of complete response was 22.3 months (range, 5.2 to 42.5 months) and none of the complete responders have relapsed. Grade 3/4 neutropenia was noted in 13% of the cycles of therapy. Another approach to reduce toxicity was to use sequential therapy with fludarabine (25 mg/m^2 on days 1 through 5 for 6 cycles) followed by consolidation with Cyclophosphamide (3,000 mg/m^2 administered every 3 weeks for three cycles) and then by Rituximab 375 mg/m^2 for four cycles (F→C→R). [51] In 36 previously untreated CLL patients an overall response of 89% was achieved including 61% CRs. 33% achieved a molecular CR (PCR negative) and achieving molecular CRs had an excellent prognosis with a plateau in the response duration curve, and 90% remain in clinical CR at 5 years. For the entire group, 5-year survival rate is 71% compared with a rate of 48% with the prior F→C regimen (not significant $P = .10$).

3. Trial Objectives and Purpose

The aim of this study is to evaluate the safety and efficacy of a combination of low dose Fludarabine (FLU) and Cyclophosphamide (CY) combined with standard dose Rituximab given as primary treatment for elderly patients with chronic lymphocytic leukemia.

The purpose of the study is to determine whether a lower dose FC combined with standard dose Rituximab regimen in elderly CLL patients is as effective as the standard FCR dose, given in most studies until now and at the same time to assess if it is indeed less toxic.

3.1 Primary Objective

To evaluate the efficacy (Response Rate) of the combination of lower dose Fludarabine 12.5 mg m^2/d given intravenously for three days and Cyclophosphamide 150 mg/d given intravenously for three days every 28 days, combined with standard dose Rituximab (375mg/ m^2 in cycle 1 and 500 mg/m^2 in cycles 2-6) for a total of 6 cycles.

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3.2 Secondary Objectives

1- Safety of the combination drugs given in the study population, especially in relation to: neutropenic fever, Infection rate, number of hospitalization days, thrombocytopenia ≥grade 3, neutropenia ≥ grade 3.

2- Progression Free Survival (PFS).

3- Time to Next Treatment (TNT).

4- Quality Of Life (FACIT)

4. Trial Design

4.1 Primary and secondary end points

Primary End Point

Overall response rate (ORR) (CR and PR) as defined in the protocol (see appendix I)

4.1.1 Secondary End Points

1- Safety of the study drugs especially in relation to: neutropenic fever, Infection rate and number of hospitalization days,

thrombocytopenia ≥grade 3, neutropenia ≥grade 3.

2- Progression free survival (PFS) defined as the time elapsed from first day of study drug administration to disease progression as defined in the protocol (see appendix I)

3- Time to Next Treatment (TNT) as defined in the protocol (see appendix I)

4- QOL(see appendix VI)

4.2 Overall study design

This is a national, multicenter, phase II, single arm (not controlled), open label clinical trial to evaluate the efficacy and safety of the combination of low dose Flu + Cy combined with standard dose Rituximab Flu dosage will be given at 12.5mg/m^2/d given I.V. together with CY150 mg/m^2/d given I.V. for three days every 28 days. Rituximab will be given before chemotherapy
cycle 1: 375 mg/m^2 I.V. on day 0 and in cycles 2-6: 500 mg/m^2 I.V. on day 1 in previously untreated CLL patients who are more than 65 years of age. Patients will be treated every 28 days for up to 6 cycles. Potential study subjects will sign an informed consent form prior to undergoing any study related procedure. In general, study medications should be continued until disease progression or drug intolerance but no more than 6 cycles. Treatment choices after completion of the study are at the investigators' discretion.
Up to 40 patients are planned to be enrolled during a two year period.
This study consists of 3 periods for each study subject: A pre-treatment period, the treatment period and a follow up period.

Pre-treatment period:

Patients will undergo screening for protocol eligibility within 28 days (4 weeks) of recruitment.

Subjects who meet all the inclusion criteria and who have signed an approved informed consent will be enrolled.

Written informed consent must be obtained before any study - specific medical procedures are performed which include laboratory screening assessments and physical examination as well as documentation of vital signs. If the hematology and/or biochemistry evaluations have been performed within 14 days of the first dose of study drug as part of the screening evaluation, they need not be repeated, and the data may be used as the Day 1 values. Bone marrow examination including cytogenetics (FISH whenever possible) must have been performed within 12 months of starting the first dose of the study drugs.

CT scan when done must be performed within 6 weeks of starting the first dose of study drugs.

Treatment period

Includes 6 months of combination treatment with low dose Fludarabine and Cyclophosphamide combined with standard dose of Rituximab. Flu dosage will be given at 12.5mg/m^2/d given I.V. together with CY150 mg/m^2/d I.V. for three days every 28 days. Rituximab given before chemotherapy cycle 1: 375 mg/m^2 I.V. on day 0 and in cycles 2-6: 500 mg/m^2 intravenously on day 1 every 28 days.

Premedication: antihistamines(e.g. 2mg clemastin or promethazine 25mg I.V.) and paracetamol (500-1,000 mg orally) 30 minutes before first infusion of Rituximab, and thereafter only if clinically indicated. Prednison/prednisolone 100 mg I.V. only if clinically indicated.

In the event of grade $\geq$ 3 hematological toxicity which in the investigators' opinion, is study drugs related, the administration of the next cycle should be delayed but not for more than 3 weeks and drug dose reduction of 25% of

both CY and F is recommended. Two dose reductions are permitted (FLU and CY Dose modification page 29). In cases where the delay period has been terminated but grade 3-4 toxicity persists, the patient should be removed from the protocol with no further study drug administration.

During the treatment period, all patients will attend periodic monthly study-center visits in order to assess safety and efficacy of the treatment.

Complete blood counts will be performed on day 1 and 14 of the first two cycles. In the next 4 cycles- on day 1 of each cycle, or more frequently at the investigators' discretion.

Bone marrow biopsy and aspiration examination will be performed before the start of treatment, for response documentation (3 to 6 months after the completion of treatment), for evaluation and documentation of cytopenias (disease or treatment related) and for any other clinical indication at the discretion of the investigators.

Supportive care including blood transfusions or administration of growth factors G-CSF or erythropoietin is permitted at the investigators' discretion throughout all study periods.

Follow up period

During the follow up period, of 30 months after the completion of the study drugs administration, all patients will be assessed every 3 months for evidence of progression and documentation of survival at clinic visits.

Complete blood count will be performed every 3 months, or more frequently at the investigators' discretion.

Bone marrow examination including cytogenetics, peripheral blood immunophenotyping (FACS), abdominal, pelvic and chest CT will be performed 3 to 6 months after the completion of the treatment regimen, at clinical progression and at any other time according to the investigators' discretion.

In the event of no response (no CR nor PR) in > 5 of the initial 10 subjects enrolled into the study, Fludarabine dosage will be increased for all the next

subjects enrolled into the study and will be given at the dose of 15mg/m^2/d given I.V.

In the event of no response after the above increment in > 5 of the next 10 subjects who received the increased dosages, the study will be terminated and declared inadequate and inefficient. If response is evident in ≥ 5 of the 10 patients, the same dosages will be continued throughout the study until its completion.

All further treatment decisions after completion of the study are at the discretion of the investigators.

4.2.1 Visit schedule and assessments

Visit schedule

Patient must be followed at the study center according to the visit schedule and assessments outlined in Figure 1.

All routine assessments must be performed within a time window of ± 7 days of the day indicated on the visit schedule, except visits 2-5 in which time window will be of ± 3 days .

Figure 1: Visit schedule and assessments

Week	-4 to 0	1	3	5	7	9-25 Every 4 weeks	29	37	49	61-77 every 12 weeks	89	101-161 every 12 weeks	EOS
Visit No.	**1**	**2**	**3**	**4**	**5**	**6-10**	**11**	**12**	**13**	**10-13**	**14**	**15-19**	**20**
Study period	preTx	**Treatment period**						**Follow up period**					
Inc. /Excl. criteria	X												
Informed consent	X												
Relevant medical history/conditions	X												
Disease history	X												
BMB*	X								X				
Physical exam	X	X	X	X	X	X	X	X	X	X	X	X	X
CBC	X	X	X	X	X	X	X	X	X	X	X	X	X
Blood chemistry	X	X		X		X	X	X	X	X	X	X	X
Beta-2 micro glob	X												
IEP, Ig level,	X								X				X
DAT***	X								X				X
FACS****	X								X				X
Hepatitis B *****	X												
Imaging - (CT) **	X												X
QOL	X						X				X		X
Treatment dosage	X	Ongoing data capture											
Adverse Events	X	Ongoing data capture and up to 30 days after completion or discontinuation of the study											
Concomitant medications	X	Ongoing data capture											
Comments	X	Ongoing data capture											
Study completion	X	Complete at any time if study drug is discontinued											

* Bone marrow examination will be performed at any clinically suspicion of progression/ leukemia transformation, cytopenia evaluation, or for any CR confirmation at investigator discretion.

** Abdominal, pelvic and chest CT will be performed at any clinical suspicion of progression.

*** DAT will be performed at any clinical suspicion of hemolysis.

**** Will be performed at any clinical suspicion of progression/ leukemia transformation, or for CR confirmation at investigators' discretion.

*****At screening and at investigator discretion for hepatitis treatment evaluation.

4.3 Trial treatment

4.3.1 Investigational therapy

Fludarabine is supplied to the study investigators by the Medical Health Organization (MHO) as routine first line treatment in all CLL patients. Fludarabine is supplied as 10 mg tablets packaged in blister packs, or as 50 mg/vial, as lyophilized powder that has to be dissolved in 2 Ml of water for injection, than diluted in 100 ml of saline (0/9%) given in 30 minutes.

Cyclophosphamide is supplied to the study investigators by the MHO as a routine first line treatment in CLL patients. Cyclophosphamide (Endoxan) is supplied as 50 mg tablets packed in blister packs or as 500 mg/vial (24 mg/ml) diluted in 100 ml of saline (0/9%) given in 30 minutes.
Rituximab is supplied to the study investigators by the MHO as a routine first line treatment in CLL patients. Rituximab (Mabthera) is supplied as 100mg of rituximab in 10ml (10mg/ml) pack of 2 vials or 500mg of rituximab in 50ml (10mg/ml) pack of 1 vial. The antibody should be diluted into a final volume of normal saline or 5% D-glucose in water to allow for a maximal concentration of 4 mg/ml. Rituximab should be administrated before FC chemotherapy.

First Infusion: Recommended initial rate is 50mg/hr; after the first 30 minutes increase by 50mg/hr every 30 minutes to maximum of 400 mg/hr. *Subsequent Infusions:* Initial rate 100mg/hr; increase by 100mg/hr every 30 minutes to a maximum of 400mg/hr

Use extreme caution and closely monitor first infusion when treating patients with ≥ $25x10^9$/l circulating malignant cells or high tumor burden (higher risk of severe cytokine release syndrome (CRS). It is recommended to administer prednisone/prednisolone 100 mg I.V. shortly before infusion with Rituximab to decrease the rate and severity of acute IRR/CRS for these patients. consider reduced rate for first infusion or a split dosing over two days during the first cycle. Severe CRS: may be associated with some features of tumor lysis syndrome e.g. hyperuricaemia, hyperkalaemia, hypocalcaemia, hypophosphataemia, acute renal failure, elevated LDH and may be

associated with acute respiratory failure and death. If severe CRS manifests stop infusion immediately and start aggressive symptomatic treatment.
If the first dose of rituximab is well tolerated, the starting flow rate for administration of the second and subsequent infusions will be 100 mg/hour and then increased gradually by 100 mg/hour intervals not to exceed 400 mg/hour.
If any grade 1 or 2 infusion related reactions occur during an infusion, the infusion should be slowed or interrupted (at the discretion of the Investigator) and supportive treatment instituted. The infusion rate can be increased or restarted on resolution of the symptoms.
If grade 3 or 4 infusion related reactions occur during an infusion, the infusion has to be discontinued immediately and not restarted until resolution of the symptoms. Treatment can be reinitiated (at half the original infusion rate) at the discretion of the Investigator, but if the same adverse event appears again with the same severity, treatment must be permanently discontinued.
Should bronchospasm or dyspnea occur in the patient during the infusion, the infusion should be stopped immediately. Medication such as anti-histamines or steroids should be given for symptomatic relief. The infusion should not be re-started until symptoms have resolved completely and should be given at half the original infusion rate.
In the event of infusion related reactions to rituximab:
• Stop rituximab infusion, treat symptoms aggressively if necessary
• NaCl 0.9 % i.v., if necessary
• Prednisone/prednisolone 100 mg i.v., if necessary
• Pethidine i.v. if necessary
• Bronchodilators/antihistamines if necessary
• If side effects occur stop Rituximab-infusion, re-try the next day

Table 2: Reduction of Infusion Rate

Drop in systolic blood	Mucosal congestion, edema	Rigors	Fever	Infusion rate
> 30 mm Hg	Mild/moderate	Mild/moderate	> 38.5°C	Decrease to ½ if any of these events are seen

Following the infusion the I.V. line should be kept open for medications as needed. If there are no complications, the line may be discontinued after one hour of observation. If complications occur during the rituximab infusion, the patient should be observed for 2 hours after the completion of the infusion.

Infusion reactions to rituximab tend to be more common and more severe with the first infusion. In patients who were unable to tolerate the first infusion, cautious reintroduction of rituximab may be tried at the second cycle, at the investigator's discretion. The recommended premedication should be given and then the patient carefully re-exposed to a small amount of rituximab (slow infusion of 10 mg I.V.) If a severe reaction occurs, the rituximab must be permanently discontinued. In such case patient will be treated with FC in the next cycles and data will be collected throughout the study period)

If no severe reaction occurs after 30 minutes, the full dose of rituximab infusion should be given.

Labels will comply with the legal requirements in Israel and be printed in Hebrew and English. All study drugs containing infusion bags will be labeled. Rituximab containing infusion bags will be labeled by the sponsor and collected for drug accountability. Storage conditions for study drug will be described on the medication label. Patients will receive the study drug combination for an exposure period of up to 6 months provided that in the opinion of the investigator the patient is benefiting from treatment with this study drug combination, and in the absence of any safety concerns. Treatment will be withheld only in the case of limiting toxicities (see below).

As administration of cyclophosphamide may cause hemorrhagic cystitis and even urinary bladder malignancy, patients will be instructed to drink at least 10 glasses of fluids on the days they receive cyclophosphamide and urinate at minimal ceasing of bladder distention.

DRUG ACCOUNTABILITY

Accountability and patient compliance will be assessed by maintaining adequate "drug dispensing log" on Rituximab.

The "drug dispensing" log for Rituximab must be kept current and should contain the following information:

- The identification of the patient (patient number) to whom Rituximab was administered.
- The date(s) and quantity of Rituximab, used for the patients' treatment.
- The date(s) and quantity of Rituximab, of empty, partial or unused medication.

All accountability logs must be available for inspection by the monitor. Drug supplies (partly used and empty Rituximab infusion bags) must be available for inspection, at every monitoring visit. Used supplies will be destructed according to local site guidelines.

4.3.2 Dose modification for hematological toxicity

Dose modifications will not be made during the first cycle of therapy. During the flowing cycles modifications of the treatment schedule will only be made as follows:

• If at day 1 of any cycle, there is a grade ≥ 3 cytopenia (See hematological toxicity section 8), NOT RELATED TO BONE MARROW INFILTRATION DUE TO CLL, treatment should be delayed for up to three weeks and then given with a 25% dose reduction of fludarabine and cyclophosphamide in the following cycles. There is no dose reduction for Rituximab in this study, but drug administration will be delayed for the same time period as for fludarabine and cyclophosphamide.

• If after three weeks, the grade ≥ 3 cytopenia, **NOT RELATED TO** CLL **BONE MARROW INFILTRATION**, still prevails, the patient should be removed from the protocol with no further study drug administration.

• If there is further grade ≥ 3 cytopenia in subsequent cycles, despite the first 25% dose reduction treatment should again be delayed for up to three weeks and the dose of fludarabine and cyclophosphamide further reduced to 50% of the full initial dose.

• If there is further grade ≥ 3 cytopenia in subsequent cycles despite the 50% drugs dose reduction the patient should be removed from the protocol with no further treatment.

• If there is any grade ≥ 3 neutropenia with infection during any cycle, G-CSF should be administered and G-CSF should than be given in all subsequent cycles prophylactically.

4.3.3 Dose modification for impaired renal function

Fludarabine is partly (40-60%) excreted by the kidneys. If the calculated (see appendix III) or measured creatinine clearance is reduced to 30-60 ml/min, the fludarabine dose should be reduced to 50%. Patients with a creatinine clearance below 30 ml/min are excluded from the study. Patients who develop renal dysfunction (serum creatinine ≥ 1.5 mg/dL or creatinine clearance < 30 ml/min) during treatment should go off protocol treatment.

4.3.4 Dose modification for other non-hematological toxicity (non renal).

• If other grade ≥ 3 non-hematological, non-renal toxicity occurs during any cycle, study drugs treatment should be delayed until recovery to grade ≤ 2, for up to three weeks. All subsequent cycles should then be given with a 25% dose reduction of fludarabine and cyclophosphamide.
• If after three weeks, the grade ≥ 3 non-hematological, non-renal toxicity still persists, the patient should be removed from the protocol .
• If there is further grade ≥ 3 non-hematological, non-renal toxicity in subsequent cycles despite the first 25% dose reduction treatment should again be delayed for up to three weeks and the dose of fludarabine and cyclophosphamide further reduced to 50% of the initial full dose.
• If there is further grade ≥ 3 non-hematological, non-renal toxicity in subsequent cycles despite 50% dose reduction the patient should be removed from the protocol treatment and the study.

4.3.5 Treatment assignment

Informed consent must be obtained before any testing for the purpose of determining a patient's eligibility is performed. Each patient will be assigned a unique patient number. Once assigned, numbers for any non-evaluable or discontinued patient will not be reutilized.

4.3.6 Blinding

This is an open label trial.

4.4 Study drug interruption or discontinuation

4.4.1 Discontinuation of study drugs

Events that will be considered as reasons for study drugs discontinuation are described in section 5.3

In addition, patient follow-up information must still be recorded (also in the event of study drug discontinuation) during and at the end of the study period.

4.4.2 Study Discontinuation

Events that will be considered as reasons for study discontinuation are described in section 5.3

4.5 Accountability procedures for the investigational products

Study drug will be prepared and dispensed by the pharmacist at the investigator's institution.

5. Selection and Withdrawal of Subjects

5.1 Inclusion Criteria

1. Diagnosis of B-CLL is established by the presence of: an absolute lymphocyte count > $5X10^9$ L, and typical CLL cell morphology in the peripheral blood smear as seen by light microscopy. CLL cells must show CD5/CD19/CD23 positivity with light chain restriction as recorded by flow cytometry. The levels of surface immunoglobulin and CD 79b are characteristically low and FMC7 is expected to be negative (optional).
2. Previously untreated B-CLL patients ≥ 65 years old.
3. Patients requiring treatment (active Binet classification stage A and B, all stage C disease).(appendixes IV and V)
4. Written informed consent

5.2 Exclusion Criteria

1. Calculated or measured creatinine clearance < 30 ml/min
2. Hepatic enzymes or bilirubin ≥ 2X ULN, unless thought to be due to CLL.
3. Suspected or documented CNS involvement by CLL
4. Known HIV positivity
5. Active or uncontrolled infections, including opportunistic infection and active hepatitis B / C
6. Various concomitant diseases requiring chronic steroid administration
7. Active Coomb's positive hemolytic anemia.
8. Prior treatment for CLL
9. CLL in transformation (Richter syndrome).
10. Presence of active second neoplasia (excluding non-melanoma skin cancer, or in situ cervix carcinoma or breast carcinoma) < 2 years prior to the study.

11. Use of other investigational agents or participation in any other clinical trials < 30 days prior to the study.
12. Low compliance.
13. Performance status: ECOG performance status ≥4. (see appendix II)
14. Patients who are mentally or physically unable to comply with all aspects of the study.

5.3 Interruption or Discontinuation of Treatment

5.3.1 Discontinuation of study drugs

The following events will be considered as reasons for discontinuation.

- Interruption of study drug/s for longer than 3 consecutive weeks.
- Adverse events, including study drugs induced active autoimmune haemolytic anemia.
- Patient with large cell transformation (Richter syndrome).
- Disease progression as defined at any time during study drug therapy.
- Treatment with other chemotherapeutic or investigational anti-neoplastic drugs
- Intolerable adverse effects that are judged by the investigator to be either physically or psychologically detrimental to the patient. Unresolved or recurrent (more than twice) grade 3 or 4 toxicity.
- The reason for discontinuation should be recorded in the CRF and in the subject's medical records. The sponsor is to be notified of all discontinuations related to study medication.
- In addition, patient follow-up information will be recorded (also in the event of study drug discontinuation) during and at the end of the study period.
- In the case of a severe reaction to Rituximab, Rituximab will be permanently discontinued. Patient will be treated with FC in the next cycles and data will be recorded throughout the study period.

5.3.2 Interruption of study drugs

In the event of grade 3 or 4 toxicity, or increase of creatinine>1.5 or GFR of less than 30ml/min study drug will be interrupted for no more than 3 weeks (see sections 4.3.2-4.3.4)

5.4 Study Discontinuation

The following events are considered as study discontinuation:

- Death
- Participation in another investigational drug trial
- Loss to follow-up
- Patient withdrawal of consent
- Major violation of the study protocol- such as eligibility criteria.
  In these cases, no patient follow-up will be recorded.
- Administrative problems
- In the event of no response after dosage increment in > 5 of the next 10 subjects who received the increased dosage.(see chapter 4.2).

The section for "Study Completion" in the CRF must be completed for all patients. The reason for early discontinuation should be given, even if the patient refuses to return for a final visit. Patients who discontinue prematurely due to significant study drug related adverse events (AEs) should continue to be followed until resolution of the AE or up to 30 days after discontinuation. The relevant sections of the CRF should be completed as appropriate.

Patients who discontinue the study drugs for any reason should be scheduled for a visit within 30 days from drug discontinuation at which time all the assessments listed for the final visit should be performed (see figure 1).

Patients lost to follow up should be recorded as such on the CRF. Patients who discontinue study treatment should be followed for tumor assessments as per standard of care, until disease progression or new cancer therapy is initiated. If patients refuse to return for these visits or are unable to do so, the subject should be considered off-study, but a Study Evaluation Completion form should be completed.

6. Treatment of Subjects

6.1 Treatment regimen

Patients will receive FLU , CY and Rituximab combination for an exposure period of up to 6 months- defined as the treatment period. Dosage, dose regimen and route of administration are detailed in 4.3-4.4

6.1.1 Treatment assignment

Informed consent must be obtained before any testing for the purpose of determining a patient's eligibility is performed. Each patient will be assigned a unique patient number. Once assigned, numbers for any non-evaluable or discontinued patient will not be reused.

6.2 Concomitant therapy

In general, concomitant medications and therapies deemed necessary for the supportive care and safety of the patient are permitted in this study, provided their use is documented in the patient records and on the appropriate Case Report Form. The administration of any anticancer agents including chemotherapy and biologic agents is NOT permitted. Similarly, the use of other concurrent investigational drugs is not allowed. The use of blood products (irradiated) transfusions, immunoglobulins or erythroid/myeloid growth factors are permitted at investigator's discretion.

Transfusion-associated graft versus host disease prevention using irradiated blood products is mandatory.

Steroids administration are allowed exclusively for patients with autoimmune hemolytic anemia and or autoimmune thrombocytopenia or as premedication for Rituximab .

Premedication: Any patient considered to be at risk of infusion related reaction (IRR) or TLS (=patients with lymphocytosis > 25,000mm^3) should receive:

Appropriate hydration starting 12-24 hours prior to initiating treatment, and thereafter until the risk of IRR/TLS is ruled out. Urine alkalization is at investigator's discretion.

Antihistamines (e.g. 2 mg clemastin or promethazine 25mg i.v.) and paracetamol (500-1,000 mg orally) 30 min before first infusion of Rituximab, and thereafter only if clinically indicated. Prednisone/prednisolone 100 mg I.V. should be given only if clinically indicated (increased risk of IRR).
Pneumocystis carinii pneumonitis prophylaxis with trimethoprim 160mg-sulfamethoxasole 800 mg twice a day, twice a week, for the treatment period and for the next 6 months of follow up are recommended. For prevention of hepatitis B reactivation that needs antiviral treatment Lamivudine 100mg/day will be administrated for the entire duration of chemotherapy and for the six months afterwards.

Hepatitis B serology will be: HBsAg+, Anti HBcore and anti-HBsAg

Treatment is recommended for: HBsAg+ (carriers), or HBVsAg (-), anti HBcore (+) and anti-HBVsAg (-).

Any use of concomitant medication must be captured in the concomitant medication CRF. Administration of Blood products must also be captured as concomitant medication on CRF.

Steroids if given (see above) are allowed for no more than 3 consecutive days. Excluding patients with autoimmune cytopenia, in which case prolonged period steroid administration is permitted (see above).

6.3 Treatment compliance

Number of FLU and CY vials administrated I.V to every subject in every study site, will be captured on CRF.
Number of Rituximab containing infusion bags administrated to every subject in every study site, will be captured on CRF

7. Assessment of efficacy

The primary efficacy parameter to be assessed will be response rate.

Response rate include CR and PR; all others (eg. stable disease, non response, progressive disease or death from any cause should be rated as treatment failure.)

An overall objective assessment of all measurable and non-measurable response parameters will be performed according to the Visit Schedules (see Figure 1).

Response rate will be assessed according the updated (2008) NCI-WG guidelines defined in appendix I: [46].

- Peripheral blood immunophenotyping by FACS for absence of clonal lymphocytes**.**
- Physical examination - lymph node examination and measurements for absence of significant (>1.5 cm in diameter), as well as the presence of hepatomegaly and splenomegaly.
- CT scan***** for CLL patients with a CT found to be abnormal before therapy or alternatively if physical examination is inconclusive.
- Complete blood count for ANC, platelets and hemoglobin levels. Blood counts have to be above the following values: ANC> 1.5 X 10^9/L. platelets > 100X 10^9/L. Hemoglobin > 11.0 g/dL.
- Bone marrow aspiration and biopsy to assess degree of bone marrow involvement by CLL using FACS and immunohistochemistry, should be performed at least 3 months after the last treatment if the above 4 clinical and laboratory results demonstrate that CR has been achieved. In some cases, it is necessary to postpone BMB until after all the other criteria used to define a CR have been satisfied but, this time interval should not exceed 6 months after the last treatment.
- Patient interview for the presence of constitutional symptoms throughout the study.

A single laboratory should perform all evaluations for all patients recruited in the same site. All the above studies must account for disease parameters that

were present at baseline and must use the same techniques as used at baseline. All the above mentioned assessments should be performed within 7 days of the scheduled day of assessment (see Figure 1).

All above mentioned efficacy (response) parameters will be performed at screening and at least 3 months after completing study drug administration for response documentation.

Bone marrow examination can be postponed until after the above clinical and laboratory examinations used to define a CR have been satisfied. However, this time interval should not exceed 6 months after the last treatment.

All known disease (measurable, and non measurable) parameters must be accounted for when assessing objective response rate. Current laboratory and physical examination results will be captured on the response forms in the CRF.

Progression free survival is defined as the interval from the first study drug treatment day to the first sign of disease progression.

Progressive disease (PD) will be assessed according the updated (2008) NCI-WG guidelines defined in appendix I. If needed, BMB will be performed for suspected progression at the investigator discretion. Progression will be captured on the response form CRF.

Time to next treatment is defined as the interval between the first study drug treatment day to the first day of starting the next treatment.

In general, second-line treatment decisions follow the same indications as those used for initiation of first-line treatment.

Re-treatment day will be captured on the treatment form CRF.
QOL questionnaires (FACIT) will be completed by study subjects at:
screening visit and at visits number: 11,14 and at the end of study.
Questionnaires will be captured on the QOL forms CRF.
CT Scan – In order to prevent contrast nephropathy, patients with GFR < 60ml/min or diabetes mellitus, hydration is recommended and if possible acetylcystein (600 mg in bolus pre CT and 1,200 mg orally twice a day for 48 hours after CT) should be given . Hospitalization for this procedure is recommended.

8. Assessment of Safety

Safety assessments will consist of evaluating adverse events and serious adverse events, laboratory parameters including hematology, chemistry, vital signs, physical examinations, and documentation of all concomitant medications and/or therapies.

8.1 Adverse events

According to the International Conference of Harmonization [ICH], an AE is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign [including an abnormal laboratory finding], symptom, or disease temporally associated with the use of a medicinal [investigational] product, whether or not considered related to the medicinal [investigational] product. Pre-existing conditions which worsen during a study are to be reported as AEs.

Information about all adverse events, whether volunteered by the patient, discovered by investigator questioning, or detected through physical examination, laboratory test or other means, will be collected and recorded on the Adverse Event Case Report Form and followed as appropriate. An adverse event is any undesirable sign, symptom or medical condition occurring after starting study treatment, even if the event is not considered to be treatment-related.

Medical conditions/diseases present before starting study treatment are only considered adverse events if they worsen after starting study treatment. Clinical events occurring before starting study treatment but after signing the informed consent form are recorded on the Medical History/Current Medical Conditions Case Report Form only if the patient receives study treatment. Abnormal laboratory values or test results constitute adverse events only if they induce clinical signs or symptoms or require therapy, when they are recorded on the Adverse Events Case Report Form under the signs, symptoms or diagnosis associated with them.

8.1.2 Grading and reporting of adverse events

All adverse events will be graded according the NCI-CTC scale version 3.0 Anemia and thrombocytopenia should be graded according to NCI-CTC scale version 2.0- the criteria for patients in leukemia studies (see assessment of toxicity below for details), and reported in detail as indicated on the CRF

Adverse events not listed on the CTCAE should be graded as follows:

Definition	Equivalent To:	CTC Grade
Discomfort noticed but no disruption of normal daily activity	Mild	Grade 1
Discomfort sufficient to reduce or affect daily activity; no treatment or medical intervention is indicated although this could improve the overall well-being or symptoms of the subject	Moderate	Grade 2
Inability to work or perform normal daily activity; treatment or medical intervention is indicated in order to improve the overall well-being or symptoms; delaying the onset of treatment is not putting the survival of the subject at direct risk.	Severe	Grade 3
An immediate threat to life or leading to a permanent mental or physical conditions that prevents work or performing normal daily activities; treatment or medical intervention is required in order to maintain survival.	Life threatening/ disabling	Grade 4
AE resulting in death	Death	Grade 5

8.1.3 Assessment of Toxicity

The evaluation of potential treatment-induced toxicity in patients with advanced CLL may be quite difficult requiring careful consideration of both the manifestations of the underlying disease, as well as adverse reactions to the therapy under study. Some of the conventional criteria for toxicity are not applicable especially under circumstances of progressive bone marrow failure from the CLL itself.

8.1.4 Hematological Toxicity

The evaluation of hematological toxicity in patients with CLL must consider the high frequency of hematological compromise already present at the initiation of therapy. Therefore the standard criteria used for solid tumors cannot be applied directly; many patients would be considered to have grade II to IV hematological toxicity at presentation.

As a consequence, **for grading of anemia and thrombocytopenia,** we plan to apply the NCI**-CTC version 2.0 criteria for patients in leukemia studies, but for grading of neutropenia, the standard NCI-CTC version 3.0 criteria,** as given below.

Table 3: NCI-CTC Grading scale for hematological toxicity for patients in this study

ANC mm^3 § (nadir)	Grade	Decrease in platelets*or Hb# from pretreatment value (%).
≥2,000	0	No change-9%
≥1,500 and <2,000	1	10-24%
≥1,000 and <1,500	2	25-49%
≥500 and <1000	3	50-74%
<500	4	≥75%

Grades: 1 = mild, 2 = moderate, 3 = severe, 4 = life-threatening

* if platelets were < 20,000mm^3 [<20 x10^9/L] prior to therapy, the patient is inevaluable for toxicity in platelets

baseline and subsequent Hb determinations must be performed before any given infusion

§ if ANC was < 1,000 prior to therapy, the patient is inevaluable for toxicity in ANC.

Platelets:

If at any level of decrease the platelet count is < 20,000 mm^3, this is considered grade 4 toxicity, unless a severe or life-threatening decrease in the initial platelet count (e.g. < 20,000 mm^3) was present before treatment in which case the patient is inevaluable for toxicity referable to platelet counts.

Hemoglobin:

Baseline and subsequent Hb determinations must be performed before any given infusions.

Fludarabine has been reported to exacerbate or precipitate autoimmune hemolytic anemia and patients should be monitored carefully for this condition. If a rapid decrease of hemoglobin occurs during therapy, the

possibility of fludarabine- or autoantibody-induced hemolysis should be considered and appropriate diagnostic tests (LDH, bilirubin, α2-haptoglobin, reticulocytes, Coombs-test) be performed. **In particular, a Coomb's test should be performed for:**

- **Any grade 3 or 4 anemia (Hb < 8 g/dL)**
- **A Hb < 10 g/dL sustained for > 2 weeks**
- **A drop in Hb of 3 g/dL in one week (while pts are receiving weekly blood tests)**
- **Any patient requiring a blood transfusion**

If, in the judgment of the treating physician, there is evidence of hemolytic anemia secondary to fludarabine, study treatment should be promptly withdrawn. Full details of the hemolytic anemia should be recorded on the adverse event pages of the CRF.

Neutrophils:

Absolute neutrophil count (ANC) is used to assess the toxicity of the myeloid lineage. Other decreases in the white blood cell count, or in circulating lymphocytes, are not to be considered, since a decrease in the white blood cell count is a desired therapeutic end point. If the ANC was < 1,000/ mm^3 [<1 $x10^9$/L] prior to therapy, the patient is inevaluable for toxicity referable to the ANC.

Any Adverse Event occurring by the time of study completion (within four weeks of last drug intake) must be recorded on the Adverse Event CRF page.

8.1.5 Drug-Adverse event relationship

The causality relationship of study drug to the adverse event will be assessed by the investigator as either:

Yes or No

If there is a reasonable suspected causal relationship to the study medication, i.e. there are facts (evidence) or arguments to suggest a causal relationship, drug-event relationship should be assessed as **Yes**.

The following criteria should be considered in order to assess the relationship as **Yes:**

- Reasonable temporal association with drug administration

- It may or may not have been produced by the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject.
- Known response pattern to suspected drug
- Disappears or decreases on cessation or reduction in dose
- Reappears on rechallenge

The following criteria should be considered in order to assess the relationship as **No**:

- It does not follow a reasonable temporal sequence from administration of the drug.
- It may readily have been produced by the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject.
- It does not follow a known pattern of response to the suspected drug.
- It does not reappear or worsen when the drug is readministered.

8.2 Serious adverse events

A serious adverse event is any experience that suggests a significant hazard, contraindication, side effect or precaution. It is any Adverse Event that at any dose fulfils at least one of the following criteria:

- is fatal; (results in **death**; NOTE: death is an outcome, not an event)
- is Life-Threatening (NOTE: the term "Life-Threatening" refers to an event in which the subject was at immediate risk of death at the time of the event; it does not refer to an event which could hypothetically have caused a death had it been more severe).
- required in-patient hospitalization or prolongation of existing hospitalization;
- results in persistent or significant disability/incapacity;
- is a congenital anomaly/birth defect;
- is medically significant or requires intervention to prevent one or other of the outcomes listed above

****The term sudden death should be used only when the cause is of a cardiac origin as per standard definition. The terms death and sudden death are clearly distinct and must not be used interchangeably**.

The study will comply with all local regulatory requirements and adhere to the full requirements of the **ICH Guideline for Clinical Safety Data Management, Definitions and Standards for Expedited Reporting, Topic E2**

Other safety findings may require expedited reporting depending on local legislation, e.g. a major safety finding from a newly completed animal study (such as carcinogenicity).

Progression of underlying malignancy is not reported as an adverse event if it is clearly consistent with the suspected progression of the underlying cancer as defined by RECIST criteria, or other criteria as determined by protocol. Hospitalization due solely to the progression of underlying malignancy should NOT be reported as a serious adverse event. Clinical symptoms of progression may be reported as adverse events if the symptom cannot be determined as exclusively due to the progression of the underlying malignancy, or does not fit the expected pattern of progression for the disease under study.

Symptomatic deterioration may occur in some subjects. In this situation, progression is evident in the subject's clinical symptoms, but is not supported by the tumor measurements. Or, the disease progression is so evident that the investigator may elect not to perform further disease assessments. In such cases, the determination of clinical progression is based on symptomatic deterioration. These determinations should be a rare exception as every effort should be made to document the objective progression of underlying malignancy.

If there is any uncertainty about an adverse event being due only to the disease under study, it should be reported as an AE or SAE.

8.2.1 Reporting of Serious Adverse Events (immediately reportable)

Any clinical adverse event or abnormal laboratory test value that is *serious* and which occurs during the course of the study (as defined in section 7.1.1.3 above), regardless of the treatment arm, occurring from the enrollment visit (start of study screening procedures), including long term follow-up (LTFU) must be reported to Roche **within *one* working day** of the investigator becoming aware of the event (expedited reporting). The investigator must complete the *SAE Reporting Form* [redacted] *]* and forward it to the SAE Responsible. All SAEs occurring from the enrollment period must be reported, (start of study screening procedures),

Related Serious Adverse Events ***MUST*** be collected and reported regardless of the time elapsed from the last study drug administration, even if the study has been closed. Suspected Unexpected Serious Adverse Reactions (SUSARs) are reported to investigators at each site and associated IRB/IEC when the following conditions occur:

- The event must be a SAE.
- There must be a certain degree of probability that the event is an adverse reaction from the administered drug.
- The adverse reaction must be unexpected, that is to say, not foreseen in the SPC text (Summary of Product Characteristics (for an authorized medicinal product) or the Investigator's Brochure (for an unauthorized medicinal product).

When all subjects at a particular site are off treatment as defined by the protocol:

- only individual SUSAR reports originating in that particular trial will be forwarded to the site and associated IRB/IEC on an expedited basis;
- individual SUSARs considered to be a significant safety issue and/or which result in Roche recommending a change to the

Informed Consent Form (ICF), will be reported in an expedited manner to all investigators and IRBs/IECs;

- SUSAR reports originating from other trials using the same IMP will be provided as six monthly SUSAR Reports (SSRs) to investigators and IRBs/IECs where long-term follow-up studies are carried out, unless they are considered significant..

Unrelated Serious Adverse Events must be collected and reported during the study and for up to 30 days after the last dose of study medication.

This study adheres to the definition and reporting requirements of **ICH Guideline for Clinical Safety Data Management, Definitions and Standards for Expedited Reporting, Topic E2.**

8.2.2 Treatment and Follow-up of AEs

During the study period and up to 30 days after completion or discontinuation of the study, continue to follow up AEs as follows:

Related AEs: Follow until one of the following occurs:

- Resolved or improved to baseline
- Relationship is reassessed as unrelated
- Death
- Start of new anti-cancer regimen
- Investigator confirms that no further improvement can be expected
- Clinical or safety data will no longer be collected, or final database closure

Unrelated severe or life threatening AEs: Follow until one of the following occurs:

- Resolved or improved to baseline
- Severity improved to Grade 2
- Death
- Start of new anti-cancer regimen
- Investigator confirms that no further improvement can be expected
- Clinical or safety data will no longer be collected, or final database closure

Unrelated Grade 1 or Grade 2 AEs: Follow up to 30 days after completion or discontinuation of the study.

The final outcome of each adverse event must be recorded on the CRF

8.2.2 Laboratory evaluations

Laboratory test results will be recorded on the laboratory results form of the CRF, or appear on electronically produced laboratory reports submitted directly from the central laboratory, if applicable.

Any laboratory result abnormality fulfilling the criteria for a serious adverse event (SAE) should be reported as such, in addition to being recorded as an AE in the CRF.

Any treatment-emergent abnormal laboratory result which is clinically significant, i.e., meeting one or more of the following conditions, should be recorded as a single diagnosis on the adverse event page in the CRF:

- Accompanied by clinical symptoms
- Leading to a change in study medication (e.g. dose modification, interruption or permanent discontinuation)
- Requiring a change in concomitant therapy (e.g. addition of, interruption of, discontinuation of, or any other change in a concomitant medication, therapy or treatment)

No special laboratory or tests are required to be performed for the purpose of the study. Measurement of Complete Blood Counts, chemistry and immunological tests such as: serum Ig level, serum immunoellectrophoresis, DAT, peripheral and bone marrow FACS are in fact regarded as current clinical practice for CLL patients, and should therefore be collected according to the Visit Schedules (see Figure 1). In case of suspected hemolysis - DAT, reticulocyte count, serum haptoglobin, bilirubin and LDH are recommended.

The institution will perform laboratory analyses according to the Visit Schedules (see Figure 1). The sponsor must be provided with a copy of the

laboratory's certification, and a tabulation of the normal ranges for each parameter required. Additionally, if at any time a patient has laboratory parameters obtained from a different outside laboratory, the sponsor must be provided with a copy of the certification and a tabulation of the normal ranges for that laboratory.

At any time during the study, abnormal laboratory parameters which are clinically relevant (e.g. require dose modification and/or interruption of study drug, lead to clinical symptoms or signs or require therapeutic intervention), whether specifically requested in the protocol or not, must be recorded on the appropriate Comment CRF page in addition to the appropriate laboratory CRF page. When abnormal laboratory values or test results constitute an adverse event (i.e., induces clinical signs/symptoms or requires therapy) they must be recorded on the Adverse Events CRF.

When, in the opinion of the investigator, other clinical laboratory evaluations may be relevant for assessing the patient's status, they will be completed and entered into the database as appropriate.

8.3 Special tests:

PET-CT will be performed in case of suspected Richter transformation.

Lymph node biopsy is recommended for definitive diagnosis of Richter transformation and for any rapidly progressive disease or unusual phenomena which may be suspicious of alteration in the of the disease or suspicious for another disease or a different type of lymphoma.

8.4 Physical examinations/vital signs

A physical examination including vital signs will be performed according to the Visit Schedules (see Figure 1). Information about the physical examination and vital signs must be present in the source documentation at the study site. Significant findings present prior to the start of study drug must be included in the Relevant Medical History/Current Medical Conditions CRF. Significant findings made after the start of study drug which meet the definition of an adverse event must be recorded on the Adverse Event Case Report Form. There are no case report forms to capture routine normal findings from physical examinations and vital signs assessments.

8.5 Laboratory examinations

8.5.1 Hematology

Hematological evaluations (CBC) are regarded as clinical practice for CLL patients and should therefore be collected according to the Visit Schedules (see Figure 1).

8.5.2 Blood chemistry

The following blood chemistry results are regarded as clinical practice for CLL patients and should therefore be collected according to the Visit Schedules (see Figure 1).

Serum creatinine, ALT, AST, alkaline Phosphatase, LDH, bilirubin. globulin, serum immunoellectrophoresis and beta-2 microglobulin.

8.6 Physical examinations/vital signs

A physical examination including vital signs will be performed according to the Visit Schedules (see Figure 1). Information about the physical examination and vital signs must be present in the source documentation at the study site. Significant findings present prior to the start of study drug must be included in the Relevant Medical History/Current Medical Conditions CRF. Significant findings made after the start of study drug which meet the definition of an adverse event must be recorded on the Adverse Event Case Report Form. There are no Case Report Forms to capture routine normal findings from physical examinations and vital signs assessments.

8.6.1 Warning and Precautions

No evidence available at the time of the approval of this study protocol indicated that special warnings or precautions were appropriate regarding Rituximab, other than those noted in the Investigators' Brochure.

Safety information regarding Fludarabine and Cyclophosphamide can be found in the respective prescription information.

9. Statistics

Subjects' age, weight, height and other continuous demographic and baseline variables will be summarized using descriptive statistics (mean, standard deviation, minimum and maximum), while performance status, gender, and other categorical variables will be summarized with frequency tabulations.

9.1 Sample size

This is a single arm phase II study. The number of subjects planed to be enrolled into the study is 40. Such a low number of patients as in most phase II studies) constitute an underpowered study for revealing any statistical significance, but is indeed clinically meaningful. So, the sample size chosen for this study was based on clinical considerations.

Those patients achieving PR or CR by the study medication will be regarded as responders. All the others (stable disease, progressive disease or death from any cause) will be regarded as non responders. Determining the ratio of responders versus non responders, even if not statistically significant, will still be clinically meaningful.

9.1.1 Data analysis

Categorical data will be presented as frequencies and percentages. For continuous data, mean, standard deviation, median, 25^{th} and 75^{th} percentiles, minimum, and maximum will be presented.

9.2 Efficacy and Safety evaluation

9.2.1 Efficacy analysis:

All analyses will be according to the intention to treat principle.

9.2.2 Response rate

The primary efficacy parameter to be assessed will be response rate determined by objective overall response rate.

The primary endpoint is a binary variable where each patient is classified as a responder or not responder.

Response rate include CR and PR; all other situations (eg. stable disease, non response, progressive disease or death from any cause) will be rated as treatment failure and therefore as non responders.

Response rate will be assessed according the updated (2008) NCI-WG guidelines defined in appendix 1:

- Peripheral blood FACS for lymphocytes clonality.
- Physical examination for lymph node examination and measurements, and documentation of the presence of hepatomegaly and splenomegaly.
- Blood count for ANC, platelets and hemoglobin levels.
- Bone marrow aspiration and biopsy for bone marrow involvement by CLL cells using FACS immunophenotyping or immunohistochemistry.
- Patient interview for the presence of constitutional symptoms throughout the study.

All above mentioned efficacy (response) parameters will be performed at screening and at least 3 months after completing study drug administration for response documentation.

All known disease (measurable, and non measurable) parameters must be accounted for when assessing objective response rate.

Progression free survival is defined as the interval between the first treatment day to the first sign of disease progression.

Progressive disease (PD) will be assessed according the updated (2008) NCI-WG guidelines defined in appendix I. If needed, BMB will be performed for suspected progression at the investigator's discretion. Progression will be captured on the response form CRF.

A Kaplan-Meier method will be used to estimate median PFS and 90% confidence interval.

QOL questionnaires will be completed by study subjects at: screening visit and visits number: 11, 14, and end of study. Questionnaires will be captured on the QOL forms CRF.

Summary statistics (standard deviation, median, minimum and maximum) will be provided for the relevant variables. ANOVA will be used to analyse the changes in the components and total score of the FACIT QOL assessment.

9.3 Safety Analysis:

Data from all subjects who receive any study drug will be included in the safety analyses. The severity of the toxicities will be graded according to the NCI CTCAE v 3.0 , or v 2.0 for cytopenia, whenever possible. In the by-subject analysis, a subject experiencing the same event more than once will be counted only once. Adverse events will be defined and summarized according to the worst NCI CTC grade. Adverse events leading to death or to discontinuation of treatment, events classified as NCI CTCAE Grade 3 or Grade 4, study-drug-related events, and serious adverse events will be summarized separately. Laboratory data will be graded according to NCI CTCAE severity grade. ANOVA will be used to analyse the changes in the components and total score of the FACIT QOL assessments.

10. Quality Control and Quality Assurance

Data from the CRFs are entered into the study database by Contract Research Organization staff following their own internal standard operating procedures that have been reviewed and approved by the sponsor.

Subsequently, the entered data are systematically checked by Data Management staff, using error messages printed from validation programs and database listings. Obvious errors are corrected by Data Management personnel. Other errors or omissions are entered on Data Query Forms, which are returned to the investigational site for resolution. The signed original and resolved Data Query Forms are kept with the CRFs at the investigator site, and a copy is sent to the sponsor so the resolutions can be entered into the database. Quality control audits of all key safety and efficacy data in the database are made prior to locking the database.

11. Direct Access to Source Data/Documents

11.1 Study monitoring

The monitoring visits will enable the sponsor or the monitor acting on his behalf, to assess the study's progress status, to verify data accuracy and degree of completeness of the CRFs, to ensure the protocol as well as local regulations are being followed, that the investigator is fulfilling his obligations and to correct errors in the CRFs compared to the source documents. The investigators' will authorize the monitor, at a mutually convenient time during the study to periodically review all of the CRFs and the related parts of administrative, medical and laboratory files of each participant in the study. The CRFs must be filled out before the monitoring visits.

11.2 Audits

During the study, people belonging to the sponsor quality assurance group may visit the investigator site in order to audit the study. The purpose of this visit is to check that the study is carried out according to Good Clinical Practices. Before conducting an audit, the monitor will contact the investigator in order to agree upon a mutually suitable date. The investigator and his team are required to cooperate with the auditors and to grant them access to the patients' medical files and to the study documents (CRFs and investigator's binders).

11.3 IRB and regulatory inspections

A regulatory authority may also wish to conduct an inspection (during the study or even after its completion). If an inspection is requested by a regulatory authority, the investigator must inform the sponsor immediately that this request has been made.

12. Ethics

12.1 Institutional Review Board

Before implementing this study, the protocol, the proposed informed consent form and other information to subjects, must be reviewed by a properly constituted Institutional Review Board (IRB). A signed and dated statement that the protocol and informed consent have been approved by the IRB must be given to the sponsor before study initiation. The name and occupation of the chairman and the members of the IRB must be supplied to the sponsor. Any amendments to the protocol, other than administrative ones, must be approved by this committee.

12.2 Informed consent

The investigator must explain to each subject (or legally authorized representative) the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits involved and any discomfort it may entail. Each subject must be informed that participation in the study is voluntary and that he/she may withdraw from the study at any time and that withdrawal of consent will not affect his/her subsequent medical treatment or relationship with the treating physician.

This informed consent should be given by means of a standard written statement, written in non-technical language. The subject should read and consider the statement before signing and dating it, and should be given a copy of the signed document. If the subject cannot read or sign the documents, oral presentation may be made or signature given by the subject's legally appointed representative, if witnessed by a person not involved in the study, mentioning that the patient could not read or sign the documents. No patient can enter the study before his/her informed consent has been obtained.

The informed consent form is considered to be part of the protocol, and must be submitted by the investigator with it for IRB approval.

12.3 Declaration of Helsinki

The investigator must conduct the trial in accordance with the principles of the Declaration of Helsinki which can be accessed via the website of the World Medical Association at http://www.wma.net/e/policy/17-c_e.html.

13. Data Handling and Record Keeping

The information obtained from the execution of the protocol will be listed on the CRFs provided by the sponsor.
The CRFs must be filled out on a timely basis, signed and dated by the investigator or a co-investigator named by the former and clinically responsible for the patient during the study.

The completed CRFs will be collected by the sponsor. The investigator must remit one CRF for every participant in his center for a time period of 15 years.

14. Financing and Insurance

Financing and insurance will be addressed in the future in a separate agreement.

15. Publication Policy

Any formal presentation or publication of data from this trial will be considered as a joint publication by the investigator(s) and appropriate sponsor's personnel. Authorship will be determined by mutual agreement taking in consideration that the group of PI's will prepare the manuscript and write it initially and decide which member of steering committee and which other active investigators who provided cases will be involved.

It is a multicenter study, and it is mandatory that the first publication is based on data from all centers, analyzed as stipulated in the protocol, by the sponsor statisticians, and not by the investigators. Investigators participating in multicenter studies agree not to present data gathered from one center or a small group of centers before the full publication, unless formally agreed to by all other investigators and the PI's and steering committee.

The investigator may be required to sign the clinical study report, if it is to be used in a registration submission to the health authorities of some countries. For multicenter studies only the coordinating (principle) investigator nominated by the sponsor and the steering committee at the start of the trial would provide any needed signature.

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16. Supplements

Appendix I Response definitions

CR (complete remission) = requires **all** of the following criteria as assessed at least 3 months after completion of therapy:

Absence of clonal lymphocytes in peripheral blood.

Absence of significant lymphadenopathy. (lymph nodes > 1.5 cm in diameter) by physical examination. CT is desirable for not palpable lymph nodes.

No hepatomegaly or splenomegaly by physical examination. (Abdominal, pelvix and thorax CT for subjects found to be abnormal before therapy or if physical examination is inconclusive.

Absence of constitutional symptoms.

Blood counts above the following values: ANC> 1.5 x 10^9/L . Platelets > 100 x10^9/L . Hemoglobin > 11.0 g/dL; untransfused).

Bone marrow aspirate and biopsy should be performed at least 3 months after the last treatment and if the above 5 clinical and laboratory results demonstrate that CR has been achieved.
Bone marrow should be analyzed by FACS/or immunohistochemistry to demonstrate marrow free of B-CLL cells.
In some cases, it is necessary to postpone BMB until after all the other criteria to define a CR have been satisfied. However, this time interval should not exceed 6 months after the last treatment.

Parameter	CR	PR	PD	SD
Group A				
Lymphadenopathy*	None more than 1.5 cm	Decrease ≥ 50%	Increase ≥ 50%	Change of −49% to +49%
Liver and/or spleen size	Normal size	Decrease ≥ 50%	Increase ≥ 50%	Change of −49% to +49%
Constitutional symptoms	None	Any	Any	Any
Polymorphonuclear leukocytes	>1500/μL	>1500/μL or > 50% improvement over baseline	Any	Any
Circulating clonal B lymphocytes	None	Decrease ≥ 50% from baseline	Increase ≥ 50% over baseline	Change of −49% to +49%
Group B				
Platelet count	>100 000/μL	>100 000/μL or increase ≥ 50% over baseline	Decrease of ≥ 50% from baseline secondary to CLL	Change of −49 to +49%
Hemoglobin	>11.0 g/dL (untransfused and without erythropoietin)	>11 g/dL or increase ≥ 50% over baseline	Decrease of >2 g/dL from baseline secondary to CLL	Increase ≤11.0 g/dL or <50' over baseline, or decrease <2 g/dL
Marrow	Normocellular, <30% lymphocytes, no B-lymphoid nodules; hypocellular marrow defines CRi	≥ 30% lymphocytes, or B-lymphoid nodules, or not done	Increase of lymphocytes to more than 30% from normal	No change in marrow infiltrat

Values in SI units for leukocytes are 1.5 × 10^9/L; for platelets 100 × 10^9/L; and for hemoglobin 110 g/L and 20 g/L.

CR indicates complete remission (all of the criteria have to be met); PR, partial remission (at least one of the criteria of group A and one of the criteria of group B have to be met); PD, progressive disease (at least one of the criteria of group A or one of the criteria of group B have to be met); SD, stable disease (all of the above criteria have to be met).

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

Relapse = a patient who has previously achieved CR or PR but after a period of 6 or more months, demonstrates evidence of disease progression

Refractory disease = defined as treatment failure or disease progression within 6 months from the last antileukemic therapy.

Progression = see above table under PD.

Progression free survival (PFS) = defined as the time from start of treatment to progression.

Time to next treatment (TNT) = defined as the time from start of treatment to start of second-line treatment

Appendix II ECOG Performance status

ECOG	Grade
Fully active, able to carry on all pre-disease performance without restriction	0
Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work	1
Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours	2
Capable of only limited self-care, confined to bed or chair more than 50% of waking hours	3
Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair	4
Dead	5

Appendix III calculated creatinine clearance

$$\text{GFR (mL/min)} = \frac{(140\text{-age}) \text{ X weight (Kg)}}{\text{Serum cr (mg/dL) X } 72} \text{ X } 0.85 \text{ (for women)}$$

Appendix IV Active disease

At least one of the following criteria should be met:

1. Evidence of progressive marrow failure as manifested by the development of, or worsening of, anemia and/or thrombocytopenia
2. Massive (ie, > 6 cm below the left costal margin) or progressive or symptomatic splenomegaly.
3. Massive nodes (ie, > 10 cm in longest diameter) or progressive or symptomatic lymphadenopathy.
4. Progressive lymphocytosis with an increase of more than 50% over a 2-month period or lymphocyte doubling time of less than 6 months; patients with initial blood lymphocyte counts of less than $30X10^9$/L (30 000/μL) may require a longer observation period to determine the lymphocyte doubling time. In addition, factors contributing to lymphocytosis or lymphadenopathy other than CLL (eg, infections) should be excluded.
5. Autoimmune anemia and/or thrombocytopenia poorly responsive to corticosteroides or other standard therapy.
6. A minimum of any of the following disease-related symptoms must be present: a- Unintentional weight loss more than or equal to 10% within the previous 6 months. b- Significant fatigue (ie, ECOG PS 2 or worse; cannot work or unable to perform usual activities). c- Fevers of greater than 38.0^0C for 2 or more weeks without other evidence of infection. d- Night sweats for more than 1 month without evidence of infection.

Appendix V Binet staging system

The areas of involvement considered for staging are as follows: 1- Head and neck, including the Waldeyer ring. 2- Axillae 3- Groins 4- Palpable spleen 5- Palpable liver.

Stage A. Hb 10 g/dL or more and platelets 100X 10^9/L or more and up to 2 of the above areas involved.

Stage B. Hb 10 g/dL or more and platelets 100X 10^9/L or more and organomegaly greater than that defined for stage A (3 or more areas).

Stage C. All patients who have Hb less than 10 g/dL and/or a platelets count less than 100X 10^9/L, irrespective of organomegaly.

Appendix VI FACIT-QOL

FACIT – Fatigue Scale (Version 4)

Below is a list of statements that other people with your illness have said are important. **By circling one (1) number per line, please indicate how true each statement has been for you during the past 7 days.**

		Not At all	A little bit	Some what	Quite a bit	Very much
HI7	I feel fatigued	0	1	2	3	4
HI12	I feel weak all over	0	1	2	3	4
An1	I feel listless ("washed out") 0	1	2	3	4	
An2	I feel tired	0	1	2	3	4
An3	I have trouble starting things because I am tired 0	1	2	3	4	
An4	I have trouble finishing things because I am tired 0	1	2	3	4	
An5	I have energy	0	1	2	3	4
An7	I am able to do my usual activities 0	1	2	3	4	
An8	I need to sleep during the day 0	1	2	3	4	
An12	I am too tired to eat	0	1	2	3	4
An14	I need help doing my usual activities	0	1	2	3	4
An15	I am frustrated by being too tired to do the things I want to do	0	1	2	3	4
A 16	I have to limit my social activity because I am tired 0	1	2	3	4	

US English

F. HOFFMANN-LA ROCHE LTD

CLINICAL STUDY PROTOCOL

PROTOCOL NUMBER ML 25464 (FCR LITE)

A PHASE II, MULTICENTER, SINGLE ARM STUDY TO DETERMINE THE EFFICACY AND SAFETY OF LOW DOSE FLUDARABINE AND CYCLOPHOSPHAMIDE COMBINED WITH STANDARD DOSE RITUXIMAB AS PRIMARY THERAPY IN ELDERLY UNTREATED PATIENTS (≥65 YEARS OLD) WITH CHRONIC LYMPHOCYTIC LEUKEMIA

PROTOCOL APPROVAL

Protocol : ML25464 Version B

Date: **January 10, 2011**

Protocol approved by:

Medical Manager: [redacted]

Project Statistician: [redacted]

[redacted]

This protocol is intended for use in a life-threatening indication: Yes ☒ No ☐

A PHASE II, MULTICENTER, SINGLE ARM STUDY TO DETERMINE THE EFFICACY AND SAFETY OF LOW DOSE FLUDARABINE AND CYCLOPHOSPHAMIDE COMBINED WITH STANDARD DOSE RITUXIMAB AS PRIMARY THERAPY IN ELDERLY UNTREATED PATIENTS (≥65 YEARS OLD) WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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[redacted] M.D.

[redacted] M.D.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AE	Adverse Event
ALT	Alanine Aminotransferase/GPT
ANC	Absolute Neutrophil Count
AST	Aspartate Aminotransferase/SGOT
ALK PHOSP	Alkaline Phosphatase
CBC	Complete Blood Count
CLL	Chronic Lymphatic Leukemia
CR	Complete Remission
CRF	Case Report/Record Form
CRO	Contract Research Organization
CT	Computerized Tomography
CY	Cyclophosphamide
FACT-CTC	Functional Assessment of Cancer Therapy - Common Terminology Criteria for cancer Adverse Events
FACS	Flow Cytometry (Fluorescence-Activated Cell Sorting)
DAT	Direct Coombs Test
FLU	Fludarabine
G-CSF	Granulocyte-Colony Stimulating Factor
Hb	Hemoglobin
HIV	Human Immune deficiency Virus
IEP	Immuno-ellectrophoresis
I.V.	Intra-Venously
Ig	Immunoglobulin
IRB	Institutional Review Board
IRR	Infusion Related Reaction
LDH	Lactic Dehydrogenase
MHO	Medical Health Organization
ORR	Overall Response Rate
PD	Progressive Disease
PET-CT	Positron Emission Tomography- Computerized Tomography
PFS	Progression Free Survival
P.O.	Per Os/ by mouth/orally
PR	Partial Response
QOL	Quality Of Life
SAE	Serious Adverse Event
TLS	Tumor Lysis Syndrome
TNT	Time to Next Treatment
ULN	Upper Limit of Normal

SYNOPSIS

A PHASE II, MULTICENTER, SINGLE ARM STUDY TO DETERMINE THE EFFICACY AND SAFETY OF LOW DOSE FLUDARABINE AND CYCLOPHOSPHAMIDE COMBINED WITH STANDARD DOSE RITUXIMAB AS PRIMARY THERAPY IN ELDERLY UNTREATED PATIENTS (≥65 YEARS OLD) WITH CHRONIC LYMPHOCYTIC LEUKEMIA

PROTOCOL NUMBER:	ML25464
DATE PROTOCOL FINAL:	January 10, 2011
STUDY DRUG:	FLUDARABINE, CYCLOPHOSPHAMIDE, RITUXIMAB
INDICATION:	CHRONIC LYMPHOCYTIC LEUKEMIA
STUDY PHASE:	II

Background and rationale Chronic lymphocytic leukemia (CLL) is characteristically diagnosed in the older population with a median age at diagnosis ranging between 65 and 72 years. Because the prevalence of co morbidity increases with age, concomitant disease is often present in this age group. Additional factors which may also adversely affect treatment outcome at standard doses in elderly patients include: altered pharmacokinetics and poorer host tissue tolerance ; changes in the bone marrow hematopoietic reserve and microenvironment leading to increased treatment related myelotoxicity and higher rates of treatment related complications such as infections. As a result, elderly patients are often excluded from clinical trials or even from routine treatment on the basis of their age and / or comorbidity. Furthermore when these patients are treated, the drug doses are often markedly reduced in an attempt to avoid causing toxicity and complications. The challenge in treating elderly CLL patients relates to finding the correct balance between drug efficacy and toxicity and because of this optimal treatment for this group of patients is often hard to achieve.

Study Objectives

The aim of this study is to evaluate the safety and efficacy (response rate) of a combination of low dose Fludarabine (FLU) and Cyclophosphamide (CY) combined with standard dose Rituximab given as primary treatment for elderly patients with chronic lymphocytic leukemia.

Primary end point: -Response rate

Secondary end points:

- Safety - Special issues: incidence of neutropenic fever, hospitalizations (no^{o}- days), - Thrombocytopenia ≥grade 3, neutropenia ≥grade 3, Infection rate.
- Progression Free Survival (PFS).
- Time to Next Treatment (TNT).
- QOL

STUDY DESIGN:

This is a multicenter, phase II, single arm study designed to evaluate the efficacy and safety of the combination of low dose Fludarabine (FLU) and Cyclophosphamide (CY) combined with standard dose Rituximab in elderly previously untreated CLL patients ($\geq$ 65 years of age). Potential study subjects will sign an informed consent prior to undergoing any study related procedure. Number of patients to be enrolled 40.

Treatment plan: Flu 12.5mg/m^2/d will be given intravenously (I.V.) for days 1-3 every 28 days, for a total of 6 cycles.

CY will be given I.V. at the dose of 150 mg/ m^2/d on days 1-3 every 28 days, for a total of 6 cycles.

Rituximab given before chemotherapy cycle1: 375 mg/m^2 I.V. on day 0, cycles 2-6: 500 mg/m^2 I.V. on day 1 every 28 days.

Dose reduction or delays are recommended in case of grade $\geq$3 (NCI-CTC) adverse events.

This study consists of 3 periods for each study subject: Pre-treatment, Treatment and Follow up.

Pre-treatment period: patients will undergo screening for protocol eligibility within 28 days (4 weeks) of recruitment.

Subjects who meet all the inclusion criteria and have signed an approved informed consent will be enrolled.

Treatment period FLU 12.5mg/m^2/d I.V. will be given on days 1-3 every 28 days, for a total of 6 cycles. CY will be given (I.V.) 150 mg/ m^2/d on days 1-3 every 28 days, for a total of 6 cycles. Rituximab given before chemotherapy cycle 1: 375 mg/m^2 intravenously on day 0, cycles 2-6: 500 mg/m^2 I.V. on day 1 every 28 days.

Premedication: antihistamines (e.g. 2 mg clemastin or promethazine 25 mg i.v.) and paracetamol (500-1000 mg orally) 30 min before first infusion of Rituximab, and thereafter only if clinically indicated. Hydrocortisone 100-500 mg I.V. (according with local common practice) only if clinically indicated.

Dose reduction or delay in case of drug induced hematological toxicity grade≥ 3 level.

During the treatment period, all patients will attend periodic monthly study center visits in order to assess the safety and efficacy of the treatment.

Complete blood count will be performed on day 1 and 14 of each of the first two cycles, and on day 1 in the other 4 cycles, or more frequently at the investigators discretion.

Bone marrow aspiration and biopsy and CT scan will be performed within 3-to 6 months after the completion of the treatment period.

Follow up period: All patients will be assessed for progression and survival every 3 months via clinic visits, for a total of 30 months.

Complete blood count and chemistry will be performed every 3 months, or more frequently at the investigators discretion.
In the event of suspected progression or relapse BMB and or CT scan will be performed at the treating physicians' discretion.

Duration of study

The duration of the treatment period is approximately 6 months. This time period is required to complete the therapy, to determine the safety profile of the drug combination and the response rate to therapy. The duration of the follow up period will be 30 months. The occurrence of PD will determine the duration of progression-free survival of each patient.

Recruitment period will be 24 months.

Inclusion criteria:

- Diagnosis of B-CLL is established by the presence of: An absolute lymphocyte count> $5X10^9$/L, typical morphology in the peripheral blood smear as seen by light microscopy, CLL cells must show CD5/CD19/CD23 positivity with light chain restriction as recorded by flow cytometry. Low surface immunoglobulin/CD 79b expression is also expected to be evident on these cells while FMC7 should be negative-are both additional typical features on flow cytometry (optional).
- Previously untreated B-CLL patients $\geq$ 65 years old.
- Patients requiring treatment (active Binet stage A and B, all stage C disease).
- Written informed consent

Exclusion criteria:

- Calculated or measured creatinine clearance < 30 ml/min
- Hepatic enzymes or bilirubin > 2X ULN, unless thought to be due to CLL
- Suspected or documented CNS involvement by CLL
- Known HIV positivity
- Active or uncontrolled infections, including opportunistic infection, active hepatitis B / C -positive Hepatitis B Surface Ag or Hepatitis B Core Ab
- Various concomitant diseases requiring chronic steroid administration
- Active Coombs positive hemolytic anemia
- Prior treatment for CLL
- CLL with transformation (Richter syndrome).
- Presence of active second neoplasia (excluding non-melanoma skin cancer, or in situ cervix or breast carcinoma) < 2 years prior to the study.
- Use of other investigational agents or participation in any other clinical trials < 30 days prior to the study.
- Low compliance.
- Performance status: ECOG performance status $\geq$4.
- Patients who mentally or physically are unable to comply with all aspects of the study.

Assessments:

Efficacy

The following evaluations will be done to record the efficacy of the treatment regimen:

- Assessment of disease response (revised International Response Criteria: updated NCI- IWG 2008).[46].
- Date of documentation of disease progression.
- Bone marrow biopsy examination
- Peripheral blood immunophenotyping by FACS.
- Changes in ANC, Hb levels and platelets counts from baseline blood counts and those done during the study period.
- Chest, abdominal and pelvic computerized tomography
- Physical examination for lymphadenopathy and hepato-splenomegaly

Safety

The following evaluations will be recorded to assess the safety of the regimen:

- Complete physical examination including vital signs.
- Clinical laboratory evaluations (hematology, biochemistry)
- Concomitant medication and procedures
- Adverse events
- Health outcomes assessment (any extra medical appointment)

Efficacy analysis:

Primary efficacy will be determined by objective overall response rate. Exact tests for assessing these will be used to compare objective overall response rates. Kaplan Meier procedure will be used to characterize the duration of response for PFS and TNT. Summary statistics (standard deviation, mean, median, minimum and maximum) will be provided for the relevant variables

Safety Analysis:

Data from all subjects who receive any study drug will be included in the safety analyses. The severity of the toxicities will be graded according to the NCI CTC v3 or v2- for cytopenia, whenever possible. In the by-subject analysis, a subject experiencing the same event more than once will be counted only once.

Adverse events will be defined and summarized by the worst NCI CTC grade. Adverse events leading to death or to discontinuation of treatment, events classified as NCI CTC Grade 3 or Grade 4, study-drug-related events, and serious adverse events will be summarized separately. Laboratory data will be graded according to NCI CTC severity grade.

STUDY DURATION: five years **TOTAL SAMPLE SIZE**: 40

2. Background Information

Chronic lymphocytic leukemia (CLL) is characteristically diagnosed in older people with a median age at diagnosis ranging between 65 and 72 years. Because the prevalence of co morbidity increases with age, concomitant disease is often evident in this age group. Goede et al [29] reported that increasing numbers of more severe concomitant comorbid illnesses are associated with a shortened survival in patients with CLL. Most recently Thurmes and colleagues [1] have shown that these comorbid conditions are in fact less important than both the age of the patient and the disease stage, in predicting prognosis in untreated patients.

Additional factors which may also adversely affect treatment outcome in elderly patients include: altered pharmacokinetics and poorer host tissue tolerance ; changes in bone marrow hematopoietic reserve and microenvironment which may lead to increased treatment related myelotoxicity and higher rates complications such as infections [21,26]. As a result, elderly patients are often excluded from clinical trials or even from routine treatment at standard doses of drugs, on the basis of their age and / or comorbidity and when treated the drug doses are often markedly reduced when given.

The basic challenge in treating elderly CLL patients appears to be related to finding the correct balance between treatment efficacy and drug toxicity and because of this, optimal treatment for older patients is hard to achieve and remains an open question and topic of debate.

Purine analogues drugs which inhibit DNA synthesis are very effective in CLL and fludarabine, cladribine and pentostatin have all been used effectively in the treatment of CLL [14]. Fludarabine is the most extensively studied purine analogue in CLL and is available in tablet form as well as for intravenous use. When used in combination with cyclophosphamide, fludarabine has been shown to be more effective than when used alone. [13,14,27,28]. In this respect the U.K. MRC LRF CLL4 trial reported in 2007 [11] compared therapy with chlorambucil alone vs fludarabine vs fludarabine (F) alone +

cyclophosphamide (FC) in 777 newly diagnosed CLL patients, and showed that FC achieved superior response rates and better progression-free survival for all CLL patients . Because of this, FC was proposed as the gold standard treatment of all CLL patients irrespective of age [11]. Similar results were published by the German CLL Study Group and the North American Intergroup [14].

However although Fludarabine - based regimes are most efficacious, they also appear to be more toxic, inducing more prolonged myelosuppression with more infections.

Pollizzotto et al [15] investigated the influence of increasing age on the ability to deliver fludarabine based regimens as well as the toxicity obtained thereafter, in patients with indolent lymphoproliferative disorders. They reported a gradual increase in toxicity with advancing age especially in patients age ≥ 70 years but still concluded that regimens were generally well tolerated and could be delivered safely to older patients who had a good performance status (PS). They noted a modestly increased degree of myelosuppression but no increase in the incidence of severe infectious complications or treatment-related mortality.

Table 1

PR	CR	OR	Patients age	Oral Cyclophosphamide dose	Oral Fludarabine dose	Disease status	Journal
NHL-59% CLL-100%	NHL-35% CLL-0%	NHL-94% CLL-100%	61-85 Median 73.5	150mg/m^2 X 4 days Every 28 days , 4 courses	25mg/m^2 (total 40 mg) X 4 days	Mostly relapsed refractory CLPD-28 Patients CLL-9 p	Hematology 2004 (16)
All-44% SLL-4/8	All-40% SLL-3/8	All-84% SLL-7/8	66-85 Med 74	150mg/m^2 X 4 days	25mg/m^2 (total 40 mg) X 4 days	Untreated 25 patients SLL-8	BJH 2007 (17)

				Every 28 days ; 4 courses			
40%	54%	94%	30% above age 70	150mg/m^2 X 5 days Every 28 dX6	24mg/m^2 X5 days	Untreated (196)	CLL4 (11)

2.1 Rituximab

Since the introduction of Rituximab (Rituxan™, IDEC) (FDA Approval Letter Nov. 26, 1997) in the USA and shortly thereafter in Europe in 1998 (MabThera™ (Hoffmann-LaRoche AG) for use in the treatment of CLL and indolent non-Hodgkins' lymphoma (NHL), it has been used extensively in the treatment of indolent and aggressive lymphoma, both as a single agent or more often in combination with chemotherapy. Today Rituximab is already being used to treat a variety of autoimmune disorders and by now has also been accepted as the model monoclonal antibody for the treatment of cancer. Rituximab is a chimeric mouse variable heavy and light chains (IDEC 2B8) with a human IgG_1 constant kappa antibody (C2B8) that reacts specifically with the CD20 antigen found on the surface of malignant and normal B cells and established B-cell lines [31,33]. The antigen is expressed at lower levels on chronic lymphocytic leukemia (CLL) and plasma cells [31]. CD20 is not shed from the surface of CD20+ cells after antibody binding and it is not internalized or downregulated [34]. Compared with its murine counterpart, Rituximab has a longer half-life in humans, interacts with human effector cells [34, 35], and is less immunogenic [36].

Reduced FC doses

There are only a few published reports (including relatively small numbers of CLL/SLL patients which describe the results of an FC regimen in elderly CLL patients-(≥ 70 years). These are listed in Table1.

2.2 Use of Rituximab and Combination of Rituximab and Fludarabine in CLL

Although initial responses to single agent treatment with Rituximab were low and partial responses and not complete remissions, interest continued in the use of this agent in CLL [37,39,40]. The original studies employed 375 mg/m^2 of Rituximab once a week for four weeks. Subsequent studies suggested using this dose three times a week for four weeks for a total of 12 doses instead of 4 doses which resulted in better overall responses [41]. Dose limiting toxicities of Rituximab have not been demonstrated perhaps due to the cost of the drug. In this respect it should be noted that in the initial studies using Rituximab to treat follicular NHL, responses were much higher than in CLL. This was attributed to the lower level of CD20 expression on CLL lymphocytes and the presence of circulating CD20 antigen. These differences were partially overcome when either the dose of Rituximab was increased or the frequency of the standard dose of 375 mg/m2 was increased [41, 44].

After establishing the safety and efficacy of standard dose Rituximab 375 mg/m2 three times a week for four weeks for a total of twelve doses, Byrd et al went on, to compare sequential and concurrent therapy with RF [43].

The concurrent group was therefore treated with 11 doses of Rituximab while the sequential cohort only received four doses. This was a randomized phase II trial of concurrent versus sequential Fludarabine and Rituximab with 51 and 53 patients per arm, respectively. The overall response rate in the concurrent arm was 90%, including CR in 47%while in the sequential arm the overall response rate was 77%, with CR in 28%. The disease-free and overall median survival has yet to be reached yet, but will be in excess of 23 months [44]. Three major side effects were observed: infusion related toxicity, myelosuppression, and infections. Infusion related toxicity were greatest in concurrent treated patients (20%) and almost not existent in the sequentially treated patients. In a phase II study, the German CLL Study Group (GCLLSG) [44] reported the treatment of 20 untreated and 11 relapsed CLL patients. Treatment consisted of Fludarabine administered at standard doses

(25mg/m2/d; days 1-5, 29-33, 57-61, 85-89) and Rituximab (375mg/m2/d) given on days 57, 85. The overall response rate (CR and PR) was 87% with a CR/CRu of 33%. The median duration of response was 75 weeks. Severe anemia (grade III/IV) was observed in 10% of patients, neutropenia in 42%, and thrombocytopenia in 9%, respectively. The most frequent non-hematologic side effects were infections (52%) and infusion-related symptoms. The majority of infections were respiratory and herpes virus infections.

A retrospective comparative analysis of the CALGB 9712 [47] (Rituximab plus Fludarabine) [48] and the CALGB 9011 (Fludarabine only) studies demonstrated that the addition of Rituximab to Fludarabine resulted in a significantly better 2 years progression free survival and overall survival compared to patients treated with Fludarabine alone (67% *Vs* 45% and 93% *Vs* 81%, respectively) and in both treatment approaches the infectious toxicity was similar [45].

The MD Anderson Cancer Center group reported a single arm study that utilized the combination of Rituximab, Fludarabine and Cyclophosphamide (FCR) in untreated and in relapsed and refractory CLL patients (224 and 177 patients, respectively) [24, 25]. In the untreated patients the overall response rate was 95% with a CR rate of 70%, a nodular PR rate of 10% and a PR rate of 15%. Although, during the follow-up period the time to treatment was not reached, a calculated analysis showed that 69% of patients were projected to relapse at 4 years. The major toxicities were myelosupression, predominantly of grade 3 and 4, neutropenia (in 52% of all courses) and infections.

In the relapsed and refractory patients the overall response was 73% with 25% CR, 16% nodular PR and 32% PR and a third of the complete responders achieved molecular remission. The median time to progression in responding patients was 28 months with an overall median survival time of 42 months. About 46% of the patients completed all six intended courses and myelosupression was the major cause for discontinuing treatment (66% and 18% grade 3-4 neutropenia and thrombocytopenia, respectively) with 16 % of the patients having major infection.

Recently, two pivotal phase III studies (CLL8 and REACH) [49,50] evaluated 6 cycles of rituximab (375mg/m^2 on cycle-1 and 500mg/m^2 on cycle 2-6) plus fludarabine (25mg/m^2 d1-3) and cyclophosphamide (250mg/m^2 d1-3) chemotherapy compared with 6 cycles of fludarabine and cyclophosphamide alone. The primary endpoint of both studies was progression-free survival. Secondary endpoints for both studies included overall survival, event-free survival, duration of response, response rate and complete response. CLL8 involved 817 patients with previously untreated (first-line) CLL and good physical fitness. With a median observation time of 37.7 months, statistically significant differences were observed in OS between the two treatment arms. The OS rate was 84.1% in the FCR arm versus 79 % in the FC arm (p=0.01). In both arms, the median OS has still not been reached. Patients who received rituximab together with chemotherapy had a median progression free survival (PFS) of 51.8 months compared to 32.3 months for those who received chemotherapy alone. (hazard ratio 0.56; p < 0.001). The overall response rate in patients receiving rituximab plus chemotherapy as first-line therapy was 95.1% vs. 88.4% in those receiving chemotherapy alone and with more complete remissions in the FCR arm (44.1 vs 21.8%; p<0.001). Severe (grade 3 or greater) adverse events that occurred more often in the rituximab arm included haematologic toxicity (56% vs. 39%), neutropenia (34% vs. 21%) and leukocytopenia (24% vs. 12%).

The REACH study[49] involved 552 patients with previously treated CLL (second line therapy). Patients who received rituximab plus chemotherapy had a median PFS of 30.6 months compared to 20.6 months for those who received chemotherapy alone (hazard ratio 0.65; p=0.0001). The overall response rate in the Rituximab-containing arm was 70% vs. 58% in those receiving chemotherapy alone without Rituximab. Grade 3 or greater events such as neutropenia (42% vs. 40%), febrile neutropenia (12% vs. 12%) and neoplasms (7% vs. 3%) occurred more often in the rituximab plus chemotherapy arm. Based on the CLL8 and REACH results the FDA approved the use of rituximab as first- and second-line treatment of CLL. In Europe, Rituximab is also approved for treatment of first and relapsing CLL patients.

In the light of the above literature, the combined use of Rituximab and Fludarabine and cyclophosphamide (FCR) is currently regarded as the gold standard and backbone treatment for physically fit patients with CLL.

2.3 Fludarabine Cyclophosphamide Rituximab (FCR) regimens for elderly patients with CLL

Due to major concerns of myleotoxicity and infection complications, this chemo-immunotherapy combination regimen cannot be applied safely to all elderly CLL patients. To address this topic efforts are being made to establish novel FCR-based protocol that will be useful for the average elderly CLL patient. In this regard, Foon and colleagues [50] reported a phase II single arm study of reduced dosages of Fludarabine and Cyclophosphamide (FCR-lite). Fifty untreated CLL patients were treated with 6 cycles of Fludarabine ($20mg/m^2$) and Cyclophosphamide ($150mg/mg^2$) combined with high–dose Rituximab ($500mg/m^2$) given every other week during induction and later as maintenance every 3 months for two years. The OR and CR rates were 100% and 79%, respectively. Median duration of complete response was 22.3 months (range, 5.2 to 42.5 months) and none of the complete responders have relapsed. Grade 3/4 neutropenia was noted in 13% of the cycles of therapy. Another approach to reduce toxicity was to use sequential therapy with fludarabine (25 mg/m^2 on days 1 through 5 for 6 cycles) followed by consolidation with Cyclophosphamide (3,000 mg/m^2 administered every 3 weeks for three cycles) and then by Rituximab 375 mg/m^2 for four cycles (F→C→R). [51] In 36 previously untreated CLL patients an overall response of 89% was achieved including 61% CRs. 33% achieved a molecular CR (PCR negative) and achieving molecular CRs had an excellent prognosis with a plateau in the response duration curve, and 90% remain in clinical CR at 5 years. For the entire group, 5-year survival rate is 71% compared with a rate of 48% with the prior F→C regimen (not significant $P = .10$).

3. Trial Objectives and Purpose

The aim of this study is to evaluate the safety and efficacy of a combination of low dose Fludarabine (FLU) and Cyclophosphamide (CY) combined with standard dose Rituximab given as primary treatment for elderly patients with chronic lymphocytic leukemia.

The purpose of the study is to determine whether a lower dose FC combined with standard dose Rituximab regimen in elderly CLL patients is as effective as the standard FCR dose, given in most studies until now and at the same time to assess if it is indeed less toxic.

3.1 Primary Objective

To evaluate the efficacy (Response Rate) of the combination of lower dose Fludarabine 12.5 mg m^2/d given intravenously for three days and Cyclophosphamide 150 mg/d given intravenously for three days every 28 days, combined with standard dose Rituximab (375mg/ m^2 in cycle 1 and 500 mg/m^2 in cycles 2-6) for a total of 6 cycles.

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3.2 Secondary Objectives

1- Safety of the combination drugs given in the study population, especially in relation to: neutropenic fever, Infection rate, number of hospitalization days, thrombocytopenia ≥grade 3, neutropenia ≥ grade 3.

2- Progression Free Survival (PFS).

3- Time to Next Treatment (TNT).

4- Quality Of Life (FACIT)

4. Trial Design

4.1 Primary and secondary end points

Primary End Point

Overall response rate (ORR) (CR and PR) as defined in the protocol (see appendix I)

4.1.1 Secondary End Points

1- Safety of the study drugs especially in relation to: neutropenic fever, Infection rate and number of hospitalization days,

thrombocytopenia ≥grade 3, neutropenia ≥grade 3.

2- Progression free survival (PFS) defined as the time elapsed from first day of study drug administration to disease progression as defined in the protocol (see appendix I)

3- Time to Next Treatment (TNT) as defined in the protocol (see appendix I)

4- QOL(see appendix VI)

4.2 Overall study design

This is a national, multicenter, phase II, single arm (not controlled), open label clinical trial to evaluate the efficacy and safety of the combination of low dose Flu + Cy combined with standard dose Rituximab Flu dosage will be given at 12.5mg/m^2/d given I.V. together with CY150 mg/m^2/d given I.V. for three days every 28 days. Rituximab will be given before chemotherapy
cycle 1: 375 mg/m^2 I.V. on day 0 and in cycles 2-6: 500 mg/m^2 I.V. on day 1 in previously untreated CLL patients who are more than 65 years of age. Patients will be treated every 28 days for up to 6 cycles. Potential study subjects will sign an informed consent form prior to undergoing any study related procedure. In general, study medications should be continued until disease progression or drug intolerance but no more than 6 cycles. Treatment choices after completion of the study are at the investigators' discretion.
Up to 40 patients are planned to be enrolled during a two year period.
This study consists of 3 periods for each study subject: A pre-treatment period, the treatment period and a follow up period.

Pre-treatment period:

Patients will undergo screening for protocol eligibility within 28 days (4 weeks) of recruitment.

Subjects who meet all the inclusion criteria and who have signed an approved informed consent will be enrolled.

Written informed consent must be obtained before any study - specific medical procedures are performed which include laboratory screening assessments and physical examination as well as documentation of vital signs. If the hematology and/or biochemistry evaluations have been performed within 14 days of the first dose of study drug as part of the screening evaluation, they need not be repeated, and the data may be used as the Day 1 values. Bone marrow examination including cytogenetics (FISH whenever possible) must have been performed within 12 months of starting the first dose of the study drugs.

CT scan when done, must be performed within 6 weeks of starting the first dose of study drugs.

Treatment period

Includes 6 months of combination treatment with low dose Fludarabine and Cyclophosphamide combined with standard dose of Rituximab. Flu dosage will be given at 12.5mg/m^2/d given I.V. together with CY150 mg/m^2/d I.V. for three days every 28 days. Rituximab given before chemotherapy cycle 1: 375 mg/m^2 I.V. on day 0 and in cycles 2-6: 500 mg/m^2 intravenously on day 1 every 28 days.

Premedication: antihistamines(e.g. 2mg clemastin or promethazine 25mg I.V.) and paracetamol (500-1,000 mg orally) 30 minutes before first infusion of Rituximab, and thereafter only if clinically indicated. Hydrocortisone 100 - 500mg I.V.(according with local common practice only if clinically indicated.

In the event of grade $\geq$ 3 hematological toxicity which in the investigators' opinion, is study drugs related, the administration of the next cycle should be delayed but not for more than 3 weeks and drug dose reduction of 25% of

both CY and F is recommended. Two dose reductions are permitted (FLU and CY Dose modification page 29). In cases where the delay period has been terminated but grade 3-4 toxicity persists, the patient should be removed from the protocol with no further study drug administration.

During the treatment period, all patients will attend periodic monthly study-center visits in order to assess safety and efficacy of the treatment.

Complete blood counts will be performed on day 1 and 14 of the first two cycles. In the next 4 cycles- on day 1 of each cycle, or more frequently at the investigators' discretion.

Bone marrow biopsy and aspiration examination will be performed before the start of treatment, for response documentation (3 to 6 months after the completion of treatment), for evaluation of cytopenias (disease or treatment related) and for any other clinical indication at the discretion of the investigators.

Supportive care including blood transfusions or administration of growth factors G-CSF or erythropoietin is permitted at the investigators' discretion throughout all study periods.

Follow up period

During the follow up period, of 30 months after the completion of the study drugs administration, all patients will be assessed every 3 months for evidence of progression and documentation of survival at clinic visits.

Complete blood count will be performed every 3 months, or more frequently at the investigators' discretion.

Bone marrow examination including cytogenetics, peripheral blood immunophenotyping (FACS), abdominal, pelvic and chest CT will be performed 3 to 6 months after the completion of the treatment regimen, at clinical progression and at any other time according to the investigators' discretion.

In the event of no response (no CR nor PR) in > 5 of the initial 10 subjects enrolled into the study, Fludarabine dosage will be increased for all the next

subjects enrolled into the study and will be given at the dose of 15mg/m^2/d given I.V.

In the event of no response after the above increment in > 5 of the next 10 subjects who received the increased dosages, the study will be terminated and declared inadequate and inefficient. If response is evident in ≥ 5 of the 10 patients, the same dosages will be continued throughout the study until its completion.

All further treatment decisions after completion of the study are at the discretion of the investigators.

4.2.1 Visit schedule and assessments

Visit schedule

Patient must be followed at the study center according to the visit schedule and assessments outlined in Figure 1.

All routine assessments must be performed within a time window of ± 7 days of the day indicated on the visit schedule, except visits 2-5 in which time window will be of ± 3 days .

Figure 1: Visit schedule and assessments

Week	-4 to 0	1	3	5	7	9-25 Every 4 weeks	29	37	49	61-77 every 12 weeks	89	101-161 every 12 weeks
Visit No.	**1**	**2**	**3**	**4**	**5**	**6-10**	**11**	**12**	**13**	**10-13**	**14**	**15-19**
Study period	preTx	**Treatment period**						**Follow up period**				
Inc. /Excl. criteria	X											
Informed consent	X											
Relevant medical history/conditions	X											
Disease history	X											
BMB*	X								X			
Physical exam	X	X	X	X	X	X	X	X	X	X	X	X
CBC	X	X	X	X	X	X	X	X	X	X	X	X
Blood chemistry	X	X		X		X	X	X	X	X	X	X
Beta-2 micro glob	X											
IEP, Ig level,	X								X			
DAT***	X								X			
FACS****	X								X			
Hepatitis B *****	X											
Imaging - (CT) **	X								X			
QOL	X						X				X	
Treatment dosage	X	Ongoing data capture										
Adverse Events	X	Ongoing data capture and up to 30 days after completion or discontinuati the study										
Concomitant medications	X	Ongoing data capture										
Comments	X	Ongoing data capture										
Study completion	X	Complete at any time if study drug is discontinued										

* Bone marrow examination will be performed at any clinically suspicion of progression/ leukemia transformation, cytopenia evaluation, or for any CR confirmation at investigator discretion.

** Abdominal, pelvic and chest CT will be performed at any clinical suspicion of progression.

*** DAT will be performed at any clinical suspicion of hemolysis.

**** Will be performed at any clinical suspicion of progression/ leukemia transformation, or for CR confirmation at investigators' discretion.

***** At screening and at investigator discretion for hepatitis treatment evaluation.

4.3 Trial treatment

4.3.1 Investigational therapy

Fludarabine is supplied to the study investigators by the Medical Health Organization (MHO) as routine first line treatment in all CLL patients. Fludarabine is supplied as 10 mg tablets packaged in blister packs, or as 50 mg/vial, as lyophilized powder that has to be dissolved in 2 Ml of water for injection, than diluted in 100 ml of saline (0/9%) given in 30 minutes.

Cyclophosphamide is supplied to the study investigators by the MHO as a routine first line treatment in CLL patients. Cyclophosphamide (Endoxan) is supplied as 50 mg tablets packed in blister packs or as 500 mg/vial (24 mg/ml) diluted in 100 ml of saline (0/9%) given in 30 minutes.

Rituximab is supplied to the study investigators by the MHO as a routine first line treatment in CLL patients. Rituximab (Mabthera) is supplied as 100mg of rituximab in 10ml (10mg/ml) pack of 2 vials or 500mg of rituximab in 50ml (10mg/ml) pack of 1 vial. The antibody should be diluted into a final volume of normal saline or 5% D-glucose in water to allow for a maximal concentration of 4 mg/ml. Rituximab should be administrated before FC chemotherapy.

First Infusion: Recommended initial rate is 50mg/hr; after the first 30 minutes increase by 50mg/hr every 30 minutes to maximum of 400 mg/hr. *Subsequent Infusions:* Initial rate 100mg/hr; increase by 100mg/hr every 30 minutes to a maximum of 400mg/hr

Use extreme caution and closely monitor first infusion when treating patients with ≥ $25x10^9$/l circulating malignant cells or high tumor burden (higher risk of severe cytokine release syndrome (CRS). It is recommended to administer Hydrocortisone 100-500 mg I.V. (according local common practice) shortly before infusion with Rituximab to decrease the rate and severity of acute IRR/CRS for these patients. consider reduced rate for first infusion or a split dosing over two days during the first cycle. Severe CRS: may be associated with some features of tumor lysis syndrome e.g. hyperuricaemia, hyperkalaemia, hypocalcaemia, hypophosphataemia, acute renal failure, elevated LDH and may be associated with acute respiratory failure and death.

If severe CRS manifests stop infusion immediately and start aggressive symptomatic treatment.

If the first dose of rituximab is well tolerated, the starting flow rate for administration of the second and subsequent infusions will be 100 mg/hour and then increased gradually by 100 mg/hour intervals not to exceed 400 mg/hour.

If any grade 1 or 2 infusion related reactions occur during an infusion, the infusion should be slowed or interrupted (at the discretion of the Investigator) and supportive treatment instituted. The infusion rate can be increased or restarted on resolution of the symptoms.

If grade 3 or 4 infusion related reactions occur during an infusion, the infusion has to be discontinued immediately and not restarted until resolution of the symptoms. Treatment can be reinitiated (at half the original infusion rate) at the discretion of the Investigator, but if the same adverse event appears again with the same severity, treatment must be permanently discontinued.

Should bronchospasm or dyspnea occur in the patient during the infusion, the infusion should be stopped immediately. Medication such as anti-histamines or steroids should be given for symptomatic relief. The infusion should not be re-started until symptoms have resolved completely and should be given at half the original infusion rate.

In the event of infusion related reactions to rituximab:

• Stop rituximab infusion, treat symptoms aggressively if necessary

• NaCl 0.9 % i.v., if necessary

• Hydrocortisone 100-500 mg I.V. (according local common practice), if necessary

• Pethidine i.v. if necessary

• Bronchodilators/antihistamines if necessary

• If side effects occur stop Rituximab-infusion, re-try the next day

Table 2: Reduction of Infusion Rate

Drop in systolic blood	Mucosal congestion, edema	Rigors	Fever	Infusion rate

> 30 mm Hg	Mild/moderate	Mild/moderate	> 38.5°C	Decrease to ½ if any of these events are seen

Following the infusion the I.V. line should be kept open for medications as needed. If there are no complications, the line may be discontinued after one hour of observation. If complications occur during the rituximab infusion, the patient should be observed for 2 hours after the completion of the infusion.

Infusion reactions to rituximab tend to be more common and more severe with the first infusion. In patients who were unable to tolerate the first infusion, cautious reintroduction of rituximab may be tried at the second cycle, at the investigator's discretion. The recommended premedication should be given and then the patient carefully re-exposed to a small amount of rituximab (slow infusion of 10 mg I.V.) If a severe reaction occurs, the rituximab must be permanently discontinued. In such case patient will be treated with FC in the next cycles and data will be collected throughout the study period)

If no severe reaction occurs after 30 minutes, the full dose of rituximab infusion should be given.

Labels will comply with the legal requirements in Israel and be printed in Hebrew and English. All study drugs containing infusion bags will be labeled. Rituximab containing infusion bags will be labeled by the sponsor and collected for drug accountability. Storage conditions for study drug will be described on the medication label. Patients will receive the study drug combination for an exposure period of up to 6 months provided that in the opinion of the investigator the patient is benefiting from treatment with this study drug combination, and in the absence of any safety concerns. Treatment will be withheld only in the case of limiting toxicities (see below).

As administration of cyclophosphamide may cause hemorrhagic cystitis and even urinary bladder malignancy, patients will be instructed to drink at least 10 glasses of fluids on the days they receive cyclophosphamide and urinate at minimal ceasing of bladder distention.

DRUG ACCOUNTABILITY

Accountability and patient compliance will be assessed by maintaining adequate "drug dispensing log" on Rituximab.

The "drug dispensing" log for Rituximab must be kept current and should contain the following information:

- The identification of the patient (patient number) to whom Rituximab was administered.
- The date(s) and quantity of Rituximab, used for the patients' treatment.
- The date(s) and quantity of Rituximab, of empty, partial or unused medication.

All accountability logs must be available for inspection by the monitor. Drug supplies (partly used and empty Rituximab infusion bags) must be available for inspection, at every monitoring visit. Used supplies will be destructed according to local site guidelines.

4.3.2 Dose modification for hematological toxicity

Dose modifications will not be made during the first cycle of therapy. During the flowing cycles modifications of the treatment schedule will only be made as follows:

• If at day 1 of any cycle, there is a grade ≥ 3 cytopenia (See hematological toxicity section 8), NOT RELATED TO BONE MARROW INFILTRATION DUE TO CLL, treatment should be delayed for up to three weeks and then given with a 25% dose reduction of fludarabine and cyclophosphamide in the following cycles. There is no dose reduction for Rituximab in this study, but drug administration will be delayed for the same time period as for fludarabine and cyclophosphamide.

• If after three weeks, the grade ≥ 3 cytopenia, NOT RELATED TO CLL BONE MARROW INFILTRATION, still prevails, the patient should be removed from the protocol with no further study drug administration.

• If there is further grade ≥ 3 cytopenia in subsequent cycles, despite the first 25% dose reduction treatment should again be delayed for up to three weeks and the dose of fludarabine and cyclophosphamide further reduced to 50% of the full initial dose.

• If there is further grade ≥ 3 cytopenia in subsequent cycles despite the 50% drugs dose reduction the patient should be removed from the protocol with no further treatment.
• If there is any grade ≥ 3 neutropenia with infection during any cycle, G-CSF should be administered and G-CSF should than be given in all subsequent cycles prophylactically.

4.3.3 Dose modification for impaired renal function

Fludarabine is partly (40-60%) excreted by the kidneys. If the calculated (see appendix III) or measured creatinine clearance is reduced to 30-60 ml/min, the fludarabine dose should be reduced to 50%. Patients with a creatinine clearance below 30 ml/min are excluded from the study. Patients who develop renal dysfunction (serum creatinine ≥ 1.5 mg/dL or creatinine clearance < 30 ml/min) during treatment should go off protocol treatment.

4.3.4 Dose modification for other non-hematological toxicity (non renal).

• If other grade ≥ 3 non-hematological, non-renal toxicity occurs during any cycle, study drugs treatment should be delayed until recovery to grade ≤ 2, for up to three weeks. All subsequent cycles should then be given with a 25% dose reduction of fludarabine and cyclophosphamide.
• If after three weeks, the grade ≥ 3 non-hematological, non-renal toxicity still persists, the patient should be removed from the protocol .
• If there is further grade ≥ 3 non-hematological, non-renal toxicity in subsequent cycles despite the first 25% dose reduction treatment should again be delayed for up to three weeks and the dose of fludarabine and cyclophosphamide further reduced to 50% of the initial full dose.
• If there is further grade ≥ 3 non-hematological, non-renal toxicity in subsequent cycles despite 50% dose reduction the patient should be removed from the protocol treatment and the study.

4.3.5 Treatment assignment

Informed consent must be obtained before any testing for the purpose of determining a patient's eligibility is performed. Each patient will be assigned a

unique patient number. Once assigned, numbers for any non-evaluable or discontinued patient will not be reutilized.

4.3.6 Blinding

This is an open label trial.

4.4 Study drug interruption or discontinuation

4.4.1 Discontinuation of study drugs

Events that will be considered as reasons for study drugs discontinuation are described in section 5.3

In addition, patient follow-up information must still be recorded (also in the event of study drug discontinuation) during and at the end of the study period.

4.4.2 Study Discontinuation

Events that will be considered as reasons for study discontinuation are described in section 5.3

4.5 Accountability procedures for the investigational products

Study drug will be prepared and dispensed by the pharmacist at the investigator's institution.

5. Selection and Withdrawal of Subjects

5.1 Inclusion Criteria

1. Diagnosis of B-CLL is established by the presence of: an absolute lymphocyte count > $5X10^9$ L, and typical CLL cell morphology in the peripheral blood smear as seen by light microscopy. CLL cells must show CD5/CD19/CD23 positivity with light chain restriction as recorded by flow cytometry. The levels of surface immunoglobulin and CD 79b are characteristically low and FMC7 is expected to be negative (optional).
2. Previously untreated B-CLL patients ≥ 65 years old.
3. Patients requiring treatment (active Binet classification stage A and B, all stage C disease).(appendixes IV and V)
4. Written informed consent

5.2 Exclusion Criteria

1. Calculated or measured creatinine clearance < 30 ml/min
2. Hepatic enzymes or bilirubin ≥ 2X ULN, unless thought to be due to CLL.
3. Suspected or documented CNS involvement by CLL
4. Known HIV positivity
5. Active or uncontrolled infections, including opportunistic infection and active hepatitis B / C
6. Positive Hepatitis B Surface Ag or Hepatitis B Core Ab
7. Various concomitant diseases requiring chronic steroid administration
8. Active Coomb's positive hemolytic anemia.
9. Prior treatment for CLL
10. CLL in transformation (Richter syndrome).
11. Presence of active second neoplasia (excluding non-melanoma skin cancer, or in situ cervix carcinoma or breast carcinoma) < 2 years prior to the study.

12. Use of other investigational agents or participation in any other clinical trials < 30 days prior to the study.
13. Low compliance.
14. Performance status: ECOG performance status ≥4. (see appendix II)
15. Patients who are mentally or physically unable to comply with all aspects of the study.

5.3 Interruption or Discontinuation of Treatment

5.3.1 Discontinuation of study drugs

The following events will be considered as reasons for discontinuation.

- Interruption of study drug/s for longer than 3 consecutive weeks.
- Adverse events, including study drugs induced active autoimmune haemolytic anemia.
- Patient with large cell transformation (Richter syndrome).
- Disease progression as defined at any time during study drug therapy.
- Treatment with other chemotherapeutic or investigational anti-neoplastic drugs
- Intolerable adverse effects that are judged by the investigator to be either physically or psychologically detrimental to the patient. Unresolved or recurrent (more than twice) grade 3 or 4 toxicity.
- The reason for discontinuation should be recorded in the CRF and in the subject's medical records. The sponsor is to be notified of all discontinuations related to study medication.
- In addition, patient follow-up information will be recorded (also in the event of study drug discontinuation) during and at the end of the study period.
- In the case of a severe reaction to Rituximab, Rituximab will be permanently discontinued. Patient will be treated with FC in the next cycles and data will be recorded throughout the study period.

5.3.2 Interruption of study drugs

In the event of grade 3 or 4 toxicity, or increase of creatinine>1.5 or GFR of less than 30ml/min study drug will be interrupted for no more than 3 weeks (see sections 4.3.2-4.3.4)

5.4 Study Discontinuation

The following events are considered as study discontinuation:

- Death
- Participation in another investigational drug trial
- Loss to follow-up
- Patient withdrawal of consent
- Major violation of the study protocol- such as eligibility criteria.
  In these cases, no patient follow-up will be recorded.
- Administrative problems
- In the event of no response after dosage increment in > 5 of the next 10 subjects who received the increased dosage.(see chapter 4.2).

The section for “Study Completion” in the CRF must be completed for all patients. The reason for early discontinuation should be given, even if the patient refuses to return for a final visit. Patients who discontinue prematurely due to significant study drug related adverse events (AEs) should continue to be followed until resolution of the AE or up to 30 days after discontinuation. The relevant sections of the CRF should be completed as appropriate.

Patients who discontinue the study drugs for any reason should be scheduled for a visit within 30 days from drug discontinuation at which time all the assessments listed for the final visit should be performed (see figure 1).

Patients lost to follow up should be recorded as such on the CRF. Patients who discontinue study treatment should be followed for tumor assessments as per standard of care, until disease progression or new cancer therapy is initiated. If patients refuse to return for these visits or are unable to do so, the subject should be considered off-study, but a Study Evaluation Completion form should be completed.

6. Treatment of Subjects

6.1 Treatment regimen

Patients will receive FLU , CY and Rituximab combination for an exposure period of up to 6 months- defined as the treatment period. Dosage, dose regimen and route of administration are detailed in 4.3-4.4

6.1.1 Treatment assignment

Informed consent must be obtained before any testing for the purpose of determining a patient's eligibility is performed. Each patient will be assigned a unique patient number. Once assigned, numbers for any non-evaluable or discontinued patient will not be reused.

6.2 Concomitant therapy

In general, concomitant medications and therapies deemed necessary for the supportive care and safety of the patient are permitted in this study, provided their use is documented in the patient records and on the appropriate Case Report Form. The administration of any anticancer agents including chemotherapy and biologic agents is NOT permitted. Similarly, the use of other concurrent investigational drugs is not allowed. The use of blood products (irradiated) transfusions, immunoglobulins or erythroid/myeloid growth factors are permitted at investigator's discretion.

Transfusion-associated graft versus host disease prevention using irradiated blood products is mandatory.

Steroids administration are allowed exclusively for patients with autoimmune hemolytic anemia and or autoimmune thrombocytopenia or as premedication for Rituximab .

Premedication: Any patient considered to be at risk of infusion related reaction (IRR) or TLS (=patients with lymphocytosis > 25,000mm^3) should receive:

Appropriate hydration starting 12-24 hours prior to initiating treatment, and thereafter until the risk of IRR/TLS is ruled out. Urine alkalization is at investigator's discretion.

Antihistamines (e.g. 2 mg clemastin or promethazine 25mg i.v.) and paracetamol (500-1,000 mg orally) 30 min before first infusion of Rituximab, and thereafter only if clinically indicated. Hydrocortisone 100-500 mg I.V. (according local common practice) should be given only if clinically indicated (increased risk of IRR).

Pneumocystis carinii pneumonitis prophylaxis with trimethoprim 160mg-sulfamethoxasole 800 mg twice a day, twice a week, for the treatment period and for the next 6 months of follow up are recommended. For prevention of hepatitis B reactivation that needs antiviral treatment Lamivudine 100mg/day will be administrated for the entire duration of chemotherapy and for the six months afterwards.

Hepatitis B serology will be: HBsAg+, Anti HBcore and anti-HBsAg

Treatment is recommended for: HBsAg+ (carriers), or HBVsAg (-), anti HBcore (+) and anti-HBVsAg (-).

Any use of concomitant medication must be captured in the concomitant medication CRF. Administration of Blood products must also be captured as concomitant medication on CRF.

Steroids if given (see above) are allowed for no more than 3 consecutive days. Excluding patients with autoimmune cytopenia, in which case prolonged period steroid administration is permitted (see above).

6.3 Treatment compliance

Number of FLU and CY vials administrated I.V to every subject in every study site, will be captured on CRF.

Number of Rituximab containing infusion bags administrated to every subject in every study site, will be captured on CRF

7. Assessment of efficacy

The primary efficacy parameter to be assessed will be response rate.

Response rate include CR and PR; all others (eg. stable disease, non response, progressive disease or death from any cause should be rated as treatment failure.)

An overall objective assessment of all measurable and non-measurable response parameters will be performed according to the Visit Schedules (see Figure 1).

Response rate will be assessed according the updated (2008) NCI-WG guidelines defined in appendix I: [46].

- Peripheral blood immunophenotyping by FACS for absence of clonal lymphocytes**.**
- Physical examination - lymph node examination and measurements for absence of significant (>1.5 cm in diameter), as well as the presence of hepatomegaly and splenomegaly.
- CT scan for CLL patients with a CT found to be abnormal before therapy or alternatively if physical examination is inconclusive.
- Complete blood count for ANC, platelets and hemoglobin levels. Blood counts have to be above the following values: ANC> 1.5 X 10^9/L. platelets > 100X 10^9/L. Hemoglobin > 11.0 g/dL.
- Bone marrow aspiration and biopsy to assess degree of bone marrow involvement by CLL using FACS and immunohistochemistry, should be performed at least 3 months after the last treatment if the above 4 clinical and laboratory results demonstrate that CR has been achieved. In some cases, it is necessary to postpone BMB until after all the other criteria used to define a CR have been satisfied but, this time interval should not exceed 6 months after the last treatment.
- Patient interview for the presence of constitutional symptoms throughout the study.

A single laboratory should perform all evaluations for all patients recruited in the same site. All the above studies must account for disease parameters that

were present at baseline and must use the same techniques as used at baseline. All the above mentioned assessments should be performed within 7 days of the scheduled day of assessment (see Figure 1).

All above mentioned efficacy (response) parameters will be performed at screening and at least 3 months after completing study drug administration for response documentation.

Bone marrow examination can be postponed until after the above clinical and laboratory examinations used to define a CR have been satisfied. However, this time interval should not exceed 6 months after the last treatment.

All known disease (measurable, and non measurable) parameters must be accounted for when assessing objective response rate. Current laboratory and physical examination results will be captured on the response forms in the CRF.

Progression free survival is defined as the interval from the first study drug treatment day to the first sign of disease progression.

Progressive disease (PD) will be assessed according the updated (2008) NCI-WG guidelines defined in appendix I. If needed, BMB will be performed for suspected progression at the investigator discretion. Progression will be captured on the response form CRF.

Time to next treatment is defined as the interval between the first study drug treatment day to the first day of starting the next treatment.

In general, second-line treatment decisions follow the same indications as those used for initiation of first-line treatment.

Re-treatment day will be captured on the treatment form CRF.
QOL questionnaires (FACIT) will be completed by study subjects at:
screening visit and at visits number: 11,14 and at the end of study.
Questionnaires will be captured on the QOL forms CRF.
CT Scan – In order to prevent contrast nephropathy, patients with GFR < 60ml/min or diabetes mellitus, hydration is recommended and if possible acetylcystein (600 mg in bolus pre CT and 1,200 mg orally twice a day for 48 hours after CT) should be given . Hospitalization for this procedure is recommended.

8. Assessment of Safety

Safety assessments will consist of evaluating adverse events and serious adverse events, laboratory parameters including hematology, chemistry, vital signs, physical examinations, and documentation of all concomitant medications and/or therapies.

8.1 Adverse events

According to the International Conference of Harmonization [ICH], an AE is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign [including an abnormal laboratory finding], symptom, or disease temporally associated with the use of a medicinal [investigational] product, whether or not considered related to the medicinal [investigational] product. Pre-existing conditions which worsen during a study are to be reported as AEs.

Information about all adverse events, whether volunteered by the patient, discovered by investigator questioning, or detected through physical examination, laboratory test or other means, will be collected and recorded on the Adverse Event Case Report Form and followed as appropriate. An adverse event is any undesirable sign, symptom or medical condition occurring after starting study treatment, even if the event is not considered to be treatment-related.

Medical conditions/diseases present before starting study treatment are only considered adverse events if they worsen after starting study treatment. Clinical events occurring before starting study treatment but after signing the informed consent form are recorded on the Medical History/Current Medical Conditions Case Report Form only if the patient receives study treatment. Abnormal laboratory values or test results constitute adverse events only if they induce clinical signs or symptoms or require therapy, when they are recorded on the Adverse Events Case Report Form under the signs, symptoms or diagnosis associated with them.

8.1.2 Grading and reporting of adverse events

All adverse events will be graded according the NCI-CTC scale version 3.0 Anemia and thrombocytopenia should be graded according to NCI-CTC scale version 2.0- the criteria for patients in leukemia studies (see assessment of toxicity below for details), and reported in detail as indicated on the CRF

Adverse events not listed on the CTCAE should be graded as follows:

Definition	Equivalent To:	CTC Grade
Discomfort noticed but no disruption of normal daily activity	Mild	Grade 1
Discomfort sufficient to reduce or affect daily activity; no treatment or medical intervention is indicated although this could improve the overall well-being or symptoms of the subject	Moderate	Grade 2
Inability to work or perform normal daily activity; treatment or medical intervention is indicated in order to improve the overall well-being or symptoms; delaying the onset of treatment is not putting the survival of the subject at direct risk.	Severe	Grade 3
An immediate threat to life or leading to a permanent mental or physical conditions that prevents work or performing normal daily activities; treatment or medical intervention is required in order to maintain survival.	Life threatening/ disabling	Grade 4
AE resulting in death	Death	Grade 5

8.1.3 Assessment of Toxicity

The evaluation of potential treatment-induced toxicity in patients with advanced CLL may be quite difficult requiring careful consideration of both the manifestations of the underlying disease, as well as adverse reactions to the therapy under study. Some of the conventional criteria for toxicity are not applicable especially under circumstances of progressive bone marrow failure from the CLL itself.

8.1.4 Hematological Toxicity

The evaluation of hematological toxicity in patients with CLL must consider the high frequency of hematological compromise already present at the initiation of therapy. Therefore the standard criteria used for solid tumors cannot be applied directly; many patients would be considered to have grade II to IV hematological toxicity at presentation.

As a consequence, **for grading of anemia and thrombocytopenia,** we plan to apply the NCI**-CTC version 2.0 criteria for patients in leukemia studies, but for grading of neutropenia, the standard NCI-CTC version 3.0 criteria,** as given below.

Table 3: NCI-CTC Grading scale for hematological toxicity for patients in this study

ANC mm^3 § (nadir)	Grade	Decrease in platelets*or Hb# from pretreatment value (%).
≥2,000	0	No change-9%
≥1,500 and <2,000	1	10-24%
≥1,000 and <1,500	2	25-49%
≥500 and <1000	3	50-74%
<500	4	≥75%

Grades: 1 = mild, 2 = moderate, 3 = severe, 4 = life-threatening

* if platelets were < 20,000mm^3 [<20 x10^9/L] prior to therapy, the patient is inevaluable for toxicity in platelets

baseline and subsequent Hb determinations must be performed before any given infusion

§ if ANC was < 1,000 prior to therapy, the patient is inevaluable for toxicity in ANC.

Platelets:

If at any level of decrease the platelet count is < 20,000 mm^3, this is considered grade 4 toxicity, unless a severe or life-threatening decrease in the initial platelet count (e.g. < 20,000 mm^3) was present before treatment in which case the patient is inevaluable for toxicity referable to platelet counts.

Hemoglobin:

Baseline and subsequent Hb determinations must be performed before any given infusions.

Fludarabine has been reported to exacerbate or precipitate autoimmune hemolytic anemia and patients should be monitored carefully for this condition. If a rapid decrease of hemoglobin occurs during therapy, the

possibility of fludarabine- or autoantibody-induced hemolysis should be considered and appropriate diagnostic tests (LDH, bilirubin, α2-haptoglobin, reticulocytes, Coombs-test) be performed. **In particular, a Coomb's test should be performed for:**

- **Any grade 3 or 4 anemia (Hb < 8 g/dL)**
- **A Hb < 10 g/dL sustained for > 2 weeks**
- **A drop in Hb of 3 g/dL in one week (while pts are receiving weekly blood tests)**
- **Any patient requiring a blood transfusion**

If, in the judgment of the treating physician, there is evidence of hemolytic anemia secondary to fludarabine, study treatment should be promptly withdrawn. Full details of the hemolytic anemia should be recorded on the adverse event pages of the CRF.

Neutrophils:

Absolute neutrophil count (ANC) is used to assess the toxicity of the myeloid lineage. Other decreases in the white blood cell count, or in circulating lymphocytes, are not to be considered, since a decrease in the white blood cell count is a desired therapeutic end point. If the ANC was < 1,000/ mm^3 [<1 $x10^9$/L] prior to therapy, the patient is inevaluable for toxicity referable to the ANC.

Any Adverse Event occurring by the time of study completion (within four weeks of last drug intake) must be recorded on the Adverse Event CRF page.

8.1.5 Drug-Adverse event relationship

The causality relationship of study drug to the adverse event will be assessed by the investigator as either:

Yes or No

If there is a reasonable suspected causal relationship to the study medication, i.e. there are facts (evidence) or arguments to suggest a causal relationship, drug-event relationship should be assessed as **Yes**.

The following criteria should be considered in order to assess the relationship as **Yes:**

– Reasonable temporal association with drug administration

- It may or may not have been produced by the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject.
- Known response pattern to suspected drug
- Disappears or decreases on cessation or reduction in dose
- Reappears on rechallenge

The following criteria should be considered in order to assess the relationship as **No**:

- It does not follow a reasonable temporal sequence from administration of the drug.
- It may readily have been produced by the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject.
- It does not follow a known pattern of response to the suspected drug.
- It does not reappear or worsen when the drug is readministered.

8.2 Serious adverse events

A serious adverse event is any experience that suggests a significant hazard, contraindication, side effect or precaution. It is any Adverse Event that at any dose fulfils at least one of the following criteria:

- is fatal; (results in **death**; NOTE: death is an outcome, not an event)
- is Life-Threatening (NOTE: the term "Life-Threatening" refers to an event in which the subject was at immediate risk of death at the time of the event; it does not refer to an event which could hypothetically have caused a death had it been more severe).
- required in-patient hospitalization or prolongation of existing hospitalization;
- results in persistent or significant disability/incapacity;
- is a congenital anomaly/birth defect;
- is medically significant or requires intervention to prevent one or other of the outcomes listed above

****The term sudden death should be used only when the cause is of a cardiac origin as per standard definition. The terms death and sudden death are clearly distinct and must not be used interchangeably**.

The study will comply with all local regulatory requirements and adhere to the full requirements of the **ICH Guideline for Clinical Safety Data Management, Definitions and Standards for Expedited Reporting, Topic E2**

Other safety findings may require expedited reporting depending on local legislation, e.g. a major safety finding from a newly completed animal study (such as carcinogenicity).

Progression of underlying malignancy is not reported as an adverse event if it is clearly consistent with the suspected progression of the underlying cancer as defined by RECIST criteria, or other criteria as determined by protocol. Hospitalization due solely to the progression of underlying malignancy should NOT be reported as a serious adverse event. Clinical symptoms of progression may be reported as adverse events if the symptom cannot be determined as exclusively due to the progression of the underlying malignancy, or does not fit the expected pattern of progression for the disease under study.

Symptomatic deterioration may occur in some subjects. In this situation, progression is evident in the subject's clinical symptoms, but is not supported by the tumor measurements. Or, the disease progression is so evident that the investigator may elect not to perform further disease assessments. In such cases, the determination of clinical progression is based on symptomatic deterioration. These determinations should be a rare exception as every effort should be made to document the objective progression of underlying malignancy.

If there is any uncertainty about an adverse event being due only to the disease under study, it should be reported as an AE or SAE.

8.2.1 Reporting of Serious Adverse Events (immediately reportable)

Any clinical adverse event or abnormal laboratory test value that is *serious* and which occurs during the course of the study (as defined in section 7.1.1.3 above), regardless of the treatment arm, occurring from the enrollment visit (start of study screening procedures), including long term follow-up (LTFU) must be reported to Roche **within *one* working day** of the investigator becoming aware of the event (expedited reporting). The investigator must complete the *SAE Reporting Form [redacted]* and forward it to the SAE Responsible. All SAEs occurring from the enrollment period must be reported, (start of study screening procedures),

Related Serious Adverse Events ***MUST*** be collected and reported regardless of the time elapsed from the last study drug administration, even if the study has been closed. Suspected Unexpected Serious Adverse Reactions (SUSARs) are reported to investigators at each site and associated IRB/IEC when the following conditions occur:

- The event must be a SAE.
- There must be a certain degree of probability that the event is an adverse reaction from the administered drug.
- The adverse reaction must be unexpected, that is to say, not foreseen in the SPC text (Summary of Product Characteristics (for an authorized medicinal product) or the Investigator's Brochure (for an unauthorized medicinal product).

When all subjects at a particular site are off treatment as defined by the protocol:

- only individual SUSAR reports originating in that particular trial will be forwarded to the site and associated IRB/IEC on an expedited basis;
- individual SUSARs considered to be a significant safety issue and/or which result in Roche recommending a change to the

Informed Consent Form (ICF), will be reported in an expedited manner to all investigators and IRBs/IECs;

- SUSAR reports originating from other trials using the same IMP will be provided as six monthly SUSAR Reports (SSRs) to investigators and IRBs/IECs where long-term follow-up studies are carried out, unless they are considered significant..

Unrelated Serious Adverse Events must be collected and reported during the study and for up to 30 days after the last dose of study medication.

This study adheres to the definition and reporting requirements of **ICH Guideline for Clinical Safety Data Management, Definitions and Standards for Expedited Reporting, Topic E2.**

8.2.2 Treatment and Follow-up of AEs

During the study period and up to 30 days after completion or discontinuation of the study, continue to follow up AEs as follows:

Related AEs: Follow until one of the following occurs:

- Resolved or improved to baseline
- Relationship is reassessed as unrelated
- Death
- Start of new anti-cancer regimen
- Investigator confirms that no further improvement can be expected
- Clinical or safety data will no longer be collected, or final database closure

Unrelated severe or life threatening AEs: Follow until one of the following occurs:

- Resolved or improved to baseline
- Severity improved to Grade 2
- Death
- Start of new anti-cancer regimen
- Investigator confirms that no further improvement can be expected
- Clinical or safety data will no longer be collected, or final database closure

Unrelated Grade 1 or Grade 2 AEs: Follow up to 30 days after completion or discontinuation of the study.

The final outcome of each adverse event must be recorded on the CRF

8.2.2 Laboratory evaluations

Laboratory test results will be recorded on the laboratory results form of the CRF, or appear on electronically produced laboratory reports submitted directly from the central laboratory, if applicable.

Any laboratory result abnormality fulfilling the criteria for a serious adverse event (SAE) should be reported as such, in addition to being recorded as an AE in the CRF.

Any treatment-emergent abnormal laboratory result which is clinically significant, i.e., meeting one or more of the following conditions, should be recorded as a single diagnosis on the adverse event page in the CRF:

- Accompanied by clinical symptoms
- Leading to a change in study medication (e.g. dose modification, interruption or permanent discontinuation)
- Requiring a change in concomitant therapy (e.g. addition of, interruption of, discontinuation of, or any other change in a concomitant medication, therapy or treatment)

No special laboratory or tests are required to be performed for the purpose of the study. Measurement of Complete Blood Counts, chemistry and immunological tests such as: serum Ig level, serum immunoellectrophoresis, DAT, peripheral and bone marrow FACS are in fact regarded as current clinical practice for CLL patients, and should therefore be collected according to the Visit Schedules (see Figure 1). In case of suspected hemolysis - DAT, reticulocyte count, serum haptoglobin, bilirubin and LDH are recommended.

The institution will perform laboratory analyses according to the Visit Schedules (see Figure 1). The sponsor must be provided with a copy of the laboratory's

certification, and a tabulation of the normal ranges for each parameter required. Additionally, if at any time a patient has laboratory parameters obtained from a different outside laboratory, the sponsor must be provided with a copy of the certification and a tabulation of the normal ranges for that laboratory.

At any time during the study, abnormal laboratory parameters which are clinically relevant (e.g. require dose modification and/or interruption of study drug, lead to clinical symptoms or signs or require therapeutic intervention), whether specifically requested in the protocol or not, must be recorded on the appropriate Comment CRF page in addition to the appropriate laboratory CRF page. When abnormal laboratory values or test results constitute an adverse event (i.e., induces clinical signs/symptoms or requires therapy) they must be recorded on the Adverse Events CRF.

When, in the opinion of the investigator, other clinical laboratory evaluations may be relevant for assessing the patient's status, they will be completed and entered into the database as appropriate.

8.3 Special tests:

PET-CT will be performed in case of suspected Richter transformation.

Lymph node biopsy is recommended for definitive diagnosis of Richter transformation and for any rapidly progressive disease or unusual phenomena which may be suspicious of alteration in the of the disease or suspicious for another disease or a different type of lymphoma.

8.4 Physical examinations/vital signs

A physical examination including vital signs will be performed according to the Visit Schedules (see Figure 1). Information about the physical examination and vital signs must be present in the source documentation at the study site. Significant findings present prior to the start of study drug must be included in the Relevant Medical History/Current Medical Conditions CRF. Significant findings made after the start of study drug which meet the definition of an adverse event must be recorded on the Adverse Event Case Report Form. There are no case report forms to capture routine normal findings from physical examinations and vital signs assessments.

8.5 Laboratory examinations

8.5.1 Hematology

Hematological evaluations (CBC) are regarded as clinical practice for CLL patients and should therefore be collected according to the Visit Schedules (see Figure 1).

8.5.2 Blood chemistry

The following blood chemistry results are regarded as clinical practice for CLL patients and should therefore be collected according to the Visit Schedules (see Figure 1).

Serum creatinine, ALT, AST, alkaline Phosphatase, LDH, bilirubin. globulin, serum immunoellectrophoresis and beta-2 microglobulin.

8.6 Physical examinations/vital signs

A physical examination including vital signs will be performed according to the Visit Schedules (see Figure 1). Information about the physical examination and vital signs must be present in the source documentation at the study site. Significant findings present prior to the start of study drug must be included in the Relevant Medical History/Current Medical Conditions CRF. Significant findings made after the start of study drug which meet the definition of an adverse event must be recorded on the Adverse Event Case Report Form. There are no Case Report Forms to capture routine normal findings from physical examinations and vital signs assessments.

8.6.1 Warning and Precautions

No evidence available at the time of the approval of this study protocol indicated that special warnings or precautions were appropriate regarding Rituximab, other than those noted in the Investigators' Brochure.

Safety information regarding Fludarabine and Cyclophosphamide can be found in the respective prescription information.

9. Statistics

Subjects' age, weight, height and other continuous demographic and baseline variables will be summarized using descriptive statistics (mean, standard deviation, minimum and maximum), while performance status, gender, and other categorical variables will be summarized with frequency tabulations.

9.1 Sample size

This is a single arm phase II study. The number of subjects planed to be enrolled into the study is 40. Such a low number of patients as in most phase II studies) constitute an underpowered study for revealing any statistical significance, but is indeed clinically meaningful. So, the sample size chosen for this study was based on clinical considerations.

Those patients achieving PR or CR by the study medication will be regarded as responders. All the others (stable disease, progressive disease or death from any cause) will be regarded as non responders. Determining the ratio of responders versus non responders, even if not statistically significant, will still be clinically meaningful.

9.1.1 Data analysis

Categorical data will be presented as frequencies and percentages. For continuous data, mean, standard deviation, median, 25^{th} and 75^{th} percentiles, minimum, and maximum will be presented.

9.2 Efficacy and Safety evaluation

9.2.1 Efficacy analysis:

All analyses will be according to the intention to treat principle.

9.2.2 Response rate

The primary efficacy parameter to be assessed will be response rate determined by objective overall response rate.

The primary endpoint is a binary variable where each patient is classified as a responder or not responder.

Response rate include CR and PR; all other situations (eg. stable disease, non response, progressive disease or death from any cause) will be rated as treatment failure and therefore as non responders.

Response rate will be assessed according the updated (2008) NCI-WG guidelines defined in appendix 1:

- Peripheral blood FACS for lymphocytes clonality.
- Physical examination for lymph node examination and measurements, and documentation of the presence of hepatomegaly and splenomegaly.
- Blood count for ANC, platelets and hemoglobin levels.
- Bone marrow aspiration and biopsy for bone marrow involvement by CLL cells using FACS immunophenotyping or immunohistochemistry.
- Patient interview for the presence of constitutional symptoms throughout the study.

All above mentioned efficacy (response) parameters will be performed at screening and at least 3 months after completing study drug administration for response documentation.

All known disease (measurable, and non measurable) parameters must be accounted for when assessing objective response rate.

Progression free survival is defined as the interval between the first treatment day to the first sign of disease progression.

Progressive disease (PD) will be assessed according the updated (2008) NCI-WG guidelines defined in appendix I. If needed, BMB will be performed for suspected progression at the investigator's discretion. Progression will be captured on the response form CRF.

A Kaplan-Meier method will be used to estimate median PFS and 90% confidence interval.

QOL questionnaires will be completed by study subjects at: screening visit and visits number: 11, 14, and end of study. Questionnaires will be captured on the QOL forms CRF.

Summary statistics (standard deviation, median, minimum and maximum) will be provided for the relevant variables. ANOVA will be used to analyse the changes in the components and total score of the FACIT QOL assessment.

9.3 Safety Analysis:

Data from all subjects who receive any study drug will be included in the safety analyses. The severity of the toxicities will be graded according to the NCI CTCAE v 3.0 , or v 2.0 for cytopenia, whenever possible. In the by-subject analysis, a subject experiencing the same event more than once will be counted only once. Adverse events will be defined and summarized according to the worst NCI CTC grade. Adverse events leading to death or to discontinuation of treatment, events classified as NCI CTCAE Grade 3 or Grade 4, study-drug-related events, and serious adverse events will be summarized separately. Laboratory data will be graded according to NCI CTCAE severity grade. ANOVA will be used to analyse the changes in the components and total score of the FACIT QOL assessments.

10. Quality Control and Quality Assurance

Data from the CRFs are entered into the study database by Contract Research Organization staff following their own internal standard operating procedures that have been reviewed and approved by the sponsor.

Subsequently, the entered data are systematically checked by Data Management staff, using error messages printed from validation programs and database listings. Obvious errors are corrected by Data Management personnel. Other errors or omissions are entered on Data Query Forms, which are returned to the investigational site for resolution. The signed original and resolved Data Query Forms are kept with the CRFs at the investigator site, and a copy is sent to the sponsor so the resolutions can be entered into the database. Quality control audits of all key safety and efficacy data in the database are made prior to locking the database.

11. Direct Access to Source Data/Documents

11.1 Study monitoring

The monitoring visits will enable the sponsor or the monitor acting on his behalf, to assess the study's progress status, to verify data accuracy and degree of completeness of the CRFs, to ensure the protocol as well as local regulations are being followed, that the investigator is fulfilling his obligations and to correct errors in the CRFs compared to the source documents. The investigators' will authorize the monitor, at a mutually convenient time during the study to periodically review all of the CRFs and the related parts of administrative, medical and laboratory files of each participant in the study. The CRFs must be filled out before the monitoring visits.

11.2 Audits

During the study, people belonging to the sponsor quality assurance group may visit the investigator site in order to audit the study. The purpose of this visit is to check that the study is carried out according to Good Clinical Practices. Before conducting an audit, the monitor will contact the investigator in order to agree upon a mutually suitable date. The investigator and his team are required to cooperate with the auditors and to grant them access to the patients' medical files and to the study documents (CRFs and investigator's binders).

11.3 IRB and regulatory inspections

A regulatory authority may also wish to conduct an inspection (during the study or even after its completion). If an inspection is requested by a regulatory authority, the investigator must inform the sponsor immediately that this request has been made.

12. Ethics

12.1 Institutional Review Board

Before implementing this study, the protocol, the proposed informed consent form and other information to subjects, must be reviewed by a properly constituted Institutional Review Board (IRB). A signed and dated statement that the protocol and informed consent have been approved by the IRB must be given to the sponsor before study initiation. The name and occupation of the chairman and the members of the IRB must be supplied to the sponsor. Any amendments to the protocol, other than administrative ones, must be approved by this committee.

12.2 Informed consent

The investigator must explain to each subject (or legally authorized representative) the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits involved and any discomfort it may entail. Each subject must be informed that participation in the study is voluntary and that he/she may withdraw from the study at any time and that withdrawal of consent will not affect his/her subsequent medical treatment or relationship with the treating physician.

This informed consent should be given by means of a standard written statement, written in non-technical language. The subject should read and consider the statement before signing and dating it, and should be given a copy of the signed document. If the subject cannot read or sign the documents, oral presentation may be made or signature given by the subject's legally appointed representative, if witnessed by a person not involved in the study, mentioning that the patient could not read or sign the documents. No patient can enter the study before his/her informed consent has been obtained.

The informed consent form is considered to be part of the protocol, and must be submitted by the investigator with it for IRB approval.

12.3 Declaration of Helsinki

The investigator must conduct the trial in accordance with the principles of the Declaration of Helsinki which can be accessed via the website of the World Medical Association at http://www.wma.net/e/policy/17-c_e.html.

13. Data Handling and Record Keeping

The information obtained from the execution of the protocol will be listed on the CRFs provided by the sponsor.
The CRFs must be filled out on a timely basis, signed and dated by the investigator or a co-investigator named by the former and clinically responsible for the patient during the study.

The completed CRFs will be collected by the sponsor. The investigator must remit one CRF for every participant in his center for a time period of 15 years.

14. Financing and Insurance

Financing and insurance will be addressed in the future in a separate agreement.

15. Publication Policy

Any formal presentation or publication of data from this trial will be considered as a joint publication by the investigator(s) and appropriate sponsor's personnel. Authorship will be determined by mutual agreement taking in consideration that the group of PI's will prepare the manuscript and write it initially and decide which member of steering committee and which other active investigators who provided cases will be involved.

It is a multicenter study, and it is mandatory that the first publication is based on data from all centers, analyzed as stipulated in the protocol, by the sponsor statisticians, and not by the investigators. Investigators participating in multicenter studies agree not to present data gathered from one center or a small group of centers before the full publication, unless formally agreed to by all other investigators and the PI's and steering committee.

The investigator may be required to sign the clinical study report, if it is to be used in a registration submission to the health authorities of some countries. For multicenter studies only the coordinating (principle) investigator nominated by the sponsor and the steering committee at the start of the trial would provide any needed signature.

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16. Supplements

Appendix I Response definitions

CR (complete remission) = requires **all** of the following criteria as assessed at least 3 months after completion of therapy:

Absence of clonal lymphocytes in peripheral blood.

Absence of significant lymphadenopathy. (lymph nodes > 1.5 cm in diameter) by physical examination. CT is desirable for not palpable lymph nodes.

No hepatomegaly or splenomegaly by physical examination. (Abdominal, pelvix and thorax CT for subjects found to be abnormal before therapy or if physical examination is inconclusive.

Absence of constitutional symptoms.

Blood counts above the following values: ANC> 1.5 x 10^9/L . Platelets > 100 x10^9/L . Hemoglobin > 11.0 g/dL; untransfused).

Bone marrow aspirate and biopsy should be performed at least 3 months after the last treatment and if the above 5 clinical and laboratory results demonstrate that CR has been achieved.
Bone marrow should be analyzed by FACS/or immunohistochemistry to demonstrate marrow free of B-CLL cells.
In some cases, it is necessary to postpone BMB until after all the other criteria to define a CR have been satisfied. However, this time interval should not exceed 6 months after the last treatment.

Parameter	CR	PR	PD	SD
Group A				
Lymphadenopathy*	None more than 1.5 cm	Decrease ≥ 50%	Increase ≥ 50%	Change of −49% to +49%
Liver and/or spleen size	Normal size	Decrease ≥ 50%	Increase ≥ 50%	Change of −49% to +49%
Constitutional symptoms	None	Any	Any	Any
Polymorphonuclear leukocytes	>1500/μL	>1500/μL or > 50% improvement over baseline	Any	Any
Circulating clonal B lymphocytes	None	Decrease ≥ 50% from baseline	Increase ≥ 50% over baseline	Change of −49% to +49%
Group B				
Platelet count	>100 000/μL	>100 000/μL or increase ≥ 50% over baseline	Decrease of ≥ 50% from baseline secondary to CLL	Change of −49 to +49%
Hemoglobin	>11.0 g/dL (untransfused and without erythropoietin)	>11 g/dL or increase ≥ 50% over baseline	Decrease of >2 g/dL from baseline secondary to CLL	Increase ≤11.0 g/dL or <50 over baseline, or decrease <2 g/dL
Marrow	Normocellular, <30% lymphocytes, no B-lymphoid nodules; hypocellular marrow defines CRi	≥ 30% lymphocytes, or B-lymphoid nodules, or not done	Increase of lymphocytes to more than 30% from normal	No change in marrow infiltrat

Values in SI units for leukocytes are 1.5 × 10^9/L; for platelets 100 × 10^9/L; and for hemoglobin 110 g/L and 20 g/L.

CR indicates complete remission (all of the criteria have to be met); PR, partial remission (at least one of the criteria of group A and one of the criteria of group B have to be met); PD, progressive disease (at least one of the criteria of group A or one of the criteria of group B have to be met); SD, stable disease (all of the above criteria have to be met).

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

Relapse = a patient who has previously achieved CR or PR but after a period of 6 or more months, demonstrates evidence of disease progression

Refractory disease = defined as treatment failure or disease progression within 6 months from the last antileukemic therapy.

Progression = see above table under PD.

Progression free survival (PFS) = defined as the time from start of treatment to progression.

Time to next treatment (TNT) = defined as the time from start of treatment to start of second-line treatment

Appendix II ECOG Performance status

ECOG	Grade
Fully active, able to carry on all pre-disease performance without restriction	0
Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work	1
Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours	2
Capable of only limited self-care, confined to bed or chair more than 50% of waking hours	3
Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair	4
Dead	5

Appendix III calculated creatinine clearance

$$\text{GFR (mL/min)} = \frac{(140\text{-age}) \text{ X weight (Kg)}}{\text{Serum cr (mg/dL) X } 72} \text{ X } 0.85 \text{ (for women)}$$

Appendix IV Active disease

At least one of the following criteria should be met:

1. Evidence of progressive marrow failure as manifested by the development of, or worsening of, anemia and/or thrombocytopenia
2. Massive (ie, > 6 cm below the left costal margin) or progressive or symptomatic splenomegaly.
3. Massive nodes (ie, > 10 cm in longest diameter) or progressive or symptomatic lymphadenopathy.
4. Progressive lymphocytosis with an increase of more than 50% over a 2-month period or lymphocyte doubling time of less than 6 months; patients with initial blood lymphocyte counts of less than $30X10^9$/L (30 000/μL) may require a longer observation period to determine the lymphocyte doubling time. In addition, factors contributing to lymphocytosis or lymphadenopathy other than CLL (eg, infections) should be excluded.
5. Autoimmune anemia and/or thrombocytopenia poorly responsive to corticosteroides or other standard therapy.
6. A minimum of any of the following disease-related symptoms must be present: a- Unintentional weight loss more than or equal to 10% within the previous 6 months. b- Significant fatigue (ie, ECOG PS 2 or worse; cannot work or unable to perform usual activities). c- Fevers of greater than 38.0^0C for 2 or more weeks without other evidence of infection. d- Night sweats for more than 1 month without evidence of infection.

Appendix V Binet staging system

The areas of involvement considered for staging are as follows: 1- Head and neck, including the Waldeyer ring. 2- Axillae 3- Groins 4- Palpable spleen 5- Palpable liver.

Stage A. Hb 10 g/dL or more and platelets 100X 10^9/L or more and up to 2 of the above areas involved.

Stage B. Hb 10 g/dL or more and platelets 100X 10^9/L or more and organomegaly greater than that defined for stage A (3 or more areas).

Stage C. All patients who have Hb less than 10 g/dL and/or a platelets count less than 100X 10^9/L, irrespective of organomegaly.

Appendix VI FACIT-QOL

FACIT − Fatigue Scale (Version 4)

Below is a list of statements that other people with your illness have said are important. **By circling one (1) number per line, please indicate how true each statement has been for you during the past 7 days.**

		Not At all	A little bit	Some what	Quite a bit	Very much
HI7	I feel fatigued	0	1	2	3	4
HI12	I feel weak all over	0	1	2	3	4
An1	I feel listless ("washed out") 0	1	2	3	4	
An2	I feel tired	0	1	2	3	4
An3	I have trouble starting things because I am tired 0	1	2	3	4	
An4	I have trouble finishing things because I am tired 0	1	2	3	4	
An5	I have energy	0	1	2	3	4
An7	I am able to do my usual activities 0	1	2	3	4	
An8	I need to sleep during the day 0	1	2	3	4	
An12	I am too tired to eat	0	1	2	3	4
An14	I need help doing my usual activities	0	1	2	3	4
An15	I am frustrated by being too tired to do the things I want to do	0	1	2	3	4
An16	I have to limit my social activity because I am tired 0	1	2	3	4	

F. HOFFMANN-LA ROCHE LTD

CLINICAL STUDY PROTOCOL

PROTOCOL NUMBER ML 25464 (FCR LITE)

A PHASE II, MULTICENTER, SINGLE ARM STUDY TO DETERMINE THE EFFICACY AND SAFETY OF LOW DOSE FLUDARABINE AND CYCLOPHOSPHAMIDE COMBINED WITH STANDARD DOSE RITUXIMAB AS PRIMARY THERAPY IN ELDERLY UNTREATED PATIENTS (≥65 YEARS OLD) WITH CHRONIC LYMPHOCYTIC LEUKEMIA

PROTOCOL APPROVAL

Protocol : ML25464 Version C

Date: **January 23, 2011**

Protocol approved by:
Medical Manager: [redacted]

Project Statistician: [redacted]

[redacted]

This protocol is intended for use in a life-threatening indication: Yes ☒ No ☐

A PHASE II, MULTICENTER, SINGLE ARM STUDY TO DETERMINE THE EFFICACY AND SAFETY OF LOW DOSE FLUDARABINE AND CYCLOPHOSPHAMIDE COMBINED WITH STANDARD DOSE RITUXIMAB AS PRIMARY THERAPY IN ELDERLY UNTREATED PATIENTS (≥65 YEARS OLD) WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AE	Adverse Event
ALT	Alanine Aminotransferase/GPT
ANC	Absolute Neutrophil Count
AST	Aspartate Aminotransferase/SGOT
ALK PHOSP	Alkaline Phosphatase
CBC	Complete Blood Count
CLL	Chronic Lymphatic Leukemia
CR	Complete Remission
CRF	Case Report/Record Form
CRO	Contract Research Organization
CT	Computerized Tomography
CY	Cyclophosphamide
FACT-CTC	Functional Assessment of Cancer Therapy - Common Terminology Criteria for cancer Adverse Events
FACS	Flow Cytometry (Fluorescence-Activated Cell Sorting)
DAT	Direct Coombs Test
FLU	Fludarabine
G-CSF	Granulocyte-Colony Stimulating Factor
Hb	Hemoglobin
HIV	Human Immune deficiency Virus
IEP	Immuno-ellectrophoresis
I.V.	Intra-Venously
Ig	Immunoglobulin
IRB	Institutional Review Board
IRR	Infusion Related Reaction
LDH	Lactic Dehydrogenase
MHO	Medical Health Organization
ORR	Overall Response Rate
PD	Progressive Disease
PET-CT	Positron Emission Tomography- Computerized Tomography
PFS	Progression Free Survival
P.O.	Per Os/ by mouth/orally
PR	Partial Response
QOL	Quality Of Life
SAE	Serious Adverse Event
TLS	Tumor Lysis Syndrome
TNT	Time to Next Treatment
ULN	Upper Limit of Normal

SYNOPSIS

A PHASE II, MULTICENTER, SINGLE ARM STUDY TO DETERMINE THE EFFICACY AND SAFETY OF LOW DOSE FLUDARABINE AND CYCLOPHOSPHAMIDE COMBINED WITH STANDARD DOSE RITUXIMAB AS PRIMARY THERAPY IN ELDERLY UNTREATED PATIENTS (≥65 YEARS OLD) WITH CHRONIC LYMPHOCYTIC LEUKEMIA

PROTOCOL NUMBER:	ML25464
DATE PROTOCOL FINAL:	10 January 2011
STUDY DRUG:	FLUDARABINE, CYCLOPHOSPHAMIDE, RITUXIMAB
INDICATION:	CHRONIC LYMPHOCYTIC LEUKEMIA
STUDY PHASE:	II

Background and rationale Chronic lymphocytic leukemia (CLL) is characteristically diagnosed in the older population with a median age at diagnosis ranging between 65 and 72 years. Because the prevalence of co morbidity increases with age, concomitant disease is often present in this age group. Additional factors which may also adversely affect treatment outcome at standard doses in elderly patients include: altered pharmacokinetics and poorer host tissue tolerance ; changes in the bone marrow hematopoietic reserve and microenvironment leading to increased treatment related myelotoxicity and higher rates of treatment related complications such as infections. As a result, elderly patients are often excluded from clinical trials or even from routine treatment on the basis of their age and / or comorbidity. Furthermore when these patients are treated, the drug doses are often markedly reduced in an attempt to avoid causing toxicity and complications. The challenge in treating elderly CLL patients relates to finding the correct balance between drug efficacy and toxicity and because of this optimal treatment for this group of patients is often hard to achieve.

Study Objectives

The aim of this study is to evaluate the safety and efficacy (response rate) of a combination of low dose Fludarabine (FLU) and Cyclophosphamide (CY) combined with standard dose Rituximab given as primary treatment for elderly patients with chronic lymphocytic leukemia.

Primary end point: -Response rate

Secondary end points:

- Safety - Special issues: incidence of neutropenic fever, hospitalizations (no^{o}- days),
- Thrombocytopenia ≥grade 3, neutropenia ≥grade 3, Infection rate.
- Progression Free Survival (PFS).
- Time to Next Treatment (TNT).
- QOL

STUDY DESIGN:

This is a multicenter, phase II, single arm study designed to evaluate the efficacy and safety of the combination of low dose Fludarabine (FLU) and Cyclophosphamide (CY) combined with standard dose Rituximab in elderly previously untreated CLL patients ($\geq$ 65 years of age). Potential study subjects will sign an informed consent prior to undergoing any study related procedure. Number of patients to be enrolled 40.

Treatment plan: Flu 12.5mg/m^2/d will be given intravenously (I.V.) for days 1-3 every 28 days, for a total of 6 cycles.

CY will be given I.V. at the dose of 150 mg/ m^2/d on days 1-3 every 28 days, for a total of 6 cycles.

Rituximab given before chemotherapy cycle1: 375 mg/m^2 I.V. on day 0, cycles 2-6: 500 mg/m^2 I.V. on day 1 every 28 days.

Dose reduction or delays are recommended in case of grade $\geq$3 (NCI-CTC) adverse events.

This study consists of 3 periods for each study subject: Pre-treatment, Treatment and Follow up.

Pre-treatment period: patients will undergo screening for protocol eligibility within 28 days (4 weeks) of recruitment.

Subjects who meet all the inclusion criteria and have signed an approved informed consent will be enrolled.

Treatment period FLU 12.5mg/m^2/d I.V. will be given on days 1-3 every 28 days, for a total of 6 cycles. CY will be given (I.V.) 150 mg/ m^2/d on days 1-3 every 28 days, for a total of 6 cycles. Rituximab given before chemotherapy cycle 1: 375 mg/m^2 intravenously on day 0, cycles 2-6: 500 mg/m^2 I.V. on day 1 every 28 days.

Premedication: antihistamines (e.g. 2 mg clemastin or promethazine 25 mg i.v.) and paracetamol (500-1000 mg orally) 30 min before first infusion of Rituximab, and thereafter only if clinically indicated. Hydrocortisone 100-500 mg I.V. (according with local common practice) only if clinically indicated.

Dose reduction or delay in case of drug induced hematological toxicity grade≥ 3 level.

During the treatment period, all patients will attend twice a month in the first 2 months and thereafter periodic monthly study center visits in order to assess the safety and efficacy of the treatment.

Complete blood count will be performed on day 1 and 14 of each of the first two cycles, and on day 1 in the other 4 cycles, or more frequently at the investigators discretion.

Bone marrow aspiration and biopsy and CT scan will be performed within 3-to 6 months after the completion of the treatment period.

Follow up period: All patients will be assessed for progression and survival every 3 months via clinic visits, for a total of 30 months.

Complete blood count and chemistry will be performed every 3 months, or more frequently at the investigators discretion.
In the event of suspected progression or relapse BMB and or CT scan will be performed at the treating physicians' discretion.

Duration of study

The duration of the treatment period is approximately 6 months. This time period is required to complete the therapy, to determine the safety profile of the drug combination and the response rate to therapy. The duration of the follow up period will be 30 months. The occurrence of PD will determine the duration of progression-free survival of each patient.

Recruitment period will be 24 months.

Inclusion criteria:

- Diagnosis of B-CLL is established by the presence of: An absolute lymphocyte count> $5X10^9$/L, typical morphology in the peripheral blood smear as seen by light microscopy, CLL cells must show CD5/CD19/CD23 positivity with light chain restriction as recorded by flow cytometry. Low surface immunoglobulin/CD 79b expression is also expected to be evident on these cells while FMC7 should be negative-are both additional typical features on flow cytometry (optional).
- Previously untreated B-CLL patients $\geq$ 65 years old.
- Patients requiring treatment (active Binet stage A and B, all stage C disease).
- Written informed consent

Exclusion criteria:

- Calculated or measured creatinine clearance < 30 ml/min
- Hepatic enzymes or bilirubin > 2X ULN, unless thought to be due to CLL
- Suspected or documented CNS involvement by CLL
- Known HIV positivity
- Active or uncontrolled infections, including opportunistic infection, active hepatitis B / C -positive Hepatitis B Surface Ag or Hepatitis B Core Ab
- Various concomitant diseases requiring chronic steroid administration
- Active Coombs positive hemolytic anemia
- Prior treatment for CLL
- CLL with transformation (Richter syndrome).
- Presence of active second neoplasia (excluding non-melanoma skin cancer, or in situ cervix or breast carcinoma) < 2 years prior to the study.
- Use of other investigational agents or participation in any other clinical trials < 30 days prior to the study.
- Low compliance.
- Performance status: ECOG performance status $\geq$4.
- Patients who mentally or physically are unable to comply with all aspects of the study. Upon investigator discretion, a psycho-geriatric evaluation will be performed for patients ≥ 80 years old.

Assessments:

Efficacy

The following evaluations will be done to record the efficacy of the treatment regimen:

- Assessment of disease response (revised International Response Criteria: updated NCI-IWG 2008).[46].
- Date of documentation of disease progression.
- Bone marrow biopsy examination
- Peripheral blood immunophenotyping by FACS.
- Changes in ANC, Hb levels and platelets counts from baseline blood counts and those done during the study period.
- Chest, abdominal and pelvic computerized tomography
- Physical examination for lymphadenopathy and hepato-splenomegaly

Safety

The following evaluations will be recorded to assess the safety of the regimen:

- Complete physical examination including vital signs.
- Clinical laboratory evaluations (hematology, biochemistry)
- Concomitant medication and procedures
- Adverse events
- Health outcomes assessment (any extra medical appointment)

Efficacy analysis:

Primary efficacy will be determined by objective overall response rate. Exact tests for assessing these will be used to compare objective overall response rates. Kaplan Meier procedure will be used to characterize the duration of response for PFS and TNT. Summary statistics (standard deviation, mean, median, minimum and maximum) will be provided for the relevant variables

Safety Analysis:

Data from all subjects who receive any study drug will be included in the safety analyses. The severity of the toxicities will be graded according to the NCI CTC v3 or v2- for cytopenia, whenever possible. In the by-subject analysis, a subject experiencing the same event more than once will be counted only once.

Adverse events will be defined and summarized by the worst NCI CTC grade. Adverse events leading to death or to discontinuation of treatment, events classified as NCI CTC Grade 3 or Grade 4, study-drug-related events, and serious adverse events will be summarized separately. Laboratory data will be graded according to NCI CTC severity grade.

STUDY DURATION: five years **TOTAL SAMPLE SIZE**: 40

2. Background Information

Chronic lymphocytic leukemia (CLL) is characteristically diagnosed in older people with a median age at diagnosis ranging between 65 and 72 years. Because the prevalence of co morbidity increases with age, concomitant disease is often evident in this age group. Goede et al [29] reported that increasing numbers of more severe concomitant comorbid illnesses are associated with a shortened survival in patients with CLL. Most recently Thurmes and colleagues [1] have shown that these comorbid conditions are in fact less important than both the age of the patient and the disease stage, in predicting prognosis in untreated patients.

Additional factors which may also adversely affect treatment outcome in elderly patients include: altered pharmacokinetics and poorer host tissue tolerance ; changes in bone marrow hematopoietic reserve and microenvironment which may lead to increased treatment related myelotoxicity and higher rates complications such as infections [21,26]. As a result, elderly patients are often excluded from clinical trials or even from routine treatment at standard doses of drugs, on the basis of their age and / or comorbidity and when treated the drug doses are often markedly reduced when given.

The basic challenge in treating elderly CLL patients appears to be related to finding the correct balance between treatment efficacy and drug toxicity and because of this, optimal treatment for older patients is hard to achieve and remains an open question and topic of debate.

Purine analogues drugs which inhibit DNA synthesis are very effective in CLL and fludarabine, cladribine and pentostatin have all been used effectively in the treatment of CLL [14]. Fludarabine is the most extensively studied purine analogue in CLL and is available in tablet form as well as for intravenous use. When used in combination with cyclophosphamide, fludarabine has been shown to be more effective than when used alone. [13,14,27,28]. In this respect the U.K. MRC LRF CLL4 trial reported in 2007 [11] compared therapy with chlorambucil alone vs fludarabine vs fludarabine (F) alone +

cyclophosphamide (FC) in 777 newly diagnosed CLL patients, and showed that FC achieved superior response rates and better progression-free survival for all CLL patients . Because of this, FC was proposed as the gold standard treatment of all CLL patients irrespective of age [11]. Similar results were published by the German CLL Study Group and the North American Intergroup [14].

However although Fludarabine - based regimes are most efficacious, they also appear to be more toxic, inducing more prolonged myelosuppression with more infections.

Pollizzotto et al [15] investigated the influence of increasing age on the ability to deliver fludarabine based regimens as well as the toxicity obtained thereafter, in patients with indolent lymphoproliferative disorders. They reported a gradual increase in toxicity with advancing age especially in patients age ≥ 70 years but still concluded that regimens were generally well tolerated and could be delivered safely to older patients who had a good performance status (PS). They noted a modestly increased degree of myelosuppression but no increase in the incidence of severe infectious complications or treatment-related mortality.

Table 1

PR	CR	OR	Patients age	Oral Cyclophosphamide dose	Oral Fludarabine dose	Disease status	Journal
NHL-59% CLL-100%	NHL-35% CLL-0%	NHL-94% CLL-100%	61-85 Median 73.5	150mg/m^2 X 4 days Every 28 days , 4 courses	25mg/m^2 (total 40 mg) X 4 days	Mostly relapsed refractory CLPD-28 Patients CLL-9 p	Hematology 2004 (16)
All-44% SLL-4/8	All-40% SLL-3/8	All-84% SLL-7/8	66-85 Med 74	150mg/m^2 X 4 days	25mg/m^2 (total 40 mg) X 4 days	Untreated 25 patients SLL-8	BJH 2007 (17)

				Every 28 days ; 4 courses			
40%	54%	94%	30% above age 70	150mg/m^2 X 5 days Every 28 dX6	24mg/m^2 X5 days	Untreated (196)	CLL4 (11)

2.1 Rituximab

Since the introduction of Rituximab (Rituxan™, IDEC) (FDA Approval Letter Nov. 26, 1997) in the USA and shortly thereafter in Europe in 1998 (MabThera™ (Hoffmann-LaRoche AG) for use in the treatment of CLL and indolent non-Hodgkins' lymphoma (NHL), it has been used extensively in the treatment of indolent and aggressive lymphoma, both as a single agent or more often in combination with chemotherapy. Today Rituximab is already being used to treat a variety of autoimmune disorders and by now has also been accepted as the model monoclonal antibody for the treatment of cancer. Rituximab is a chimeric mouse variable heavy and light chains (IDEC 2B8) with a human IgG_1 constant kappa antibody (C2B8) that reacts specifically with the CD20 antigen found on the surface of malignant and normal B cells and established B-cell lines [31,33]. The antigen is expressed at lower levels on chronic lymphocytic leukemia (CLL) and plasma cells [31]. CD20 is not shed from the surface of CD20+ cells after antibody binding and it is not internalized or downregulated [34]. Compared with its murine counterpart, Rituximab has a longer half-life in humans, interacts with human effector cells [34, 35], and is less immunogenic [36].

Reduced FC doses

There are only a few published reports (including relatively small numbers of CLL/SLL patients which describe the results of an FC regimen in elderly CLL patients-(≥ 70 years). These are listed in Table1.

2.2 Use of Rituximab and Combination of Rituximab and Fludarabine in CLL

Although initial responses to single agent treatment with Rituximab were low and partial responses and not complete remissions, interest continued in the use of this agent in CLL [37,39,40]. The original studies employed 375 mg/m^2 of Rituximab once a week for four weeks. Subsequent studies suggested using this dose three times a week for four weeks for a total of 12 doses instead of 4 doses which resulted in better overall responses [41]. Dose limiting toxicities of Rituximab have not been demonstrated perhaps due to the cost of the drug. In this respect it should be noted that in the initial studies using Rituximab to treat follicular NHL, responses were much higher than in CLL. This was attributed to the lower level of CD20 expression on CLL lymphocytes and the presence of circulating CD20 antigen. These differences were partially overcome when either the dose of Rituximab was increased or the frequency of the standard dose of 375 mg/m2 was increased [41, 44].

After establishing the safety and efficacy of standard dose Rituximab 375 mg/m2 three times a week for four weeks for a total of twelve doses, Byrd et al went on, to compare sequential and concurrent therapy with RF [43].

The concurrent group was therefore treated with 11 doses of Rituximab while the sequential cohort only received four doses. This was a randomized phase II trial of concurrent versus sequential Fludarabine and Rituximab with 51 and 53 patients per arm, respectively. The overall response rate in the concurrent arm was 90%, including CR in 47%while in the sequential arm the overall response rate was 77%, with CR in 28%. The disease-free and overall median survival has yet to be reached yet, but will be in excess of 23 months [44]. Three major side effects were observed: infusion related toxicity, myelosuppression, and infections. Infusion related toxicity were greatest in concurrent treated patients (20%) and almost not existent in the sequentially treated patients. In a phase II study, the German CLL Study Group (GCLLSG) [44] reported the treatment of 20 untreated and 11 relapsed CLL patients. Treatment consisted of Fludarabine administered at standard doses

(25mg/m2/d; days 1-5, 29-33, 57-61, 85-89) and Rituximab (375mg/m2/d) given on days 57, 85. The overall response rate (CR and PR) was 87% with a CR/CRu of 33%. The median duration of response was 75 weeks. Severe anemia (grade III/IV) was observed in 10% of patients, neutropenia in 42%, and thrombocytopenia in 9%, respectively. The most frequent non-hematologic side effects were infections (52%) and infusion-related symptoms. The majority of infections were respiratory and herpes virus infections.

A retrospective comparative analysis of the CALGB 9712 [47] (Rituximab plus Fludarabine) [48] and the CALGB 9011 (Fludarabine only) studies demonstrated that the addition of Rituximab to Fludarabine resulted in a significantly better 2 years progression free survival and overall survival compared to patients treated with Fludarabine alone (67% *Vs* 45% and 93% *Vs* 81%, respectively) and in both treatment approaches the infectious toxicity was similar [45].

The MD Anderson Cancer Center group reported a single arm study that utilized the combination of Rituximab, Fludarabine and Cyclophosphamide (FCR) in untreated and in relapsed and refractory CLL patients (224 and 177 patients, respectively) [24, 25]. In the untreated patients the overall response rate was 95% with a CR rate of 70%, a nodular PR rate of 10% and a PR rate of 15%. Although, during the follow-up period the time to treatment was not reached, a calculated analysis showed that 69% of patients were projected to relapse at 4 years. The major toxicities were myelosupression, predominantly of grade 3 and 4, neutropenia (in 52% of all courses) and infections.

In the relapsed and refractory patients the overall response was 73% with 25% CR, 16% nodular PR and 32% PR and a third of the complete responders achieved molecular remission. The median time to progression in responding patients was 28 months with an overall median survival time of 42 months. About 46% of the patients completed all six intended courses and myelosupression was the major cause for discontinuing treatment (66% and 18% grade 3-4 neutropenia and thrombocytopenia, respectively) with 16 % of the patients having major infection.

Recently, two pivotal phase III studies (CLL8 and REACH) [49,50] evaluated 6 cycles of rituximab (375mg/m^2 on cycle-1 and 500mg/m^2 on cycle 2-6) plus fludarabine (25mg/m^2 d1-3) and cyclophosphamide (250mg/m^2 d1-3) chemotherapy compared with 6 cycles of fludarabine and cyclophosphamide alone. The primary endpoint of both studies was progression-free survival. Secondary endpoints for both studies included overall survival, event-free survival, duration of response, response rate and complete response. CLL8 involved 817 patients with previously untreated (first-line) CLL and good physical fitness. With a median observation time of 37.7 months, statistically significant differences were observed in OS between the two treatment arms. The OS rate was 84.1% in the FCR arm versus 79 % in the FC arm (p=0.01). In both arms, the median OS has still not been reached. Patients who received rituximab together with chemotherapy had a median progression free survival (PFS) of 51.8 months compared to 32.3 months for those who received chemotherapy alone. (hazard ratio 0.56; $p < 0.001$). The overall response rate in patients receiving rituximab plus chemotherapy as first-line therapy was 95.1% vs. 88.4% in those receiving chemotherapy alone and with more complete remissions in the FCR arm (44.1 vs 21.8%; $p<0.001$). Severe (grade 3 or greater) adverse events that occurred more often in the rituximab arm included haematologic toxicity (56% vs. 39%), neutropenia (34% vs. 21%) and leukocytopenia (24% vs. 12%).

The REACH study[49] involved 552 patients with previously treated CLL (second line therapy). Patients who received rituximab plus chemotherapy had a median PFS of 30.6 months compared to 20.6 months for those who received chemotherapy alone (hazard ratio 0.65; p=0.0001). The overall response rate in the Rituximab-containing arm was 70% vs. 58% in those receiving chemotherapy alone without Rituximab. Grade 3 or greater events such as neutropenia (42% vs. 40%), febrile neutropenia (12% vs. 12%) and neoplasms (7% vs. 3%) occurred more often in the rituximab plus chemotherapy arm. Based on the CLL8 and REACH results the FDA approved the use of rituximab as first- and second-line treatment of CLL. In Europe, Rituximab is also approved for treatment of first and relapsing CLL patients.

In the light of the above literature, the combined use of Rituximab and Fludarabine and cyclophosphamide (FCR) is currently regarded as the gold standard and backbone treatment for physically fit patients with CLL.

2.3 Fludarabine Cyclophosphamide Rituximab (FCR) regimens for elderly patients with CLL

Due to major concerns of myleotoxicity and infection complications, this chemo-immunotherapy combination regimen cannot be applied safely to all elderly CLL patients. To address this topic efforts are being made to establish novel FCR-based protocol that will be useful for the average elderly CLL patient. In this regard, Foon and colleagues [50] reported a phase II single arm study of reduced dosages of Fludarabine and Cyclophosphamide (FCR-lite). Fifty untreated CLL patients were treated with 6 cycles of Fludarabine (20mg/m^2) and Cyclophosphamide (150mg/mg^2) combined with high–dose Rituximab (500mg/m^2) given every other week during induction and later as maintenance every 3 months for two years. The OR and CR rates were 100% and 79%, respectively. Median duration of complete response was 22.3 months (range, 5.2 to 42.5 months) and none of the complete responders have relapsed. Grade 3/4 neutropenia was noted in 13% of the cycles of therapy. Another approach to reduce toxicity was to use sequential therapy with fludarabine (25 mg/m^2 on days 1 through 5 for 6 cycles) followed by consolidation with Cyclophosphamide (3,000 mg/m^2 administered every 3 weeks for three cycles) and then by Rituximab 375 mg/m^2 for four cycles (F→C→R). [51] In 36 previously untreated CLL patients an overall response of 89% was achieved including 61% CRs. 33% achieved a molecular CR (PCR negative) and achieving molecular CRs had an excellent prognosis with a plateau in the response duration curve, and 90% remain in clinical CR at 5 years. For the entire group, 5-year survival rate is 71% compared with a rate of 48% with the prior F→C regimen (not significant $P = .10$).

3. Trial Objectives and Purpose

The aim of this study is to evaluate the safety and efficacy of a combination of low dose Fludarabine (FLU) and Cyclophosphamide (CY) combined with standard dose Rituximab given as primary treatment for elderly patients with chronic lymphocytic leukemia.
The purpose of the study is to determine whether a lower dose FC combined with standard dose Rituximab regimen in elderly CLL patients is as effective as the standard FCR dose, given in most studies until now and at the same time to assess if it is indeed less toxic.

3.1 Primary Objective

To evaluate the efficacy (Response Rate) of the combination of lower dose Fludarabine 12.5 mg m^2/d given intravenously for three days and Cyclophosphamide 150 mg/d given intravenously for three days every 28 days, combined with standard dose Rituximab (375mg/ m^2 in cycle 1 and 500 mg/m^2 in cycles 2-6) for a total of 6 cycles.

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3.2 Secondary Objectives

1- Safety of the combination drugs given in the study population, especially in relation to: neutropenic fever, Infection rate, number of hospitalization days, thrombocytopenia ≥grade 3, neutropenia ≥ grade 3.

2- Progression Free Survival (PFS).

3- Time to Next Treatment (TNT).

4- Quality Of Life (FACIT)

4. Trial Design

4.1 Primary and secondary end points

Primary End Point

Overall response rate (ORR) (CR and PR) as defined in the protocol (see appendix I)

4.1.1 Secondary End Points

1- Safety of the study drugs especially in relation to: neutropenic fever, Infection rate and number of hospitalization days,

thrombocytopenia ≥grade 3, neutropenia ≥grade 3.

2- Progression free survival (PFS) defined as the time elapsed from first day of study drug administration to disease progression as defined in the protocol (see appendix I)

3- Time to Next Treatment (TNT) as defined in the protocol (see appendix I)

4- QOL(see appendix VI)

4.2 Overall study design

This is a national, multicenter, phase II, single arm (not controlled), open label clinical trial to evaluate the efficacy and safety of the combination of low dose Flu + Cy combined with standard dose Rituximab Flu dosage will be given at 12.5mg/m^2/d given I.V. together with CY150 mg/m^2/d given I.V. for three days every 28 days. Rituximab will be given before chemotherapy
cycle 1: 375 mg/m^2 I.V. on day 0 and in cycles 2-6: 500 mg/m^2 I.V. on day 1 in previously untreated CLL patients who are more than 65 years of age. Patients will be treated every 28 days for up to 6 cycles. Potential study subjects will sign an informed consent form prior to undergoing any study related procedure. In general, study medications should be continued until disease progression or drug intolerance but no more than 6 cycles. Treatment choices after completion of the study are at the investigators' discretion.
Up to 40 patients are planned to be enrolled during a two year period.
This study consists of 3 periods for each study subject: A pre-treatment period, the treatment period and a follow up period.

Pre-treatment period:

Patients will undergo screening for protocol eligibility within 28 days (4 weeks) of recruitment.

Subjects who meet all the inclusion criteria and who have signed an approved informed consent will be enrolled.

Written informed consent must be obtained before any study - specific medical procedures are performed which include laboratory screening assessments and physical examination as well as documentation of vital signs. If the hematology and/or biochemistry evaluations have been performed within 14 days of the first dose of study drug as part of the screening evaluation, they need not be repeated, and the data may be used as the Day 1 values. Bone marrow examination including cytogenetics (FISH whenever possible) must have been performed within 12 months of starting the first dose of the study drugs.

CT scan when done, must be performed within 6 weeks of starting the first dose of study drugs.

Treatment period

Includes 6 months of combination treatment with low dose Fludarabine and Cyclophosphamide combined with standard dose of Rituximab. Flu dosage will be given at 12.5mg/m^2/d given I.V. together with CY150 mg/m^2/d I.V. for three days every 28 days. Rituximab given before chemotherapy cycle 1: 375 mg/m^2 I.V. on day 0 and in cycles 2-6: 500 mg/m^2 intravenously on day 1 every 28 days.

Premedication: antihistamines(e.g. 2mg clemastin or promethazine 25mg I.V.) and paracetamol (500-1,000 mg orally) 30 minutes before first infusion of Rituximab, and thereafter only if clinically indicated. Hydrocortisone 100 - 500mg I.V.(according with local common practice only if clinically indicated.

In the event of grade $\geq$ 3 hematological toxicity which in the investigators' opinion, is study drugs related, the administration of the next cycle should be delayed but not for more than 3 weeks and drug dose reduction of 25% of

both CY and F is recommended. Two dose reductions are permitted (FLU and CY Dose modification page 29). In cases where the delay period has been terminated but grade 3-4 toxicity persists, the patient should be removed from the protocol with no further study drug administration.

During the treatment period, all patients will attend periodic twice a month in the first 2 months and thereafter monthly study- center visits in order to assess safety and efficacy of the treatment.

Complete blood counts will be performed on day 1 and 14 of the first two cycles. In the next 4 cycles- on day 1 of each cycle, or more frequently at the investigators' discretion.

Bone marrow biopsy and aspiration examination will be performed before the start of treatment, for response documentation (3 to 6 months after the completion of treatment), for evaluation of cytopenias (disease or treatment related) and for any other clinical indication at the discretion of the investigators.

Supportive care including blood transfusions or administration of growth factors G-CSF or erythropoietin is permitted at the investigators' discretion throughout all study periods.

Follow up period

During the follow up period, of 30 months after the completion of the study drugs administration, all patients will be assessed every 3 months for evidence of progression and documentation of survival at clinic visits.

Complete blood count will be performed every 3 months, or more frequently at the investigators' discretion.

Bone marrow examination including cytogenetics, peripheral blood immunophenotyping (FACS), abdominal, pelvic and chest CT will be performed 3 to 6 months after the completion of the treatment regimen, at clinical progression and at any other time according to the investigators' discretion.

In the event of no response (no CR nor PR) in > 5 of the initial 10 subjects enrolled into the study, Fludarabine dosage will be increased for all the next subjects enrolled into the study and will be given at the dose of 15mg/m^2/d given I.V.

In the event of no response after the above increment in > 5 of the next 10 subjects who received the increased dosages, the study will be terminated and declared inadequate and inefficient. If response is evident in $\geq$ 5 of the 10 patients, the same dosages will be continued throughout the study until its completion.

All further treatment decisions after completion of the study are at the discretion of the investigators.

4.2.1 Visit schedule and assessments

Visit schedule

Patient must be followed at the study center according to the visit schedule and assessments outlined in Figure 1.

All routine assessments must be performed within a time window of $\pm$ 7 days of the day indicated on the visit schedule, except visits 2-5 in which time window will be of $\pm$ 3 days .

Figure 1: Visit schedule and assessments

Week	-4 to 0	1	3	5	7	9-25 Every 4 weeks	29	37	49	61	73-145 every 12 weeks	157 EOS
Visit No.	**1**	**2**	**3**	**4**	**5**	**6-10**	**11**	**12**	**13**	**14**	**15-21**	**22**
Study period	preTx	**Treatment period**						**Follow up period**				
Inc. /Excl. criteria	X											
Informed consent	X											
Relevant medical history/conditions	X											
Disease history	X											
BMB*	X								X			
Physical exam	X	X	X	X	X	X	X	X	X	X	X	X
CBC	X	X	X	X	X	X	X	X	X	X	X	X
Blood chemistry	X	X		X		X	X	X	X	X	X	X
Beta-2 micro glob	X											
IEP, Ig level,	X								X			X
DAT***	X								X			
FACS******	X								X			X
Hepatitis B *******	X											
Imaging - (CT) **	X								X			
QOL	X						X			X		X
Treatment dosage	X	Ongoing data capture										
Adverse Events	X	Ongoing data capture and up to 30 days after completion or discontinuation of the study										
Concomitant medications	X	Ongoing data capture										
Comments	X	Ongoing data capture										
Study completion	X	Complete at any time if study drug is discontinued										

progression/ leukemia transformation, cytopenia evaluation, or for any CR confirmation at investigator discretion.

** Abdominal, pelvic and chest CT will be performed at any clinical suspicion of progression.

*** DAT will be performed at any clinical suspicion of hemolysis.

**** Will be performed at any clinical suspicion of progression/ leukemia transformation, or for CR confirmation at investigators' discretion.

*****At screening and at investigator discretion for hepatitis treatment evaluation.

4.3 Trial treatment

4.3.1 Investigational therapy

Fludarabine is supplied to the study investigators by the Medical Health Organization (MHO) as routine first line treatment in all CLL patients. Fludarabine is supplied as 10 mg tablets packaged in blister packs, or as 50 mg/vial, as lyophilized powder that has to be dissolved in 2 Ml of water for injection, than diluted in 100 ml of saline (0/9%) given in 30 minutes.

Cyclophosphamide is supplied to the study investigators by the MHO as a routine first line treatment in CLL patients. Cyclophosphamide (Endoxan) is supplied as 50 mg tablets packed in blister packs or as 500 mg/vial (24 mg/ml) diluted in 100 ml of saline (0/9%) given in 30 minutes.
Rituximab is supplied to the study investigators by the MHO as a routine first line treatment in CLL patients. Rituximab (Mabthera) is supplied as 100mg of rituximab in 10ml (10mg/ml) pack of 2 vials or 500mg of rituximab in 50ml (10mg/ml) pack of 1 vial. The antibody should be diluted into a final volume of normal saline or 5% D-glucose in water to allow for a maximal concentration of 4 mg/ml. Rituximab should be administrated before FC chemotherapy.

First Infusion: Recommended initial rate is 50mg/hr; after the first 30 minutes increase by 50mg/hr every 30 minutes to maximum of 400 mg/hr. *Subsequent Infusions:* Initial rate 100mg/hr; increase by 100mg/hr every 30 minutes to a maximum of 400mg/hr

Use extreme caution and closely monitor first infusion when treating patients with ≥ $25x10^9$/l circulating malignant cells or high tumor burden (higher risk of severe cytokine release syndrome (CRS). It is recommended to administer Hydrocortisone 100-500 mg I.V. (according local common practice) shortly before infusion with Rituximab to decrease the rate and severity of acute IRR/CRS for these patients. consider reduced rate for first infusion or a split dosing over two days during the first cycle. Severe CRS: may be associated with some features of tumor lysis syndrome e.g. hyperuricaemia, hyperkalaemia, hypocalcaemia, hypophosphataemia, acute renal failure,

elevated LDH and may be associated with acute respiratory failure and death. If severe CRS manifests stop infusion immediately and start aggressive symptomatic treatment.

If the first dose of rituximab is well tolerated, the starting flow rate for administration of the second and subsequent infusions will be 100 mg/hour and then increased gradually by 100 mg/hour intervals not to exceed 400 mg/hour.

If any grade 1 or 2 infusion related reactions occur during an infusion, the infusion should be slowed or interrupted (at the discretion of the Investigator) and supportive treatment instituted. The infusion rate can be increased or restarted on resolution of the symptoms.

If grade 3 or 4 infusion related reactions occur during an infusion, the infusion has to be discontinued immediately and not restarted until resolution of the symptoms. Treatment can be reinitiated (at half the original infusion rate) at the discretion of the Investigator, but if the same adverse event appears again with the same severity, treatment must be permanently discontinued.

Should bronchospasm or dyspnea occur in the patient during the infusion, the infusion should be stopped immediately. Medication such as anti-histamines or steroids should be given for symptomatic relief. The infusion should not be re-started until symptoms have resolved completely and should be given at half the original infusion rate.

In the event of infusion related reactions to rituximab:

• Stop rituximab infusion, treat symptoms aggressively if necessary

• NaCl 0.9 % i.v., if necessary

• Hydrocortisone 100-500 mg I.V. (according local common practice), if necessary

• Pethidine i.v. if necessary

• Bronchodilators/antihistamines if necessary

• If side effects occur stop Rituximab-infusion, re-try the next day

Table 2: Reduction of Infusion Rate

Drop in systolic blood	Mucosal congestion, edema	Rigors	Fever	Infusion rate

> 30 mm Hg	Mild/moderate	Mild/moderate	> 38.5°C	Decrease to ½ if any of these events are seen

Following the infusion the I.V. line should be kept open for medications as needed. If there are no complications, the line may be discontinued after one hour of observation. If complications occur during the rituximab infusion, the patient should be observed for 2 hours after the completion of the infusion.

Infusion reactions to rituximab tend to be more common and more severe with the first infusion. In patients who were unable to tolerate the first infusion, cautious reintroduction of rituximab may be tried at the second cycle, at the investigator's discretion. The recommended premedication should be given and then the patient carefully re-exposed to a small amount of rituximab (slow infusion of 10 mg I.V.) If a severe reaction occurs, the rituximab must be permanently discontinued. In such case patient will be treated with FC in the next cycles and data will be collected throughout the study period)

If no severe reaction occurs after 30 minutes, the full dose of rituximab infusion should be given.

Labels will comply with the legal requirements in Israel and be printed in Hebrew and English. All study drugs containing infusion bags will be labeled. All infusion bags will be labeled by the study nurse prior to administration and only the empty Rituximab infusion bags will be collected for drug accountability by the study monitor. Storage conditions for study drug will be described on the medication label. Patients will receive the study drug combination for an exposure period of up to 6 months provided that in the opinion of the investigator the patient is benefiting from treatment with this study drug combination, and in the absence of any safety concerns. Treatment will be withheld only in the case of limiting toxicities (see below).

As administration of cyclophosphamide may cause hemorrhagic cystitis and even urinary bladder malignancy, patients will be instructed to drink at least 10 glasses of fluids on the days they receive cyclophosphamide and urinate at minimal ceasing of bladder distention.

DRUG ACCOUNTABILITY

Accountability and patient compliance will be assessed by maintaining adequate "drug dispensing log" on **study medication**.

The "drug dispensing" log for the **study medication** must be kept current and should contain the following information:

- The identification of the patient (patient number) to whom the **study medication** was administered.
- The date(s) and quantity of **study medication**, used for the patients' treatment.
- The date(s) and quantity of **study medication**, of empty, partial or unused medication.

All accountability logs must be available for inspection by the monitor. Drug supplies (partly used and empty Rituximab infusion bags) must be available for inspection, at every monitoring visit. Used supplies will be destructed according to local site guidelines.

4.3.2 Dose modification for hematological toxicity

Dose modifications will not be made during the first cycle of therapy. During the flowing cycles modifications of the treatment schedule will only be made as follows:

• If at day 1 of any cycle, there is a grade ≥ 3 cytopenia (See hematological toxicity section 8), NOT RELATED TO BONE MARROW INFILTRATION DUE TO CLL, treatment should be delayed for up to three weeks and then given with a 25% dose reduction of fludarabine and cyclophosphamide in the following cycles. There is no dose reduction for Rituximab in this study, but drug administration will be delayed for the same time period as for fludarabine and cyclophosphamide.

• If after three weeks, the grade ≥ 3 cytopenia, **NOT RELATED TO** CLL **BONE MARROW INFILTRATION**, still prevails, the patient should be removed from the protocol with no further study drug administration.
• If there is further grade ≥ 3 cytopenia in subsequent cycles, despite the first 25% dose reduction treatment should again be delayed for up to three weeks and the dose of fludarabine and cyclophosphamide further reduced to 50% of the full initial dose.
• If there is further grade ≥ 3 cytopenia in subsequent cycles despite the 50% drugs dose reduction the patient should be removed from the protocol with no further treatment.
• If there is any grade ≥ 3 neutropenia with infection during any cycle, G-CSF should be administered and G-CSF should than be given in all subsequent cycles prophylactically.

4.3.3 Dose modification for impaired renal function

Fludarabine is partly (40-60%) excreted by the kidneys. If the calculated (see appendix III) or measured creatinine clearance is reduced to 30-60 ml/min, the fludarabine dose should be reduced to 50%. Patients with a creatinine clearance below 30 ml/min are excluded from the study. Patients who develop renal dysfunction (serum creatinine ≥ 1.5 mg/dL or creatinine clearance < 30 ml/min) during treatment should go off protocol treatment.

4.3.4 Dose modification for other non-hematological toxicity (non renal).

• If other grade ≥ 3 non-hematological, non-renal toxicity occurs during any cycle, study drugs treatment should be delayed until recovery to grade ≤ 2, for up to three weeks. All subsequent cycles should then be given with a 25% dose reduction of fludarabine and cyclophosphamide.
• If after three weeks, the grade ≥ 3 non-hematological, non-renal toxicity still persists, the patient should be removed from the protocol .
• If there is further grade ≥ 3 non-hematological, non-renal toxicity in subsequent cycles despite the first 25% dose reduction treatment should again be delayed for up to three weeks and the dose of fludarabine and cyclophosphamide further reduced to 50% of the initial full dose.

• If there is further grade ≥ 3 non-hematological, non-renal toxicity in subsequent cycles despite 50% dose reduction the patient should be removed from the protocol treatment and the study.

4.3.5 Treatment assignment

Informed consent must be obtained before any testing for the purpose of determining a patient's eligibility is performed. Each patient will be assigned a unique patient number. Once assigned, numbers for any non-evaluable or discontinued patient will not be reutilized.

4.3.6 Blinding

This is an open label trial.

4.4 Study drug interruption or discontinuation

4.4.1 Discontinuation of study drugs

Events that will be considered as reasons for study drugs discontinuation are described in section 5.3

In addition, patient follow-up information must still be recorded (also in the event of study drug discontinuation) during and at the end of the study period.

4.4.2 Study Discontinuation

Events that will be considered as reasons for study discontinuation are described in section 5.3

4.5 Accountability procedures for the investigational products

Study drug will be prepared and dispensed by the pharmacist at the investigator's institution.

5. Selection and Withdrawal of Subjects

5.1 Inclusion Criteria

1. Diagnosis of B-CLL is established by the presence of: an absolute lymphocyte count > $5X10^9$ L, and typical CLL cell morphology in the peripheral blood smear as seen by light microscopy. CLL cells must show CD5/CD19/CD23 positivity with light chain restriction as recorded by flow cytometry. The levels of surface immunoglobulin and CD 79b are characteristically low and FMC7 is expected to be negative (optional). ZAP 70 and CD38 are optional.
2. Previously untreated B-CLL patients ≥ 65 years old.
3. Patients requiring treatment (active Binet classification stage A and B, all stage C disease).(appendixes IV and V)
4. Written informed consent

5.2 Exclusion Criteria

1. Calculated or measured creatinine clearance < 30 ml/min
2. Hepatic enzymes or bilirubin ≥ 2X ULN, unless thought to be due to CLL.
3. Suspected or documented CNS involvement by CLL
4. Known HIV positivity
5. Active or uncontrolled infections, including opportunistic infection and active hepatitis B / C
6. Positive Hepatitis B Surface Ag or Hepatitis B Core Ab
7. Various concomitant diseases requiring chronic steroid administration
8. Active Coomb's positive hemolytic anemia.
9. Prior treatment for CLL
10. CLL in transformation (Richter syndrome).
11. Presence of active second neoplasia (excluding non-melanoma skin cancer, or in situ cervix carcinoma or breast carcinoma) < 2 years prior to the study.

12. Use of other investigational agents or participation in any other clinical trials < 30 days prior to the study.
13. Low compliance.
14. Performance status: ECOG performance status ≥4. (see appendix II)
15. Patients who are mentally or physically unable to comply with all aspects of the study. **Upon investigator discretion, a psycho-geriatric evaluation will be performed for patients ≥ 80 years old.**

5.3 Interruption or Discontinuation of Treatment

5.3.1 Discontinuation of study drugs

The following events will be considered as reasons for discontinuation.

- Interruption of study drug/s for longer than 3 consecutive weeks.
- Adverse events, including study drugs induced active autoimmune haemolytic anemia.
- Patient with large cell transformation (Richter syndrome).
- Disease progression as defined at any time during study drug therapy.
- Treatment with other chemotherapeutic or investigational anti-neoplastic drugs
- Intolerable adverse effects that are judged by the investigator to be either physically or psychologically detrimental to the patient. Unresolved or recurrent (more than twice) grade 3 or 4 toxicity.
- The reason for discontinuation should be recorded in the CRF and in the subject's medical records. The sponsor is to be notified of all discontinuations related to study medication.
- In addition, patient follow-up information will be recorded (also in the event of study drug discontinuation) during and at the end of the study period.
- In the case of a severe reaction to Rituximab, Rituximab will be permanently discontinued. Patient will be treated with FC in the next cycles and data will be recorded throughout the study period.

5.3.2 Interruption of study drugs

In the event of grade 3 or 4 toxicity, or increase of creatinine>1.5 or GFR of less than 30ml/min study drug will be interrupted for no more than 3 weeks (see sections 4.3.2-4.3.4)

5.4 Study Discontinuation

The following events are considered as study discontinuation:

- Death
- Participation in another investigational drug trial
- Loss to follow-up
- Patient withdrawal of consent
- Major violation of the study protocol- such as eligibility criteria.
  In these cases, no patient follow-up will be recorded.
- Administrative problems
- In the event of no response after dosage increment in > 5 of the next 10 subjects who received the increased dosage.(see chapter 4.2).

The section for "Study Completion" in the CRF must be completed for all patients. The reason for early discontinuation should be given, even if the patient refuses to return for a final visit. Patients who discontinue prematurely due to significant study drug related adverse events (AEs) should continue to be followed until resolution of the AE or up to 30 days after discontinuation. The relevant sections of the CRF should be completed as appropriate.

Patients who discontinue the study drugs for any reason should be scheduled for a visit within 30 days from drug discontinuation at which time all the assessments listed for the final visit should be performed (see figure 1).

Patients lost to follow up should be recorded as such on the CRF. Patients who discontinue study treatment should be followed for tumor assessments as per standard of care, until disease progression or new cancer therapy is initiated. If patients refuse to return for these visits or are unable to do so, the

subject should be considered off-study, but a Study Evaluation Completion form should be completed.

6. Treatment of Subjects

6.1 Treatment regimen

Patients will receive FLU , CY and Rituximab combination for an exposure period of up to 6 months- defined as the treatment period. Dosage, dose regimen and route of administration are detailed in 4.3-4.4

6.1.1 Treatment assignment

Informed consent must be obtained before any testing for the purpose of determining a patient's eligibility is performed. Each patient will be assigned a unique patient number. Once assigned, numbers for any non-evaluable or discontinued patient will not be reused.

6.2 Concomitant therapy

In general, concomitant medications and therapies deemed necessary for the supportive care and safety of the patient are permitted in this study, provided their use is documented in the patient records and on the appropriate Case Report Form. The administration of any anticancer agents including chemotherapy and biologic agents is NOT permitted. Similarly, the use of other concurrent investigational drugs is not allowed. The use of blood products (irradiated) transfusions, immunoglobulins or erythroid/myeloid growth factors are permitted at investigator's discretion.

Transfusion-associated graft versus host disease prevention using irradiated blood products is mandatory.

Steroids administration are allowed exclusively for patients with autoimmune hemolytic anemia and or autoimmune thrombocytopenia or as premedication for Rituximab .

Premedication: Any patient considered to be at risk of infusion related reaction (IRR) or TLS (=patients with lymphocytosis > 25,000mm^3) should receive:

Appropriate hydration starting 12-24 hours prior to initiating treatment, and thereafter until the risk of IRR/TLS is ruled out. Urine alkalization is at investigator's discretion.

Antihistamines (e.g. 2 mg clemastin or promethazine 25mg i.v.) and paracetamol (500-1,000 mg orally) 30 min before first infusion of Rituximab, and thereafter only if clinically indicated. Hydrocortisone 100-500 mg I.V. (according local common practice) should be given only if clinically indicated (increased risk of IRR).

Pneumocystis carinii pneumonitis prophylaxis with trimethoprim 160mg-sulfamethoxasole 800 mg twice a day, twice a week, for the treatment period and for the next 6 months of follow up are recommended. For prevention of hepatitis B reactivation that needs antiviral treatment Lamivudine 100mg/day will be administrated for the entire duration of chemotherapy and for the six months afterwards.

Hepatitis B serology will be: HBsAg+, Anti HBcore and anti-HBsAg

Treatment is recommended for: HBsAg+ (carriers), or HBVsAg (-), anti HBcore (+) and anti-HBVsAg (-).

Any use of concomitant medication must be captured in the concomitant medication CRF. Administration of Blood products must also be captured as concomitant medication on CRF.

Steroids if given (see above) are allowed for no more than 3 consecutive days. Excluding patients with autoimmune cytopenia, in which case prolonged period steroid administration is permitted (see above).

6.3 Treatment compliance

Number of FLU and CY vials administrated I.V to every subject in every study site, will be captured on CRF.

Number of Rituximab containing infusion bags administrated to every subject in every study site, will be captured on CRF

7. Assessment of efficacy

The primary efficacy parameter to be assessed will be response rate.

Response rate include CR and PR; all others (eg. stable disease, non response, progressive disease or death from any cause should be rated as treatment failure.)

An overall objective assessment of all measurable and non-measurable response parameters will be performed according to the Visit Schedules (see Figure 1).

Response rate will be assessed according the updated (2008) NCI-WG guidelines defined in appendix I: [46].

- Peripheral blood immunophenotyping by FACS for absence of clonal lymphocytes**.**
- Physical examination - lymph node examination and measurements for absence of significant (>1.5 cm in diameter), as well as the presence of hepatomegaly and splenomegaly.
- CT scan for CLL patients with a CT found to be abnormal before therapy or alternatively if physical examination is inconclusive.
- Complete blood count for ANC, platelets and hemoglobin levels. Blood counts have to be above the following values: ANC> 1.5 X 10^9/L. platelets > 100X 10^9/L. Hemoglobin > 11.0 g/dL.
- Bone marrow aspiration and biopsy to assess degree of bone marrow involvement by CLL using FACS and immunohistochemistry, should be performed at least 3 months after the last treatment if the above 4 clinical and laboratory results demonstrate that CR has been achieved. In some cases, it is necessary to postpone BMB until after all the other criteria used to define a CR have been satisfied but, this time interval should not exceed 6 months after the last treatment.
- Patient interview for the presence of constitutional symptoms throughout the study.

A single laboratory should perform all evaluations for all patients recruited in the same site. All the above studies must account for disease parameters that

were present at baseline and must use the same techniques as used at baseline. All the above mentioned assessments should be performed within 7 days of the scheduled day of assessment (see Figure 1).

All above mentioned efficacy (response) parameters will be performed at screening and at least 3 months after completing study drug administration for response documentation.

Bone marrow examination can be postponed until after the above clinical and laboratory examinations used to define a CR have been satisfied. However, this time interval should not exceed 6 months after the last treatment.

All known disease (measurable, and non measurable) parameters must be accounted for when assessing objective response rate. Current laboratory and physical examination results will be captured on the response forms in the CRF.

Progression free survival is defined as the interval from the first study drug treatment day to the first sign of disease progression.

Progressive disease (PD) will be assessed according the updated (2008) NCI-WG guidelines defined in appendix I. If needed, BMB will be performed for suspected progression at the investigator discretion. Progression will be captured on the response form CRF.

Time to next treatment is defined as the interval between the first study drug treatment day to the first day of starting the next treatment.

In general, second-line treatment decisions follow the same indications as those used for initiation of first-line treatment.

Re-treatment day will be captured on the treatment form CRF.
QOL questionnaires (FACIT) will be completed by study subjects at:
screening visit and at visits number: 11,14 and at the end of study.
Questionnaires will be captured on the QOL forms CRF.
CT Scan – In order to prevent contrast nephropathy, patients with GFR < 60ml/min or diabetes mellitus, hydration is recommended and if possible acetylcystein (600 mg in bolus pre CT and 1,200 mg orally twice a day for 48 hours after CT) should be given . Hospitalization for this procedure is recommended.

8. Assessment of Safety

Safety assessments will consist of evaluating adverse events and serious adverse events, laboratory parameters including hematology, chemistry, vital signs, physical examinations, and documentation of all concomitant medications and/or therapies.

8.1 Adverse events

According to the International Conference of Harmonization [ICH], an AE is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign [including an abnormal laboratory finding], symptom, or disease temporally associated with the use of a medicinal [investigational] product, whether or not considered related to the medicinal [investigational] product. Pre-existing conditions which worsen during a study are to be reported as AEs.

Information about all adverse events, whether volunteered by the patient, discovered by investigator questioning, or detected through physical examination, laboratory test or other means, will be collected and recorded on the Adverse Event Case Report Form and followed as appropriate. An adverse event is any undesirable sign, symptom or medical condition occurring after starting study treatment, even if the event is not considered to be treatment-related.

Medical conditions/diseases present before starting study treatment are only considered adverse events if they worsen after starting study treatment. Clinical events occurring before starting study treatment but after signing the informed consent form are recorded on the Medical History/Current Medical Conditions Case Report Form only if the patient receives study treatment. Abnormal laboratory values or test results constitute adverse events only if they induce clinical signs or symptoms or require therapy, when they are recorded on the Adverse Events Case Report Form under the signs, symptoms or diagnosis associated with them.

8.1.2 Grading and reporting of adverse events

All adverse events will be graded according the NCI-CTC scale version 3.0 Anemia and thrombocytopenia should be graded according to NCI-CTC scale version 2.0- the criteria for patients in leukemia studies (see assessment of toxicity below for details), and reported in detail as indicated on the CRF

Adverse events not listed on the CTCAE should be graded as follows:

Definition	Equivalent To:	CTC Grade
Discomfort noticed but no disruption of normal daily activity	Mild	Grade 1
Discomfort sufficient to reduce or affect daily activity; no treatment or medical intervention is indicated although this could improve the overall well-being or symptoms of the subject	Moderate	Grade 2
Inability to work or perform normal daily activity; treatment or medical intervention is indicated in order to improve the overall well-being or symptoms; delaying the onset of treatment is not putting the survival of the subject at direct risk.	Severe	Grade 3
An immediate threat to life or leading to a permanent mental or physical conditions that prevents work or performing normal daily activities; treatment or medical intervention is required in order to maintain survival.	Life threatening/ disabling	Grade 4
AE resulting in death	Death	Grade 5

8.1.3 Assessment of Toxicity

The evaluation of potential treatment-induced toxicity in patients with advanced CLL may be quite difficult requiring careful consideration of both the manifestations of the underlying disease, as well as adverse reactions to the therapy under study. Some of the conventional criteria for toxicity are not applicable especially under circumstances of progressive bone marrow failure from the CLL itself.

8.1.4 Hematological Toxicity

The evaluation of hematological toxicity in patients with CLL must consider the high frequency of hematological compromise already present at the initiation of therapy. Therefore the standard criteria used for solid tumors cannot be applied directly; many patients would be considered to have grade II to IV hematological toxicity at presentation.

As a consequence, **for grading of anemia and thrombocytopenia,** we plan to apply the NCI**-CTC version 2.0 criteria for patients in leukemia studies, but for grading of neutropenia, the standard NCI-CTC version 3.0 criteria,** as given below.

Table 3: NCI-CTC Grading scale for hematological toxicity for patients in this study

ANC mm^3 § (nadir)	Grade	Decrease in platelets*or Hb# from pretreatment value (%).
≥2,000	0	No change-9%
≥1,500 and <2,000	1	10-24%
≥1,000 and <1,500	2	25-49%
≥500 and <1000	3	50-74%
<500	4	≥75%

Grades: 1 = mild, 2 = moderate, 3 = severe, 4 = life-threatening

* if platelets were < 20,000mm^3 [<20 x10^9/L] prior to therapy, the patient is inevaluable for toxicity in platelets

baseline and subsequent Hb determinations must be performed before any given infusion

§ if ANC was < 1,000 prior to therapy, the patient is inevaluable for toxicity in ANC.

Platelets:

If at any level of decrease the platelet count is < 20,000 mm^3, this is considered grade 4 toxicity, unless a severe or life-threatening decrease in the initial platelet count (e.g. < 20,000 mm^3) was present before treatment in which case the patient is inevaluable for toxicity referable to platelet counts.

Hemoglobin:

Baseline and subsequent Hb determinations must be performed before any given infusions.

Fludarabine has been reported to exacerbate or precipitate autoimmune hemolytic anemia and patients should be monitored carefully for this condition. If a rapid decrease of hemoglobin occurs during therapy, the

possibility of fludarabine- or autoantibody-induced hemolysis should be considered and appropriate diagnostic tests (LDH, bilirubin, α 2-haptoglobin, reticulocytes, Coombs-test) be performed. **In particular, a Coomb's test should be performed for:**

- **Any grade 3 or 4 anemia (Hb < 8 g/dL)**
- **A Hb < 10 g/dL sustained for > 2 weeks**
- **A drop in Hb of 3 g/dL in one week (while pts are receiving weekly blood tests)**
- **Any patient requiring a blood transfusion**

If, in the judgment of the treating physician, there is evidence of hemolytic anemia secondary to fludarabine, study treatment should be promptly withdrawn. Full details of the hemolytic anemia should be recorded on the adverse event pages of the CRF.

Neutrophils:

Absolute neutrophil count (ANC) is used to assess the toxicity of the myeloid lineage. Other decreases in the white blood cell count, or in circulating lymphocytes, are not to be considered, since a decrease in the white blood cell count is a desired therapeutic end point. If the ANC was < 1,000/ mm^3 [<1 $x10^9$/L] prior to therapy, the patient is inevaluable for toxicity referable to the ANC.

Any Adverse Event occurring by the time of study completion (within four weeks of last drug intake) must be recorded on the Adverse Event CRF page.

8.1.5 Drug-Adverse event relationship

The causality relationship of study drug to the adverse event will be assessed by the investigator as either:

Yes or No

If there is a reasonable suspected causal relationship to the study medication, i.e. there are facts (evidence) or arguments to suggest a causal relationship, drug-event relationship should be assessed as **Yes**.

The following criteria should be considered in order to assess the relationship as **Yes:**

- Reasonable temporal association with drug administration

- It may or may not have been produced by the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject.
- Known response pattern to suspected drug
- Disappears or decreases on cessation or reduction in dose
- Reappears on rechallenge

The following criteria should be considered in order to assess the relationship as **No**:

- It does not follow a reasonable temporal sequence from administration of the drug.
- It may readily have been produced by the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject.
- It does not follow a known pattern of response to the suspected drug.
- It does not reappear or worsen when the drug is readministered.

8.2 Serious adverse events

A serious adverse event is any experience that suggests a significant hazard, contraindication, side effect or precaution. It is any Adverse Event that at any dose fulfils at least one of the following criteria:

- is fatal; (results in **death**; NOTE: death is an outcome, not an event)
- is Life-Threatening (NOTE: the term "Life-Threatening" refers to an event in which the subject was at immediate risk of death at the time of the event; it does not refer to an event which could hypothetically have caused a death had it been more severe).
- required in-patient hospitalization or prolongation of existing hospitalization;
- results in persistent or significant disability/incapacity;
- is a congenital anomaly/birth defect;
- is medically significant or requires intervention to prevent one or other of the outcomes listed above

****The term sudden death should be used only when the cause is of a cardiac origin as per standard definition. The terms death and sudden death are clearly distinct and must not be used interchangeably**.

The study will comply with all local regulatory requirements and adhere to the full requirements of the **ICH Guideline for Clinical Safety Data Management, Definitions and Standards for Expedited Reporting, Topic E2**

Other safety findings may require expedited reporting depending on local legislation, e.g. a major safety finding from a newly completed animal study (such as carcinogenicity).

Progression of underlying malignancy is not reported as an adverse event if it is clearly consistent with the suspected progression of the underlying cancer as defined by RECIST criteria, or other criteria as determined by protocol. Hospitalization due solely to the progression of underlying malignancy should NOT be reported as a serious adverse event. Clinical symptoms of progression may be reported as adverse events if the symptom cannot be determined as exclusively due to the progression of the underlying malignancy, or does not fit the expected pattern of progression for the disease under study.

Symptomatic deterioration may occur in some subjects. In this situation, progression is evident in the subject's clinical symptoms, but is not supported by the tumor measurements. Or, the disease progression is so evident that the investigator may elect not to perform further disease assessments. In such cases, the determination of clinical progression is based on symptomatic deterioration. These determinations should be a rare exception as every effort should be made to document the objective progression of underlying malignancy.

If there is any uncertainty about an adverse event being due only to the disease under study, it should be reported as an AE or SAE.

8.2.1 Reporting of Serious Adverse Events (immediately reportable)

Any clinical adverse event or abnormal laboratory test value that is *serious* and which occurs during the course of the study (as defined in section 7.1.1.3 above), occurring from the enrollment visit (start of study screening procedures), including long term follow-up (LTFU) must be reported to Roche **within *one* working day** of the investigator becoming aware of the event (expedited reporting). The investigator must complete the *SAE Reporting Form [redacted]* and forward it to the SAE Responsible. All SAEs occurring from the enrollment period must be reported, (start of study screening procedures),

Related Serious Adverse Events ***MUST*** be collected and reported regardless of the time elapsed from the last study drug administration, even if the study has been closed. Suspected Unexpected Serious Adverse Reactions (SUSARs) are reported to investigators at each site and associated IRB/IEC when the following conditions occur:

- The event must be a SAE.
- There must be a certain degree of probability that the event is an adverse reaction from the administered drug.
- The adverse reaction must be unexpected, that is to say, not foreseen in the SPC text (Summary of Product Characteristics (for an authorized medicinal product) or the Investigator's Brochure (for an unauthorized medicinal product).

When all subjects at a particular site are off treatment as defined by the protocol:

- only individual SUSAR reports originating in that particular trial will be forwarded to the site and associated IRB/IEC on an expedited basis;
- individual SUSARs considered to be a significant safety issue and/or which result in Roche recommending a change to the

Informed Consent Form (ICF), will be reported in an expedited manner to all investigators and IRBs/IECs;

- SUSAR reports originating from other trials using the same IMP will be provided as six monthly SUSAR Reports (SSRs) to investigators and IRBs/IECs where long-term follow-up studies are carried out, unless they are considered significant..

Unrelated Serious Adverse Events must be collected and reported during the study and for up to 30 days after the last dose of study medication.

This study adheres to the definition and reporting requirements of **ICH Guideline for Clinical Safety Data Management, Definitions and Standards for Expedited Reporting, Topic E2.**

8.2.2 Treatment and Follow-up of AEs

During the study period and up to 30 days after completion or discontinuation of the study, continue to follow up AEs as follows:

Related AEs: Follow until one of the following occurs:

- Resolved or improved to baseline
- Relationship is reassessed as unrelated
- Death
- Start of new anti-cancer regimen
- Investigator confirms that no further improvement can be expected
- Clinical or safety data will no longer be collected, or final database closure

Unrelated severe or life threatening AEs: Follow until one of the following occurs:

- Resolved or improved to baseline
- Severity improved to Grade 2
- Death
- Start of new anti-cancer regimen
- Investigator confirms that no further improvement can be expected
- Clinical or safety data will no longer be collected, or final database closure

Unrelated Grade 1 or Grade 2 AEs: Follow up to 30 days after completion or discontinuation of the study.

The final outcome of each adverse event must be recorded on the CRF

8.2.2 Laboratory evaluations

Laboratory test results will be recorded on the laboratory results form of the CRF, or appear on electronically produced laboratory reports submitted directly from the central laboratory, if applicable.

Any laboratory result abnormality fulfilling the criteria for a serious adverse event (SAE) should be reported as such, in addition to being recorded as an AE in the CRF.

Any treatment-emergent abnormal laboratory result which is clinically significant, i.e., meeting one or more of the following conditions, should be recorded as a single diagnosis on the adverse event page in the CRF:

- Accompanied by clinical symptoms
- Leading to a change in study medication (e.g. dose modification, interruption or permanent discontinuation)
- Requiring a change in concomitant therapy (e.g. addition of, interruption of, discontinuation of, or any other change in a concomitant medication, therapy or treatment)

No special laboratory or tests are required to be performed for the purpose of the study. Measurement of Complete Blood Counts, chemistry and immunological tests such as: serum Ig level, serum immunoellectrophoresis, DAT, peripheral and bone marrow FACS and hepatitis B serology are in fact regarded as current clinical practice for CLL patients, and should therefore be collected according to the Visit Schedules (see Figure 1). In case of suspected hemolysis - DAT, reticulocyte count, serum haptoglobin, bilirubin and LDH are recommended.

The institution will perform laboratory analyses according to the Visit Schedules (see Figure 1). The sponsor must be provided with a copy of the laboratory's certification, and a tabulation of the normal ranges for each parameter required. Additionally, if at any time a patient has laboratory parameters obtained from a different outside laboratory, the sponsor must be provided with a copy of the certification and a tabulation of the normal ranges for that laboratory.

At any time during the study, abnormal laboratory parameters which are clinically relevant (e.g. require dose modification and/or interruption of study drug, lead to clinical symptoms or signs or require therapeutic intervention), whether specifically requested in the protocol or not, must be recorded on the appropriate Comment CRF page in addition to the appropriate laboratory CRF page. When abnormal laboratory values or test results constitute an adverse event (i.e., induces clinical signs/symptoms or requires therapy) they must be recorded on the Adverse Events CRF.

When, in the opinion of the investigator, other clinical laboratory evaluations may be relevant for assessing the patient's status, they will be completed and entered into the database as appropriate.

8.3 Special tests:

PET-CT will be performed in case of suspected Richter transformation.

Lymph node biopsy is recommended for definitive diagnosis of Richter transformation and for any rapidly progressive disease or unusual phenomena which may be suspicious of alteration in the normal behavior of the disease or suspicious for another disease or a different type of lymphoma.

8.4 Physical examinations/vital signs

A physical examination including vital signs will be performed according to the Visit Schedules (see Figure 1). Information about the physical examination and vital signs must be present in the source documentation at the study site. Significant findings present prior to the start of study drug must be included in the Relevant Medical History/Current Medical Conditions CRF. Significant findings made after the start of study drug which meet the definition of an adverse event must be recorded on the Adverse Event Case Report Form. There are no case report forms to capture routine normal findings from physical examinations and vital signs assessments.

8.5 Laboratory examinations

8.5.1 Hematology

Hematological evaluations (CBC) are regarded as clinical practice for CLL patients and should therefore be collected according to the Visit Schedules (see Figure 1).

8.5.2 Blood chemistry

The following blood chemistry results are regarded as clinical practice for CLL patients and should therefore be collected according to the Visit Schedules (see Figure 1).

Serum creatinine, ALT, AST, alkaline Phosphatase, LDH, bilirubin. globulin, serum immunoellectrophoresis and beta-2 microglobulin.

8.6 Physical examinations/vital signs

A physical examination including vital signs will be performed according to the Visit Schedules (see Figure 1). Information about the physical examination and vital signs must be present in the source documentation at the study site. Significant findings present prior to the start of study drug must be included in the Relevant Medical History/Current Medical Conditions CRF. Significant findings made after the start of study drug which meet the definition of an adverse event must be recorded on the Adverse Event Case Report Form. There are no Case Report Forms to capture routine normal findings from physical examinations and vital signs assessments.

8.6.1 Warning and Precautions

No evidence available at the time of the approval of this study protocol indicated that special warnings or precautions were appropriate regarding Rituximab, other than those noted in the Investigators' Brochure.

Safety information regarding Fludarabine and Cyclophosphamide can be found in the respective prescription information.

9. Statistics

Subjects' age, weight, height and other continuous demographic and baseline variables will be summarized using descriptive statistics (mean, standard deviation, minimum and maximum), while performance status, gender, and other categorical variables will be summarized with frequency tabulations.

9.1 Sample size

This is a single arm phase II study. The number of subjects planed to be enrolled into the study is 40. Such a low number of patients as in most phase II studies) constitute an underpowered study for revealing any statistical significance, but is indeed clinically meaningful. So, the sample size chosen for this study was based on clinical considerations.

Those patients achieving PR or CR by the study medication will be regarded as responders. All the others (stable disease, progressive disease or death from any cause) will be regarded as non responders. Determining the ratio of responders versus non responders, even if not statistically significant, will still be clinically meaningful.

9.1.1 Data analysis

Categorical data will be presented as frequencies and percentages. For continuous data, mean, standard deviation, median, 25^{th} and 75^{th} percentiles, minimum, and maximum will be presented.

9.2 Efficacy and Safety evaluation

9.2.1 Efficacy analysis:

All analyses will be according to the intention to treat principle.

9.2.2 Response rate

The primary efficacy parameter to be assessed will be response rate determined by objective overall response rate.

The primary endpoint is a binary variable where each patient is classified as a responder or not responder.

Response rate include CR and PR; all other situations (eg. stable disease, non response, progressive disease or death from any cause) will be rated as treatment failure and therefore as non responders.

Response rate will be assessed according the updated (2008) NCI-WG guidelines defined in appendix 1:

- Peripheral blood FACS for lymphocytes clonality.
- Physical examination for lymph node examination and measurements, and documentation of the presence of hepatomegaly and splenomegaly.
- Blood count for ANC, platelets and hemoglobin levels.
- Bone marrow aspiration and biopsy for bone marrow involvement by CLL cells using FACS immunophenotyping or immunohistochemistry.
- Patient interview for the presence of constitutional symptoms throughout the study.

All above mentioned efficacy (response) parameters will be performed at screening and at least 3 months after completing study drug administration for response documentation.

All known disease (measurable, and non measurable) parameters must be accounted for when assessing objective response rate.

Progression free survival is defined as the interval between the first treatment day to the first sign of disease progression.

Progressive disease (PD) will be assessed according the updated (2008) NCI-WG guidelines defined in appendix I. If needed, BMB will be performed for suspected progression at the investigator's discretion. Progression will be captured on the response form CRF.

A Kaplan-Meier method will be used to estimate median PFS and 90% confidence interval.

QOL questionnaires will be completed by study subjects at: screening visit and visits number: 11, 14, and end of study. Questionnaires will be captured on the QOL forms CRF.

Summary statistics (standard deviation, median, minimum and maximum) will be provided for the relevant variables. ANOVA will be used to analyse the changes in the components and total score of the FACIT QOL assessment.

9.3 Safety Analysis:

Data from all subjects who receive any study drug will be included in the safety analyses. The severity of the toxicities will be graded according to the NCI CTCAE v 3.0
http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcaev3.pdf
or v 2.0 for cytopenia, whenever possible. In the by-subject analysis, a subject experiencing the same event more than once will be counted only once. Adverse events will be defined and summarized according to the worst NCI CTC grade. Adverse events leading to death or to discontinuation of treatment, events classified as NCI CTCAE Grade 3 or Grade 4, study-drug-related events, and serious adverse events will be summarized separately. Laboratory data will be graded according to NCI CTCAE severity grade. ANOVA will be used to analyse the changes in the components and total score of the FACIT QOL assessments.

10. Quality Control and Quality Assurance

Data from the CRFs are entered into the study database by Contract Research Organization staff following their own internal standard operating procedures that have been reviewed and approved by the sponsor.

Subsequently, the entered data are systematically checked by Data Management staff, using error messages printed from validation programs and database listings. Obvious errors are corrected by Data Management personnel. Other errors or omissions are entered on Data Query Forms, which are returned to the investigational site for resolution. The signed original and resolved Data Query Forms are kept with the CRFs at the investigator site, and a copy is sent to the sponsor so the resolutions can be entered into the database. Quality control audits of all key safety and efficacy data in the database are made prior to locking the database.

11. Direct Access to Source Data/Documents

11.1 Study monitoring

The monitoring visits will enable the sponsor or the monitor acting on his behalf, to assess the study's progress status, to verify data accuracy and degree of completeness of the CRFs, to ensure the protocol as well as local regulations are being followed, that the investigator is fulfilling his obligations and to correct errors in the CRFs compared to the source documents. The investigators' will authorize the monitor, at a mutually convenient time during the study to periodically review all of the CRFs and the related parts of administrative, medical and laboratory files of each participant in the study. The CRFs must be filled out before the monitoring visits.

11.2 Audits

During the study, people belonging to the sponsor quality assurance group may visit the investigator site in order to audit the study. The purpose of this visit is to check that the study is carried out according to Good Clinical Practices. Before conducting an audit, the monitor will contact the investigator in order to agree upon a mutually suitable date. The investigator and his team are required to cooperate with the auditors and to grant them access to the patients' medical files and to the study documents (CRFs and investigator's binders).

11.3 IRB and regulatory inspections

A regulatory authority may also wish to conduct an inspection (during the study or even after its completion). If an inspection is requested by a regulatory authority, the investigator must inform the sponsor immediately that this request has been made.

12. Ethics

12.1 Institutional Review Board

Before implementing this study, the protocol, the proposed informed consent form and other information to subjects, must be reviewed by a properly constituted Institutional Review Board (IRB). A signed and dated statement that the protocol and informed consent have been approved by the IRB must be given to the sponsor before study initiation. The name and occupation of the chairman and the members of the IRB must be supplied to the sponsor. Any amendments to the protocol, other than administrative ones, must be approved by this committee.

12.2 Informed consent

The investigator must explain to each subject (or legally authorized representative) the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits involved and any discomfort it may entail. Each subject must be informed that participation in the study is voluntary and that he/she may withdraw from the study at any time and that withdrawal of consent will not affect his/her subsequent medical treatment or relationship with the treating physician.

This informed consent should be given by means of a standard written statement, written in non-technical language. The subject should read and consider the statement before signing and dating it, and should be given a copy of the signed document. If the subject cannot read or sign the documents, oral presentation may be made or signature given by the subject's legally appointed representative, if witnessed by a person not involved in the study, mentioning that the patient could not read or sign the documents. No patient can enter the study before his/her informed consent has been obtained.

The informed consent form is considered to be part of the protocol, and must be submitted by the investigator with it for IRB approval.

12.3 Declaration of Helsinki

The investigator must conduct the trial in accordance with the principles of the Declaration of Helsinki which can be accessed via the website of the World Medical Association at http://www.wma.net/e/policy/17-c_e.html.

13. Data Handling and Record Keeping

The information obtained from the execution of the protocol will be listed on the CRFs provided by the sponsor.
The CRFs must be filled out on a timely basis, signed and dated by the investigator or a co-investigator named by the former and clinically responsible for the patient during the study.

The completed CRFs will be collected by the sponsor. The investigator must remit one CRF for every participant in his center for a time period of 15 years.

14. Financing and Insurance

Financing and insurance will be addressed in the future in a separate agreement.

15. Publication Policy

Any formal presentation or publication of data from this trial will be considered as a joint publication by the investigator(s) and appropriate sponsor's personnel. Authorship will be determined by mutual agreement taking in consideration that the group of PI's will prepare the manuscript and write it initially and decide which member of steering committee and which other active investigators who provided cases will be involved.

It is a multicenter study, and it is mandatory that the first publication is based on data from all centers, analyzed as stipulated in the protocol, by the sponsor statisticians, and not by the investigators. Investigators participating in multicenter studies agree not to present data gathered from one center or a small group of centers before the full publication, unless formally agreed to by all other investigators and the PI's and steering committee.

The investigator may be required to sign the clinical study report, if it is to be used in a registration submission to the health authorities of some countries. For multicenter studies only the coordinating (principle) investigator nominated by the sponsor and the steering committee at the start of the trial would provide any needed signature.

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16. Supplements

Appendix I Response definitions

CR (complete remission) = requires **all** of the following criteria as assessed at least 3 months after completion of therapy:

Absence of clonal lymphocytes in peripheral blood.

Absence of significant lymphadenopathy. (lymph nodes > 1.5 cm in diameter) by physical examination. CT is desirable for not palpable lymph nodes.

No hepatomegaly or splenomegaly by physical examination. (Abdominal, pelvix and thorax CT for subjects found to be abnormal before therapy or if physical examination is inconclusive.

Absence of constitutional symptoms.

Blood counts above the following values: ANC> 1.5 x 10^9/L . Platelets > 100 x10^9/L . Hemoglobin > 11.0 g/dL; untransfused).

Bone marrow aspirate and biopsy should be performed at least 3 months after the last treatment and if the above 5 clinical and laboratory results demonstrate that CR has been achieved.
Bone marrow should be analyzed by FACS/or immunohistochemistry to demonstrate marrow free of B-CLL cells.
In some cases, it is necessary to postpone BMB until after all the other criteria to define a CR have been satisfied. However, this time interval should not exceed 6 months after the last treatment.

Parameter	CR	PR	PD	SD
Group A				
Lymphadenopathy*	None more than 1.5 cm	Decrease ≥ 50%	Increase ≥ 50%	Change of −49% to +49%
Liver and/or spleen size	Normal size	Decrease ≥ 50%	Increase ≥ 50%	Change of −49% to +49%
Constitutional symptoms	None	Any	Any	Any
Polymorphonuclear leukocytes	>1500/μL	>1500/μL or > 50% improvement over baseline	Any	Any
Circulating clonal B lymphocytes	None	Decrease ≥ 50% from baseline	Increase ≥ 50% over baseline	Change of −49% to +49%
Group B				
Platelet count	>100 000/μL	>100 000/μL or increase ≥ 50% over baseline	Decrease of ≥ 50% from baseline secondary to CLL	Change of −49 to +49%
Hemoglobin	>11.0 g/dL (untransfused and without erythropoietin)	>11 g/dL or increase ≥ 50% over baseline	Decrease of >2 g/dL from baseline secondary to CLL	Increase ≤11.0 g/dL or <50' over baseline, or decrease <2 g/dL
Marrow	Normocellular, <30% lymphocytes, no B-lymphoid nodules; hypocellular marrow defines CRi	≥ 30% lymphocytes, or B-lymphoid nodules, or not done	Increase of lymphocytes to more than 30% from normal	No change in marrow infiltrat

Values in SI units for leukocytes are 1.5 × 10^9/L; for platelets 100 × 10^9/L; and for hemoglobin 110 g/L and 20 g/L.

CR indicates complete remission (all of the criteria have to be met); PR, partial remission (at least one of the criteria of group A and one of the criteria of group B have to be met); PD, progressive disease (at least one of the criteria of group A or one of the criteria of group B have to be met); SD, stable disease (all of the above criteria have to be met).

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

Relapse = a patient who has previously achieved CR or PR but after a period of 6 or more months, demonstrates evidence of disease progression

Refractory disease = defined as treatment failure or disease progression within 6 months from the last antileukemic therapy.

Progression = see above table under PD.

Progression free survival (PFS) = defined as the time from start of treatment to progression.

Time to next treatment (TNT) = defined as the time from start of treatment to start of second-line treatment

Appendix II ECOG Performance status

ECOG	Grade
Fully active, able to carry on all pre-disease performance without restriction	0
Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work	1
Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours	2
Capable of only limited self-care, confined to bed or chair more than 50% of waking hours	3
Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair	4
Dead	5

Appendix III calculated creatinine clearance

$$\text{GFR (mL/min)} = \frac{(140\text{-age}) \times \text{weight (Kg)}}{\text{Serum cr (mg/dL)} \times 72} \times 0.85 \text{ (for women)}$$

Appendix IV Active disease

At least one of the following criteria should be met:

1. Evidence of progressive marrow failure as manifested by the development of, or worsening of, anemia and/or thrombocytopenia
2. Massive (ie, > 6 cm below the left costal margin) or progressive or symptomatic splenomegaly.
3. Massive nodes (ie, > 10 cm in longest diameter) or progressive or symptomatic lymphadenopathy.
4. Progressive lymphocytosis with an increase of more than 50% over a 2-month period or lymphocyte doubling time of less than 6 months; patients with initial blood lymphocyte counts of less than $30X10^9$/L (30 000/μL) may require a longer observation period to determine the lymphocyte doubling time. In addition, factors contributing to lymphocytosis or lymphadenopathy other than CLL (eg, infections) should be excluded.
5. Autoimmune anemia and/or thrombocytopenia poorly responsive to corticosteroides or other standard therapy.
6. A minimum of any of the following disease-related symptoms must be present: a- Unintentional weight loss more than or equal to 10% within the previous 6 months. b- Significant fatigue (ie, ECOG PS 2 or worse; cannot work or unable to perform usual activities). c- Fevers of greater than 38.0^0C for 2 or more weeks without other evidence of infection. d- Night sweats for more than 1 month without evidence of infection.

Appendix V Binet staging system

The areas of involvement considered for staging are as follows: 1- Head and neck, including the Waldeyer ring. 2- Axillae 3- Groins 4- Palpable spleen 5- Palpable liver.

Stage A. Hb 10 g/dL or more and platelets 100X 10^9/L or more and up to 2 of the above areas involved.

Stage B. Hb 10 g/dL or more and platelets 100X 10^9/L or more and organomegaly greater than that defined for stage A (3 or more areas).

Stage C. All patients who have Hb less than 10 g/dL and/or a platelets count less than 100X 10^9/L, irrespective of organomegaly.

Appendix VI FACIT-QOL

FACIT – Fatigue Scale (Version 4)

Below is a list of statements that other people with your illness have said are important. **By circling one (1) number per line, please indicate how true each statement has been for you during the past 7 days.**

		Not At all	A little bit	Some what	Quite a bit	Very much
HI7	I feel fatigued	0	1	2	3	4
HI12	I feel weak all over	0	1	2	3	4
An1	I feel listless ("washed out") 0	1	2	3	4	
An2	I feel tired	0	1	2	3	4
An3	I have trouble starting things because I am tired 0	1	2	3	4	
An4	I have trouble finishing things because I am tired 0	1	2	3	4	
An5	I have energy	0	1	2	3	4
An7	I am able to do my usual activities 0	1	2	3	4	
An8	I need to sleep during the day 0	1	2	3	4	
An12	I am too tired to eat	0	1	2	3	4
An14	I need help doing my usual activities	0	1	2	3	4
An15	I am frustrated by being too tired to do the things I want to do	0	1	2	3	4
A 16	I have to limit my social activity because I am tired 0	1	2	3	4	

US English

AMENDMENT HISTORY FOR PROTOCOL ML25464
PROTOCOL AMENDMENT 1
Date of Amendment 1: January 30, 2011

Version: C

Subject: Protocol Title (also synopsis)

Reason:

Adding a protocol nick name

Section: Cover page and synopsis

New text

F. HOFFMANN-LA ROCHE LTD
CLINICAL STUDY PROTOCOL
PROTOCOL NUMBER ML 25464 (FCR LITE)

PROTOCOL APPROVAL

ML25464 Version C
Date: **January 23, 2011**

Old text

F. HOFFMANN-LA ROCHE LTD
CLINICAL STUDY PROTOCOL
PROTOCOL NUMBER ML 25464

PROTOCOL APPROVAL

ML25464 Version A and B
Date: **January 10, 2011** **August 30 2010**

Subject: changes visit schedule

Reason for change:

Corrections of protocol discrepancies between figure 1 and text in protocol design - section 4 and synopsis

Section: 4.2.1 visit schedule and assessments

New text

Figure 1: Visit schedule and assessments

Week	-4 to 0	1	3	5	7	9-25 Every 4 weeks	29	37	49	61	73-145 every 12 weeks	157 EOS
Visit No.	1	2	3	4	5	6-10	11	12	13	14	15-21	22
Study period	preTx	Treatment period						Follow up period				
Inc. /Excl. criteria	X											
Informed consent	X											
Relevant medical history/conditions	X											
Disease history	X											
BMB*	X								X			
Physical exam	X	X	X	X	X	X	X	X	X	X	X	X
CBC	X	X	X	X	X	X	X	X	X	X	X	X
Blood chemistry	X	X		X		X	X	X	X	X	X	X
Beta-2 micro glob	X											
IEP, Ig level,	X								X			X
DAT***	X								X			
FACS****	X								X			X
Hepatitis B *****	X											
Imaging - (CT) **	X								X			
QOL	X						X			X		X
Treatment dosage	X	Ongoing data capture										
Adverse Events	X	Ongoing data capture and up to 30 days after completion or discontinuation of the study										
Concomitant medications	X	Ongoing data capture										
Comments	X	Ongoing data capture										
Study completion	X	Complete at any time if study drug is discontinued										

* Bone marrow examination will be performed at any clinically suspicion of progression/ leukemia transformation, cytopenia evaluation, or for any CR confirmation at investigator discretion.

** Abdominal, pelvic and chest CT will be performed at any clinical suspicion of progression.

*** DAT will be performed at any clinical suspicion of hemolysis.

**** Will be performed at any clinical suspicion of progression/ leukemia transformation, or for CR confirmation at investigators' discretion.

*****At screening and at investigator discretion for hepatitis treatment evaluation.

Old text

Figure 1: Visit schedule and assessments

Week	-4 to 0	1	3	5	7	9-25 Every 4 weeks	29	37	49	61-77 every 12 weeks	89	101-161 every 12 weeks	EOS
Visit No.	**1**	**2**	**3**	**4**	**5**	**6-10**	**11**	**12**	**13**	**10-13**	**14**	**15-19**	**20**
Study period	preTx	**Treatment period**						**Follow up period**					
Inc. /Excl. criteria	X												
Informed consent	X												
Relevant medical history/conditions	X												
Disease history	X												
BMB*	X								X				
Physical exam	X	X	X	X	X	X	X	X	X	X	X	X	X
CBC	X	X	X	X	X	X	X	X	X	X	X	X	X
Blood chemistry	X	X		X		X	X	X	X	X	X	X	X
Beta-2 micro glob	X												
IEP, Ig level,	X								X				X
DAT***	X								X				X
FACS**	X								X				X
Hepatitis B ***	X												
Imaging - (CT) **	X												X
QOL	X						X				X		X
Treatment dosage	X	Ongoing data capture											

Adverse Events	X	Ongoing data capture and up to 30 days after completion or discontinuation of the study
Concomitant medications	X	Ongoing data capture
Comments	X	Ongoing data capture
Study completion	X	Complete at any time if study drug is discontinued

* Bone marrow examination will be performed at any clinically suspicion of progression/ leukemia transformation, cytopenia evaluation, or for any CR confirmation at investigator discretion.

** Abdominal, pelvic and chest CT will be performed at any clinical suspicion of progression.

*** DAT will be performed at any clinical suspicion of hemolysis.

**** Will be performed at any clinical suspicion of progression/ leukemia transformation, or for CR confirmation at investigators' discretion.

*****At screening and at investigator discretion for hepatitis treatment evaluation.

Subject: changes to steroids type

Reason for change:

Adaptation to steroids used in Israel

Sections: synopsis, section 4.2 overall study design, section 4.3.1 investigational therapy, section 6.2 concomitant therapy

New text

Premedication: antihistamines (e.g. 2 mg clemastin or promethazine 25 mg i.v.) and paracetamol (500-1000 mg orally) 30 min before first infusion of Rituximab, and thereafter only if clinically indicated. Hydrocortisone 100-500 mg I.V. (according local common practice) only if clinically indicated.

Old text

Premedication: antihistamines (e.g. 2 mg clemastin or promethazine 25 mg i.v.) and paracetamol (500-1000 mg orally) 30 min before first infusion of Rituximab, and thereafter only if clinically indicated. Prednisone/prednisolone 500 mg I.V. only if clinically indicated.

Subject: Exclusion criteria- Hepatitis B carriers

Reason for change:

Recent knowledge about the risk of life threatening hepatitis B reactivation in persons treated with Rituximab

Section 5.2 Exclusion criteria and synopsis

New text

- Active or uncontrolled infections, including opportunistic infection, active hepatitis B / C -positive Hepatitis B Surface Ag or Hepatitis B Core Ab

Old text

Active or uncontrolled infections, including opportunistic infection, active hepatitis B / C

Subject: Cytopenia documentation by BMB

Reason for change:

Typo error correction (deletion).

Section 4.2 Overall study design

New text

Bone marrow biopsy and aspiration examination will be performed before the start of treatment, for response documentation (3 to 6 months after the completion of treatment), for evaluation of cytopenias (disease or treatment related) and for any other clinical indication at the discretion of the investigators.

Old text

Bone marrow biopsy and aspiration examination will be performed before the start of treatment, for response documentation (3 to 6 months after the completion of treatment), for evaluation and documentation of cytopenias (disease or treatment related) and for any other clinical indication at the discretion of the investigators.

Subject: Infusion bags labeling and accountability

Section:4.3.1 Investigational therapy

Reason for change:

Correction according GCP rules and sponsor SOP

New text

Labels will comply with the legal requirements in Israel and be printed in Hebrew and English. All study drugs containing infusion bags will be labeled. All infusion bags will be labeled by the study nurse prior to administration and only the empty Rituximab infusion bags will be collected for drug accountability by the study monitor.

Old text

Labels will comply with the legal requirements in Israel and be printed in Hebrew and English. All study drugs containing infusion bags will be labeled. Rituximab infusion bags will be labeled by the sponsor and will be collected for drug accountability .

Section 4.2.1 Drug accountability

New text

Accountability and patient compliance will be assessed by maintaining adequate "drug dispensing log" on **study medication**.

The "drug dispensing" log for the **study medication** must be kept current and should contain the following information:

- The identification of the patient (patient number) to whom the **study medication** was administered.
- The date(s) and quantity of **study medication**, used for the patients' treatment.
- The date(s) and quantity of **study medication**, of empty, partial or unused medication.

Old text

Accountability and patient compliance will be assessed by maintaining adequate "drug dispensing log" on **Rituximab**.

The "drug dispensing" log for **Rituximab** must be kept current and should contain the following information:

- The identification of the patient (patient number) to whom **Rituximab** was administered.
- The date(s) and quantity of **Rituximab**, used for the patients' treatment.
- The date(s) and quantity of **Rituximab**, of empty, partial or unused medication.

Subject: Reporting of Serious Adverse Events

Reason for change:
Deletion of typo error

Section: **8.2.1 Reporting of Serious Adverse Events (immediately reportable)**

New text

Any clinical adverse event or abnormal laboratory test value that is *serious* and which occurs during the course of the study (as defined in section 7.1.1.3 above occurring from the enrollment visit (start of study screening procedures), including long term follow-up (LTFU) must be reported to Roche **within *one* working day** of the investigator becoming aware of the event (expedited reporting).

Old text
Any clinical adverse event or abnormal laboratory test value that is *serious* and which occurs during the course of the study (as defined in section 7.1.1.3 above), regardless of the treatment arm occurring from the enrollment visit (start of study screening procedures), including long term follow-up (LTFU) must be reported to Roche **within *one* working day** of the investigator becoming aware of the event (expedited reporting).

Subject: Missing wording

Reason for change:
Missing wording

Section 8.3 special tests

New text

Lymph node biopsy is recommended for definitive diagnosis of Richter transformation and for any rapidly progressive disease or unusual phenomena which may be suspicious of alteration in the normal behavior of the disease or suspicious for another disease or a different type of lymphoma.

Old text

Lymph node biopsy is recommended for definitive diagnosis of Richter transformation and for any rapidly progressive disease or unusual phenomena which may be suspicious of alteration in the of the disease or suspicious for another disease or a different type of lymphoma.

Subject: Geriatric evaluation

Reason for change:

Increasing safety of geriatric age patient recruitment

Section 5.2 exclusion criteria (and synopsis)

New text

Patients who are mentally or physically unable to comply with all aspects of the study. Upon investigator discretion, a psycho-geriatric evaluation will be performed for patients ≥ 80 years old.

Old text

Patients who are mentally or physically unable to comply with all aspects of the study.

Subject: ZAP70 and CD38 as optional tests

Reason for change:

To allow more information collection about possible risk factors influencing response

Section: 5.1 Inclusion criteria and synopsis

New text

1. Diagnosis of B-CLL is established by the presence of: an absolute lymphocyte count > $5X10^9$ L, and typical CLL cell morphology in the peripheral blood smear as seen by light microscopy. CLL cells must show CD5/CD19/CD23 positivity with light chain restriction as recorded by flow cytometry. The levels of surface immunoglobulin and CD 79b are characteristically low and FMC7 is expected to be negative (optional). ZAP 70 and CD38 are optional.

Old text

1. Diagnosis of B-CLL is established by the presence of: an absolute lymphocyte count > $5X10^9$ L, and typical CLL cell morphology in the peripheral blood smear as seen by light microscopy. CLL cells must show CD5/CD19/CD23 positivity with light chain restriction as recorded by flow cytometry. The levels of surface immunoglobulin and CD 79b are characteristically low and FMC7 is expected to be negative (optional).

Subject: CTCAE V.3

Reason for change:

Better determination(version number) of CTCAE and giving reference to CTCAE details.

Section 9.3 Safety Analysis

New text

Data from all subjects who receive any study drug will be included in the safety analyses. The severity of the toxicities will be graded according to the NCI CTCAE v 3.0 http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcaev3.pdf

Old text

Data from all subjects who receive any study drug will be included in the safety analyses. The severity of the toxicities will be graded according to the NCI CTCAE v 3.0

AMENDMENT HISTORY FOR PROTOCOL ML25464 VERSION D FCR-LITE DATED 06 OCT 2011

1. Subject: Cytogenetics in bone marrow

Reason for change:

Cytogenetics in bone marrow is not performed routinely in medical centers and is not part of CLL assessment.

Section 4.2 Overall study design

New text:

Pre-treatment period:… Bone marrow **biopsy** (FISH whenever possible) must have been performed within 12 months of starting the first dose of the study drugs.

Old text:

Pre-treatment period:…Bone marrow examination **including cytogenetics** (FISH whenever possible) must have been performed within 12 months of starting the first dose of the study drugs.

New text:

Follow up period:…Bone marrow examination, peripheral blood immunophenotyping (FACS), abdominal, pelvic and chest CT will be performed 3 to 6 months after the completion of the treatment regimen, at clinical progression and at any other time according to the investigators' discretion.

Old text:

Follow up period:…Bone marrow examination **including cytogenetics**, peripheral blood immunophenotyping (FACS), abdominal, pelvic and chest CT will be performed 3 to 6 months after the completion of the treatment regimen, at clinical progression and at any other time according to the investigators' discretion.

Section 7 Assessment of efficacy

New text:

Bone marrow aspiration and biopsy to assess degree of bone marrow involvement by CLL using FACS and immunohistochemistry, should be performed at least 3 months after the last treatment, if the above **four** clinical and laboratory results **support** that CR has been achieved.

Old text:

Bone marrow aspiration and biopsy to assess degree of bone marrow involvement by CLL using FACS and immunohistochemistry, should be performed at least 3 months after the last treatment if the above 4 clinical and laboratory results demonstrate that CR has been achieved

2. SUBJECT: Visit schedule and assessments

Reason for change:

The schedule was corrected to fit the text of the protocol: One pre-treatment visit, 8 treatment visits (composed of 2 examination visits [these are titled visits 3 and 5] and 6 treatment visits). The Follow-up period will consist of 12 follow-up visits, once every 12 weeks.

All visits in the table during which a treatment is given are marked with an X in the new column within the visit schedule table named – "Treatment"

Section Figure 1: Visit schedule and assessments

New text:

Figure 1: Visit schedule and assessments

Week	**-4 to 0**	**1**	**3**	**5**	**7**	**9**	**13**	**17**	**21**	**33**	**45**	**57**	**69**	**81**	**93-153 every 12 weeks**	**EOS**
Visit No.	**1 Screening**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15-21**	**22**
Study period	**preTx**	**Treatment Period**								**Follow up period**						
Inc. /Excl. criteria	X															
Informed consent	X															
Relevant medical history/conditions	X															
Disease history	X															
BMB [1]	X												X			
Physical exam	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
CBC	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Blood chemistry	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Beta-2 micro glob	X															
IEP, Ig level,	X												X			X
DAT [3]	X												X			X
FACS [4]	X [4]												X			X
Hepatitis B [5]	X															
Imaging - (CT) [2]	X															X
QOL – FACIT	X										X			X		X
Treatment dosage		X		X		X	X	X	X							

Adverse Events	X	Ongoing data capture and up to 30 days after completion or discontinuation of the study
Concomitant medications	X	Ongoing data capture
Comments	X	Ongoing data capture
Study completion	X	Complete at any time if study drug is discontinued

1 - Bone marrow biopsy must be performed within 12 months before start of treatment. BMB will be performed for progression/ lymphoma transformation, cytopenia evaluation, and for treatment response within 3-6 months after completion of treatment. For any other clinical indication it is at the discretion of the investigator whether to perform BMB.

2 - CT scan when done pre-treatment, must be performed within 12 weeks prior to starting the first dose of study drugs. Post therapy a CT scan will be performed for all CLL patients who had an abnormal CT scan prior to treatment or if physical examination is inconclusive - this is an optional assessment.

3 - DAT will be performed **at screening** and at any clinical suspicion of hemolysis.

4 - **FACS results from up to 12 months prior to screening are relevant and no further test is necessary at screening. FACS will also be performed at** any clinical suspicion of progression/ **lymphoma** transformation, or for CR confirmation at investigators' discretion.

5 - At screening and at investigator discretion for hepatitis treatment evaluation.

Old Text:

Figure 1: Visit schedule and assessments

Week	-4 to 0	1	3	5	7	9-25 Every 4 weeks	29	37	49	61	73-145 every 12 weeks	157 EOS
Visit No.	1	2	3	4	5	6-10	11	12	13	14	15-21	22
Study period	preTx	Treatment period						Follow up period				
Inc. /Excl. criteria	X											
Informed consent	X											
Relevant medical history/conditions	X											
Disease history	X											
BMB*	X								X			
Physical exam	X	X	X	X	X	X	X	X	X	X	X	X
CBC	X	X	X	X	X	X	X	X	X	X	X	X
Blood chemistry	X	X		X		X	X	X	X	X	X	X
Beta-2 micro glob	X											
IEP, Ig level,	X								X			X
DAT***	X								X			
FACS****	X								X			X
Hepatitis B *****	X											
Imaging - (CT) **	X								X			
QOL	X						X			X		X
Treatment dosage	X	Ongoing data capture										
Adverse Events	X	Ongoing data capture and up to 30 days after completion or discontinuation of the study										
Concomitant	X	Ongoing data capture										
Comments	X	Ongoing data capture										
Study completion	X	Complete at any time if study drug is discontinued										

progression/ leukemia transformation, cytopenia evaluation, or for any CR confirmation at investigator discretion.

** Abdominal, pelvic and chest CT will be performed at any clinical suspicion of progression.

*** DAT will be performed at any clinical suspicion of hemolysis.

**** Will be performed at any clinical suspicion of progression/ leukemia transformation, or for CR confirmation at investigators' discretion.

*****At screening and at investigator discretion for hepatitis treatment evaluation.

Section Exams at Screening (page 23) – comments of figure 1

In the schedule (page 23) the following assessments were emphasized as being performed at screening:

New text:

3 - DAT will be performed **at screening** and at any clinical suspicion of hemolysis.

Old Text:

***DAT will be performed at any clinical suspicion of hemolysis.

New text:

1 - **Bone marrow biopsy must be performed within 12 months before start of treatment. BMB will be performed for progression/ lymphoma transformation, cytopenia evaluation and for treatment response within 3-6 months after completion of treatment. For any other clinical indication it is at the discretion of the investigator whether to perform BMB.**

Old Text:

progression/ leukemia transformation, cytopenia evaluation, or for any CR confirmation at investigator discretion.

New text:

4 - **FACS results from up to 12 months prior to screening are relevant and no further test is necessary at screening. FACS will also be performed at** any

clinical suspicion of progression/**lymphoma** transformation, or for CR confirmation at investigators' discretion.

Old Text:

****Will be performed at any clinical suspicion of progression/ leukemia transformation, or for CR confirmation at investigators' discretion.

3. SUBJECT: Dose modification for impaired renal function

Reason for change:

After a consultation with prinicpal investigators and after review of the Fludarabine investigator brochure and the CLL 8 protocol we arrived at a consensus that if the calculated creatinine clearance is in the range of 30-70 mL/min the dose of Fludarabine be reduced by 20% (to 80%) and the cyclophosphamide dose be reduced by 25% (to 75%).

SECTION 4.3.3 Dose modification for impaired renal function

New text:

Fludarabine **and cyclophosphomide are** partly (40-60%) excreted by the kidneys. If the calculated (see appendix III) or measured creatinine clearance is **at screening or during treatment is decreased to 30-70 mL/min, the dose of fludarabine should be reduced to 80% and the cyclophosphamide dose reduced to 75%**. Patients with a creatinine clearance below 30 ml/min are excluded from the study.

Old Text:

Fludarabine is partly (40-60%) excreted by the kidneys. If the calculated (see appendix III) or measured creatinine clearance is reduced to 30-60 ml/min, the fludarabine dose should be reduced to 50%. Patients with a creatinine clearance below 30 ml/min are excluded from the study.

4. SUBJECT: Rituximab discontinuation will result in study termination

Reason for change:

If it is impossible to continue treatment with Rituximab due to side effects, the patient's participation in the study will be discontinued.

SECTION 5.3.1 Discontinuation of study drugs

New text:

In the case of a severe reaction to Rituximab, Rituximab will be permanently discontinued **and the patient's participation in the study will be discontinued.**

Old Text:

In the case of a severe reaction to Rituximab, Rituximab will be permanently discontinued. **Patient will be treated with FC in the next cycles and data will be recorded throughout the study period.**

5. SUBJECT: Delaying Rituximab treatment due to high tumor burden

Reason for change:

The option of delaying the treatment with Rituximab for up to 7 days after FC administration in cycle 1 has been added in case of high tumor burden.

SECTION 4.3.1 Investigational therapy

New text:

Consider reduced rate of Rituximab for first infusion or splitting the dose over two days during the first cycle. In cycle 1 the administration of Rituximab may be delayed up to 7 days after the administration of the Fludarabine and Cyclophosphomide (FC). Rituximab will be given together with FC in cycle 2, 28 days after the administration of FC in cycle 1.

Old Text:

Consider reduced rate for first infusion or a split dosing over two days during the first cycle.

6. SUBJECT: Patients with autoimmune hemolytic anemia

Reason for change:

Patients with autoimmune hemolytic anemia are excluded from the study and thus cannot receive steroids in the study.

SECTION 6.2 Concomitant therapy

New text:

Steroid administration is allowed exclusively for patients with autoimmune thrombocytopenia or as premedication for Rituximab.

Old Text:

Steroids administration are allowed exclusively for patients with **autoimmune hemolytic anemia and or** autoimmune thrombocytopenia or as premedication for Rituximab .

New text:

Steroids if given (see above) are allowed for no more than 3 consecutive days.

Old Text:

Steroids if given (see above) are allowed for no more than 3 consecutive days.

Excluding patients with autoimmune cytopenia, in which case prolonged period steroid administration is permitted (see above).

7. SUBJECT: In situ prostate cancer does not prevent recruitment - Exclusion no. 11

Reason for change:

In addition to in situ breast and cervix cancer in situ, prostate cancer will also not prevent recruitment to the study.

SECTION 5.2 & Synopsis Exclusion Criteria no.11

New text:

- Presence of active second neoplasia (excluding non-melanoma skin cancer, or in situ cervix, breast **or prostate** carcinoma) < 2 years prior to the study.

Old Text:

- Presence of active second neoplasia (excluding non-melanoma skin cancer, or in situ cervix or breast carcinoma) < 2 years prior to the study.

8. SUBJECT: CT scan prior to treatment

Reason for change:

CT scan prior to treatment will be accepted as baseline if performed up to 3 months prior to cycle 1.

SECTION 4.2 Overall study design - Pre-treatment period

New text:

CT scan when done, must be performed **within 12 weeks prior** to starting the first dose of study drugs.

Old Text:

CT scan when done, must be performed within 6 weeks of starting the first dose of study drugs.

9. SUBJECT: CT scan post treatment is optional

Reason for change:

Visit schedule needs to be updated as to the optional use of CT scan in baseline and response assessment.

SECTION Figure 1 - comments

New text:

CT scan when done pre treatment, must be performed within 12 weeks prior to starting the first dose of study drugs. Post therapy a CT scan will be performed for all CLL patients who had an abnormal CT scan prior to treatment or if physical examination is inconclusive – this is an optional assessment.

Old Text:

Abdominal, pelvic and chest CT will be performed at any clinical suspicion of progression.

10. SUBJECT: Performance status ≥3 as an exclusion criteria

Reason for change:

Due to concerns over patients safety and the desire not to include patients who cannot undergo this protocol the PS has been lowered.

SECTION 5.2 & Synopsis Exclusion Criteria no. 14

New text:

Performance status: ECOG performance status **≥3**

Old Text:

Performance status: ECOG performance status ≥4.

11. SUBJECT: Reporting of Serious Adverse Events

Reason for change:

Clarifications were updated in the Safety section according to Roche SOPs.

SECTION 8.2.1 Reporting of Serious Adverse Events (immediately reportable)

New text:

None.

Old Text:

When all subjects at a particular site are off treatment as defined by the protocol:

- only individual SUSAR reports originating in that particular trial will be forwarded to the site and associated IRB/IEC on an expedited basis;
- individual SUSARs considered to be a significant safety issue and/or which result in Roche recommending a change to the Informed Consent Form (ICF), will be reported in an expedited manner to all investigators and IRBs/IECs;
- SUSAR reports originating from other trials using the same IMP will be provided as six monthly SUSAR Reports (SSRs) to investigators and IRBs/IECs where long-term follow-up studies are carried out, unless they are considered significant.

12. SUBJECT: Warning and Precautions

Reason for change:

Clarifications were updated in the Warning section regarding Fludarabine and Cyclophosphamide.

SECTION 8.6.1 Warning and Precautions

New text:

Safety information regarding Fludarabine and Cyclophosphamide can be found in the respective **local MoH approved product label.**

Old Text:

Safety information regarding Fludarabine and Cyclophosphamide can be found in the respective prescription information.

13. SUBJECT: Destruction Process

Reason for change:

Section 4.3.1.2 has been added in order to detail destruction process in the site.

SECTION 4.3.1.2 DESTRUCTION OF THE INVESTIGATIONAL MEDICINAL PRODUCT

New text:

After the monitor has reconciled study drug supplies, used supplies (partly used and empty Rituximab infusion bags) will be sent for destruction according to the site guidelines. The destruction process in the site should be documented.

Old Text:

None.

SECTION 4.5 Accountability procedures for the investigational products

New text:

Study drug will be prepared and dispensed by the pharmacist at the investigator's institution. **Drug accountability & destruction procedures are described in sections 4.3.1.1 & 4.3.1.2, accordingly.**

Old Text:

Study drug will be prepared and dispensed by the pharmacist at the investigator's institution.

14. SUBJECT: Statistics

Reason for change:

Changes and clarifications made by the protocol statistician.

SECTION 9.2.2 Response rate

New text:

95% Confidence Interval (CI) will be calculated to the proportions of responders.

Old Text:

None

New text:

Summary statistics (standard deviation, median, minimum and maximum) will be provided for the relevant variables**. The Paired T-test or Signed Rand Test for paired observations (as is appropriate) will be applied for analysing** the changes in the components and total score of the FACIT QOL assessment.

The data will be analyzed using the SAS ® version 9.1.3(SAS Institute, Cary North Carolina).

Old Text:

Summary statistics (standard deviation, median, minimum and maximum) will be provided for the relevant variables**. ANOVA will be used to analyse** the changes in the components and total score of the FACIT QOL assessment.

SECTION 9.3 Safety Analysis

New text:

All adverse events will be coded according to coding dictionaries (MedDRA version 12.1) and presented in tables by system organ class and preferred term.

Old Text:

None.

New text:
None.

Old Text:

ANOVA will be used to analyse the changes in the components and total score of the FACIT QOL assessments

F. HOFFMANN-LA ROCHE LTD

CLINICAL STUDY PROTOCOL

PROTOCOL NUMBER ML 25464 (FCR LITE)

A PHASE II, MULTICENTER, SINGLE ARM STUDY TO DETERMINE THE EFFICACY AND SAFETY OF LOW DOSE FLUDARABINE AND CYCLOPHOSPHAMIDE COMBINED WITH STANDARD DOSE RITUXIMAB AS PRIMARY THERAPY IN ELDERLY UNTREATED PATIENTS (≥65 YEARS OLD) WITH CHRONIC LYMPHOCYTIC LEUKEMIA

PROTOCOL APPROVAL

Protocol Number / Version: **ML25464 / Version D**

Medical Manager : [redacted] Date: 06 Oct 2011

Project statistician: [redacted] Date: 06 Oct 2011

This protocol is intended for use in a life-threatening indication: Yes ☑ No ☐

A PHASE II, MULTICENTER, SINGLE ARM STUDY TO DETERMINE THE EFFICACY AND SAFETY OF LOW DOSE FLUDARABINE AND CYCLOPHOSPHAMIDE COMBINED WITH STANDARD DOSE RITUXIMAB AS PRIMARY THERAPY IN ELDERLY UNTREATED PATIENTS (≥65 YEARS OLD) WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AE	Adverse Event
ALT	Alanine Aminotransferase/GPT
ANC	Absolute Neutrophil Count
AST	Aspartate Aminotransferase/SGOT
ALK PHOSP	Alkaline Phosphatase
CBC	Complete Blood Count
CLL	Chronic Lymphatic Leukemia
CR	Complete Remission
CRF	Case Report/Record Form
CRO	Contract Research Organization
CT	Computerized Tomography
CY	Cyclophosphamide
FACT-CTC	Functional Assessment of Cancer Therapy - Common Terminology Criteria for cancer Adverse Events
FACS	Flow Cytometry (Fluorescence-Activated Cell Sorting)
DAT	Direct Coombs Test
FLU	Fludarabine
G-CSF	Granulocyte-Colony Stimulating Factor
Hb	Hemoglobin
HIV	Human Immune deficiency Virus
IEP	Immuno-ellectrophoresis
I.V.	Intra-Venously
Ig	Immunoglobulin
IRB	Institutional Review Board
IRR	Infusion Related Reaction
LDH	Lactic Dehydrogenase
MHO	Medical Health Organization
ORR	Overall Response Rate
PD	Progressive Disease
PET-CT	Positron Emission Tomography- Computerized Tomography
PFS	Progression Free Survival
P.O.	Per Os/ by mouth/orally
PR	Partial Response
QOL	Quality Of Life
SAE	Serious Adverse Event
TLS	Tumor Lysis Syndrome
TNT	Time to Next Treatment
ULN	Upper Limit of Normal

SYNOPSIS

A PHASE II, MULTICENTER, SINGLE ARM STUDY TO DETERMINE THE EFFICACY AND SAFETY OF LOW DOSE FLUDARABINE AND CYCLOPHOSPHAMIDE COMBINED WITH STANDARD DOSE RITUXIMAB AS PRIMARY THERAPY IN ELDERLY UNTREATED PATIENTS (≥65 YEARS OLD) WITH CHRONIC LYMPHOCYTIC LEUKEMIA

PROTOCOL NUMBER:	ML25464
DATE PROTOCOL FINAL:	20 August 2011
STUDY DRUG:	FLUDARABINE, CYCLOPHOSPHAMIDE, RITUXIMAB
INDICATION:	CHRONIC LYMPHOCYTIC LEUKEMIA
STUDY PHASE:	II

Background and rationale Chronic lymphocytic leukemia (CLL) is characteristically diagnosed in the older population with a median age at diagnosis ranging between 65 and 72 years. Because the prevalence of co morbidity increases with age, concomitant disease is often present in this age group. Additional factors which may also adversely affect treatment outcome at standard doses in elderly patients include: altered pharmacokinetics and poorer host tissue tolerance ; changes in the bone marrow hematopoietic reserve and microenvironment leading to increased treatment related myelotoxicity and higher rates of treatment related complications such as infections. As a result, elderly patients are often excluded from clinical trials or even from routine treatment on the basis of their age and / or comorbidity. Furthermore when these patients are treated, the drug doses are often markedly reduced in an attempt to avoid causing toxicity and complications. The challenge in treating elderly CLL patients relates to finding the correct balance between drug efficacy and toxicity and because of this optimal treatment for this group of patients is often hard to achieve.

Study Objectives

The aim of this study is to evaluate the safety and efficacy (response rate) of a combination of low dose Fludarabine (FLU) and Cyclophosphamide (CY) combined with standard dose Rituximab given as primary treatment for elderly patients with chronic lymphocytic leukemia.

Primary end point: -Response rate

Secondary end points:

- Safety - Special issues: incidence of neutropenic fever, hospitalizations (no^{o}- days),
- Thrombocytopenia ≥grade 3, neutropenia ≥grade 3, Infection rate.
- Progression Free Survival (PFS).
- Time to Next Treatment (TNT).
- QOL

STUDY DESIGN:

This is a multicenter, phase II, single arm study designed to evaluate the efficacy and safety of the combination of low dose Fludarabine (FLU) and Cyclophosphamide (CY) combined with standard dose Rituximab in elderly previously untreated CLL patients ($\geq$ 65 years of age). Potential study subjects will sign an informed consent prior to undergoing any study related procedure. Number of patients to be enrolled is 40.

Treatment plan: Flu 12.5mg/m^2/d will be given intravenously (I.V.) for days 1-3 every 28 days, for a total of 6 cycles.

CY will be given I.V. at the dose of 150 mg/ m^2/d on days 1-3 every 28 days, for a total of 6 cycles.

Rituximab will be given before chemotherapy. In cycle1: 375 mg/m^2 I.V. on day 0, cycles 2-6: 500 mg/m^2 I.V. on day 1 every 28 days.

Dose reduction or delays are recommended in case of grade $\geq$3 (NCI-CTC) adverse events.

This study consists of 3 periods for each study subject: Pre-treatment, Treatment and Follow up.

Pre-treatment period: patients will undergo screening for protocol eligibility within 28 days (4 weeks) of recruitment.

Subjects who meet all the inclusion criteria and have signed an approved informed consent will be enrolled.

Treatment period FLU 12.5mg/m^2/d I.V. will be given on days 1-3 every 28 days, for a total of 6 cycles. CY will be given (I.V.) 150 mg/ m^2/d on days 1-3 every 28 days, for a total of 6 cycles. Rituximab given before chemotherapy cycle 1: 375 mg/m^2 intravenously on day 0, cycles 2-6: 500 mg/m^2 I.V. on day 1 every 28 days.

Premedication: antihistamines (e.g. 2 mg clemastin or promethazine 25 mg i.v.) and paracetamol (500-1000 mg orally) 30 min before first infusion of Rituximab, and thereafter only if clinically indicated. Hydrocortisone 100-500 mg I.V. (according with local common practice) only if clinically indicated.

Dose reduction or delay in case of drug induced hematological toxicity grade ≥ 3 level.

During the treatment period, all patients will attend twice a month in the first 2 months and thereafter periodic monthly study center visits in order to assess the safety and efficacy of the treatment.

Complete blood count will be performed on day 1 and 14 of each of the first two cycles, and on day 1 in the other 4 cycles, or more frequently at the investigators discretion.

Bone marrow aspiration and biopsy and CT scan (optional) will be performed within 3-to 6 months after the completion of the treatment period.

Follow up period: All patients will be assessed for progression and survival every 3 months via clinic visits, for a total of 30 months.
Complete blood count and chemistry will be performed every 3 months, or more frequently at the investigators discretion.
In the event of suspected progression or relapse BMB and or CT scan will be performed at the treating physicians' discretion.

Duration of study

The duration of the treatment period is approximately 6 months. This time period is required to complete the therapy, to determine the safety profile of the drug combination and the response rate to therapy. The duration of the follow up period will be 30 months. The occurrence of PD will determine the duration of progression-free survival of each patient.

Recruitment period will be 24 months.

Inclusion criteria:

- Diagnosis of B-CLL is established by the presence of: An absolute lymphocyte count> $5X10^9$/L, typical morphology in the peripheral blood smear as seen by light microscopy, CLL cells must show CD5/CD19/CD23 positivity with light chain restriction as recorded by flow cytometry. Low surface immunoglobulin/CD 79b expression is also expected to be evident on these cells while FMC7 should be negative-are both additional typical features on flow cytometry (optional).
- Previously untreated B-CLL patients $\geq$ 65 years old.
- Patients requiring treatment (active Binet stage A and B, all stage C disease).
- Written informed consent

Exclusion criteria:

- Calculated or measured creatinine clearance < 30 ml/min
- Hepatic enzymes or bilirubin > 2X ULN, unless thought to be due to CLL
- Suspected or documented CNS involvement by CLL
- Known HIV positivity
- Active or uncontrolled infections, including opportunistic infection, active hepatitis B / C -positive Hepatitis B Surface Ag or Hepatitis B Core Ab
- Various concomitant diseases requiring chronic steroid administration
- Active Coombs positive hemolytic anemia
- Prior treatment for CLL
- CLL with transformation (Richter syndrome).
- Presence of active second neoplasia (excluding non-melanoma skin cancer, or in situ cervix, breast **or prostate** carcinoma) < 2 years prior to the study.
- Use of other investigational agents or participation in any other clinical trials < 30 days prior to the study.
- Low compliance.
- Performance status: ECOG performance status **$\geq$3**.
- Patients who mentally or physically are unable to comply with all aspects of the study.

Assessments:

Efficacy

The following evaluations will be done to record the efficacy of the treatment regimen:

- Assessment of disease response (revised International Response Criteria: updated NCI-IWG 2008).[46].
- Date of documentation of disease progression.
- Bone marrow biopsy examination
- Peripheral blood immunophenotyping by FACS.
- Changes in ANC, Hb levels and platelets counts from baseline blood counts and those done during the study period.
- Chest, abdominal and pelvic computerized tomography
- Physical examination for lymphadenopathy and hepato-splenomegaly

Safety

The following evaluations will be recorded to assess the safety of the regimen:

- Complete physical examination including vital signs.
- Clinical laboratory evaluations (hematology, biochemistry)
- Concomitant medication and procedures
- Adverse events
- Health outcomes assessment (any extra medical appointment)

Efficacy analysis:

Primary efficacy will be determined by objective overall response rate. Exact tests for assessing these will be used to compare objective overall response rates. Kaplan Meier procedure will be used to characterize the duration of response for PFS and TNT. Summary statistics (standard deviation, mean, median, minimum and maximum) will be provided for the relevant variables

Safety Analysis:

Data from all subjects who receive any study drug will be included in the safety analyses. The severity of the toxicities will be graded according to the NCI CTC v3 or v2- for cytopenia, whenever possible. In the by-subject analysis, a subject experiencing the same event more than once will be counted only once.

Adverse events will be defined and summarized by the worst NCI CTC grade. Adverse events leading to death or to discontinuation of treatment, events classified as NCI CTC Grade 3 or Grade 4, study-drug-related events, and serious adverse events will be summarized separately. Laboratory data will be graded according to NCI CTC severity grade.

STUDY DURATION: five years **TOTAL SAMPLE SIZE**: 40

2. Background Information

Chronic lymphocytic leukemia (CLL) is characteristically diagnosed in older people with a median age at diagnosis ranging between 65 and 72 years. Because the prevalence of co morbidity increases with age, concomitant disease is often evident in this age group. Goede et al [29] reported that increasing numbers of more severe concomitant comorbid illnesses are associated with a shortened survival in patients with CLL. Most recently Thurmes and colleagues [1] have shown that these comorbid conditions are in fact less important than both the age of the patient and the disease stage, in predicting prognosis in untreated patients.

Additional factors which may also adversely affect treatment outcome in elderly patients include: altered pharmacokinetics and poorer host tissue tolerance ; changes in bone marrow hematopoietic reserve and microenvironment which may lead to increased treatment related myelotoxicity and higher rates complications such as infections [21,26]. As a result, elderly patients are often excluded from clinical trials or even from routine treatment at standard doses of drugs, on the basis of their age and / or comorbidity and when treated the drug doses are often markedly reduced when given.

The basic challenge in treating elderly CLL patients appears to be related to finding the correct balance between treatment efficacy and drug toxicity and because of this, optimal treatment for older patients is hard to achieve and remains an open question and topic of debate.

Purine analogues drugs which inhibit DNA synthesis are very effective in CLL and fludarabine, cladribine and pentostatin have all been used effectively in the treatment of CLL [14]. Fludarabine is the most extensively studied purine analogue in CLL and is available in tablet form as well as for intravenous use. When used in combination with cyclophosphamide, fludarabine has been shown to be more effective than when used alone. [13,14,27,28]. In this respect the U.K. MRC LRF CLL4 trial reported in 2007 [11] compared therapy with chlorambucil alone vs fludarabine vs fludarabine (F) alone + cyclophosphamide (FC) in 777 newly diagnosed CLL patients, and showed

that FC achieved superior response rates and better progression-free survival for all CLL patients . Because of this, FC was proposed as the gold standard treatment of all CLL patients irrespective of age [11]. Similar results were published by the German CLL Study Group and the North American Intergroup [14].

However although Fludarabine - based regimes are most efficacious, they also appear to be more toxic, inducing more prolonged myelosuppression with more infections.

Pollizzotto et al [15] investigated the influence of increasing age on the ability to deliver fludarabine based regimens as well as the toxicity obtained thereafter, in patients with indolent lymphoproliferative disorders. They reported a gradual increase in toxicity with advancing age especially in patients age ≥ 70 years but still concluded that regimens were generally well tolerated and could be delivered safely to older patients who had a good performance status (PS). They noted a modestly increased degree of myelosuppression but no increase in the incidence of severe infectious complications or treatment-related mortality.

Table 1

PR	CR	OR	Patients age	Oral Cyclophosphamide dose	Oral Fludarabine dose	Disease status	Journal
NHL-59% CLL-100%	NHL-35% CLL-0%	NHL-94% CLL-100%	61-85 Median 73.5	150mg/m^2 X 4 days Every 28 days , 4 courses	25mg/m^2 (total 40 mg) X 4 days	Mostly relapsed refractory CLPD-28 Patients CLL-9 p	Hematology 2004 (16)
All-44% SLL-4/8	All-40% SLL-3/8	All-84% SLL-7/8	66-85 Med 74	150mg/m^2 X 4 days Every 28 days ; 4 courses	25mg/m^2 (total 40 mg) X 4 days	Untreated 25 patients SLL-8	BJH 2007 (17)

40%	54%	94%	30% above age 70	150mg/m^2 X 5 days Every 28 dX6	24mg/m^2 X5 days	Untreated (196)	CLL4 (11)

2.1 Rituximab

Since the introduction of Rituximab (Rituxan™, IDEC) (FDA Approval Letter Nov. 26, 1997) in the USA and shortly thereafter in Europe in 1998 (MabThera™ (Hoffmann-LaRoche AG) for use in the treatment of CLL and indolent non-Hodgkins' lymphoma (NHL), it has been used extensively in the treatment of indolent and aggressive lymphoma, both as a single agent or more often in combination with chemotherapy. Today Rituximab is already being used to treat a variety of autoimmune disorders and by now has also been accepted as the model monoclonal antibody for the treatment of cancer. Rituximab is a chimeric mouse variable heavy and light chains (IDEC 2B8) with a human IgG_1 constant kappa antibody (C2B8) that reacts specifically with the CD20 antigen found on the surface of malignant and normal B cells and established B-cell lines [31,33]. The antigen is expressed at lower levels on chronic lymphocytic leukemia (CLL) and plasma cells [31]. CD20 is not shed from the surface of CD20+ cells after antibody binding and it is not internalized or downregulated [34]. Compared with its murine counterpart, Rituximab has a longer half-life in humans, interacts with human effector cells [34, 35], and is less immunogenic [36].

Reduced FC doses

There are only a few published reports (including relatively small numbers of CLL/SLL patients which describe the results of an FC regimen in elderly CLL patients-(≥ 70 years). These are listed in Table1.

2.2 Use of Rituximab and Combination of Rituximab and Fludarabine in CLL

Although initial responses to single agent treatment with Rituximab were low and partial responses and not complete remissions, interest continued in the use of this agent in CLL [37,39,40]. The original studies employed 375 mg/m^2 of Rituximab once a week for four weeks. Subsequent studies suggested using this dose three times a week for four weeks for a total of 12 doses instead of 4 doses which resulted in better overall responses [41]. Dose limiting toxicities of Rituximab have not been demonstrated perhaps due to the cost of the drug. In this respect it should be noted that in the initial studies using Rituximab to treat follicular NHL, responses were much higher than in CLL. This was attributed to the lower level of CD20 expression on CLL lymphocytes and the presence of circulating CD20 antigen. These differences were partially overcome when either the dose of Rituximab was increased or the frequency of the standard dose of 375 mg/m2 was increased [41, 44].

After establishing the safety and efficacy of standard dose Rituximab 375 mg/m2 three times a week for four weeks for a total of twelve doses, Byrd et al went on, to compare sequential and concurrent therapy with RF [43]. The concurrent group was therefore treated with 11 doses of Rituximab while the sequential cohort only received four doses. This was a randomized phase II trial of concurrent versus sequential Fludarabine and Rituximab with 51 and 53 patients per arm, respectively. The overall response rate in the concurrent arm was 90%, including CR in 47%while in the sequential arm the overall response rate was 77%, with CR in 28%. The disease-free and overall median survival has yet to be reached yet, but will be in excess of 23 months [44]. Three major side effects were observed: infusion related toxicity, myelosuppression, and infections. Infusion related toxicity were greatest in concurrent treated patients (20%) and almost not existent in the sequentially treated patients. In a phase II study, the German CLL Study Group (GCLLSG) [44] reported the treatment of 20 untreated and 11 relapsed CLL patients. Treatment consisted of Fludarabine administered at standard doses (25mg/m2/d; days 1-5, 29-33, 57-61, 85-89) and Rituximab (375mg/m2/d)

given on days 57, 85. The overall response rate (CR and PR) was 87% with a CR/CRu of 33%. The median duration of response was 75 weeks. Severe anemia (grade III/IV) was observed in 10% of patients, neutropenia in 42%, and thrombocytopenia in 9%, respectively. The most frequent non-hematologic side effects were infections (52%) and infusion-related symptoms. The majority of infections were respiratory and herpes virus infections.

A retrospective comparative analysis of the CALGB 9712 [47] (Rituximab plus Fludarabine) [48] and the CALGB 9011 (Fludarabine only) studies demonstrated that the addition of Rituximab to Fludarabine resulted in a significantly better 2 years progression free survival and overall survival compared to patients treated with Fludarabine alone (67% *Vs* 45% and 93% *Vs* 81%, respectively) and in both treatment approaches the infectious toxicity was similar [45].

The MD Anderson Cancer Center group reported a single arm study that utilized the combination of Rituximab, Fludarabine and Cyclophosphamide (FCR) in untreated and in relapsed and refractory CLL patients (224 and 177 patients, respectively) [24, 25]. In the untreated patients the overall response rate was 95% with a CR rate of 70%, a nodular PR rate of 10% and a PR rate of 15%. Although, during the follow-up period the time to treatment was not reached, a calculated analysis showed that 69% of patients were projected to relapse at 4 years. The major toxicities were myelosupression, predominantly of grade 3 and 4, neutropenia (in 52% of all courses) and infections.

In the relapsed and refractory patients the overall response was 73% with 25% CR, 16% nodular PR and 32% PR and a third of the complete responders achieved molecular remission. The median time to progression in responding patients was 28 months with an overall median survival time of 42 months. About 46% of the patients completed all six intended courses and myelosupression was the major cause for discontinuing treatment (66% and 18% grade 3-4 neutropenia and thrombocytopenia, respectively) with 16 % of the patients having major infection.

Recently, two pivotal phase III studies (CLL8 and REACH) [49,50] evaluated 6 cycles of rituximab (375mg/m^2 on cycle-1 and 500mg/m^2 on cycle 2-6) plus

fludarabine (25mg/m^2 d1-3) and cyclophosphamide (250mg/m^2 d1-3) chemotherapy compared with 6 cycles of fludarabine and cyclophosphamide alone. The primary endpoint of both studies was progression-free survival. Secondary endpoints for both studies included overall survival, event-free survival, duration of response, response rate and complete response. CLL8 involved 817 patients with previously untreated (first-line) CLL and good physical fitness. With a median observation time of 37.7 months, statistically significant differences were observed in OS between the two treatment arms. The OS rate was 84.1% in the FCR arm versus 79 % in the FC arm (p=0.01). In both arms, the median OS has still not been reached. Patients who received rituximab together with chemotherapy had a median progression free survival (PFS) of 51.8 months compared to 32.3 months for those who received chemotherapy alone. (hazard ratio 0.56; p < 0.001). The overall response rate in patients receiving rituximab plus chemotherapy as first-line therapy was 95.1% vs. 88.4% in those receiving chemotherapy alone and with more complete remissions in the FCR arm (44.1 vs 21.8%; p<0.001). Severe (grade 3 or greater) adverse events that occurred more often in the rituximab arm included haematologic toxicity (56% vs. 39%), neutropenia (34% vs. 21%) and leukocytopenia (24% vs. 12%).

The REACH study[49] involved 552 patients with previously treated CLL (second line therapy). Patients who received rituximab plus chemotherapy had a median PFS of 30.6 months compared to 20.6 months for those who received chemotherapy alone (hazard ratio 0.65; p=0.0001). The overall response rate in the Rituximab-containing arm was 70% vs. 58% in those receiving chemotherapy alone without Rituximab. Grade 3 or greater events such as neutropenia (42% vs. 40%), febrile neutropenia (12% vs. 12%) and neoplasms (7% vs. 3%) occurred more often in the rituximab plus chemotherapy arm. Based on the CLL8 and REACH results the FDA approved the use of rituximab as first- and second-line treatment of CLL. In Europe, Rituximab is also approved for treatment of first and relapsing CLL patients.

In the light of the above literature, the combined use of Rituximab and Fludarabine and cyclophosphamide (FCR) is currently regarded as the gold standard and backbone treatment for physically fit patients with CLL.

2.3 Fludarabine Cyclophosphamide Rituximab (FCR) regimens for elderly patients with CLL

Due to major concerns of myleotoxicity and infection complications, this chemo-immunotherapy combination regimen cannot be applied safely to all elderly CLL patients. To address this topic efforts are being made to establish novel FCR-based protocol that will be useful for the average elderly CLL patient. In this regard, Foon and colleagues [50] reported a phase II single arm study of reduced dosages of Fludarabine and Cyclophosphamide (FCR-lite). Fifty untreated CLL patients were treated with 6 cycles of Fludarabine (20mg/m^2) and Cyclophosphamide (150mg/mg^2) combined with high–dose Rituximab (500mg/m^2) given every other week during induction and later as maintenance every 3 months for two years. The OR and CR rates were 100% and 79%, respectively. Median duration of complete response was 22.3 months (range, 5.2 to 42.5 months) and none of the complete responders have relapsed. Grade 3/4 neutropenia was noted in 13% of the cycles of therapy. Another approach to reduce toxicity was to use sequential therapy with fludarabine (25 mg/m^2 on days 1 through 5 for 6 cycles) followed by consolidation with Cyclophosphamide (3,000 mg/m^2 administered every 3 weeks for three cycles) and then by Rituximab 375 mg/m^2 for four cycles (F→C→R). [51] In 36 previously untreated CLL patients an overall response of 89% was achieved including 61% CRs. 33% achieved a molecular CR (PCR negative) and achieving molecular CRs had an excellent prognosis with a plateau in the response duration curve, and 90% remain in clinical CR at 5 years. For the entire group, 5-year survival rate is 71% compared with a rate of 48% with the prior F→C regimen (not significant *P* = .10).

3. Trial Objectives and Purpose

The aim of this study is to evaluate the safety and efficacy of a combination of low dose Fludarabine (FLU) and Cyclophosphamide (CY) combined with standard dose Rituximab given as primary treatment for elderly patients with chronic lymphocytic leukemia.

The purpose of the study is to determine whether a lower dose FC combined with standard dose Rituximab regimen in elderly CLL patients is as effective as the standard FCR dose, given in most studies until now and at the same time to assess if it is indeed less toxic.

3.1 Primary Objective

To evaluate the efficacy (Response Rate) of the combination of lower dose Fludarabine 12.5 mg m^2/d given intravenously for three days and Cyclophosphamide 150 mg/d given intravenously for three days every 28 days, combined with standard dose Rituximab (375mg/ m^2 in cycle 1 and 500 mg/m^2 in cycles 2-6) for a total of 6 cycles.

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3.2 Secondary Objectives

1- Safety of the combination drugs given in the study population, especially in relation to: neutropenic fever, Infection rate, number of hospitalization days, thrombocytopenia ≥grade 3, neutropenia ≥ grade 3.

2- Progression Free Survival (PFS).

3- Time to Next Treatment (TNT).

4- Quality Of Life (FACIT)

4. Trial Design

4.1 Primary and secondary end points

Primary End Point

Overall response rate (ORR) (CR and PR) as defined in the protocol (see appendix I)

4.1.1 Secondary End Points

1- Safety of the study drugs especially in relation to: neutropenic fever, Infection rate and number of hospitalization days,

thrombocytopenia ≥grade 3, neutropenia ≥grade 3.

2- Progression free survival (PFS) defined as the time elapsed from first day of study drug administration to disease progression as defined in the protocol (see appendix I)

3- Time to Next Treatment (TNT) as defined in the protocol (see appendix I)

4- QOL(see appendix VI)

4.2 Overall study design

This is a national, multicenter, phase II, single arm (not controlled), open label clinical trial to evaluate the efficacy and safety of the combination of low dose Flu + Cy combined with standard dose Rituximab Flu dosage will be given at 12.5mg/m^2/d given I.V. together with CY150 mg/m^2/d given I.V. for three days every 28 days. Rituximab will be given before chemotherapy
cycle 1: 375 mg/m^2 I.V. on day 0 and in cycles 2-6: 500 mg/m^2 I.V. on day 1 in previously untreated CLL patients who are more than 65 years of age. Patients will be treated every 28 days for up to 6 cycles. Potential study subjects will sign an informed consent form prior to undergoing any study related procedure. In general, study medications should be continued until disease progression or drug intolerance but no more than 6 cycles. Treatment choices after completion of the study are at the investigators' discretion.
Up to 40 patients are planned to be enrolled during a two year period.
This study consists of 3 periods for each study subject: A pre-treatment period, the treatment period and a follow up period.

Pre-treatment period:

Patients will undergo screening for protocol eligibility within 28 days (4 weeks) of recruitment.

Subjects who meet all the inclusion criteria and who have signed an approved informed consent will be enrolled.

Written informed consent must be obtained before any study - specific medical procedures are performed which include laboratory screening assessments and physical examination as well as documentation of vital signs. If the hematology and/or biochemistry evaluations have been performed within 14 days of the first dose of study drug as part of the screening evaluation, they need not be repeated, and the data may be used as the Day 1 values. **Bone marrow biopsy** (FISH whenever possible) must have been performed within 12 months of starting the first dose of the study drugs.

CT scan when done, must be performed **within 12 weeks prior** to starting the first dose of study drugs.

Treatment period

Includes 6 months of combination treatment with low dose Fludarabine and Cyclophosphamide combined with standard dose of Rituximab. Flu dosage will be given at 12.5mg/m^2/d given I.V. together with CY150 mg/m^2/d I.V. for three days every 28 days. Rituximab given before chemotherapy cycle 1: 375 mg/m^2 I.V. on day 0 and in cycles 2-6: 500 mg/m^2 intravenously on day 1 every 28 days.

Premedication: antihistamines(e.g. 2mg clemastin or promethazine 25mg I.V.) and paracetamol (500-1,000 mg orally) 30 minutes before first infusion of Rituximab, and thereafter only if clinically indicated. Hydrocortisone 100 - 500mg I.V.(according with local common practice only if clinically indicated.

In the event of grade $\geq$ 3 hematological toxicity which in the investigators' opinion, is study drugs related, the administration of the next cycle should be delayed but not for more than 3 weeks and drug dose reduction of 25% of

both CY and F is recommended. Two dose reductions are permitted (FLU and CY Dose modification page 29). In cases where the delay period has been terminated but grade 3-4 toxicity persists, the patient should be removed from the protocol with no further study drug administration.

During the treatment period, all patients will attend periodic twice a month in the first 2 months and thereafter monthly study- center visits in order to assess safety and efficacy of the treatment.

Complete blood counts will be performed on day 1 and 14 of the first two cycles. In the next 4 cycles- on day 1 of each cycle, or more frequently at the investigators' discretion.

Bone marrow biopsy and aspiration examination will be performed before the start of treatment, for response documentation (3 to 6 months after the completion of treatment), for evaluation of cytopenias (disease or treatment related) and for any other clinical indication at the discretion of the investigators.

Supportive care including blood transfusions or administration of growth factors G-CSF or erythropoietin is permitted at the investigators' discretion throughout all study periods.

Follow up period

During the follow up period, of 30 months after the completion of the study drugs administration, all patients will be assessed every 3 months for evidence of progression and documentation of survival at clinic visits.

Complete blood count will be performed every 3 months, or more frequently at the investigators' discretion.

Bone marrow examination, peripheral blood immunophenotyping (FACS), abdominal, pelvic and chest CT will be performed 3 to 6 months after the completion of the treatment regimen, at clinical progression and at any other time according to the investigators' discretion.

In the event of no response (no CR nor PR) in > 5 of the initial 10 subjects enrolled into the study, Fludarabine dosage will be increased for all the next

subjects enrolled into the study and will be given at the dose of 15mg/m^2/d given I.V.

In the event of no response after the above increment in > 5 of the next 10 subjects who received the increased dosages, the study will be terminated and declared inadequate and inefficient. If response is evident in ≥ 5 of the 10 patients, the same dosages will be continued throughout the study until its completion.

All further treatment decisions after completion of the study are at the discretion of the investigators.

4.2.1 Visit schedule and assessments

Visit schedule

Patient must be followed at the study center according to the visit schedule and assessments outlined in Figure 1.

All routine assessments must be performed within a time window of ± 7 days of the day indicated on the visit schedule, except visits 2-5 in which time window will be of ± 3 days .

Figure 1: Visit schedule and assessments

Week	-4 to 0	1	3	5	7	9	13	17	21	33	45	57	69	81	93-153 every 12 weeks	EOS
Visit No.	1 Screening	2	3	4	5	6	7	8	9	10	11	12	13	14	15-21	22
Study period	preTx	Treatment Period								Follow up period						
Inc. /Excl. criteria	X															
Informed consent	X															
Relevant medical history/conditions	X															
Disease history	X															
BMB [1]	X												X			
Physical exam	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
CBC	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Blood chemistry	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Beta-2 micro glob	X															
IEP, Ig level,	X												X			X
DAT [3]	X												X			X
FACS [4]	X [4]												X			X
Hepatitis B [5]	X															
Imaging - (CT) [2]	X															X
QOL – FACIT	X										X			X		X
Treatment dosage		X		X		X	X	X	X							
Adverse Events	X	Ongoing data capture and up to 30 days after completion or discontinuation of the study														
Concomitant medications	X	Ongoing data capture														
Comments	X	Ongoing data capture														
Study completion	X	Complete at any time if study drug is discontinued														

1 - Bone marrow examination must be performed within 12 months before start of treatment. BMB will be performed for progression/ lymphoma transformation, cytopenia evaluation, and for treatment response within 3-6 months after completion of treatment. For any other clinical indication it is at the discretion of the investigator whether to perform BMB.

2 - CT scan when done pre-treatment, must be performed within 12 weeks prior to starting the first dose of study drugs. Post therapy a CT scan will be performed for all CLL patients who had an abnormal CT scan prior to treatment or if physical examination is inconclusive - this is an optional assessment.

3 - DAT will be performed **at screening** and at any clinical suspicion of hemolysis.

4 - **FACS results from up to 12 months prior to screening are relevant and no further test is necessary at screening. FACS will also be performed at** any clinical suspicion of progression/ **lymphoma** transformation, or for CR confirmation at investigators' discretion.

5 - At screening and at investigator discretion for hepatitis treatment evaluation.

4.3 Trial treatment

4.3.1 Investigational therapy

Fludarabine is supplied to the study investigators by the Medical Health Organization (MHO) as routine first line treatment in all CLL patients. Fludarabine is supplied as 10 mg tablets packaged in blister packs, or as 50 mg/vial, as lyophilized powder that has to be dissolved in 2 Ml of water for injection, than diluted in 100 ml of saline (0/9%) given in 30 minutes.

Cyclophosphamide is supplied to the study investigators by the MHO as a routine first line treatment in CLL patients. Cyclophosphamide (Endoxan) is supplied as 50 mg tablets packed in blister packs or as 500 mg/vial (24 mg/ml) diluted in 100 ml of saline (0/9%) given in 30 minutes.
Rituximab is supplied to the study investigators by the MHO as a routine first line treatment in CLL patients. Rituximab (Mabthera) is supplied as 100mg of rituximab in 10ml (10mg/ml) pack of 2 vials or 500mg of rituximab in 50ml (10mg/ml) pack of 1 vial. The antibody should be diluted into a final volume of normal saline or 5% D-glucose in water to allow for a maximal concentration of 4 mg/ml. Rituximab should be administrated before FC chemotherapy.

First Infusion: Recommended initial rate is 50mg/hr; after the first 30 minutes increase by 50mg/hr every 30 minutes to maximum of 400 mg/hr. *Subsequent Infusions:* Initial rate 100mg/hr; increase by 100mg/hr every 30 minutes to a maximum of 400mg/hr

Use extreme caution and closely monitor first infusion when treating patients with ≥ $25x10^{9}$/l circulating malignant cells or high tumor burden (higher risk of severe cytokine release syndrome (CRS). It is recommended to administer Hydrocortisone 100-500 mg I.V. (according local common practice) shortly before infusion with Rituximab to decrease the rate and severity of acute IRR/CRS for these patients. **Consider reduced rate of Rituximab for first infusion or splitting the dose over two days during the first cycle. In cycle 1 the administration of Rituximab may be delayed up to 7 days after the administration of the Fludarabine and Cyclophosphomide (FC).**

Rituximab will be given together with FC in cycle 2, 28 days after the administration of FC in cycle 1. Severe CRS: may be associated with some features of tumor lysis syndrome e.g. hyperuricaemia, hyperkalaemia, hypocalcaemia, hypophosphataemia, acute renal failure, elevated LDH and may be associated with acute respiratory failure and death. If severe CRS manifests stop infusion immediately and start aggressive symptomatic treatment.

If the first dose of rituximab is well tolerated, the starting flow rate for administration of the second and subsequent infusions will be 100 mg/hour and then increased gradually by 100 mg/hour intervals not to exceed 400 mg/hour.

If any grade 1 or 2 infusion related reactions occur during an infusion, the infusion should be slowed or interrupted (at the discretion of the Investigator) and supportive treatment instituted. The infusion rate can be increased or restarted on resolution of the symptoms.

If grade 3 or 4 infusion related reactions occur during an infusion, the infusion has to be discontinued immediately and not restarted until resolution of the symptoms. Treatment can be reinitiated (at half the original infusion rate) at the discretion of the Investigator, but if the same adverse event appears again with the same severity, treatment must be permanently discontinued.

Should bronchospasm or dyspnea occur in the patient during the infusion, the infusion should be stopped immediately. Medication such as anti-histamines or steroids should be given for symptomatic relief. The infusion should not be re-started until symptoms have resolved completely and should be given at half the original infusion rate.

In the event of infusion related reactions to rituximab:

• Stop rituximab infusion, treat symptoms aggressively if necessary
• NaCl 0.9 % i.v., if necessary
• Hydrocortisone 100-500 mg I.V. (according local common practice), if necessary
• Pethidine i.v. if necessary
• Bronchodilators/antihistamines if necessary
• If side effects occur stop Rituximab-infusion, re-try the next day

Table 2: Reduction of Infusion Rate

Drop in systolic blood	Mucosal congestion, edema	Rigors	Fever	Infusion rate
> 30 mm Hg	Mild/moderate	Mild/moderate	> 38.5°C	Decrease to ½ if any of these events are seen

Following the infusion the I.V. line should be kept open for medications as needed. If there are no complications, the line may be discontinued after one hour of observation. If complications occur during the rituximab infusion, the patient should be observed for 2 hours after the completion of the infusion.

Infusion reactions to rituximab tend to be more common and more severe with the first infusion. In patients who were unable to tolerate the first infusion, cautious reintroduction of rituximab may be tried at the second cycle, at the investigator's discretion. The recommended premedication should be given and then the patient carefully re-exposed to a small amount of rituximab (slow infusion of 10 mg I.V.) If a severe reaction occurs, the rituximab must be permanently discontinued. In such case patient will be treated with FC in the next cycles and data will be collected throughout the study period)
If no severe reaction occurs after 30 minutes, the full dose of rituximab infusion should be given.

Labels will comply with the legal requirements in Israel and be printed in Hebrew and English. All study drugs containing infusion bags will be labeled. All infusion bags will be labeled by the study nurse prior to administration and only the empty Rituximab infusion bags will be collected for drug accountability by the study monitor. Storage conditions for study drug will be described on the medication label. Patients will receive the study drug combination for an exposure period of up to 6 months provided that in the opinion of the investigator the patient is benefiting from treatment with this study drug combination, and in the absence of any safety concerns. Treatment will be withheld only in the case of limiting toxicities (see below).

As administration of cyclophosphamide may cause hemorrhagic cystitis and even urinary bladder malignancy, patients will be instructed to drink at least 10 glasses of fluids on the days they receive cyclophosphamide and urinate at minimal ceasing of bladder distention.

4.3.1.1 DRUG ACCOUNTABILITY

Accountability and patient compliance will be assessed by maintaining adequate "drug dispensing log" on study medication.

The "drug dispensing" log for the study medication must be kept current and should contain the following information:

- The identification of the patient (patient number) to whom the study medication was administered.
- The date(s) and quantity of study medication, used for the patients' treatment.
- The date(s) and quantity of study medication, of empty, partial or unused medication.

All accountability logs must be available for inspection by the monitor. Drug supplies (partly used and empty Rituximab infusion bags) must be available for inspection, at every monitoring visit. Used supplies will be destructed according to local site guidelines.

4.3.1.2 DESTRUCTION OF THE INVESTIGATIONAL MEDICINAL PRODUCT

After the monitor has reconciled study drug supplies, used supplies (partly used and empty Rituximab infusion bags) will be sent for destruction according to the site guidelines. The destruction process in the site should be documented.

4.3.2 Dose modification for hematological toxicity

Dose modifications will not be made during the first cycle of therapy. During the flowing cycles modifications of the treatment schedule will only be made as follows:

• If at day 1 of any cycle, there is a grade ≥ 3 cytopenia (See hematological toxicity section 8), NOT RELATED TO BONE MARROW INFILTRATION DUE TO CLL, treatment should be delayed for up to three weeks and then given with a 25% dose reduction of fludarabine and cyclophosphamide in the following cycles. There is no dose reduction for Rituximab in this study, but

drug administration will be delayed for the same time period as for fludarabine and cyclophosphamide.

• If after three weeks, the grade ≥ 3 cytopenia, NOT RELATED TO CLL BONE MARROW INFILTRATION, still prevails, the patient should be removed from the protocol with no further study drug administration.

• If there is further grade ≥ 3 cytopenia in subsequent cycles, despite the first 25% dose reduction treatment should again be delayed for up to three weeks and the dose of fludarabine and cyclophosphamide further reduced to 50% of the full initial dose.

• If there is further grade ≥ 3 cytopenia in subsequent cycles despite the 50% drugs dose reduction the patient should be removed from the protocol with no further treatment.

• If there is any grade ≥ 3 neutropenia with infection during any cycle, G-CSF should be administered and G-CSF should than be given in all subsequent cycles prophylactically.

4.3.3 Dose modification for impaired renal function

Fludarabine **and cyclophosphomide are** partly (40-60%) excreted by the kidneys. If the calculated (see appendix III) or measured creatinine clearance is **at screening or during treatment reduced to 30-70 mL/min, the fludarabine dose should be reduced to 80% and the cyclophosphamide dose will be reduced to 75%.** Patients with a creatinine clearance below 30 ml/min are excluded from the study. Patients who develop renal dysfunction (serum creatinine ≥ 1.5 mg/dL or creatinine clearance < 30 ml/min) during treatment should go off protocol treatment.

4.3.4 Dose modification for other non-hematological toxicity (non renal).

• If other grade ≥ 3 non-hematological, non-renal toxicity occurs during any cycle, study drugs treatment should be delayed until recovery to grade ≤ 2, for up to three weeks. All subsequent cycles should then be given with a 25% dose reduction of fludarabine and cyclophosphamide.

• If after three weeks, the grade ≥ 3 non-hematological, non-renal toxicity still persists, the patient should be removed from the protocol.
• If there is further grade ≥ 3 non-hematological, non-renal toxicity in subsequent cycles despite the first 25% dose reduction treatment should again be delayed for up to three weeks and the dose of fludarabine and cyclophosphamide further reduced to 50% of the initial full dose.
• If there is further grade ≥ 3 non-hematological, non-renal toxicity in subsequent cycles despite 50% dose reduction the patient should be removed from the protocol treatment and the study.

4.3.5 Treatment assignment

Informed consent must be obtained before any testing for the purpose of determining a patient's eligibility is performed. Each patient will be assigned a unique patient number. Once assigned, numbers for any non-evaluable or discontinued patient will not be reutilized.

4.3.6 Blinding

This is an open label trial.

4.4 Study drug interruption or discontinuation

4.4.1 Discontinuation of study drugs

Events that will be considered as reasons for study drugs discontinuation are described in section 5.3
In addition, patient follow-up information must still be recorded (also in the event of study drug discontinuation) during and at the end of the study period.

4.4.2 Study Discontinuation

Events that will be considered as reasons for study discontinuation are described in section 5.3

4.5 Accountability procedures for the investigational products

Study drug will be prepared and dispensed by the pharmacist at the investigator's institution. **Drug accountability & destruction procedures are described in sections 4.3.1.1 & 4.3.1.2, accordingly.**

5. Selection and Withdrawal of Subjects

5.1 Inclusion Criteria

1. Diagnosis of B-CLL is established by the presence of: an absolute lymphocyte count > $5X10^9$ L, and typical CLL cell morphology in the peripheral blood smear as seen by light microscopy. CLL cells must show CD5/CD19/CD23 positivity with light chain restriction as recorded by flow cytometry. The levels of surface immunoglobulin and CD 79b are characteristically low and FMC7 is expected to be negative (optional). ZAP 70 and CD38 are optional.
2. Previously untreated B-CLL patients ≥ 65 years old.
3. Patients requiring treatment (active Binet classification stage A and B, all stage C disease).(appendixes IV and V)
4. Written informed consent

5.2 Exclusion Criteria

1. Calculated or measured creatinine clearance < 30 ml/min
2. Hepatic enzymes or bilirubin ≥ 2X ULN, unless thought to be due to CLL.
3. Suspected or documented CNS involvement by CLL
4. Known HIV positivity
5. Active or uncontrolled infections, including opportunistic infection and active hepatitis B / C
6. Positive Hepatitis B Surface Ag or Hepatitis B Core Ab
7. Various concomitant diseases requiring chronic steroid administration
8. Active Coomb's positive hemolytic anemia.
9. Prior treatment for CLL
10. CLL in transformation (Richter syndrome).
11. Presence of active second neoplasia (excluding non-melanoma skin cancer, or in situ cervix, breast **or prostate** carcinoma) < 2 years prior to the study.

12. Use of other investigational agents or participation in any other clinical trials < 30 days prior to the study.
13. Low compliance.
14. Performance status: ECOG performance status **≥3**. (see appendix II)
15. Patients who are mentally or physically unable to comply with all aspects of the study. Upon investigator discretion, a psycho-geriatric evaluation will be performed for patients ≥ 80 years old.

5.3 Interruption or Discontinuation of Treatment

5.3.1 Discontinuation of study drugs

The following events will be considered as reasons for discontinuation.

- Interruption of study drug/s for longer than 3 consecutive weeks.
- Adverse events, including study drugs induced active autoimmune haemolytic anemia.
- Patient with large cell transformation (Richter syndrome).
- Disease progression as defined at any time during study drug therapy.
- Treatment with other chemotherapeutic or investigational anti-neoplastic drugs
- Intolerable adverse effects that are judged by the investigator to be either physically or psychologically detrimental to the patient. Unresolved or recurrent (more than twice) grade 3 or 4 toxicity.
- The reason for discontinuation should be recorded in the CRF and in the subject's medical records. The sponsor is to be notified of all discontinuations related to study medication.
- In addition, patient follow-up information will be recorded (also in the event of study drug discontinuation) during and at the end of the study period.
- In the case of a severe reaction to Rituximab, Rituximab will be permanently discontinued **and the patient's participation in the study will be discontinued.**

5.3.2 Interruption of study drugs

In the event of grade 3 or 4 toxicity, or increase of creatinine>1.5 or GFR of less than 30ml/min study drug will be interrupted for no more than 3 weeks (see sections 4.3.2-4.3.4).

5.4 Study Discontinuation

The following events are considered as study discontinuation:

- Death
- Participation in another investigational drug trial
- Loss to follow-up
- Patient withdrawal of consent
- Major violation of the study protocol- such as eligibility criteria. In these cases, no patient follow-up will be recorded.
- Administrative problems
- In the event of no response after dosage increment in > 5 of the next 10 subjects who received the increased dosage.(see chapter 4.2).

The section for “Study Completion” in the CRF must be completed for all patients. The reason for early discontinuation should be given, even if the patient refuses to return for a final visit. Patients who discontinue prematurely due to significant study drug related adverse events (AEs) should continue to be followed until resolution of the AE or up to 30 days after discontinuation. The relevant sections of the CRF should be completed as appropriate.

Patients who discontinue the study drugs for any reason should be scheduled for a visit within 30 days from drug discontinuation at which time all the assessments listed for the final visit should be performed (see figure 1).

Patients lost to follow up should be recorded as such on the CRF. Patients who discontinue study treatment should be followed for tumor assessments as per standard of care, until disease progression or new cancer therapy is initiated. If patients refuse to return for these visits or are unable to do so, the subject should be considered off-study, but a Study Evaluation Completion form should be completed.

6. Treatment of Subjects

6.1 Treatment regimen

Patients will receive FLU , CY and Rituximab combination for an exposure period of up to 6 months- defined as the treatment period. Dosage, dose regimen and route of administration are detailed in 4.3-4.4

6.1.1 Treatment assignment

Informed consent must be obtained before any testing for the purpose of determining a patient's eligibility is performed. Each patient will be assigned a unique patient number. Once assigned, numbers for any non-evaluable or discontinued patient will not be reused.

6.2 Concomitant therapy

In general, concomitant medications and therapies deemed necessary for the supportive care and safety of the patient are permitted in this study, provided their use is documented in the patient records and on the appropriate Case Report Form. The administration of any anticancer agents including chemotherapy and biologic agents is NOT permitted. Similarly, the use of other concurrent investigational drugs is not allowed. The use of blood products (irradiated) transfusions, immunoglobulins or erythroid/myeloid growth factors are permitted at investigator's discretion.

Transfusion-associated graft versus host disease prevention using irradiated blood products is mandatory.

Steroids administration is allowed exclusively for patients with autoimmune thrombocytopenia or as premedication for Rituximab.

Premedication: Any patient considered to be at risk of infusion related reaction (IRR) or TLS (=patients with lymphocytosis > 25,000mm^3) should receive:

Appropriate hydration starting 12-24 hours prior to initiating treatment, and thereafter until the risk of IRR/TLS is ruled out. Urine alkalization is at investigator's discretion.

Antihistamines (e.g. 2 mg clemastin or promethazine 25mg i.v.) and paracetamol (500-1,000 mg orally) 30 min before first infusion of Rituximab, and thereafter only if clinically indicated. Hydrocortisone 100-500 mg I.V. (according local common practice) should be given only if clinically indicated (increased risk of IRR).
Pneumocystis carinii pneumonitis prophylaxis with trimethoprim 160mg-sulfamethoxasole 800 mg twice a day, twice a week, for the treatment period and for the next 6 months of follow up are recommended. For prevention of hepatitis B reactivation that needs antiviral treatment Lamivudine 100mg/day will be administrated for the entire duration of chemotherapy and for the six months afterwards.

Hepatitis B serology will be: HBsAg+, Anti HBcore and anti-HBsAg

Treatment is recommended for: HBsAg+ (carriers), or HBVsAg (-), anti HBcore (+) and anti-HBVsAg (-).

Any use of concomitant medication must be captured in the concomitant medication CRF. Administration of Blood products must also be captured as concomitant medication on CRF.

Steroids, if given (see above) are allowed for no more than 3 consecutive days.

6.3 Treatment compliance

Number of FLU and CY vials administrated I.V to every subject in every study site, will be captured on CRF.
Number of Rituximab containing infusion bags administrated to every subject in every study site, will be captured on CRF

7. Assessment of efficacy

The primary efficacy parameter to be assessed will be response rate.

Response rate include CR and PR; all others (eg. stable disease, non response, progressive disease or death from any cause should be rated as treatment failure.)

An overall objective assessment of all measurable and non-measurable response parameters will be performed according to the Visit Schedules (see Figure 1).

Response rate will be assessed according the updated (2008) NCI-WG guidelines defined in appendix I: [46].

- Peripheral blood immunophenotyping by FACS for absence of clonal lymphocytes**.**
- Physical examination - lymph node examination and measurements for absence of significant (>1.5 cm in diameter), as well as the presence of hepatomegaly and splenomegaly.
- CT scan for CLL patients with a CT found to be abnormal before therapy or alternatively if physical examination is inconclusive.
- Complete blood count for ANC, platelets and hemoglobin levels. Blood counts have to be above the following values: ANC> 1.5 X 10^9/L. platelets > 100X 10^9/L. Hemoglobin > 11.0 g/dL.
- Bone marrow aspiration and biopsy to assess degree of bone marrow involvement by CLL using FACS and immunohistochemistry, should be performed at least 3 months after the last treatment, if the above **four** clinical and laboratory results **support** that CR has been achieved. In some cases, it is necessary to postpone BMB until after all the other criteria used to define a CR have been satisfied but, this time interval should not exceed 6 months after the last treatment.
- Patient interview for the presence of constitutional symptoms throughout the study.

A single laboratory should perform all evaluations for all patients recruited in the same site. All the above studies must account for disease parameters that

were present at baseline and must use the same techniques as used at baseline. All the above mentioned assessments should be performed within 7 days of the scheduled day of assessment (see Figure 1).

All above mentioned efficacy (response) parameters will be performed at screening and at least 3 months after completing study drug administration for response documentation.

Bone marrow examination can be postponed until after the above clinical and laboratory examinations used to define a CR have been satisfied. However, this time interval should not exceed 6 months after the last treatment.

All known disease (measurable, and non measurable) parameters must be accounted for when assessing objective response rate. Current laboratory and physical examination results will be captured on the response forms in the CRF.

Progression free survival is defined as the interval from the first study drug treatment day to the first sign of disease progression.

Progressive disease (PD) will be assessed according the updated (2008) NCI-WG guidelines defined in appendix I. If needed, BMB will be performed for suspected progression at the investigator discretion. Progression will be captured on the response form CRF.

Time to next treatment is defined as the interval between the first study drug treatment day to the first day of starting the next treatment.

In general, second-line treatment decisions follow the same indications as those used for initiation of first-line treatment.

Re-treatment day will be captured on the treatment form CRF.
QOL questionnaires (FACIT) will be completed by study subjects at: screening visit and at visits number: 11,14 and at the end of study. Questionnaires will be captured on the QOL forms CRF.
CT Scan – In order to prevent contrast nephropathy, patients with GFR < 60ml/min or diabetes mellitus, hydration is recommended and if possible acetylcystein (600 mg in bolus pre CT and 1,200 mg orally twice a day for 48 hours after CT) should be given . Hospitalization for this procedure is recommended.

8. Assessment of Safety

Safety assessments will consist of evaluating adverse events and serious adverse events, laboratory parameters including hematology, chemistry, vital signs, physical examinations, and documentation of all concomitant medications and/or therapies.

8.1 Adverse events

According to the International Conference of Harmonization [ICH], an AE is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign [including an abnormal laboratory finding], symptom, or disease temporally associated with the use of a medicinal [investigational] product, whether or not considered related to the medicinal [investigational] product. Pre-existing conditions which worsen during a study are to be reported as AEs.

Information about all adverse events, whether volunteered by the patient, discovered by investigator questioning, or detected through physical examination, laboratory test or other means, will be collected and recorded on the Adverse Event Case Report Form and followed as appropriate. An adverse event is any undesirable sign, symptom or medical condition occurring after starting study treatment, even if the event is not considered to be treatment-related.

Medical conditions/diseases present before starting study treatment are only considered adverse events if they worsen after starting study treatment. Clinical events occurring before starting study treatment but after signing the informed consent form are recorded on the Medical History/Current Medical Conditions Case Report Form only if the patient receives study treatment. Abnormal laboratory values or test results constitute adverse events only if they induce clinical signs or symptoms or require therapy, when they are recorded on the Adverse Events Case Report Form under the signs, symptoms or diagnosis associated with them.

8.1.2 Grading and reporting of adverse events

All adverse events will be graded according the NCI-CTC scale version 3.0 Anemia and thrombocytopenia should be graded according to NCI-CTC scale version 2.0- the criteria for patients in leukemia studies (see assessment of toxicity below for details), and reported in detail as indicated on the CRF

Adverse events not listed on the CTCAE should be graded as follows:

Definition	Equivalent To:	CTC Grade
Discomfort noticed but no disruption of normal daily activity	Mild	Grade 1
Discomfort sufficient to reduce or affect daily activity; no treatment or medical intervention is indicated although this could improve the overall well-being or symptoms of the subject	Moderate	Grade 2
Inability to work or perform normal daily activity; treatment or medical intervention is indicated in order to improve the overall well-being or symptoms; delaying the onset of treatment is not putting the survival of the subject at direct risk.	Severe	Grade 3
An immediate threat to life or leading to a permanent mental or physical conditions that prevents work or performing normal daily activities; treatment or medical intervention is required in order to maintain survival.	Life threatening/ disabling	Grade 4
AE resulting in death	Death	Grade 5

8.1.3 Assessment of Toxicity

The evaluation of potential treatment-induced toxicity in patients with advanced CLL may be quite difficult requiring careful consideration of both the manifestations of the underlying disease, as well as adverse reactions to the therapy under study. Some of the conventional criteria for toxicity are not applicable especially under circumstances of progressive bone marrow failure from the CLL itself.

8.1.4 Hematological Toxicity

The evaluation of hematological toxicity in patients with CLL must consider the high frequency of hematological compromise already present at the initiation of therapy. Therefore the standard criteria used for solid tumors

cannot be applied directly; many patients would be considered to have grade II to IV hematological toxicity at presentation.
As a consequence, for grading of anemia and thrombocytopenia, we plan to apply the NCI-CTC version 2.0 criteria for patients in leukemia studies, but for grading of neutropenia, the standard NCI-CTC version 3.0 criteria, as given below.

Table 3: NCI-CTC Grading scale for hematological toxicity for patients in this study

ANC mm^3 § (nadir)	Grade	Decrease in platelets*or Hb# from pretreatment value (%).
≥2,000	0	No change-9%
≥1,500 and <2,000	1	10-24%
≥1,000 and <1,500	2	25-49%
≥500 and <1000	3	50-74%
<500	4	≥75%

Grades: 1 = mild, 2 = moderate, 3 = severe, 4 = life-threatening
* if platelets were < 20,000mm^3 [<20 x10^9/L] prior to therapy, the patient is inevaluable for toxicity in platelets
baseline and subsequent Hb determinations must be performed before any given infusion
§ if ANC was < 1,000 prior to therapy, the patient is inevaluable for toxicity in ANC.

Platelets:
If at any level of decrease the platelet count is < 20,000 mm^3, this is considered grade 4 toxicity, unless a severe or life-threatening decrease in the initial platelet count (e.g. < 20,000 mm^3) was present before treatment in which case the patient is inevaluable for toxicity referable to platelet counts.
Hemoglobin:
Baseline and subsequent Hb determinations must be performed before any given infusions.

Fludarabine has been reported to exacerbate or precipitate autoimmune hemolytic anemia and patients should be monitored carefully for this condition. If a rapid decrease of hemoglobin occurs during therapy, the possibility of fludarabine- or autoantibody-induced hemolysis should be considered and appropriate diagnostic tests (LDH, bilirubin, α 2-haptoglobin, reticulocytes, Coombs-test) be performed. In particular, a Coomb's test should be performed for:

- Any grade 3 or 4 anemia (Hb < 8 g/dL)
- A Hb < 10 g/dL sustained for > 2 weeks
- A drop in Hb of 3 g/dL in one week (while pts are receiving weekly blood tests)
- Any patient requiring a blood transfusion

If, in the judgment of the treating physician, there is evidence of hemolytic anemia secondary to fludarabine, study treatment should be promptly withdrawn. Full details of the hemolytic anemia should be recorded on the adverse event pages of the CRF.

Neutrophils:

Absolute neutrophil count (ANC) is used to assess the toxicity of the myeloid lineage. Other decreases in the white blood cell count, or in circulating lymphocytes, are not to be considered, since a decrease in the white blood cell count is a desired therapeutic end point. If the ANC was < 1,000/ mm^3 [<1 $x10^9$/L] prior to therapy, the patient is inevaluable for toxicity referable to the ANC.

Any Adverse Event occurring by the time of study completion (within four weeks of last drug intake) must be recorded on the Adverse Event CRF page.

8.1.5 Drug-Adverse event relationship

The causality relationship of study drug to the adverse event will be assessed by the investigator as either:

Yes or No

If there is a reasonable suspected causal relationship to the study medication, i.e. there are facts (evidence) or arguments to suggest a causal relationship, drug-event relationship should be assessed as **Yes**.

The following criteria should be considered in order to assess the relationship as **Yes:**

- Reasonable temporal association with drug administration
- It may or may not have been produced by the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject.
- Known response pattern to suspected drug
- Disappears or decreases on cessation or reduction in dose
- Reappears on rechallenge

The following criteria should be considered in order to assess the relationship as **No**:

- It does not follow a reasonable temporal sequence from administration of the drug.
- It may readily have been produced by the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject.
- It does not follow a known pattern of response to the suspected drug.
- It does not reappear or worsen when the drug is readministered.

8.2 Serious adverse events

A serious adverse event is any experience that suggests a significant hazard, contraindication, side effect or precaution. It is any Adverse Event that at any dose fulfils at least one of the following criteria:

- is fatal; (results in **death**; NOTE: death is an outcome, not an event)
- is Life-Threatening (NOTE: the term "Life-Threatening" refers to an event in which the subject was at immediate risk of death at the time of the event; it does not refer to an event which could hypothetically have caused a death had it been more severe).
- required in-patient hospitalization or prolongation of existing hospitalization;
- results in persistent or significant disability/incapacity;
- is a congenital anomaly/birth defect;
- is medically significant or requires intervention to prevent one or other of the outcomes listed above

****The term sudden death should be used only when the cause is of a cardiac origin as per standard definition. The terms death and sudden death are clearly distinct and must not be used interchangeably**.

The study will comply with all local regulatory requirements and adhere to the full requirements of the ICH Guideline for Clinical Safety Data Management, Definitions and Standards for Expedited Reporting, Topic E2

Other safety findings may require expedited reporting depending on local legislation, e.g. a major safety finding from a newly completed animal study (such as carcinogenicity).

Progression of underlying malignancy is not reported as an adverse event if it is clearly consistent with the suspected progression of the underlying cancer as defined by RECIST criteria, or other criteria as determined by protocol. Hospitalization due solely to the progression of underlying malignancy should NOT be reported as a serious adverse event. Clinical symptoms of progression may be reported as adverse events if the symptom cannot be determined as exclusively due to the progression of the underlying malignancy, or does not fit the expected pattern of progression for the disease under study.

Symptomatic deterioration may occur in some subjects. In this situation, progression is evident in the subject's clinical symptoms, but is not supported by the tumor measurements. Or, the disease progression is so evident that the investigator may elect not to perform further disease assessments. In such cases, the determination of clinical progression is based on symptomatic deterioration. These determinations should be a rare exception as every effort should be made to document the objective progression of underlying malignancy.

If there is any uncertainty about an adverse event being due only to the disease under study, it should be reported as an AE or SAE.

8.2.1 Reporting of Serious Adverse Events (immediately reportable)

Any clinical adverse event or abnormal laboratory test value that is *serious* and which occurs during the course of the study (as defined in section 7.1.1.3 above), occurring from the enrollment visit (start of study screening procedures), including long term follow-up (LTFU) must be reported to Roche **within *one* working day** of the investigator becoming aware of the event (expedited reporting). The investigator must complete the *SAE Reporting Form [redacted]* and forward it to the SAE Responsible. All SAEs occurring from the enrolment period must be reported, (start of study screening procedures),

Related Serious Adverse Events ***MUST*** be collected and reported regardless of the time elapsed from the last study drug administration, even if the study has been closed. Suspected Unexpected Serious Adverse Reactions (SUSARs) are reported to investigators at each site and associated IRB/IEC when the following conditions occur:

- The event must be a SAE.
- There must be a certain degree of probability that the event is an adverse reaction from the administered drug.
- The adverse reaction must be unexpected, that is to say, not foreseen in the SPC text (Summary of Product Characteristics (for an authorized medicinal product) or the Investigator's Brochure (for an unauthorized medicinal product).

Unrelated Serious Adverse Events must be collected and reported during the study and for up to 30 days after the last dose of study medication.

This study adheres to the definition and reporting requirements of **ICH Guideline for Clinical Safety Data Management, Definitions and Standards for Expedited Reporting, Topic E2.**

8.2.2 Treatment and Follow-up of AEs

During the study period and up to 30 days after completion or discontinuation of the study, continue to follow up AEs as follows:

Related AEs: Follow until one of the following occurs:

- Resolved or improved to baseline
- Relationship is reassessed as unrelated
- Death
- Start of new anti-cancer regimen
- Investigator confirms that no further improvement can be expected
- Clinical or safety data will no longer be collected, or final database closure

Unrelated severe or life threatening AEs: Follow until one of the following occurs:

- Resolved or improved to baseline
- Severity improved to Grade 2
- Death
- Start of new anti-cancer regimen
- Investigator confirms that no further improvement can be expected
- Clinical or safety data will no longer be collected, or final database closure

Unrelated Grade 1 or Grade 2 AEs: Follow up to 30 days after completion or discontinuation of the study.

The final outcome of each adverse event must be recorded on the CRF

8.2.3 Laboratory evaluations

Laboratory test results will be recorded on the laboratory results form of the CRF, or appear on electronically produced laboratory reports submitted directly from the central laboratory, if applicable.

Any laboratory result abnormality fulfilling the criteria for a serious adverse event (SAE) should be reported as such, in addition to being recorded as an AE in the CRF.

Any treatment-emergent abnormal laboratory result which is clinically significant, i.e., meeting one or more of the following conditions, should be recorded as a single diagnosis on the adverse event page in the CRF:

- Accompanied by clinical symptoms
- Leading to a change in study medication (e.g. dose modification, interruption or permanent discontinuation)
- Requiring a change in concomitant therapy (e.g. addition of, interruption of, discontinuation of, or any other change in a concomitant medication, therapy or treatment)

No special laboratory or tests are required to be performed for the purpose of the study. Measurement of Complete Blood Counts, chemistry and immunological tests such as: serum Ig level, serum immunoellectrophoresis, DAT, peripheral and bone marrow FACS and hepatitis B serology are in fact regarded as current clinical practice for CLL patients, and should therefore be collected according to the Visit Schedules (see Figure 1). In case of suspected hemolysis - DAT, reticulocyte count, serum haptoglobin, bilirubin and LDH are recommended.

The institution will perform laboratory analyses according to the Visit Schedules (see Figure 1). The sponsor must be provided with a copy of the laboratory's certification, and a tabulation of the normal ranges for each parameter required. Additionally, if at any time a patient has laboratory parameters obtained from a different outside laboratory, the sponsor must be provided with a copy of the certification and a tabulation of the normal ranges for that laboratory.

At any time during the study, abnormal laboratory parameters which are clinically relevant (e.g. require dose modification and/or interruption of study drug, lead to clinical symptoms or signs or require therapeutic intervention), whether specifically requested in the protocol or not, must be recorded on the appropriate Comment CRF page in addition to the appropriate laboratory CRF page. When abnormal laboratory values or test results constitute an adverse

event (i.e., induces clinical signs/symptoms or requires therapy) they must be recorded on the Adverse Events CRF.

When, in the opinion of the investigator, other clinical laboratory evaluations may be relevant for assessing the patient's status, they will be completed and entered into the database as appropriate.

8.3 Special tests

PET-CT will be performed in case of suspected Richter transformation.

Lymph node biopsy is recommended for definitive diagnosis of Richter transformation and for any rapidly progressive disease or unusual phenomena which may be suspicious of alteration in the normal behavior of the disease or suspicious for another disease or a different type of lymphoma.

8.4 Physical examinations/vital signs

A physical examination including vital signs will be performed according to the Visit Schedules (see Figure 1). Information about the physical examination and vital signs must be present in the source documentation at the study site. Significant findings present prior to the start of study drug must be included in the Relevant Medical History/Current Medical Conditions CRF. Significant findings made after the start of study drug which meet the definition of an adverse event must be recorded on the Adverse Event Case Report Form. There are no case report forms to capture routine normal findings from physical examinations and vital signs assessments.

8.5 Laboratory examinations

8.5.1 Hematology

Hematological evaluations (CBC) are regarded as clinical practice for CLL patients and should therefore be collected according to the Visit Schedules (see Figure 1).

8.5.2 Blood chemistry

The following blood chemistry results are regarded as clinical practice for CLL patients and should therefore be collected according to the Visit Schedules (see Figure 1).

Serum creatinine, ALT, AST, alkaline Phosphatase, LDH, bilirubin. globulin, serum immunoellectrophoresis and beta-2 microglobulin.

8.6 Physical examinations/vital signs

A physical examination including vital signs will be performed according to the Visit Schedules (see Figure 1). Information about the physical examination and vital signs must be present in the source documentation at the study site. Significant findings present prior to the start of study drug must be included in the Relevant Medical History/Current Medical Conditions CRF. Significant findings made after the start of study drug which meet the definition of an adverse event must be recorded on the Adverse Event Case Report Form. There are no Case Report Forms to capture routine normal findings from physical examinations and vital signs assessments.

8.6.1 Warning and Precautions

No evidence available at the time of the approval of this study protocol indicated that special warnings or precautions were appropriate regarding Rituximab, other than those noted in the Investigators' Brochure.

Safety information regarding Fludarabine and Cyclophosphamide can be found in the respective **local MoH approved product label**.

9. Statistics

Subjects' age, weight, height and other continuous demographic and baseline variables will be summarized using descriptive statistics (mean, standard deviation, minimum and maximum), while performance status, gender, and other categorical variables will be summarized with frequency tabulations.

9.1 Sample size

This is a single arm phase II study. The number of subjects planed to be enrolled into the study is 40. Such a low number of patients as in most phase II studies) constitute an underpowered study for revealing any statistical significance, but is indeed clinically meaningful. So, the sample size chosen for this study was based on clinical considerations.

Those patients achieving PR or CR by the study medication will be regarded as responders. All the others (stable disease, progressive disease or death from any cause) will be regarded as non responders. Determining the ratio of responders versus non responders, even if not statistically significant, will still be clinically meaningful.

9.1.1 Data analysis

Categorical data will be presented as frequencies and percentages. For continuous data, mean, standard deviation, median, 25^{th} and 75^{th} percentiles, minimum, and maximum will be presented.

9.2 Efficacy and Safety evaluation

9.2.1 Efficacy analysis:

All analyses will be according to the intention to treat principle.

9.2.2 Response rate

The primary efficacy parameter to be assessed will be response rate determined by objective overall response rate.

The primary endpoint is a binary variable where each patient is classified as a responder or not responder.

95% Confidence Interval (CI) will be calculated to the proportions of responders.

Response rate include CR and PR; all other situations (eg. stable disease, non response, progressive disease or death from any cause) will be rated as treatment failure and therefore as non responders.

Response rate will be assessed according the updated (2008) NCI-WG guidelines defined in appendix 1:

- Peripheral blood FACS for lymphocytes clonality.
- Physical examination for lymph node examination and measurements, and documentation of the presence of hepatomegaly and splenomegaly.
- Blood count for ANC, platelets and hemoglobin levels.
- Bone marrow aspiration and biopsy for bone marrow involvement by CLL cells using FACS immunophenotyping or immunohistochemistry.
- Patient interview for the presence of constitutional symptoms throughout the study.

All above mentioned efficacy (response) parameters will be performed at screening and at least 3 months after completing study drug administration for response documentation.

All known disease (measurable, and non measurable) parameters must be accounted for when assessing objective response rate.

Progression free survival is defined as the interval between the first treatment day to the first sign of disease progression.

Progressive disease (PD) will be assessed according the updated (2008) NCI-WG guidelines defined in appendix I. If needed, BMB will be performed for suspected progression at the investigator's discretion. Progression will be captured on the response form CRF.

A Kaplan-Meier method will be used to estimate median PFS and 90% confidence interval.

QOL questionnaires will be completed by study subjects at: screening visit and visits number: 11, 14, and end of study. Questionnaires will be captured on the QOL forms CRF.

Summary statistics (standard deviation, median, minimum and maximum) will be provided for the relevant variables**. The Paired T-test or Signed Rand Test for paired observations (as is appropriate) will be applied for**

analysing the changes in the components and total score of the FACIT QOL assessment.

The data will be analyzed using the SAS ® version 9.1.3 (SAS Institute, Cary North Carolina).

9.3 Safety Analysis

Data from all subjects who receive any study drug will be included in the safety analyses. The severity of the toxicities will be graded according to the NCI CTCAE v 3.0
http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcaev3.pdf
or v 2.0 for cytopenia, whenever possible.

All adverse events will be coded according to coding dictionaries (MedDRA version 12.1) and presented in tables by system organ class and preferred term.

In the by-subject analysis, a subject experiencing the same event more than once will be counted only once. Adverse events will be defined and summarized according to the worst NCI CTC grade. Adverse events leading to death or to discontinuation of treatment, events classified as NCI CTCAE Grade 3 or Grade 4, study-drug-related events, and serious adverse events will be summarized separately. Laboratory data will be graded according to NCI CTCAE severity grade.

10. Quality Control and Quality Assurance

Data from the CRFs are entered into the study database by Contract Research Organization staff following their own internal standard operating procedures that have been reviewed and approved by the sponsor.

Subsequently, the entered data are systematically checked by Data Management staff, using error messages printed from validation programs and database listings. Obvious errors are corrected by Data Management personnel. Other errors or omissions are entered on Data Query Forms, which are returned to the investigational site for resolution. The signed original and resolved Data Query Forms are kept with the CRFs at the investigator site, and a copy is sent to the sponsor so the resolutions can be entered into the database. Quality control audits of all key safety and efficacy data in the database are made prior to locking the database.

11. Direct Access to Source Data/Documents

11.1 Study monitoring

The monitoring visits will enable the sponsor or the monitor acting on his behalf, to assess the study's progress status, to verify data accuracy and degree of completeness of the CRFs, to ensure the protocol as well as local regulations are being followed, that the investigator is fulfilling his obligations and to correct errors in the CRFs compared to the source documents. The investigators' will authorize the monitor, at a mutually convenient time during the study to periodically review all of the CRFs and the related parts of administrative, medical and laboratory files of each participant in the study. The CRFs must be filled out before the monitoring visits.

11.2 Audits

During the study, people belonging to the sponsor quality assurance group may visit the investigator site in order to audit the study. The purpose of this visit is to check that the study is carried out according to Good Clinical Practices. Before conducting an audit, the monitor will contact the investigator in order to agree upon a mutually suitable date. The investigator and his team are required to cooperate with the auditors and to grant them access to the patients' medical files and to the study documents (CRFs and investigator's binders).

11.3 IRB and regulatory inspections

A regulatory authority may also wish to conduct an inspection (during the study or even after its completion). If an inspection is requested by a regulatory authority, the investigator must inform the sponsor immediately that this request has been made.

12. Ethics

12.1 Institutional Review Board

Before implementing this study, the protocol, the proposed informed consent form and other information to subjects, must be reviewed by a properly constituted Institutional Review Board (IRB). A signed and dated statement that the protocol and informed consent have been approved by the IRB must be given to the sponsor before study initiation. The name and occupation of the chairman and the members of the IRB must be supplied to the sponsor. Any amendments to the protocol, other than administrative ones, must be approved by this committee.

12.2 Informed consent

The investigator must explain to each subject (or legally authorized representative) the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits involved and any discomfort it may entail. Each subject must be informed that participation in the study is voluntary and that he/she may withdraw from the study at any time and that withdrawal of consent will not affect his/her subsequent medical treatment or relationship with the treating physician.

This informed consent should be given by means of a standard written statement, written in non-technical language. The subject should read and consider the statement before signing and dating it, and should be given a copy of the signed document. If the subject cannot read or sign the documents, oral presentation may be made or signature given by the subject's legally appointed representative, if witnessed by a person not involved in the study, mentioning that the patient could not read or sign the documents. No patient can enter the study before his/her informed consent has been obtained.

The informed consent form is considered to be part of the protocol, and must be submitted by the investigator with it for IRB approval.

12.3 Declaration of Helsinki

The investigator must conduct the trial in accordance with the principles of the Declaration of Helsinki which can be accessed via the website of the World Medical Association at http://www.wma.net/e/policy/17-c_e.html.

13. Data Handling and Record Keeping

The information obtained from the execution of the protocol will be listed on the CRFs provided by the sponsor.
The CRFs must be filled out on a timely basis, signed and dated by the investigator or a co-investigator named by the former and clinically responsible for the patient during the study.

The completed CRFs will be collected by the sponsor. The investigator must remit one CRF for every participant in his center for a time period of 15 years.

14. Financing and Insurance

Financing and insurance will be addressed in the future in a separate agreement.

15.Publication Policy

Any formal presentation or publication of data from this trial will be considered as a joint publication by the investigator(s) and appropriate sponsor's personnel. Authorship will be determined by mutual agreement taking in consideration that the group of PI's will prepare the manuscript and write it initially and decide which member of steering committee and which other active investigators who provided cases will be involved.

It is a multicenter study, and it is mandatory that the first publication is based on data from all centers, analyzed as stipulated in the protocol, by the sponsor statisticians, and not by the investigators. Investigators participating in multicenter studies agree not to present data gathered from one center or a small group of centers before the full publication, unless formally agreed to by all other investigators and the PI's and steering committee.

The investigator may be required to sign the clinical study report, if it is to be used in a registration submission to the health authorities of some countries. For multicenter studies only the coordinating (principle) investigator nominated by the sponsor and the steering committee at the start of the trial would provide any needed signature.

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16. Supplements

Appendix I Response definitions

CR (complete remission) = requires **all** of the following criteria as assessed at least 3 months after completion of therapy:

Absence of clonal lymphocytes in peripheral blood.

Absence of significant lymphadenopathy. (lymph nodes > 1.5 cm in diameter) by physical examination. CT is desirable for not palpable lymph nodes.

No hepatomegaly or splenomegaly by physical examination. (Abdominal, pelvix and thorax CT for subjects found to be abnormal before therapy or if physical examination is inconclusive.

Absence of constitutional symptoms.

Blood counts above the following values: ANC> 1.5 x 10^9/L . Platelets > 100 x10^9/L . Hemoglobin > 11.0 g/dL; untransfused).

Bone marrow aspirate and biopsy should be performed at least 3 months after the last treatment and if the above 5 clinical and laboratory results demonstrate that CR has been achieved.
Bone marrow should be analyzed by FACS/or immunohistochemistry to demonstrate marrow free of B-CLL cells.
In some cases, it is necessary to postpone BMB until after all the other criteria to define a CR have been satisfied. However, this time interval should not exceed 6 months after the last treatment.

Parameter	CR	PR	PD	SD
Group A				
Lymphadenopathy*	None more than 1.5 cm	Decrease ≥ 50%	Increase ≥ 50%	Change of −49% to +49%
Liver and/or spleen size	Normal size	Decrease ≥ 50%	Increase ≥ 50%	Change of −49% to +49%
Constitutional symptoms	None	Any	Any	Any
Polymorphonuclear leukocytes	>1500/μL	>1500/μL or > 50% improvement over baseline	Any	Any
Circulating clonal B lymphocytes	None	Decrease ≥ 50% from baseline	Increase ≥ 50% over baseline	Change of −49% to +49%
Group B				
Platelet count	>100 000/μL	>100 000/μL or increase ≥ 50% over baseline	Decrease of ≥ 50% from baseline secondary to CLL	Change of −49 to +49%
Hemoglobin	>11.0 g/dL (untransfused and without erythropoietin)	>11 g/dL or increase ≥ 50% over baseline	Decrease of >2 g/dL from baseline secondary to CLL	Increase ≤11.0 g/dL or <50' over baseline, or decrease <2 g/dL
Marrow	Normocellular, <30% lymphocytes, no B-lymphoid nodules; hypocellular marrow defines CRi	≥ 30% lymphocytes, or B-lymphoid nodules, or not done	Increase of lymphocytes to more than 30% from normal	No change in marrow infiltrat

Values in SI units for leukocytes are 1.5 × 10^9/L; for platelets 100 × 10^9/L; and for hemoglobin 110 g/L and 20 g/L.

CR indicates complete remission (all of the criteria have to be met); PR, partial remission (at least one of the criteria of group A and one of the criteria of group B have to be met); PD, progressive disease (at least one of the criteria of group A or one of the criteria of group B have to be met); SD, stable disease (all of the above criteria have to be met).

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

Relapse = a patient who has previously achieved CR or PR but after a period of 6 or more months, demonstrates evidence of disease progression

Refractory disease = defined as treatment failure or disease progression within 6 months from the last antileukemic therapy.

Progression = see above table under PD.

Progression free survival (PFS) = defined as the time from start of treatment to progression.

Time to next treatment (TNT) = defined as the time from start of treatment to start of second-line treatment

Appendix II ECOG Performance status

ECOG	Grade
Fully active, able to carry on all pre-disease performance without restriction	0
Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work	1
Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours	2
Capable of only limited self-care, confined to bed or chair more than 50% of waking hours	3
Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair	4
Dead	5

Appendix III calculated creatinine clearance

$$\text{GFR (mL/min)} = \frac{(140\text{-age}) \text{ X weight (Kg)}}{\text{Serum cr (mg/dL) X } 72} \text{ X } 0.85 \text{ (for women)}$$

Appendix IV Active disease

At least one of the following criteria should be met:

1. Evidence of progressive marrow failure as manifested by the development of, or worsening of, anemia and/or thrombocytopenia
2. Massive (ie, > 6 cm below the left costal margin) or progressive or symptomatic splenomegaly.
3. Massive nodes (ie, > 10 cm in longest diameter) or progressive or symptomatic lymphadenopathy.
4. Progressive lymphocytosis with an increase of more than 50% over a 2-month period or lymphocyte doubling time of less than 6 months; patients with initial blood lymphocyte counts of less than 30X109/L (30 000/µL) may require a longer observation period to determine the lymphocyte doubling time. In addition, factors contributing to lymphocytosis or lymphadenopathy other than CLL (eg, infections) should be excluded.
5. Autoimmune anemia and/or thrombocytopenia poorly responsive to corticosteroides or other standard therapy.
6. A minimum of any of the following disease-related symptoms must be present: a- Unintentional weight loss more than or equal to 10% within the previous 6 months. b- Significant fatigue (ie, ECOG PS 2 or worse; cannot work or unable to perform usual activities). c- Fevers of greater than 38.00C for 2 or more weeks without other evidence of infection. d- Night sweats for more than 1 month without evidence of infection.

Appendix V Binet staging system

The areas of involvement considered for staging are as follows: 1- Head and neck, including the Waldeyer ring. 2- Axillae 3- Groins 4- Palpable spleen 5- Palpable liver.

Stage A. Hb 10 g/dL or more and platelets 100X 10^9/L or more and up to 2 of the above areas involved.

Stage B. Hb 10 g/dL or more and platelets 100X 10^9/L or more and organomegaly greater than that defined for stage A (3 or more areas).

Stage C. All patients who have Hb less than 10 g/dL and/or a platelets count less than 100X 10^9/L, irrespective of organomegaly.

Appendix VI FACIT-QOL

FACIT − Fatigue Scale (Version 4)

Below is a list of statements that other people with your illness have said are important. **By circling one (1) number per line, please indicate how true each statement has been for you during the past 7 days.**

		Not At all	A little bit	Some what	Quite a bit	Very much
HI7	I feel fatigued	0	1	2	3	4
HI12	I feel weak all over	0	1	2	3	4
An1	I feel listless ("washed out") 0	1	2	3	4	
An2	I feel tired	0	1	2	3	4
An3	I have trouble starting things because I am tired 0	1	2	3	4	
An4	I have trouble finishing things because I am tired 0	1	2	3	4	
An5	I have energy	0	1	2	3	4
An7	I am able to do my usual activities 0	1	2	3	4	
An8	I need to sleep during the day 0	1	2	3	4	
An12	I am too tired to eat	0	1	2	3	4
An14	I need help doing my usual activities	0	1	2	3	4
An15	I am frustrated by being too tired to do the things I want to do	0	1	2	3	4
An16	I have to limit my social activity because I am tired 0	1	2	3	4	

US English

AMENDMENT HISTORY FOR PROTOCOL ML25464 (FCR-LITE)

VERSION E DATED 22-Apr-2013

1. **Subject: Overall study design**

Reason for change: The option to round the dose of Rituximab and chemotherapy was added to fit the common practice.

Section 4.2 Overall study design

New text:

This is a national, multicenter, phase II, single arm (not controlled), open label clinical trial to evaluate the efficacy and safety of the combination of low dose Flu + Cy combined with standard dose Rituximab Flu dosage will be given at 12.5mg/m^2/d given I.V. together with CY150 mg/m^2/d given I.V. for three days every 28 days. Rituximab will be given before chemotherapy

cycle 1: 375 mg/m^2 I.V. on day 0 and in cycles 2-6: 500 mg/m^2 I.V. on day 1 in previously untreated CLL patients who are more than 65 years of age.

Please note: All medication dosages will be rounded up to the nearest dosage according to the medical center routine administration procedures.

Old text:

This is a national, multicenter, phase II, single arm (not controlled), open label clinical trial to evaluate the efficacy and safety of the combination of low dose Flu + Cy combined with standard dose Rituximab Flu dosage will be given at 12.5mg/m^2/d given I.V. together with CY150 mg/m^2/d given I.V. for three days every 28 days. Rituximab will be given before chemotherapy

cycle 1: 375 mg/m^2 I.V. on day 0 and in cycles 2-6: 500 mg/m^2 I.V. on day 1 in previously untreated CLL patients who are more than 65 years of age.

2. Subject : Visit schedule and assessments

Reason for change: The schedule of BMB was corrected to fit the text of the protocol. Original text in the protocol: BMB will be performed for progression/ lymphoma transformation, cytopenia evaluation, and for treatment response within 3-6 months after completion of treatment.

Section 4.2.1 Visit schedule and assessments

New text:

Figure 1: Visit schedule and assessments

Week	-4 to 0	1	3	5	7	9	13	17	21	33	45	57	69	81	93-153 every 12 weeks	EOS
Visit No.	1 Screening	2	3	4	5	6	7	8	9	10	11	12	13	14	15-21	22
Study period	preTx	Treatment Period								Follow up period						
Inc. /Excl. criteria	X															
Informed consent	X															
Relevant medical history/conditions	X															
Disease history	X															
BMB [1]	X										X					
Physical exam	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
CBC	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Blood chemistry	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Beta-2 micro glob	X															
IEP, Ig level,	X												X			X
DAT [3]	X												X			X
FACS [4]	X [4]												X			X
Hepatitis B and C [5]	X															
Imaging - (CT) [2]	X															X

QOL – FACIT	X										X			X		X
Treatment dosage		X		X		X	X	X	X							
Adverse Events	X [6]	Ongoing data capture and up to 30 days after completion or discontinuation of the study														
Concomitant medications	X	Ongoing data capture														
Comments	X	Ongoing data capture														
Study completion	X	Complete at any time if study drug is discontinued														

Old text:

Week	**-4 to 0**	**1**	**3**	**5**	**7**	**9**	**13**	**17**	**21**	**33**	**45**	**57**	**69**	**81**	**93-153 every 12 weeks**	**EOS**
Visit No.	**1 Screening**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15-21**	**22**
Study period	**preTx**	**Treatment Period**								**Follow up period**						
Inc. /Excl. criteria	X															
Informed consent	X															
Relevant medical history/conditions	X															
Disease history	X															
BMB [1]	X												X			
Physical exam	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
CBC	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Blood chemistry	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Beta-2 micro glob	X															
IEP, Ig level,	X												X			X
DAT [3]	X												X			X
FACS [4]	X [4]												X			X
Hepatitis B [5]	X															
Imaging - (CT) [2]	X															X
QOL – FACIT	X										X			X		X

Treatment dosage		X		X		X	X	X	X							
Adverse Events	X	Ongoing data capture and up to 30 days after completion or discontinuation of the study														
Concomitant medications	X	Ongoing data capture														
Comments	X	Ongoing data capture														
Study completion	X	Complete at any time if study drug is discontinued														

3. **Subject: Schedule of FACS assessment**

   Reason for change: No clinical justification to perform FACS 12 mounts prior to screening.

   Section 4.2.1 Visit schedule and assessments: Figure 1 - Comment no. 4

   New text:

   4 - FACS results prior to screening are relevant and no further test is necessary at screening.

   Old text:

   4 - FACS results from up to 12 months prior to screening are relevant and no further test is necessary at screening.

4. **Subject: SAE reporting during Screening**

   Reason for change: Clarification regarding SAE reporting during Screening.

   Section 4.2.1 Visit schedule and assessments: Figure 1 - Comment no. 6

New text:

6 - After signature on informed consent, but prior to initiation of study medications, only SAEs caused by a protocol-mandated intervention will be collected. After first study medication, all SAEs must be reported.

Old text:

None

Section 8.2.1 Reporting of Serious Adverse Events (immediately reportable)

New text:

Any clinical adverse event or abnormal laboratory test value that is *serious* and which occurs during the course of the study (as defined in section **8.2** above), occurring from the enrollment visit (start of study screening procedures), including long term follow-up (LTFU) must be reported to Roche *within one* working day of the investigator becoming aware of the event (expedited reporting). The investigator must complete the *SAE Reporting Form [redacted]* and forward it to the SAE Responsible.

After signature on informed consent, but prior to initiation of study medications, only SAEs caused by a protocol-mandated intervention will be collected (e.g., SAEs related to invasive procedures such as biopsies, medication washout, or no treatment run-in). After first study medication, all SAEs must be reported.

Old text:

Any clinical adverse event or abnormal laboratory test value that is *serious* and which occurs during the course of the study (as defined in section 7.1.1.3 above), occurring from the enrollment visit (start of study screening procedures), including long term follow-up (LTFU) must be reported to Roche within *one* working day of the investigator becoming aware of the event (expedited reporting). The investigator must complete the

SAE Reporting Form [redacted] and forward it to the SAE Responsible. All SAEs occurring from the enrolment period must be reported, (start of study screening procedures).

5. **Subject: Dose modification for other non-hematological toxicity (non renal).**

Reason for change: Prevent clinically unnecessary dose reduction of fludarabine and cyclophosphamide in case of grade ≥ 3 non-hematological, non-renal toxicity, not drug related AE (after recovery to grade ≤ 2).

Section 4.3.4 Dose modification for other non-hematological toxicity (non renal)

New text:

- If other grade ≥ 3, non-hematological, non-renal toxicity occurs during any cycle, study drugs treatment should be delayed until recovery to grade ≤ 2, for up to three weeks. **In case of grade ≥ 3, non-hematological, non-renal toxicity, which in the investigators' opinion is not study drug related, dose reduction will be performed upon investigator discretion. In case of grade ≥ 3 non-hematological toxicity, which in the investigators' opinion is related to study drugs, all subsequent cycles should then be given with a 25% dose reduction of fludarabine and cyclophosphamide.**

- If after three weeks, the grade ≥ 3 non-hematological, non-renal toxicity, **drug related** still persists, the patient should be removed from the protocol.

- If there is further grade ≥ 3 non-hematological, non-renal toxicity, **drug related** in subsequent cycles despite the first 25% dose reduction treatment should again be delayed for up to three weeks and the dose of fludarabine and cyclophosphamide further reduced to 50% of the initial full dose.

- If there is further grade ≥ 3 non-hematological, non-renal toxicity, **drug related** in subsequent cycles despite 50% dose reduction the patient should be removed from the protocol treatment and the study.

Old text:

- If other grade ≥ 3, non-hematological, non-renal toxicity occurs during any cycle, study drugs treatment should be delayed until recovery to grade ≤ 2, for up to three weeks. All subsequent cycles should then be given with a 25% dose reduction of fludarabine and cyclophosphamide.

-If after three weeks, the grade ≥ 3, non-hematological, non-renal toxicity still persists, the patient should be removed from the protocol.

-If there is further grade ≥ 3 non-hematological, non-renal toxicity in subsequent cycles despite the first 25% dose reduction treatment should again be delayed for up to three weeks and the dose of fludarabine and cyclophosphamide further reduced to 50% of the initial full dose.

- If there is further grade ≥ 3 non-hematological, non-renal toxicity in subsequent cycles despite 50% dose reduction the patient should be removed from the protocol treatment and the study.

6. Subject: Dose modification for Weight Changes

Reason for change: Body weights were added to screening visit and visits 2 and 4 in order to make dose adjustment in case of more than ± 10% change in weight.

Section 4.3.5 Dose modification for Weight Changes

New text:

Dose of medication will be determined according to weight at screening.

If body weight has changed at visit 2 or visit 4 by ± 10% or more, appropriate adjustment in dose of all medications should be performed.

Old text:
None.

Section 4.2.1 Visit schedule and assessments

New text:

Figure 1: Visit schedule and assessments

Week	-4 to 0	1	3	5	7	9	13	17	21	33	45	57	69	81	93-153 every 12 weeks	EOS
Visit No.	1 Screening	2	3	4	5	6	7	8	9	10	11	12	13	14	15-21	22
Study period	preTx	Treatment Period								Follow up period						
Inc. /Excl. criteria	X															
Informed consent	X															
Relevant medical history/conditions	X															
Disease history	X															
BMB [1]	X									X						
Physical exam	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Weight [7]	X	X		X												
CBC	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Blood chemistry	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Beta-2 micro glob	X															
IEP, Ig level,	X												X			X
DAT [3]	X												X			X
FACS [4]	X [4]												X			X
Hepatitis B and C [5]	X															

Imaging - (CT) [2]	X															X
QOL – FACIT	X										X			X		X
Treatment dosage		X		X		X	X	X	X							
Adverse Events	X [6]	Ongoing data capture and up to 30 days after completion or discontinuation of the study														
Concomitant	X	Ongoing data capture														
Comments	X	Ongoing data capture														
Study completion	X	Complete at any time if study drug is discontinued														

Old text:

Week	**-4 to 0**	**1**	**3**	**5**	**7**	**9**	**13**	**17**	**21**	**33**	**45**	**57**	**69**	**81**	**93-153 every 12 weeks**	**EOS**
Visit No.	**1 Screening**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15-21**	**22**
Study period	**preTx**	**Treatment Period**								**Follow up period**						
Inc. /Excl. criteria	X															
Informed consent	X															
Relevant medical history/conditions	X															
Disease history	X															
BMB [1]	X												X			
Physical exam	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
CBC	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Blood chemistry	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Beta-2 micro glob	X															
IEP, Ig level,	X												X			X
DAT [3]	X												X			X
FACS [4]	X [4]												X			X
Hepatitis B [5]	X															
Imaging - (CT) [2]	X															X
QOL – FACIT	X										X			X		X

Treatment dosage		X		X		X	X	X	X							
Adverse Events	X	Ongoing data capture and up to 30 days after completion or discontinuation of the study														
Concomitant medications	X	Ongoing data capture														
Comments	X	Ongoing data capture														
Study completion	X	Complete at any time if study drug is discontinued														

Section 4.2.1 Visit schedule and assessments: Figure 1 - Comment no. 7

New text:

7 - Dose of medication will be determined according to weight at screening. If body weight has changed at visit 2 or visit 4 by ± 10% or more, appropriate adjustment in dose of all medications should be performed.

Old text:

None.

7. Subject: Inclusion criteria number 1.

Reason for change: In cases when atypical CLL exists the patient will only be recruited following the confirmation of a pathologist and the sponsor.

Section 5.1 Inclusion criteria & Synopsis
New text:

Diagnosis of B-CLL is established by the presence of: an absolute lymphocyte count > $5X10^9$ L, and typical CLL cell morphology in the peripheral blood smear as seen by light microscopy. CLL cells must show CD5/CD19/CD23 positivity with light chain restriction as recorded by flow cytometry. The levels of surface immunoglobulin and CD 79b are characteristically low and FMC7 is expected to be negative (optional). ZAP 70 and CD38 are optional. **Patients with different phenotype than described above can be included, in case the pathologist will give a written approval of B-CLL and with the approval of the sponsor.**

Old text:

Diagnosis of B-CLL is established by the presence of: an absolute lymphocyte count > $5X10^9$ L, and typical CLL cell morphology in the peripheral blood smear as seen by light microscopy. CLL cells must show CD5/CD19/CD23 positivity with light chain restriction as recorded by flow cytometry. The levels of surface immunoglobulin and CD 79b are characteristically low and FMC7 is expected to be negative (optional). ZAP 70 and CD38 are optional.

8. **Subject: Exclusion criteria number 6.**

Reason for change: permit enrollment of patients with previous exposure to HBV which are negative for HBS Ag and negative for presence of DNA replicates of the virus in the blood.

Section 5.2 Exclusion criteria & Synopsis

New text:

- **Patients with active hepatitis B or HBV surface antigen positive.**
- **Patients with anti-HBV core antibodies (past infection with HBV) but who are negative for HBVsAg (either anti-HBS Ab positive or negative) and are positive for HBV-DNA by PCR analysis - are not eligible.**

Old text:

- Positive Hepatitis B Surface Ag or Hepatitis B Core Ab

Section 6.2 Concomitant therapy

New text:

Patients with anti-HBV core antibodies (past infection with HBV) but who are negative for HBSAg (either anti-HBVs Ab positive or negative) and had negative baseline for HBV-DNA by PCR analysis will be given anti-viral prophylaxis during and for at least 6 months after the last cycle of treatment.

For prevention of hepatitis B reactivation that needs antiviral treatment **(prophylaxis treatment)** Lamivudine 100mg/day will be administrated for the entire duration of chemotherapy and for the six months afterwards.

Old text:

For prevention of hepatitis B reactivation that needs antiviral treatment Lamivudine 100mg/day will be administrated for the entire duration of chemotherapy and for the six months afterwards.

9. Subject: Assessments for hepatitis C

Reason for change: The assessment was mistakenly dropped in some places in the protocol.

Section 4.2.1 Visit schedule and assessments

New text:

Week	**-4 to 0**	**1**	**3**	**5**	**7**	**9**	**13**	**17**	**21**	**33**	**45**	**57**	**69**	**81**	**93-153 every 12 weeks**	**EOS**
Visit No.	**1 Screening**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15-21**	**22**
Study period	**preTx**	**Treatment Period**								**Follow up period**						
Inc. /Excl. criteria	X															
Informed consent	X															
Relevant medical history/conditions	X															
Disease history	X															
BMB [1]	X									X						
Physical exam	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
CBC	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Blood chemistry	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

Beta-2 micro glob	X														
IEP, Ig level,	X											X			X
DAT [3]	X											X			X
FACS [4]	X [4]											X			X
Hepatitis B and C [5]	X														
Imaging - (CT) [2]	X														X
QOL – FACIT	X									X			X		X
Treatment dosage		X		X		X	X	X	X						
Adverse Events	X [6]	Ongoing data capture and up to 30 days after completion or discontinuation of the study													
Concomitant medications	X	Ongoing data capture													
Comments	X	Ongoing data capture													
Study completion	X	Complete at any time if study drug is discontinued													

Old text:

Week	**-4 to 0**	**1**	**3**	**5**	**7**	**9**	**13**	**17**	**21**	**33**	**45**	**57**	**69**	**81**	**93-153 every 12 weeks**	**EOS**
Visit No.	**1 Screening**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15-21**	**22**
Study period	**preTx**	**Treatment Period**								**Follow up period**						
Inc. /Excl. criteria	X															
Informed consent	X															
Relevant medical history/conditions	X															
Disease history	X															
BMB [1]	X									X	X					
Physical exam	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
CBC	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Blood chemistry	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Beta-2 micro glob	X															
IEP, Ig level,	X												X			X

DAT [3]	X											X			X
FACS [4]	X [4]											X			X
Hepatitis B [5]	X														
Imaging - (CT) [2]	X														X
QOL – FACIT	X									X			X		X
Treatment dosage		X		X		X	X	X	X						
Adverse Events	X	Ongoing data capture and up to 30 days after completion or discontinuation of the study													
Concomitant medications	X	Ongoing data capture													
Comments	X	Ongoing data capture													
Study completion	X	Complete at any time if study drug is discontinued													

Section 8.2.3 Laboratory evaluations

New text:

Measurement of Complete Blood Counts, chemistry and immunological tests such as: serum Ig level, serum immunoellectrophoresis, DAT, peripheral and bone marrow FACS and hepatitis B **and C** serology are in fact regarded as current clinical practice for CLL patients, and should therefore be collected according to the Visit Schedules (see Figure 1).

Old text:

Measurement of Complete Blood Counts, chemistry and immunological tests such as: serum Ig level, serum immunoellectrophoresis, DAT, peripheral and bone marrow FACS and hepatitis B serology are in fact regarded as current clinical practice for CLL patients, and should therefore be collected according to the Visit Schedules (see Figure 1).

10. Subject: Cancellation of Secondary Endpoint - TNT

Reason for change: It was decided to remove secondary endpoint of Time to Next Treatment (TNT) as it was not applicable to collect the relevant data.

Section 3.2 Synopsis

New text:

Secondary end points:

- Safety - Special issues: incidence of neutropenic fever, hospitalizations (no°- days),
- Thrombocytopenia ≥grade 3, neutropenia ≥grade 3, Infection rate.

- Progression Free Survival (PFS).

- QOL

Old text:

Secondary end points:

- Safety - Special issues: incidence of neutropenic fever, hospitalizations (no°- days),
- Thrombocytopenia ≥grade 3, neutropenia ≥grade 3, Infection rate.

- Progression Free Survival (PFS).

- Time to Next Treatment (TNT).

- QOL

Section 3.2 Secondary Objectives

New text:

1- Safety of the combination drugs given in the study population, especially in relation to: neutropenic fever, Infection rate, number of hospitalization days, thrombocytopenia ≥grade 3, neutropenia ≥ grade 3.

2- Progression Free Survival (PFS).

3- Quality Of Life (FACIT)

Old text:

1- Safety of the combination drugs given in the study population, especially in relation to: neutropenic fever, Infection rate, number of hospitalization days, thrombocytopenia ≥grade 3, neutropenia ≥ grade 3.

2- Progression Free Survival (PFS).

3- Time to Next Treatment (TNT).

4- Quality Of Life (FACIT)

Section 4.1.1 Secondary End Points

New text:

1- Safety of the study drugs especially in relation to: neutropenic fever, Infection rate and number of hospitalization days,

thrombocytopenia ≥grade 3, neutropenia ≥grade 3.

2- Progression free survival (PFS) defined as the time elapsed from first day of study drug administration to disease progression as defined in the protocol (see appendix I)

3- QOL(see appendix VI)

Old text:

1- Safety of the study drugs especially in relation to: neutropenic fever, Infection rate and number of hospitalization days,

thrombocytopenia ≥grade 3, neutropenia ≥grade 3.

2- Progression free survival (PFS) defined as the time elapsed from first day of study drug administration to disease progression as defined in the protocol (see appendix I)

3- Time to Next Treatment (TNT) as defined in the protocol (see appendix I)

4- QOL(see appendix VI)

11. Subject: Drug Accountability Updates

Reason for change: Several updates were performed in sections 4.3.1, 4.3.1.1 & 4.3.1.2 in order to clarify and ease the procedure of drug accountability.

Section 4.3.1 Investigational therapy

New text:

Labels will comply with the legal requirements in Israel and be printed in Hebrew and English. All study drugs containing infusion bags will be labeled. All infusion bags will be labeled by the study nurse prior to administration and **the removable part of the labels will be attached to the drug administration log, signed and dated by the study nurse**. Storage conditions for study drug will be described on the medication label. Patients will receive the study drug combination for an exposure period of up to 6 months provided that in the opinion of the investigator the patient is benefiting from treatment with this study drug combination, and in the absence of any safety concerns. Treatment will be withheld only in the case of limiting toxicities (see below).

Old text:

Labels will comply with the legal requirements in Israel and be printed in Hebrew and English. All study drugs containing infusion bags will be labeled. All infusion bags will be labeled by the study nurse prior to administration and only the empty Rituximab infusion bags will be collected for drug accountability by the study monitor. Storage conditions for study drug will be described on the medication label. Patients will receive the study drug combination for an exposure period of

up to 6 months provided that in the opinion of the investigator the patient is benefiting from treatment with this study drug combination, and in the absence of any safety concerns. Treatment will be withheld only in the case of limiting toxicities (see below).

Section 4.3.1.1 DRUG ACCOUNTABILITY

New text:

Accountability and patient compliance will be assessed by maintaining adequate "drug **administration** log" on study medication.

The "drug **administration**" log for the study medication must be kept current and should contain the following information:

- The identification of the patient (patient number) to whom the study medication was administered.
- **Labels of used study drugs - signed and dated by the study nurse.**

All accountability **administration** logs must be available for inspection by the monitor. **Infusion bags** will be destructed according to local site guidelines.

Old text:

Accountability and patient compliance will be assessed by maintaining adequate "drug dispensing log" on study medication.

The "drug dispensing" log for the study medication must be kept current and should contain the following information:

- The identification of the patient (patient number) to whom the study medication was administered.
- The date(s) and quantity of study medication, used for the patients' treatment.

- The date(s) and quantity of study medication, of empty, partial or unused medication.

All accountability logs must be available for inspection by the monitor. Drug supplies (partly used and empty Rituximab infusion bags) must be available for inspection, at every monitoring visit. Used supplies will be destructed according to local site guidelines.

Section 4.3.1.2 DESTRUCTION OF THE INVESTIGATIONAL MEDICINAL PRODUCT

New text:

Used supplies (**infusion bags**) will be sent for destruction according to the site guidelines. The destruction process in the site should be documented.

Old text:

After the monitor has reconciled study drug supplies, used supplies (partly used and empty Rituximab infusion bags) will be sent for destruction according to the site guidelines. The destruction process in the site should be documented.

F. HOFFMANN-LA ROCHE LTD

CLINICAL STUDY PROTOCOL

PROTOCOL NUMBER ML 25464 (FCR LITE)

A PHASE II, MULTICENTER, SINGLE ARM STUDY TO DETERMINE THE EFFICACY AND SAFETY OF LOW DOSE FLUDARABINE AND CYCLOPHOSPHAMIDE COMBINED WITH STANDARD DOSE RITUXIMAB AS PRIMARY THERAPY IN ELDERLY UNTREATED PATIENTS (≥65 YEARS OLD) WITH CHRONIC LYMPHOCYTIC LEUKEMIA

PROTOCOL APPROVAL

Protocol Number / Version: **ML25464 / Version E**

Medical Manager : [redacted] Date: 22 Apr 2013

Project statistician: [redacted] Date: 22 Apr 2013

This protocol is intended for use in a life-threatening indication: Yes ☑ No ☐

A PHASE II, MULTICENTER, SINGLE ARM STUDY TO DETERMINE THE EFFICACY AND SAFETY OF LOW DOSE FLUDARABINE AND CYCLOPHOSPHAMIDE COMBINED WITH STANDARD DOSE RITUXIMAB AS PRIMARY THERAPY IN ELDERLY UNTREATED PATIENTS (≥65 YEARS OLD) WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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[redacted] MHA+M.D.

[redacted] M.D.

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LIST OF ABBREVIATIONS

AE	Adverse Event
ALT	Alanine Aminotransferase/GPT
ANC	Absolute Neutrophil Count
AST	Aspartate Aminotransferase/SGOT
ALK PHOSP	Alkaline Phosphatase
CBC	Complete Blood Count
CLL	Chronic Lymphatic Leukemia
CR	Complete Remission
CRF	Case Report/Record Form
CRO	Contract Research Organization
CT	Computerized Tomography
CY	Cyclophosphamide
FACT-CTC	Functional Assessment of Cancer Therapy - Common Terminology Criteria for cancer Adverse Events
FACS	Flow Cytometry (Fluorescence-Activated Cell Sorting)
DAT	Direct Coombs Test
FLU	Fludarabine
G-CSF	Granulocyte-Colony Stimulating Factor
Hb	Hemoglobin
HIV	Human Immune deficiency Virus
IEP	Immuno-ellectrophoresis
I.V.	Intra-Venously
Ig	Immunoglobulin
IRB	Institutional Review Board
IRR	Infusion Related Reaction
LDH	Lactic Dehydrogenase
MHO	Medical Health Organization
ORR	Overall Response Rate
PD	Progressive Disease
PET-CT	Positron Emission Tomography- Computerized Tomography
PFS	Progression Free Survival
P.O.	Per Os/ by mouth/orally
PR	Partial Response
QOL	Quality Of Life
SAE	Serious Adverse Event
TLS	Tumor Lysis Syndrome
TNT	Time to Next Treatment
ULN	Upper Limit of Normal

SYNOPSIS

A PHASE II, MULTICENTER, SINGLE ARM STUDY TO DETERMINE THE EFFICACY AND SAFETY OF LOW DOSE FLUDARABINE AND CYCLOPHOSPHAMIDE COMBINED WITH STANDARD DOSE RITUXIMAB AS PRIMARY THERAPY IN ELDERLY UNTREATED PATIENTS (≥65 YEARS OLD) WITH CHRONIC LYMPHOCYTIC LEUKEMIA

PROTOCOL NUMBER:	ML25464
DATE PROTOCOL FINAL:	20 August 2011
STUDY DRUG:	FLUDARABINE, CYCLOPHOSPHAMIDE, RITUXIMAB
INDICATION:	CHRONIC LYMPHOCYTIC LEUKEMIA
STUDY PHASE:	II

Background and rationale Chronic lymphocytic leukemia (CLL) is characteristically diagnosed in the older population with a median age at diagnosis ranging between 65 and 72 years. Because the prevalence of co morbidity increases with age, concomitant disease is often present in this age group. Additional factors which may also adversely affect treatment outcome at standard doses in elderly patients include: altered pharmacokinetics and poorer host tissue tolerance ; changes in the bone marrow hematopoietic reserve and microenvironment leading to increased treatment related myelotoxicity and higher rates of treatment related complications such as infections. As a result, elderly patients are often excluded from clinical trials or even from routine treatment on the basis of their age and / or comorbidity. Furthermore when these patients are treated, the drug doses are often markedly reduced in an attempt to avoid causing toxicity and complications. The challenge in treating elderly CLL patients relates to finding the correct balance between drug efficacy and toxicity and because of this optimal treatment for this group of patients is often hard to achieve.

Study Objectives

The aim of this study is to evaluate the safety and efficacy (response rate) of a combination of low dose Fludarabine (FLU) and Cyclophosphamide (CY) combined with standard dose Rituximab given as primary treatment for elderly patients with chronic lymphocytic leukemia.

Primary end point: -Response rate

Secondary end points:

- Safety - Special issues: incidence of neutropenic fever, hospitalizations (no^{o}- days),
- Thrombocytopenia ≥grade 3, neutropenia ≥grade 3, Infection rate.
- Progression Free Survival (PFS).
- QOL

STUDY DESIGN:

This is a multicenter, phase II, single arm study designed to evaluate the efficacy and safety of the combination of low dose Fludarabine (FLU) and Cyclophosphamide (CY) combined with standard dose Rituximab in elderly previously untreated CLL patients (≥ 65 years of age). Potential study subjects will sign an informed consent prior to undergoing any study related procedure. Number of patients to be enrolled is 40.

Treatment plan: Flu 12.5mg/m^2/d will be given intravenously (I.V.) for days 1-3 every 28 days, for a total of 6 cycles.

CY will be given I.V. at the dose of 150 mg/ m^2/d on days 1-3 every 28 days, for a total of 6 cycles.

Rituximab will be given before chemotherapy. In cycle1: 375 mg/m^2 I.V. on day 0, cycles 2-6: 500 mg/m^2 I.V. on day 1 every 28 days.

Dose reduction or delays are recommended in case of grade ≥3 (NCI-CTC) adverse events.

This study consists of 3 periods for each study subject: Pre-treatment, Treatment and Follow up.

Pre-treatment period: patients will undergo screening for protocol eligibility within 28 days (4 weeks) of recruitment.

Subjects who meet all the inclusion criteria and have signed an approved informed consent will be enrolled.

Treatment period FLU 12.5mg/m^2/d I.V. will be given on days 1-3 every 28 days, for a total of 6 cycles. CY will be given (I.V.) 150 mg/ m^2/d on days 1-3 every 28 days, for a total of 6 cycles. Rituximab given before chemotherapy cycle 1: 375 mg/m^2 intravenously on day 0, cycles 2-6: 500 mg/m^2 I.V. on day 1 every 28 days.

Premedication: antihistamines (e.g. 2 mg clemastin or promethazine 25 mg i.v.) and paracetamol (500-1000 mg orally) 30 min before first infusion of Rituximab, and thereafter only if clinically indicated. Hydrocortisone 100-500 mg I.V. (according with local common practice) only if clinically indicated.

Dose reduction or delay in case of drug induced hematological toxicity grade ≥ 3 level.

During the treatment period, all patients will attend twice a month in the first 2 months and thereafter periodic monthly study center visits in order to assess the safety and efficacy of the treatment.

Complete blood count will be performed on day 1 and 14 of each of the first two cycles, and on day 1 in the other 4 cycles, or more frequently at the investigators discretion.

Bone marrow aspiration and biopsy and CT scan (optional) will be performed within 3-to 6 months after the completion of the treatment period.

Follow up period: All patients will be assessed for progression and survival every 3 months via clinic visits, for a total of 30 months.
Complete blood count and chemistry will be performed every 3 months, or more frequently at the investigators discretion.
In the event of suspected progression or relapse BMB and or CT scan will be performed at the treating physicians' discretion.

Duration of study

The duration of the treatment period is approximately 6 months. This time period is required to complete the therapy, to determine the safety profile of the drug combination and the response rate to therapy. The duration of the follow up period will be 30 months. The occurrence of PD will determine the duration of progression-free survival of each patient.

Recruitment period will be 24 months.

Inclusion criteria:

- Diagnosis of B-CLL is established by the presence of: An absolute lymphocyte count> $5X10^9$/L, typical morphology in the peripheral blood smear as seen by light microscopy, CLL cells must show CD5/CD19/CD23 positivity with light chain restriction as recorded by flow cytometry. Low surface immunoglobulin/CD 79b expression is also expected to be evident on these cells while FMC7 should be negative-are both additional typical features on flow cytometry (optional). **Patients with different phenotype than described above can be included, in case the pathologist will give a written approval of B-CLL and with the approval of the sponsor.**
- Previously untreated B-CLL patients $\geq$ 65 years old.
- Patients requiring treatment (active Binet stage A and B, all stage C disease).
- Written informed consent

Exclusion criteria:

- Calculated or measured creatinine clearance < 30 ml/min
- Hepatic enzymes or bilirubin > 2X ULN, unless thought to be due to CLL
- Suspected or documented CNS involvement by CLL
- Known HIV positivity
- Active or uncontrolled infections, including opportunistic infection, active hepatitis B / C
- **Patients with active hepatitis B or HBV surface antigen positive**
- **Patients with anti-HBV core antibodies (past infection with HBV) but who are negative for HBVsAg (either anti-HBS Ab positive or negative) and are positive for HBV-DNA by PCR analysis - are not eligible**
- Various concomitant diseases requiring chronic steroid administration
- Active Coombs positive hemolytic anemia
- Prior treatment for CLL
- CLL with transformation (Richter syndrome).
- Presence of active second neoplasia (excluding non-melanoma skin cancer, or in situ cervix, breast or prostate carcinoma) < 2 years prior to the study.

- Use of other investigational agents or participation in any other clinical trials < 30 days prior to the study.
- Low compliance.
- Performance status: ECOG performance status ≥3.
- Patients who mentally or physically are unable to comply with all aspects of the study.

Assessments:

Efficacy

The following evaluations will be done to record the efficacy of the treatment regimen:

- Assessment of disease response (revised International Response Criteria: updated NCI-IWG 2008).[46].
- Date of documentation of disease progression.
- Bone marrow biopsy examination
- Peripheral blood immunophenotyping by FACS.
- Changes in ANC, Hb levels and platelets counts from baseline blood counts and those done during the study period.
- Chest, abdominal and pelvic computerized tomography
- Physical examination for lymphadenopathy and hepato-splenomegaly

Safety

The following evaluations will be recorded to assess the safety of the regimen:

- Complete physical examination including vital signs.
- Clinical laboratory evaluations (hematology, biochemistry)
- Concomitant medication and procedures
- Adverse events
- Health outcomes assessment (any extra medical appointment)

Efficacy analysis:

Primary efficacy will be determined by objective overall response rate. Exact tests for assessing these will be used to compare objective overall response rates. Kaplan Meier procedure will be used to characterize the duration of response for PFS and TNT. Summary statistics (standard deviation, mean, median, minimum and maximum) will be provided for the relevant variables

Safety Analysis:

Data from all subjects who receive any study drug will be included in the safety analyses. The severity of the toxicities will be graded according to the NCI CTC v3 or v2- for cytopenia, whenever possible. In the by-subject analysis, a subject experiencing the same event more than once will be counted only once.

Adverse events will be defined and summarized by the worst NCI CTC grade. Adverse events leading to death or to discontinuation of treatment, events classified as NCI CTC Grade 3 or Grade 4, study-drug-related events, and serious adverse events will be summarized separately. Laboratory data will be graded according to NCI CTC severity grade.

STUDY DURATION: five years	**TOTAL SAMPLE SIZE**: 40

2. Background Information

Chronic lymphocytic leukemia (CLL) is characteristically diagnosed in older people with a median age at diagnosis ranging between 65 and 72 years. Because the prevalence of co morbidity increases with age, concomitant disease is often evident in this age group. Goede et al [29] reported that increasing numbers of more severe concomitant comorbid illnesses are associated with a shortened survival in patients with CLL. Most recently Thurmes and colleagues [1] have shown that these comorbid conditions are in fact less important than both the age of the patient and the disease stage, in predicting prognosis in untreated patients.

Additional factors which may also adversely affect treatment outcome in elderly patients include: altered pharmacokinetics and poorer host tissue tolerance ; changes in bone marrow hematopoietic reserve and microenvironment which may lead to increased treatment related myelotoxicity and higher rates complications such as infections [21,26]. As a result, elderly patients are often excluded from clinical trials or even from routine treatment at standard doses of drugs, on the basis of their age and / or comorbidity and when treated the drug doses are often markedly reduced when given.

The basic challenge in treating elderly CLL patients appears to be related to finding the correct balance between treatment efficacy and drug toxicity and because of this, optimal treatment for older patients is hard to achieve and remains an open question and topic of debate.

Purine analogues drugs which inhibit DNA synthesis are very effective in CLL and fludarabine, cladribine and pentostatin have all been used effectively in the treatment of CLL [14]. Fludarabine is the most extensively studied purine analogue in CLL and is available in tablet form as well as for intravenous use. When used in combination with cyclophosphamide, fludarabine has been shown to be more effective than when used alone. [13,14,27,28]. In this respect the U.K. MRC LRF CLL4 trial reported in 2007 [11] compared therapy with chlorambucil alone vs fludarabine vs fludarabine (F) alone + cyclophosphamide (FC) in 777 newly diagnosed CLL patients, and showed

that FC achieved superior response rates and better progression-free survival for all CLL patients . Because of this, FC was proposed as the gold standard treatment of all CLL patients irrespective of age [11]. Similar results were published by the German CLL Study Group and the North American Intergroup [14].

However although Fludarabine - based regimes are most efficacious, they also appear to be more toxic, inducing more prolonged myelosuppression with more infections.

Pollizzotto et al [15] investigated the influence of increasing age on the ability to deliver fludarabine based regimens as well as the toxicity obtained thereafter, in patients with indolent lymphoproliferative disorders. They reported a gradual increase in toxicity with advancing age especially in patients age ≥ 70 years but still concluded that regimens were generally well tolerated and could be delivered safely to older patients who had a good performance status (PS). They noted a modestly increased degree of myelosuppression but no increase in the incidence of severe infectious complications or treatment-related mortality.

Table 1

PR	CR	OR	Patients age	Oral Cyclophosphamide dose	Oral Fludarabine dose	Disease status	Journal
NHL-59% CLL-100%	NHL-35% CLL-0%	NHL-94% CLL-100%	61-85 Median 73.5	150mg/m^2 X 4 days Every 28 days , 4 courses	25mg/m^2 (total 40 mg) X 4 days	Mostly relapsed refractory CLPD-28 Patients CLL-9 p	Hematology 2004 (16)
All-44% SLL-4/8	All-40% SLL-3/8	All-84% SLL-7/8	66-85 Med 74	150mg/m^2 X 4 days Every 28 days ; 4 courses	25mg/m^2 (total 40 mg) X 4 days	Untreated 25 patients SLL-8	BJH 2007 (17)

40%	54%	94%	30% above age 70	150mg/m^2 X 5 days Every 28 dX6	24mg/m^2 X5 days	Untreated (196)	CLL4 (11)

2.1 Rituximab

Since the introduction of Rituximab (Rituxan™, IDEC) (FDA Approval Letter Nov. 26, 1997) in the USA and shortly thereafter in Europe in 1998 (MabThera™ (Hoffmann-LaRoche AG) for use in the treatment of CLL and indolent non-Hodgkins' lymphoma (NHL), it has been used extensively in the treatment of indolent and aggressive lymphoma, both as a single agent or more often in combination with chemotherapy. Today Rituximab is already being used to treat a variety of autoimmune disorders and by now has also been accepted as the model monoclonal antibody for the treatment of cancer. Rituximab is a chimeric mouse variable heavy and light chains (IDEC 2B8) with a human IgG_1 constant kappa antibody (C2B8) that reacts specifically with the CD20 antigen found on the surface of malignant and normal B cells and established B-cell lines [31,33]. The antigen is expressed at lower levels on chronic lymphocytic leukemia (CLL) and plasma cells [31]. CD20 is not shed from the surface of CD20+ cells after antibody binding and it is not internalized or downregulated [34]. Compared with its murine counterpart, Rituximab has a longer half-life in humans, interacts with human effector cells [34, 35], and is less immunogenic [36].

Reduced FC doses

There are only a few published reports (including relatively small numbers of CLL/SLL patients which describe the results of an FC regimen in elderly CLL patients-(≥ 70 years). These are listed in Table1.

2.2 Use of Rituximab and Combination of Rituximab and Fludarabine in CLL

Although initial responses to single agent treatment with Rituximab were low and partial responses and not complete remissions, interest continued in the use of this agent in CLL [37,39,40]. The original studies employed 375 mg/m^2 of Rituximab once a week for four weeks. Subsequent studies suggested using this dose three times a week for four weeks for a total of 12 doses instead of 4 doses which resulted in better overall responses [41]. Dose limiting toxicities of Rituximab have not been demonstrated perhaps due to the cost of the drug. In this respect it should be noted that in the initial studies using Rituximab to treat follicular NHL, responses were much higher than in CLL. This was attributed to the lower level of CD20 expression on CLL lymphocytes and the presence of circulating CD20 antigen. These differences were partially overcome when either the dose of Rituximab was increased or the frequency of the standard dose of 375 mg/m2 was increased [41, 44].

After establishing the safety and efficacy of standard dose Rituximab 375 mg/m2 three times a week for four weeks for a total of twelve doses, Byrd et al went on, to compare sequential and concurrent therapy with RF [43]. The concurrent group was therefore treated with 11 doses of Rituximab while the sequential cohort only received four doses. This was a randomized phase II trial of concurrent versus sequential Fludarabine and Rituximab with 51 and 53 patients per arm, respectively. The overall response rate in the concurrent arm was 90%, including CR in 47%while in the sequential arm the overall response rate was 77%, with CR in 28%. The disease-free and overall median survival has yet to be reached yet, but will be in excess of 23 months [44]. Three major side effects were observed: infusion related toxicity, myelosuppression, and infections. Infusion related toxicity were greatest in concurrent treated patients (20%) and almost not existent in the sequentially treated patients. In a phase II study, the German CLL Study Group (GCLLSG) [44] reported the treatment of 20 untreated and 11 relapsed CLL patients. Treatment consisted of Fludarabine administered at standard doses (25mg/m2/d; days 1-5, 29-33, 57-61, 85-89) and Rituximab (375mg/m2/d)

given on days 57, 85. The overall response rate (CR and PR) was 87% with a CR/CRu of 33%. The median duration of response was 75 weeks. Severe anemia (grade III/IV) was observed in 10% of patients, neutropenia in 42%, and thrombocytopenia in 9%, respectively. The most frequent non-hematologic side effects were infections (52%) and infusion-related symptoms. The majority of infections were respiratory and herpes virus infections.

A retrospective comparative analysis of the CALGB 9712 [47] (Rituximab plus Fludarabine) [48] and the CALGB 9011 (Fludarabine only) studies demonstrated that the addition of Rituximab to Fludarabine resulted in a significantly better 2 years progression free survival and overall survival compared to patients treated with Fludarabine alone (67% *Vs* 45% and 93% *Vs* 81%, respectively) and in both treatment approaches the infectious toxicity was similar [45].

The MD Anderson Cancer Center group reported a single arm study that utilized the combination of Rituximab, Fludarabine and Cyclophosphamide (FCR) in untreated and in relapsed and refractory CLL patients (224 and 177 patients, respectively) [24, 25]. In the untreated patients the overall response rate was 95% with a CR rate of 70%, a nodular PR rate of 10% and a PR rate of 15%. Although, during the follow-up period the time to treatment was not reached, a calculated analysis showed that 69% of patients were projected to relapse at 4 years. The major toxicities were myelosupression, predominantly of grade 3 and 4, neutropenia (in 52% of all courses) and infections.

In the relapsed and refractory patients the overall response was 73% with 25% CR, 16% nodular PR and 32% PR and a third of the complete responders achieved molecular remission. The median time to progression in responding patients was 28 months with an overall median survival time of 42 months. About 46% of the patients completed all six intended courses and myelosupression was the major cause for discontinuing treatment (66% and 18% grade 3-4 neutropenia and thrombocytopenia, respectively) with 16 % of the patients having major infection.

Recently, two pivotal phase III studies (CLL8 and REACH) [49,50] evaluated 6 cycles of rituximab (375mg/m^2 on cycle-1 and 500mg/m^2 on cycle 2-6) plus

fludarabine (25mg/m^2 d1-3) and cyclophosphamide (250mg/m^2 d1-3) chemotherapy compared with 6 cycles of fludarabine and cyclophosphamide alone. The primary endpoint of both studies was progression-free survival. Secondary endpoints for both studies included overall survival, event-free survival, duration of response, response rate and complete response. CLL8 involved 817 patients with previously untreated (first-line) CLL and good physical fitness. With a median observation time of 37.7 months, statistically significant differences were observed in OS between the two treatment arms. The OS rate was 84.1% in the FCR arm versus 79 % in the FC arm (p=0.01). In both arms, the median OS has still not been reached. Patients who received rituximab together with chemotherapy had a median progression free survival (PFS) of 51.8 months compared to 32.3 months for those who received chemotherapy alone. (hazard ratio 0.56; p < 0.001). The overall response rate in patients receiving rituximab plus chemotherapy as first-line therapy was 95.1% vs. 88.4% in those receiving chemotherapy alone and with more complete remissions in the FCR arm (44.1 vs 21.8%; p<0.001). Severe (grade 3 or greater) adverse events that occurred more often in the rituximab arm included haematologic toxicity (56% vs. 39%), neutropenia (34% vs. 21%) and leukocytopenia (24% vs. 12%).

The REACH study[49] involved 552 patients with previously treated CLL (second line therapy). Patients who received rituximab plus chemotherapy had a median PFS of 30.6 months compared to 20.6 months for those who received chemotherapy alone (hazard ratio 0.65; p=0.0001). The overall response rate in the Rituximab-containing arm was 70% vs. 58% in those receiving chemotherapy alone without Rituximab. Grade 3 or greater events such as neutropenia (42% vs. 40%), febrile neutropenia (12% vs. 12%) and neoplasms (7% vs. 3%) occurred more often in the rituximab plus chemotherapy arm. Based on the CLL8 and REACH results the FDA approved the use of rituximab as first- and second-line treatment of CLL. In Europe, Rituximab is also approved for treatment of first and relapsing CLL patients.

In the light of the above literature, the combined use of Rituximab and Fludarabine and cyclophosphamide (FCR) is currently regarded as the gold standard and backbone treatment for physically fit patients with CLL.

2.3 Fludarabine Cyclophosphamide Rituximab (FCR) regimens for elderly patients with CLL

Due to major concerns of myleotoxicity and infection complications, this chemo-immunotherapy combination regimen cannot be applied safely to all elderly CLL patients. To address this topic efforts are being made to establish novel FCR-based protocol that will be useful for the average elderly CLL patient. In this regard, Foon and colleagues [50] reported a phase II single arm study of reduced dosages of Fludarabine and Cyclophosphamide (FCR-lite). Fifty untreated CLL patients were treated with 6 cycles of Fludarabine (20mg/m^2) and Cyclophosphamide (150mg/mg^2) combined with high–dose Rituximab (500mg/m^2) given every other week during induction and later as maintenance every 3 months for two years. The OR and CR rates were 100% and 79%, respectively. Median duration of complete response was 22.3 months (range, 5.2 to 42.5 months) and none of the complete responders have relapsed. Grade 3/4 neutropenia was noted in 13% of the cycles of therapy. Another approach to reduce toxicity was to use sequential therapy with fludarabine (25 mg/m^2 on days 1 through 5 for 6 cycles) followed by consolidation with Cyclophosphamide (3,000 mg/m^2 administered every 3 weeks for three cycles) and then by Rituximab 375 mg/m^2 for four cycles (F→C→R). [51] In 36 previously untreated CLL patients an overall response of 89% was achieved including 61% CRs. 33% achieved a molecular CR (PCR negative) and achieving molecular CRs had an excellent prognosis with a plateau in the response duration curve, and 90% remain in clinical CR at 5 years. For the entire group, 5-year survival rate is 71% compared with a rate of 48% with the prior F→C regimen (not significant *P* = .10).

3. Trial Objectives and Purpose

The aim of this study is to evaluate the safety and efficacy of a combination of low dose Fludarabine (FLU) and Cyclophosphamide (CY) combined with standard dose Rituximab given as primary treatment for elderly patients with chronic lymphocytic leukemia.

The purpose of the study is to determine whether a lower dose FC combined with standard dose Rituximab regimen in elderly CLL patients is as effective as the standard FCR dose, given in most studies until now and at the same time to assess if it is indeed less toxic.

3.1 Primary Objective

To evaluate the efficacy (Response Rate) of the combination of lower dose Fludarabine 12.5 mg m^2/d given intravenously for three days and Cyclophosphamide 150 mg/d given intravenously for three days every 28 days, combined with standard dose Rituximab (375mg/ m^2 in cycle 1 and 500 mg/m^2 in cycles 2-6) for a total of 6 cycles.

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3.2 Secondary Objectives

1- Safety of the combination drugs given in the study population, especially in relation to: neutropenic fever, Infection rate, number of hospitalization days, thrombocytopenia ≥grade 3, neutropenia ≥ grade 3.

2- Progression Free Survival (PFS).

3- Quality Of Life (FACIT)

4. Trial Design

4.1 Primary and secondary end points

Primary End Point

Overall response rate (ORR) (CR and PR) as defined in the protocol (see appendix I)

4.1.1 Secondary End Points

1- Safety of the study drugs especially in relation to: neutropenic fever, Infection rate and number of hospitalization days,

thrombocytopenia ≥grade 3, neutropenia ≥grade 3.

2- Progression free survival (PFS) defined as the time elapsed from first day of study drug administration to disease progression as defined in the protocol (see appendix I)

3- QOL(see appendix VI)

4.2 Overall study design

This is a national, multicenter, phase II, single arm (not controlled), open label clinical trial to evaluate the efficacy and safety of the combination of low dose Flu + Cy combined with standard dose Rituximab Flu dosage will be given at 12.5mg/m^2/d given I.V. together with CY150 mg/m^2/d given I.V. for three days every 28 days. Rituximab will be given before chemotherapy
cycle 1: 375 mg/m^2 I.V. on day 0 and in cycles 2-6: 500 mg/m^2 I.V. on day 1 in previously untreated CLL patients who are more than 65 years of age.
Please note: All medication dosages will be rounded up to the nearest dosage according to the medical center routine administration procedures.

Patients will be treated every 28 days for up to 6 cycles. Potential study subjects will sign an informed consent form prior to undergoing any study related procedure. In general, study medications should be continued until disease progression or drug intolerance but no more than 6 cycles. Treatment choices after completion of the study are at the investigators' discretion.

Up to 40 patients are planned to be enrolled during a two year period.
This study consists of 3 periods for each study subject: A pre-treatment period, the treatment period and a follow up period.

Pre-treatment period:

Patients will undergo screening for protocol eligibility within 28 days (4 weeks) of recruitment.
Subjects who meet all the inclusion criteria and who have signed an approved informed consent will be enrolled.

Written informed consent must be obtained before any study - specific medical procedures are performed which include laboratory screening assessments and physical examination as well as documentation of vital signs. If the hematology and/or biochemistry evaluations have been performed within 14 days of the first dose of study drug as part of the screening evaluation, they need not be repeated, and the data may be used as the Day 1 values. Bone marrow biopsy (FISH whenever possible) must have been performed within 12 months of starting the first dose of the study drugs.
CT scan when done, must be performed within 12 weeks prior to starting the first dose of study drugs.

Treatment period

Includes 6 months of combination treatment with low dose Fludarabine and Cyclophosphamide combined with standard dose of Rituximab. Flu dosage will be given at 12.5mg/m^2/d given I.V. together with CY150 mg/m^2/d I.V. for three days every 28 days. Rituximab given before chemotherapy cycle 1: 375 mg/m^2 I.V. on day 0 and in cycles 2-6: 500 mg/m^2 intravenously on day 1 every 28 days.
Premedication: antihistamines (e.g. 2mg clemastin or promethazine 25mg I.V.) and paracetamol (500-1,000 mg orally) 30 minutes before first infusion of Rituximab, and thereafter only if clinically indicated. Hydrocortisone 100 - 500mg I.V.(according with local common practice only if clinically indicated.
In the event of grade $\geq$ 3 hematological toxicity which in the investigators' opinion, is study drugs related, the administration of the next cycle should be

delayed but not for more than 3 weeks and drug dose reduction of 25% of both CY and F is recommended. Two dose reductions are permitted (FLU and CY Dose modification page 29). In cases where the delay period has been terminated but grade 3-4 toxicity persists, the patient should be removed from the protocol with no further study drug administration.

During the treatment period, all patients will attend periodic twice a month in the first 2 months and thereafter monthly study- center visits in order to assess safety and efficacy of the treatment.

Complete blood counts will be performed on day 1 and 14 of the first two cycles. In the next 4 cycles- on day 1 of each cycle, or more frequently at the investigators' discretion.

Bone marrow biopsy and aspiration examination will be performed before the start of treatment, for response documentation (3 to 6 months after the completion of treatment), for evaluation of cytopenias (disease or treatment related) and for any other clinical indication at the discretion of the investigators.

Supportive care including blood transfusions or administration of growth factors G-CSF or erythropoietin is permitted at the investigators' discretion throughout all study periods.

Follow up period

During the follow up period, of 30 months after the completion of the study drugs administration, all patients will be assessed every 3 months for evidence of progression and documentation of survival at clinic visits.

Complete blood count will be performed every 3 months, or more frequently at the investigators' discretion.

Bone marrow examination, peripheral blood immunophenotyping (FACS), abdominal, pelvic and chest CT will be performed 3 to 6 months after the completion of the treatment regimen, at clinical progression and at any other time according to the investigators' discretion.

In the event of no response (no CR nor PR) in > 5 of the initial 10 subjects enrolled into the study, Fludarabine dosage will be increased for all the next subjects enrolled into the study and will be given at the dose of 15mg/m^2/d given I.V.

In the event of no response after the above increment in > 5 of the next 10 subjects who received the increased dosages, the study will be terminated and declared inadequate and inefficient. If response is evident in ≥ 5 of the 10 patients, the same dosages will be continued throughout the study until its completion.

All further treatment decisions after completion of the study are at the discretion of the investigators.

4.2.1 Visit schedule and assessments

Visit schedule

Patient must be followed at the study center according to the visit schedule and assessments outlined in Figure 1.

All routine assessments must be performed within a time window of ± 7 days of the day indicated on the visit schedule, except visits 2-5 in which time window will be of ± 3 days.

Figure 1: Visit schedule and assessments

Week	-4 to 0	1	3	5	7	9	13	17	21	33	45	57	69	81	93-153 every 12 weeks	EOS
Visit No.	1 Screening	2	3	4	5	6	7	8	9	10	11	12	13	14	15-21	22
Study period	preTx	Treatment Period								Follow up period						
Inc. /Excl. criteria	X															
Informed consent	X															
Relevant medical history/conditions	X															
Disease history	X															
BMB [1]	X									X						
Physical exam	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Weight [7]	X	X		X												
CBC	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Blood chemistry	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Beta-2 micro glob	X															
IEP, Ig level,	X												X			X
DAT [3]	X												X			X
FACS [4]	X [4]												X			X
Hepatitis B and C [5]	X															
Imaging - (CT) [2]	X															X
QOL – FACIT	X										X			X		X
Treatment dosage		X		X		X	X	X	X							
Adverse Events	X [6]	Ongoing data capture and up to 30 days after completion or discontinuation of the study														
Concomitant medications	X	Ongoing data capture														
Comments	X	Ongoing data capture														

Study completion	X	Complete at any time if study drug is discontinued

1 - Bone marrow examination must be performed within 12 months before start of treatment. BMB will be performed for progression/ lymphoma transformation, cytopenia evaluation, and for treatment response within 3-6 months after completion of treatment. For any other clinical indication it is at the discretion of the investigator whether to perform BMB.

2 - CT scan when done pre-treatment, must be performed within 12 weeks prior to starting the first dose of study drugs. Post therapy a CT scan will be performed for all CLL patients who had an abnormal CT scan prior to treatment or if physical examination is inconclusive - this is an optional assessment.

3 - DAT will be performed at screening and at any clinical suspicion of hemolysis.

4 - **FACS results prior to screening are relevant and no further test is necessary at screening.** FACS will also be performed at any clinical suspicion of progression/ lymphoma transformation, or for CR confirmation at investigators' discretion.

5 - At screening and at investigator discretion for hepatitis treatment evaluation.

6 - After signature on informed consent, but prior to initiation of study medications, only SAEs caused by a protocol-mandated intervention will be collected. After first study medication, all SAEs must be reported.

7 - Dose of medication will be determined according to weight at screening. If body weight has changed at visit 2 or visit 4 by ± 10% or more, appropriate adjustment in dose of all medications should be performed.

4.3 Trial treatment

4.3.1 Investigational therapy

Fludarabine is supplied to the study investigators by the Medical Health Organization (MHO) as routine first line treatment in all CLL patients. Fludarabine is supplied as 10 mg tablets packaged in blister packs, or as 50 mg/vial, as lyophilized powder that has to be dissolved in 2 Ml of water for injection, than diluted in 100 ml of saline (0/9%) given in 30 minutes.

Cyclophosphamide is supplied to the study investigators by the MHO as a routine first line treatment in CLL patients. Cyclophosphamide (Endoxan) is supplied as 50 mg tablets packed in blister packs or as 500 mg/vial (24 mg/ml) diluted in 100 ml of saline (0/9%) given in 30 minutes.
Rituximab is supplied to the study investigators by the MHO as a routine first line treatment in CLL patients. Rituximab (Mabthera) is supplied as 100mg of rituximab in 10ml (10mg/ml) pack of 2 vials or 500mg of rituximab in 50ml (10mg/ml) pack of 1 vial. The antibody should be diluted into a final volume of normal saline or 5% D-glucose in water to allow for a maximal concentration of 4 mg/ml. Rituximab should be administrated before FC chemotherapy.

First Infusion: Recommended initial rate is 50mg/hr; after the first 30 minutes increase by 50mg/hr every 30 minutes to maximum of 400 mg/hr. *Subsequent Infusions:* Initial rate 100mg/hr; increase by 100mg/hr every 30 minutes to a maximum of 400mg/hr

Use extreme caution and closely monitor first infusion when treating patients with ≥ $25x10^9$/l circulating malignant cells or high tumor burden (higher risk of severe cytokine release syndrome (CRS). It is recommended to administer Hydrocortisone 100-500 mg I.V. (according local common practice) shortly before infusion with Rituximab to decrease the rate and severity of acute IRR/CRS for these patients. Consider reduced rate of Rituximab for first infusion or splitting the dose over two days during the first cycle. In cycle 1 the administration of Rituximab may be delayed up to 7 days after the administration of the Fludarabine and Cyclophosphomide (FC). Rituximab will be given together with FC in cycle 2, 28 days after the administration of FC in cycle 1. Severe CRS: may be associated with some features of tumor lysis

syndrome e.g. hyperuricaemia, hyperkalaemia, hypocalcaemia, hypophosphataemia, acute renal failure, elevated LDH and may be associated with acute respiratory failure and death. If severe CRS manifests stop infusion immediately and start aggressive symptomatic treatment.
If the first dose of rituximab is well tolerated, the starting flow rate for administration of the second and subsequent infusions will be 100 mg/hour and then increased gradually by 100 mg/hour intervals not to exceed 400 mg/hour.
If any grade 1 or 2 infusion related reactions occur during an infusion, the infusion should be slowed or interrupted (at the discretion of the Investigator) and supportive treatment instituted. The infusion rate can be increased or restarted on resolution of the symptoms.
If grade 3 or 4 infusion related reactions occur during an infusion, the infusion has to be discontinued immediately and not restarted until resolution of the symptoms. Treatment can be reinitiated (at half the original infusion rate) at the discretion of the Investigator, but if the same adverse event appears again with the same severity, treatment must be permanently discontinued.
Should bronchospasm or dyspnea occur in the patient during the infusion, the infusion should be stopped immediately. Medication such as anti-histamines or steroids should be given for symptomatic relief. The infusion should not be re-started until symptoms have resolved completely and should be given at half the original infusion rate.
In the event of infusion related reactions to rituximab:
• Stop rituximab infusion, treat symptoms aggressively if necessary
• NaCl 0.9 % i.v., if necessary
• Hydrocortisone 100-500 mg I.V. (according local common practice), if necessary
• Pethidine i.v. if necessary
• Bronchodilators/antihistamines if necessary
• If side effects occur stop Rituximab-infusion, re-try the next day

Table 2: Reduction of Infusion Rate

Drop in systolic blood	Mucosal congestion, edema	Rigors	Fever	Infusion rate
> 30 mm Hg	Mild/moderate	Mild/moderate	> 38.5°C	Decrease to ½ if any of these events are seen

Following the infusion the I.V. line should be kept open for medications as needed. If there are no complications, the line may be discontinued after one hour of observation. If complications occur during the rituximab infusion, the patient should be observed for 2 hours after the completion of the infusion.

Infusion reactions to rituximab tend to be more common and more severe with the first infusion. In patients who were unable to tolerate the first infusion, cautious reintroduction of rituximab may be tried at the second cycle, at the investigator's discretion. The recommended premedication should be given and then the patient carefully re-exposed to a small amount of rituximab (slow infusion of 10 mg I.V.) If a severe reaction occurs, the rituximab must be permanently discontinued. In such case patient will be treated with FC in the next cycles and data will be collected throughout the study period)

If no severe reaction occurs after 30 minutes, the full dose of rituximab infusion should be given.

Labels will comply with the legal requirements in Israel and be printed in Hebrew and English. All study drugs containing infusion bags will be labeled. All infusion bags will be labeled by the study nurse prior to administration and **the removable part of the labels will be attached to the drug administration log, signed and dated by the study nurse.** Storage conditions for study drug will be described on the medication label. Patients will receive the study drug combination for an exposure period of up to 6 months provided that in the opinion of the investigator the patient is benefiting from treatment with this study drug combination, and in the absence of any

safety concerns. Treatment will be withheld only in the case of limiting toxicities (see below).

As administration of cyclophosphamide may cause hemorrhagic cystitis and even urinary bladder malignancy, patients will be instructed to drink at least 10 glasses of fluids on the days they receive cyclophosphamide and urinate at minimal ceasing of bladder distention.

4.3.1.1 DRUG ACCOUNTABILITY

Accountability and patient compliance will be assessed by maintaining adequate "drug **administration** log" on study medication.

The "drug **administration**" log for the study medication must be kept current and should contain the following information:

- The identification of the patient (patient number) to whom the study medication was administered.
- **Labels of used study drugs - signed and dated by the study nurse.**

All accountability **administration** logs must be available for inspection by the monitor. **Infusion bags** will be destructed according to local site guidelines.

4.3.1.2 DESTRUCTION OF THE INVESTIGATIONAL MEDICINAL PRODUCT

Used supplies (**infusion bags**) will be sent for destruction according to the site guidelines. The destruction process in the site should be documented.

4.3.2 Dose modification for hematological toxicity

Dose modifications will not be made during the first cycle of therapy. During the flowing cycles modifications of the treatment schedule will only be made as follows:

• If at day 1 of any cycle, there is a grade ≥ 3 cytopenia (See hematological toxicity section 8), NOT RELATED TO BONE MARROW INFILTRATION DUE TO CLL, treatment should be delayed for up to three weeks and then given with a 25% dose reduction of fludarabine and cyclophosphamide in the

following cycles. There is no dose reduction for Rituximab in this study, but drug administration will be delayed for the same time period as for fludarabine and cyclophosphamide.

• If after three weeks, the grade ≥ 3 cytopenia, NOT RELATED TO CLL BONE MARROW INFILTRATION, still prevails, the patient should be removed from the protocol with no further study drug administration.

• If there is further grade ≥ 3 cytopenia in subsequent cycles, despite the first 25% dose reduction treatment should again be delayed for up to three weeks and the dose of fludarabine and cyclophosphamide further reduced to 50% of the full initial dose.

• If there is further grade ≥ 3 cytopenia in subsequent cycles despite the 50% drugs dose reduction the patient should be removed from the protocol with no further treatment.

• If there is any grade ≥ 3 neutropenia with infection during any cycle, G-CSF should be administered and G-CSF should than be given in all subsequent cycles prophylactically.

4.3.3 Dose modification for impaired renal function

Fludarabine and cyclophosphomide are partly (40-60%) excreted by the kidneys. If the calculated (see appendix III) or measured creatinine clearance is at screening or during treatment reduced to 30-70 mL/min, the fludarabine dose should be reduced to 80% and the cyclophosphamide dose will be reduced to 75%. Patients with a creatinine clearance below 30 ml/min are excluded from the study. Patients who develop renal dysfunction (serum creatinine ≥ 1.5 mg/dL or creatinine clearance < 30 ml/min) during treatment should go off protocol treatment.

4.3.4 Dose modification for other non-hematological toxicity (non renal).

• If other grade ≥ 3 non-hematological, non-renal toxicity occurs during any cycle, study drugs treatment should be delayed until recovery to grade ≤ 2, for up to three weeks. **In case of grade ≥ 3, non-hematological, non-renal toxicity, which in the investigators' opinion is not study drug related, dose reduction will be performed upon investigator discretion. In case**

of grade ≥ 3 non-hematological toxicity, which in the investigators' opinion is related to study drugs, all subsequent cycles should then be given with a 25% dose reduction of fludarabine and cyclophosphamide.

• If after three weeks, the grade ≥ 3 non-hematological, non-renal toxicity, **drug related** still persists, the patient should be removed from the protocol.

• If there is further grade ≥ 3 non-hematological, non-renal toxicity, **drug related** in subsequent cycles despite the first 25% dose reduction treatment should again be delayed for up to three weeks and the dose of fludarabine and cyclophosphamide further reduced to 50% of the initial full dose.

• If there is further grade ≥ 3 non-hematological, non-renal toxicity, **drug related** in subsequent cycles despite 50% dose reduction the patient should be removed from the protocol treatment and the study.

4.3.5 Dose modification for Weight Changes

Dose of medication will be determined according to weight at screening. If body weight has changed at visit 2 or visit 4 by ± 10% or more, appropriate adjustment in dose of all medications should be performed.

4.3.6 Treatment assignment

Informed consent must be obtained before any testing for the purpose of determining a patient's eligibility is performed. Each patient will be assigned a unique patient number. Once assigned, numbers for any non-evaluable or discontinued patient will not be reutilized.

4.3.7 Blinding

This is an open label trial.

4.4 Study drug interruption or discontinuation

4.4.1 Discontinuation of study drugs

Events that will be considered as reasons for study drugs discontinuation are described in section 5.3

In addition, patient follow-up information must still be recorded (also in the event of study drug discontinuation) during and at the end of the study period.

4.4.2 Study Discontinuation

Events that will be considered as reasons for study discontinuation are described in section 5.3

4.5 Accountability procedures for the investigational products

Study drug will be prepared and dispensed by the pharmacist at the investigator's institution. Drug accountability & destruction procedures are described in sections 4.3.1.1 & 4.3.1.2, accordingly.

5. Selection and Withdrawal of Subjects

5.1 Inclusion Criteria

1. Diagnosis of B-CLL is established by the presence of: an absolute lymphocyte count > $5X10^9$ L, and typical CLL cell morphology in the peripheral blood smear as seen by light microscopy. CLL cells must show CD5/CD19/CD23 positivity with light chain restriction as recorded by flow cytometry. The levels of surface immunoglobulin and CD 79b are characteristically low and FMC7 is expected to be negative (optional). ZAP 70 and CD38 are optional. **Patients with different phenotype than described above can be included, in case the pathologist will give a written approval of B-CLL and with the approval of the sponsor.**
2. Previously untreated B-CLL patients ≥ 65 years old.
3. Patients requiring treatment (active Binet classification stage A and B, all stage C disease).(appendixes IV and V)
4. Written informed consent

5.2 Exclusion Criteria

1. Calculated or measured creatinine clearance < 30 ml/min
2. Hepatic enzymes or bilirubin ≥ 2X ULN, unless thought to be due to CLL.
3. Suspected or documented CNS involvement by CLL
4. Known HIV positivity
5. Active or uncontrolled infections, including opportunistic infection and active hepatitis B / C
6. **- Patients with active hepatitis B or HBV surface antigen positive.**
   - Patients with anti-HBV core antibodies (past infection with HBV) but who are negative for HBVsAg (either anti-HBS Ab positive or negative) and are positive for HBV-DNA by PCR analysis - are not eligible.

7. Various concomitant diseases requiring chronic steroid administration
8. Active Coomb's positive hemolytic anemia.
9. Prior treatment for CLL
10. CLL in transformation (Richter syndrome).
11. Presence of active second neoplasia (excluding non-melanoma skin cancer, or in situ cervix, breast or prostate carcinoma) < 2 years prior to the study.
12. Use of other investigational agents or participation in any other clinical trials < 30 days prior to the study.
13. Low compliance.
14. Performance status: ECOG performance status ≥3. (see appendix II)
15. Patients who are mentally or physically unable to comply with all aspects of the study. Upon investigator discretion, a psycho-geriatric evaluation will be performed for patients ≥ 80 years old.

5.3 Interruption or Discontinuation of Treatment

5.3.1 Discontinuation of study drugs

The following events will be considered as reasons for discontinuation.

- Interruption of study drug/s for longer than 3 consecutive weeks.
- Adverse events, including study drugs induced active autoimmune haemolytic anemia.
- Patient with large cell transformation (Richter syndrome).
- Disease progression as defined at any time during study drug therapy.
- Treatment with other chemotherapeutic or investigational anti-neoplastic drugs
- Intolerable adverse effects that are judged by the investigator to be either physically or psychologically detrimental to the patient. Unresolved or recurrent (more than twice) grade 3 or 4 toxicity.

- The reason for discontinuation should be recorded in the CRF and in the subject's medical records. The sponsor is to be notified of all discontinuations related to study medication.
- In addition, patient follow-up information will be recorded (also in the event of study drug discontinuation) during and at the end of the study period.
- In the case of a severe reaction to Rituximab, Rituximab will be permanently discontinued and the patient's participation in the study will be discontinued.

5.3.2 Interruption of study drugs

In the event of grade 3 or 4 toxicity, or increase of creatinine>1.5 or GFR of less than 30ml/min study drug will be interrupted for no more than 3 weeks (see sections 4.3.2-4.3.4).

5.4 Study Discontinuation

The following events are considered as study discontinuation:

- Death
- Participation in another investigational drug trial
- Loss to follow-up
- Patient withdrawal of consent
- Major violation of the study protocol- such as eligibility criteria. In these cases, no patient follow-up will be recorded.
- Administrative problems
- In the event of no response after dosage increment in > 5 of the next 10 subjects who received the increased dosage.(see chapter 4.2).

The section for "Study Completion" in the CRF must be completed for all patients. The reason for early discontinuation should be given, even if the patient refuses to return for a final visit. Patients who discontinue prematurely due to significant study drug related adverse events (AEs) should continue to be followed until resolution of the AE or up to 30 days after discontinuation. The relevant sections of the CRF should be completed as appropriate.

Patients who discontinue the study drugs for any reason should be scheduled for a visit within 30 days from drug discontinuation at which time all the assessments listed for the final visit should be performed (see figure 1).

Patients lost to follow up should be recorded as such on the CRF. Patients who discontinue study treatment should be followed for tumor assessments as per standard of care, until disease progression or new cancer therapy is initiated. If patients refuse to return for these visits or are unable to do so, the subject should be considered off-study, but a Study Evaluation Completion form should be completed.

6. Treatment of Subjects

6.1 Treatment regimen

Patients will receive FLU , CY and Rituximab combination for an exposure period of up to 6 months- defined as the treatment period. Dosage, dose regimen and route of administration are detailed in 4.3-4.4

6.1.1 Treatment assignment

Informed consent must be obtained before any testing for the purpose of determining a patient's eligibility is performed. Each patient will be assigned a unique patient number. Once assigned, numbers for any non-evaluable or discontinued patient will not be reused.

6.2 Concomitant therapy

In general, concomitant medications and therapies deemed necessary for the supportive care and safety of the patient are permitted in this study, provided their use is documented in the patient records and on the appropriate Case Report Form. The administration of any anticancer agents including chemotherapy and biologic agents is NOT permitted. Similarly, the use of other concurrent investigational drugs is not allowed. The use of blood products (irradiated) transfusions, immunoglobulins or erythroid/myeloid growth factors are permitted at investigator's discretion.

Transfusion-associated graft versus host disease prevention using irradiated blood products is mandatory.

Steroids administration is allowed exclusively for patients with autoimmune thrombocytopenia or as premedication for Rituximab.

Premedication: Any patient considered to be at risk of infusion related reaction (IRR) or TLS (=patients with lymphocytosis > 25,000mm^3) should receive: Appropriate hydration starting 12-24 hours prior to initiating treatment, and thereafter until the risk of IRR/TLS is ruled out. Urine alkalization is at investigator's discretion.

Antihistamines (e.g. 2 mg clemastin or promethazine 25mg i.v.) and paracetamol (500-1,000 mg orally) 30 min before first infusion of Rituximab, and thereafter only if clinically indicated. Hydrocortisone 100-500 mg I.V. (according local common practice) should be given only if clinically indicated (increased risk of IRR).
Pneumocystis carinii pneumonitis prophylaxis with trimethoprim 160mg-sulfamethoxasole 800 mg twice a day, twice a week, for the treatment period and for the next 6 months of follow up are recommended.

Patients with anti-HBV core antibodies (past infection with HBV) but who are negative for HBSAg (either anti-HBVs Ab positive or negative) and had negative baseline for HBV-DNA by PCR analysis will be given anti-viral prophylaxis during and for at least 6 months after the last cycle of treatment.
For prevention of hepatitis B reactivation that needs antiviral treatment **(prophylaxis treatment)** Lamivudine 100mg/day will be administrated for the entire duration of chemotherapy and for the six months afterwards.

Hepatitis B serology will be: HBsAg+, Anti HBcore and anti-HBsAg

Treatment is recommended for: HBsAg+ (carriers), or HBVsAg (-), anti HBcore (+) and anti-HBVsAg (-).

Any use of concomitant medication must be captured in the concomitant medication CRF. Administration of Blood products must also be captured as concomitant medication on CRF.

Steroids, if given (see above) are allowed for no more than 3 consecutive days.

6.3 Treatment compliance

Number of FLU and CY vials administrated I.V to every subject in every study site, will be captured on CRF.
Number of Rituximab containing infusion bags administrated to every subject in every study site, will be captured on CRF

7. Assessment of efficacy

The primary efficacy parameter to be assessed will be response rate.

Response rate include CR and PR; all others (eg. stable disease, non response, progressive disease or death from any cause should be rated as treatment failure.)

An overall objective assessment of all measurable and non-measurable response parameters will be performed according to the Visit Schedules (see Figure 1).

Response rate will be assessed according the updated (2008) NCI-WG guidelines defined in appendix I: [46].

- Peripheral blood immunophenotyping by FACS for absence of clonal lymphocytes**.**
- Physical examination - lymph node examination and measurements for absence of significant (>1.5 cm in diameter), as well as the presence of hepatomegaly and splenomegaly.
- CT scan for CLL patients with a CT found to be abnormal before therapy or alternatively if physical examination is inconclusive.
- Complete blood count for ANC, platelets and hemoglobin levels. Blood counts have to be above the following values: ANC> 1.5 X 10^9/L. platelets > 100X 10^9/L. Hemoglobin > 11.0 g/dL.
- Bone marrow aspiration and biopsy to assess degree of bone marrow involvement by CLL using FACS and immunohistochemistry, should be performed at least 3 months after the last treatment, if the above four clinical and laboratory results support that CR has been achieved. In some cases, it is necessary to postpone BMB until after all the other criteria used to define a CR have been satisfied but, this time interval should not exceed 6 months after the last treatment.
- Patient interview for the presence of constitutional symptoms throughout the study.

A single laboratory should perform all evaluations for all patients recruited in the same site. All the above studies must account for disease parameters that

were present at baseline and must use the same techniques as used at baseline. All the above mentioned assessments should be performed within 7 days of the scheduled day of assessment (see Figure 1).

All above mentioned efficacy (response) parameters will be performed at screening and at least 3 months after completing study drug administration for response documentation.

Bone marrow examination can be postponed until after the above clinical and laboratory examinations used to define a CR have been satisfied. However, this time interval should not exceed 6 months after the last treatment.

All known disease (measurable, and non measurable) parameters must be accounted for when assessing objective response rate. Current laboratory and physical examination results will be captured on the response forms in the CRF.

Progression free survival is defined as the interval from the first study drug treatment day to the first sign of disease progression.

Progressive disease (PD) will be assessed according the updated (2008) NCI-WG guidelines defined in appendix I. If needed, BMB will be performed for suspected progression at the investigator discretion. Progression will be captured on the response form CRF.

Time to next treatment is defined as the interval between the first study drug treatment day to the first day of starting the next treatment.

In general, second-line treatment decisions follow the same indications as those used for initiation of first-line treatment.

Re-treatment day will be captured on the treatment form CRF.
QOL questionnaires (FACIT) will be completed by study subjects at: screening visit and at visits number: 11,14 and at the end of study. Questionnaires will be captured on the QOL forms CRF.
CT Scan – In order to prevent contrast nephropathy, patients with GFR < 60ml/min or diabetes mellitus, hydration is recommended and if possible acetylcystein (600 mg in bolus pre CT and 1,200 mg orally twice a day for 48 hours after CT) should be given . Hospitalization for this procedure is recommended.

8. Assessment of Safety

Safety assessments will consist of evaluating adverse events and serious adverse events, laboratory parameters including hematology, chemistry, vital signs, physical examinations, and documentation of all concomitant medications and/or therapies.

8.1 Adverse events

According to the International Conference of Harmonization [ICH], an AE is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign [including an abnormal laboratory finding], symptom, or disease temporally associated with the use of a medicinal [investigational] product, whether or not considered related to the medicinal [investigational] product. Pre-existing conditions which worsen during a study are to be reported as AEs.

Information about all adverse events, whether volunteered by the patient, discovered by investigator questioning, or detected through physical examination, laboratory test or other means, will be collected and recorded on the Adverse Event Case Report Form and followed as appropriate. An adverse event is any undesirable sign, symptom or medical condition occurring after starting study treatment, even if the event is not considered to be treatment-related.

Medical conditions/diseases present before starting study treatment are only considered adverse events if they worsen after starting study treatment. Clinical events occurring before starting study treatment but after signing the informed consent form are recorded on the Medical History/Current Medical Conditions Case Report Form only if the patient receives study treatment. Abnormal laboratory values or test results constitute adverse events only if they induce clinical signs or symptoms or require therapy, when they are recorded on the Adverse Events Case Report Form under the signs, symptoms or diagnosis associated with them.

8.1.2 Grading and reporting of adverse events

All adverse events will be graded according the NCI-CTC scale version 3.0 Anemia and thrombocytopenia should be graded according to NCI-CTC scale version 2.0- the criteria for patients in leukemia studies (see assessment of toxicity below for details), and reported in detail as indicated on the CRF

Adverse events not listed on the CTCAE should be graded as follows:

Definition	Equivalent To:	CTC Grade
Discomfort noticed but no disruption of normal daily activity	Mild	Grade 1
Discomfort sufficient to reduce or affect daily activity; no treatment or medical intervention is indicated although this could improve the overall well-being or symptoms of the subject	Moderate	Grade 2
Inability to work or perform normal daily activity; treatment or medical intervention is indicated in order to improve the overall well-being or symptoms; delaying the onset of treatment is not putting the survival of the subject at direct risk.	Severe	Grade 3
An immediate threat to life or leading to a permanent mental or physical conditions that prevents work or performing normal daily activities; treatment or medical intervention is required in order to maintain survival.	Life threatening/ disabling	Grade 4
AE resulting in death	Death	Grade 5

8.1.3 Assessment of Toxicity

The evaluation of potential treatment-induced toxicity in patients with advanced CLL may be quite difficult requiring careful consideration of both the manifestations of the underlying disease, as well as adverse reactions to the therapy under study. Some of the conventional criteria for toxicity are not applicable especially under circumstances of progressive bone marrow failure from the CLL itself.

8.1.4 Hematological Toxicity

The evaluation of hematological toxicity in patients with CLL must consider the high frequency of hematological compromise already present at the initiation of therapy. Therefore the standard criteria used for solid tumors

cannot be applied directly; many patients would be considered to have grade II to IV hematological toxicity at presentation.
As a consequence, for grading of anemia and thrombocytopenia, we plan to apply the NCI-CTC version 2.0 criteria for patients in leukemia studies, but for grading of neutropenia, the standard NCI-CTC version 3.0 criteria, as given below.

Table 3: NCI-CTC Grading scale for hematological toxicity for patients in this study

ANC mm^3 § (nadir)	Grade	Decrease in platelets*or Hb# from pretreatment value (%).
≥2,000	0	No change-9%
≥1,500 and <2,000	1	10-24%
≥1,000 and <1,500	2	25-49%
≥500 and <1000	3	50-74%
<500	4	≥75%

Grades: 1 = mild, 2 = moderate, 3 = severe, 4 = life-threatening
* if platelets were < 20,000mm^3 [<20 x10^9/L] prior to therapy, the patient is inevaluable for toxicity in platelets
baseline and subsequent Hb determinations must be performed before any given infusion
§ if ANC was < 1,000 prior to therapy, the patient is inevaluable for toxicity in ANC.

Platelets:
If at any level of decrease the platelet count is < 20,000 mm^3, this is considered grade 4 toxicity, unless a severe or life-threatening decrease in the initial platelet count (e.g. < 20,000 mm^3) was present before treatment in which case the patient is inevaluable for toxicity referable to platelet counts.
Hemoglobin:
Baseline and subsequent Hb determinations must be performed before any given infusions.

Fludarabine has been reported to exacerbate or precipitate autoimmune hemolytic anemia and patients should be monitored carefully for this condition. If a rapid decrease of hemoglobin occurs during therapy, the possibility of fludarabine- or autoantibody-induced hemolysis should be considered and appropriate diagnostic tests (LDH, bilirubin, α 2-haptoglobin, reticulocytes, Coombs-test) be performed. In particular, a Coomb's test should be performed for:

- Any grade 3 or 4 anemia (Hb < 8 g/dL)
- A Hb < 10 g/dL sustained for > 2 weeks
- A drop in Hb of 3 g/dL in one week (while pts are receiving weekly blood tests)
- Any patient requiring a blood transfusion

If, in the judgment of the treating physician, there is evidence of hemolytic anemia secondary to fludarabine, study treatment should be promptly withdrawn. Full details of the hemolytic anemia should be recorded on the adverse event pages of the CRF.

Neutrophils:

Absolute neutrophil count (ANC) is used to assess the toxicity of the myeloid lineage. Other decreases in the white blood cell count, or in circulating lymphocytes, are not to be considered, since a decrease in the white blood cell count is a desired therapeutic end point. If the ANC was < 1,000/ mm^3 [<1 $x10^9$/L] prior to therapy, the patient is inevaluable for toxicity referable to the ANC.

Any Adverse Event occurring by the time of study completion (within four weeks of last drug intake) must be recorded on the Adverse Event CRF page.

8.1.5 Drug-Adverse event relationship

The causality relationship of study drug to the adverse event will be assessed by the investigator as either:

Yes or No

If there is a reasonable suspected causal relationship to the study medication, i.e. there are facts (evidence) or arguments to suggest a causal relationship, drug-event relationship should be assessed as **Yes**.

The following criteria should be considered in order to assess the relationship as **Yes:**

- Reasonable temporal association with drug administration
- It may or may not have been produced by the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject.
- Known response pattern to suspected drug
- Disappears or decreases on cessation or reduction in dose
- Reappears on rechallenge

The following criteria should be considered in order to assess the relationship as **No**:

- It does not follow a reasonable temporal sequence from administration of the drug.
- It may readily have been produced by the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject.
- It does not follow a known pattern of response to the suspected drug.
- It does not reappear or worsen when the drug is readministered.

8.2 Serious adverse events

A serious adverse event is any experience that suggests a significant hazard, contraindication, side effect or precaution. It is any Adverse Event that at any dose fulfils at least one of the following criteria:

- is fatal; (results in **death**; NOTE: death is an outcome, not an event)
- is Life-Threatening (NOTE: the term "Life-Threatening" refers to an event in which the subject was at immediate risk of death at the time of the event; it does not refer to an event which could hypothetically have caused a death had it been more severe).
- required in-patient hospitalization or prolongation of existing hospitalization;
- results in persistent or significant disability/incapacity;
- is a congenital anomaly/birth defect;
- is medically significant or requires intervention to prevent one or other of the outcomes listed above

****The term sudden death should be used only when the cause is of a cardiac origin as per standard definition. The terms death and sudden death are clearly distinct and must not be used interchangeably**.

The study will comply with all local regulatory requirements and adhere to the full requirements of the ICH Guideline for Clinical Safety Data Management, Definitions and Standards for Expedited Reporting, Topic E2

Other safety findings may require expedited reporting depending on local legislation, e.g. a major safety finding from a newly completed animal study (such as carcinogenicity).

Progression of underlying malignancy is not reported as an adverse event if it is clearly consistent with the suspected progression of the underlying cancer as defined by RECIST criteria, or other criteria as determined by protocol. Hospitalization due solely to the progression of underlying malignancy should NOT be reported as a serious adverse event. Clinical symptoms of progression may be reported as adverse events if the symptom cannot be determined as exclusively due to the progression of the underlying malignancy, or does not fit the expected pattern of progression for the disease under study.

Symptomatic deterioration may occur in some subjects. In this situation, progression is evident in the subject's clinical symptoms, but is not supported by the tumor measurements. Or, the disease progression is so evident that the investigator may elect not to perform further disease assessments. In such cases, the determination of clinical progression is based on symptomatic deterioration. These determinations should be a rare exception as every effort should be made to document the objective progression of underlying malignancy.

If there is any uncertainty about an adverse event being due only to the disease under study, it should be reported as an AE or SAE.

8.2.1 Reporting of Serious Adverse Events (immediately reportable)

Any clinical adverse event or abnormal laboratory test value that is *serious* and which occurs during the course of the study (as defined in section **8.2** above), occurring from the enrollment visit (start of study screening procedures), including long term follow-up (LTFU) must be reported to Roche *within one* working day of the investigator becoming aware of the event (expedited reporting). The investigator must complete the *SAE Reporting Form [redacted]* and forward it to the SAE Responsible.

After signature on informed consent, but prior to initiation of study medications, only SAEs caused by a protocol-mandated intervention will be collected (e.g., SAEs related to invasive procedures such as biopsies, medication washout, or no treatment run-in). After first study medication, all SAEs must be reported.

Related Serious Adverse Events ***MUST*** be collected and reported regardless of the time elapsed from the last study drug administration, even if the study has been closed. Suspected Unexpected Serious Adverse Reactions (SUSARs) are reported to investigators at each site and associated IRB/IEC when the following conditions occur:

- The event must be a SAE.
- There must be a certain degree of probability that the event is an adverse reaction from the administered drug.
- The adverse reaction must be unexpected, that is to say, not foreseen in the SPC text (Summary of Product Characteristics (for an authorized medicinal product) or the Investigator's Brochure (for an unauthorized medicinal product).

Unrelated Serious Adverse Events must be collected and reported during the study and for up to 30 days after the last dose of study medication.

This study adheres to the definition and reporting requirements of **ICH Guideline for Clinical Safety Data Management, Definitions and Standards for Expedited Reporting, Topic E2.**

8.2.2 Treatment and Follow-up of AEs

During the study period and up to 30 days after completion or discontinuation of the study, continue to follow up AEs as follows:

Related AEs: Follow until one of the following occurs:

- Resolved or improved to baseline
- Relationship is reassessed as unrelated
- Death
- Start of new anti-cancer regimen
- Investigator confirms that no further improvement can be expected
- Clinical or safety data will no longer be collected, or final database closure

Unrelated severe or life threatening AEs: Follow until one of the following occurs:

- Resolved or improved to baseline
- Severity improved to Grade 2
- Death
- Start of new anti-cancer regimen
- Investigator confirms that no further improvement can be expected
- Clinical or safety data will no longer be collected, or final database closure

Unrelated Grade 1 or Grade 2 AEs: Follow up to 30 days after completion or discontinuation of the study.

The final outcome of each adverse event must be recorded on the CRF

8.2.3 Laboratory evaluations

Laboratory test results will be recorded on the laboratory results form of the CRF, or appear on electronically produced laboratory reports submitted directly from the central laboratory, if applicable.

Any laboratory result abnormality fulfilling the criteria for a serious adverse event (SAE) should be reported as such, in addition to being recorded as an AE in the CRF.

Any treatment-emergent abnormal laboratory result which is clinically significant, i.e., meeting one or more of the following conditions, should be recorded as a single diagnosis on the adverse event page in the CRF:

- Accompanied by clinical symptoms
- Leading to a change in study medication (e.g. dose modification, interruption or permanent discontinuation)
- Requiring a change in concomitant therapy (e.g. addition of, interruption of, discontinuation of, or any other change in a concomitant medication, therapy or treatment)

No special laboratory or tests are required to be performed for the purpose of the study. Measurement of Complete Blood Counts, chemistry and immunological tests such as: serum Ig level, serum immunoellectrophoresis, DAT, peripheral and bone marrow FACS and hepatitis B **and C** serology are in fact regarded as current clinical practice for CLL patients, and should therefore be collected according to the Visit Schedules (see Figure 1). In case of suspected hemolysis - DAT, reticulocyte count, serum haptoglobin, bilirubin and LDH are recommended.

The institution will perform laboratory analyses according to the Visit Schedules (see Figure 1). The sponsor must be provided with a copy of the laboratory's certification, and a tabulation of the normal ranges for each parameter required. Additionally, if at any time a patient has laboratory parameters obtained from a different outside laboratory, the sponsor must be provided with a copy of the certification and a tabulation of the normal ranges for that laboratory.

At any time during the study, abnormal laboratory parameters which are clinically relevant (e.g. require dose modification and/or interruption of study drug, lead to clinical symptoms or signs or require therapeutic intervention), whether specifically requested in the protocol or not, must be recorded on the

appropriate Comment CRF page in addition to the appropriate laboratory CRF page. When abnormal laboratory values or test results constitute an adverse event (i.e., induces clinical signs/symptoms or requires therapy) they must be recorded on the Adverse Events CRF.

When, in the opinion of the investigator, other clinical laboratory evaluations may be relevant for assessing the patient's status, they will be completed and entered into the database as appropriate.

8.3 Special tests

PET-CT will be performed in case of suspected Richter transformation.

Lymph node biopsy is recommended for definitive diagnosis of Richter transformation and for any rapidly progressive disease or unusual phenomena which may be suspicious of alteration in the normal behavior of the disease or suspicious for another disease or a different type of lymphoma.

8.4 Physical examinations/vital signs

A physical examination including vital signs will be performed according to the Visit Schedules (see Figure 1). Information about the physical examination and vital signs must be present in the source documentation at the study site. Significant findings present prior to the start of study drug must be included in the Relevant Medical History/Current Medical Conditions CRF. Significant findings made after the start of study drug which meet the definition of an adverse event must be recorded on the Adverse Event Case Report Form. There are no case report forms to capture routine normal findings from physical examinations and vital signs assessments.

8.5 Laboratory examinations

8.5.1 Hematology

Hematological evaluations (CBC) are regarded as clinical practice for CLL patients and should therefore be collected according to the Visit Schedules (see Figure 1).

8.5.2 Blood chemistry

The following blood chemistry results are regarded as clinical practice for CLL patients and should therefore be collected according to the Visit Schedules (see Figure 1).

Serum creatinine, ALT, AST, alkaline Phosphatase, LDH, bilirubin. globulin, serum immunoellectrophoresis and beta-2 microglobulin.

8.6 Physical examinations/vital signs

A physical examination including vital signs will be performed according to the Visit Schedules (see Figure 1). Information about the physical examination and vital signs must be present in the source documentation at the study site. Significant findings present prior to the start of study drug must be included in the Relevant Medical History/Current Medical Conditions CRF. Significant findings made after the start of study drug which meet the definition of an adverse event must be recorded on the Adverse Event Case Report Form. There are no Case Report Forms to capture routine normal findings from physical examinations and vital signs assessments.

8.6.1 Warning and Precautions

No evidence available at the time of the approval of this study protocol indicated that special warnings or precautions were appropriate regarding Rituximab, other than those noted in the Investigators' Brochure.

Safety information regarding Fludarabine and Cyclophosphamide can be found in the respective local MoH approved product label.

9. Statistics

Subjects' age, weight, height and other continuous demographic and baseline variables will be summarized using descriptive statistics (mean, standard deviation, minimum and maximum), while performance status, gender, and other categorical variables will be summarized with frequency tabulations.

9.1 Sample size

This is a single arm phase II study. The number of subjects planed to be enrolled into the study is 40. Such a low number of patients as in most phase II studies) constitute an underpowered study for revealing any statistical significance, but is indeed clinically meaningful. So, the sample size chosen for this study was based on clinical considerations.

Those patients achieving PR or CR by the study medication will be regarded as responders. All the others (stable disease, progressive disease or death from any cause) will be regarded as non responders. Determining the ratio of responders versus non responders, even if not statistically significant, will still be clinically meaningful.

9.1.1 Data analysis

Categorical data will be presented as frequencies and percentages. For continuous data, mean, standard deviation, median, 25^{th} and 75^{th} percentiles, minimum, and maximum will be presented.

9.2 Efficacy and Safety evaluation

9.2.1 Efficacy analysis:

All analyses will be according to the intention to treat principle.

9.2.2 Response rate

The primary efficacy parameter to be assessed will be response rate determined by objective overall response rate.

The primary endpoint is a binary variable where each patient is classified as a responder or not responder.

95% Confidence Interval (CI) will be calculated to the proportions of responders.

Response rate include CR and PR; all other situations (eg. stable disease, non response, progressive disease or death from any cause) will be rated as treatment failure and therefore as non responders.

Response rate will be assessed according the updated (2008) NCI-WG guidelines defined in appendix 1:

- Peripheral blood FACS for lymphocytes clonality.
- Physical examination for lymph node examination and measurements, and documentation of the presence of hepatomegaly and splenomegaly.
- Blood count for ANC, platelets and hemoglobin levels.
- Bone marrow aspiration and biopsy for bone marrow involvement by CLL cells using FACS immunophenotyping or immunohistochemistry.
- Patient interview for the presence of constitutional symptoms throughout the study.

All above mentioned efficacy (response) parameters will be performed at screening and at least 3 months after completing study drug administration for response documentation.

All known disease (measurable, and non measurable) parameters must be accounted for when assessing objective response rate.

Progression free survival is defined as the interval between the first treatment day to the first sign of disease progression.

Progressive disease (PD) will be assessed according the updated (2008) NCI-WG guidelines defined in appendix I. If needed, BMB will be performed for suspected progression at the investigator's discretion. Progression will be captured on the response form CRF.

A Kaplan-Meier method will be used to estimate median PFS and 90% confidence interval.

QOL questionnaires will be completed by study subjects at: screening visit and visits number: 11, 14, and end of study. Questionnaires will be captured on the QOL forms CRF.

Summary statistics (standard deviation, median, minimum and maximum) will be provided for the relevant variables**.** The Paired T-test or Signed Rand Test for paired observations (as is appropriate) will be applied for analysing the changes in the components and total score of the FACIT QOL assessment.

The data will be analyzed using the SAS ® version 9.1.3 (SAS Institute, Cary North Carolina).

9.3 Safety Analysis

Data from all subjects who receive any study drug will be included in the safety analyses. The severity of the toxicities will be graded according to the NCI CTCAE v 3.0
http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcaev3.pdf
or v 2.0 for cytopenia, whenever possible.

All adverse events will be coded according to coding dictionaries (MedDRA version 12.1) and presented in tables by system organ class and preferred term.

In the by-subject analysis, a subject experiencing the same event more than once will be counted only once. Adverse events will be defined and summarized according to the worst NCI CTC grade. Adverse events leading to death or to discontinuation of treatment, events classified as NCI CTCAE Grade 3 or Grade 4, study-drug-related events, and serious adverse events will be summarized separately. Laboratory data will be graded according to NCI CTCAE severity grade.

10. Quality Control and Quality Assurance

Data from the CRFs are entered into the study database by Contract Research Organization staff following their own internal standard operating procedures that have been reviewed and approved by the sponsor.

Subsequently, the entered data are systematically checked by Data Management staff, using error messages printed from validation programs and database listings. Obvious errors are corrected by Data Management personnel. Other errors or omissions are entered on Data Query Forms, which are returned to the investigational site for resolution. The signed original and resolved Data Query Forms are kept with the CRFs at the investigator site, and a copy is sent to the sponsor so the resolutions can be entered into the database. Quality control audits of all key safety and efficacy data in the database are made prior to locking the database.

11. Direct Access to Source Data/Documents

11.1 Study monitoring

The monitoring visits will enable the sponsor or the monitor acting on his behalf, to assess the study's progress status, to verify data accuracy and degree of completeness of the CRFs, to ensure the protocol as well as local regulations are being followed, that the investigator is fulfilling his obligations and to correct errors in the CRFs compared to the source documents. The investigators' will authorize the monitor, at a mutually convenient time during the study to periodically review all of the CRFs and the related parts of administrative, medical and laboratory files of each participant in the study. The CRFs must be filled out before the monitoring visits.

11.2 Audits

During the study, people belonging to the sponsor quality assurance group may visit the investigator site in order to audit the study. The purpose of this visit is to check that the study is carried out according to Good Clinical Practices. Before conducting an audit, the monitor will contact the investigator in order to agree upon a mutually suitable date. The investigator and his team are required to cooperate with the auditors and to grant them access to the patients' medical files and to the study documents (CRFs and investigator's binders).

11.3 IRB and regulatory inspections

A regulatory authority may also wish to conduct an inspection (during the study or even after its completion). If an inspection is requested by a regulatory authority, the investigator must inform the sponsor immediately that this request has been made.

12. Ethics

12.1 Institutional Review Board

Before implementing this study, the protocol, the proposed informed consent form and other information to subjects, must be reviewed by a properly constituted Institutional Review Board (IRB). A signed and dated statement that the protocol and informed consent have been approved by the IRB must be given to the sponsor before study initiation. The name and occupation of the chairman and the members of the IRB must be supplied to the sponsor. Any amendments to the protocol, other than administrative ones, must be approved by this committee.

12.2 Informed consent

The investigator must explain to each subject (or legally authorized representative) the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits involved and any discomfort it may entail. Each subject must be informed that participation in the study is voluntary and that he/she may withdraw from the study at any time and that withdrawal of consent will not affect his/her subsequent medical treatment or relationship with the treating physician.

This informed consent should be given by means of a standard written statement, written in non-technical language. The subject should read and consider the statement before signing and dating it, and should be given a copy of the signed document. If the subject cannot read or sign the documents, oral presentation may be made or signature given by the subject's legally appointed representative, if witnessed by a person not involved in the study, mentioning that the patient could not read or sign the documents. No patient can enter the study before his/her informed consent has been obtained.

The informed consent form is considered to be part of the protocol, and must be submitted by the investigator with it for IRB approval.

12.3 Declaration of Helsinki

The investigator must conduct the trial in accordance with the principles of the Declaration of Helsinki which can be accessed via the website of the World Medical Association at http://www.wma.net/e/policy/17-c_e.html.

13. Data Handling and Record Keeping

The information obtained from the execution of the protocol will be listed on the CRFs provided by the sponsor.
The CRFs must be filled out on a timely basis, signed and dated by the investigator or a co-investigator named by the former and clinically responsible for the patient during the study.

The completed CRFs will be collected by the sponsor. The investigator must remit one CRF for every participant in his center for a time period of 15 years.

14. Financing and Insurance

Financing and insurance will be addressed in the future in a separate agreement.

15.Publication Policy

Any formal presentation or publication of data from this trial will be considered as a joint publication by the investigator(s) and appropriate sponsor's personnel. Authorship will be determined by mutual agreement taking in consideration that the group of PI's will prepare the manuscript and write it initially and decide which member of steering committee and which other active investigators who provided cases will be involved.

It is a multicenter study, and it is mandatory that the first publication is based on data from all centers, analyzed as stipulated in the protocol, by the sponsor statisticians, and not by the investigators. Investigators participating in multicenter studies agree not to present data gathered from one center or a small group of centers before the full publication, unless formally agreed to by all other investigators and the PI's and steering committee.

The investigator may be required to sign the clinical study report, if it is to be used in a registration submission to the health authorities of some countries. For multicenter studies only the coordinating (principle) investigator nominated by the sponsor and the steering committee at the start of the trial would provide any needed signature.

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16. Supplements

Appendix I Response definitions

CR (complete remission) = requires **all** of the following criteria as assessed at least 3 months after completion of therapy:

Absence of clonal lymphocytes in peripheral blood.

Absence of significant lymphadenopathy. (lymph nodes > 1.5 cm in diameter) by physical examination. CT is desirable for not palpable lymph nodes.

No hepatomegaly or splenomegaly by physical examination. (Abdominal, pelvix and thorax CT for subjects found to be abnormal before therapy or if physical examination is inconclusive.

Absence of constitutional symptoms.

Blood counts above the following values: ANC> 1.5 x 10^9/L . Platelets > 100 x10^9/L . Hemoglobin > 11.0 g/dL; untransfused).

Bone marrow aspirate and biopsy should be performed at least 3 months after the last treatment and if the above 5 clinical and laboratory results demonstrate that CR has been achieved.
Bone marrow should be analyzed by FACS/or immunohistochemistry to demonstrate marrow free of B-CLL cells.
In some cases, it is necessary to postpone BMB until after all the other criteria to define a CR have been satisfied. However, this time interval should not exceed 6 months after the last treatment.

Parameter	CR	PR	PD	SD
Group A				
Lymphadenopathy*	None more than 1.5 cm	Decrease ≥ 50%	Increase ≥ 50%	Change of −49% to +49%
Liver and/or spleen size	Normal size	Decrease ≥ 50%	Increase ≥ 50%	Change of −49% to +49%
Constitutional symptoms	None	Any	Any	Any
Polymorphonuclear leukocytes	>1500/μL	>1500/μL or > 50% improvement over baseline	Any	Any
Circulating clonal B lymphocytes	None	Decrease ≥ 50% from baseline	Increase ≥ 50% over baseline	Change of −49% to +49%
Group B				
Platelet count	>100 000/μL	>100 000/μL or increase ≥ 50% over baseline	Decrease of ≥ 50% from baseline secondary to CLL	Change of −49 to +49%
Hemoglobin	>11.0 g/dL (untransfused and without erythropoietin)	>11 g/dL or increase ≥ 50% over baseline	Decrease of >2 g/dL from baseline secondary to CLL	Increase ≤11.0 g/dL or <50' over baseline, or decrease <2 g/dL
Marrow	Normocellular, <30% lymphocytes, no B-lymphoid nodules; hypocellular marrow defines CRi	≥ 30% lymphocytes, or B-lymphoid nodules, or not done	Increase of lymphocytes to more than 30% from normal	No change in marrow infiltrat

Values in SI units for leukocytes are 1.5 × 10^9/L; for platelets 100 × 10^9/L; and for hemoglobin 110 g/L and 20 g/L.

CR indicates complete remission (all of the criteria have to be met); PR, partial remission (at least one of the criteria of group A and one of the criteria of group B have to be met); PD, progressive disease (at least one of the criteria of group A or one of the criteria of group B have to be met); SD, stable disease (all of the above criteria have to be met).

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

Relapse = a patient who has previously achieved CR or PR but after a period of 6 or more months, demonstrates evidence of disease progression

Refractory disease = defined as treatment failure or disease progression within 6 months from the last antileukemic therapy.

Progression = see above table under PD.

Progression free survival (PFS) = defined as the time from start of treatment to progression.

Time to next treatment (TNT) = defined as the time from start of treatment to start of second-line treatment

Appendix II ECOG Performance status

ECOG	Grade
Fully active, able to carry on all pre-disease performance without restriction	0
Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work	1
Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours	2
Capable of only limited self-care, confined to bed or chair more than 50% of waking hours	3
Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair	4
Dead	5

Appendix III calculated creatinine clearance

$$\text{GFR (mL/min)} = \frac{(140\text{-age}) \text{ X weight (Kg)}}{\text{Serum cr (mg/dL) X } 72} \text{ X } 0.85 \text{ (for women)}$$

Appendix IV Active disease

At least one of the following criteria should be met:

1. Evidence of progressive marrow failure as manifested by the development of, or worsening of, anemia and/or thrombocytopenia
2. Massive (ie, > 6 cm below the left costal margin) or progressive or symptomatic splenomegaly.
3. Massive nodes (ie, > 10 cm in longest diameter) or progressive or symptomatic lymphadenopathy.
4. Progressive lymphocytosis with an increase of more than 50% over a 2-month period or lymphocyte doubling time of less than 6 months; patients with initial blood lymphocyte counts of less than 30X109/L (30 000/µL) may require a longer observation period to determine the lymphocyte doubling time. In addition, factors contributing to lymphocytosis or lymphadenopathy other than CLL (eg, infections) should be excluded.
5. Autoimmune anemia and/or thrombocytopenia poorly responsive to corticosteroides or other standard therapy.
6. A minimum of any of the following disease-related symptoms must be present: a- Unintentional weight loss more than or equal to 10% within the previous 6 months. b- Significant fatigue (ie, ECOG PS 2 or worse; cannot work or unable to perform usual activities). c- Fevers of greater than 38.00C for 2 or more weeks without other evidence of infection. d- Night sweats for more than 1 month without evidence of infection.

Appendix V Binet staging system

The areas of involvement considered for staging are as follows: 1- Head and neck, including the Waldeyer ring. 2- Axillae 3- Groins 4- Palpable spleen 5- Palpable liver.

Stage A. Hb 10 g/dL or more and platelets 100X 10^9/L or more and up to 2 of the above areas involved.

Stage B. Hb 10 g/dL or more and platelets 100X 10^9/L or more and organomegaly greater than that defined for stage A (3 or more areas).

Stage C. All patients who have Hb less than 10 g/dL and/or a platelets count less than 100X 10^9/L, irrespective of organomegaly.

Appendix VI FACIT-QOL

FACIT − Fatigue Scale (Version 4)

Below is a list of statements that other people with your illness have said are important. **By circling one (1) number per line, please indicate how true each statement has been for you during the past 7 days.**

			Not At all	A little bit	Some what	Quite a bit	Very much
HI7	I feel fatigued		0	1	2	3	4
HI12	I feel weak all over		0	1	2	3	4
An1	I feel listless ("washed out")	0	1	2	3	4	
An2	I feel tired		0	1	2	3	4
An3	I have trouble starting things because I am tired	0	1	2	3	4	
An4	I have trouble finishing things because I am tired	0	1	2	3	4	
An5	I have energy		0	1	2	3	4
An7	I am able to do my usual activities	0	1	2	3	4	
An8	I need to sleep during the day	0	1	2	3	4	
An12	I am too tired to eat		0	1	2	3	4
An14	I need help doing my usual activities		0	1	2	3	4
An15	I am frustrated by being too tired to do the things I want to do		0	1	2	3	4
An16	I have to limit my social activity because I am tired	0	1	2	3	4	

US English

AMENDMENT HISTORY FOR PROTOCOL ML25464 (FCR-LITE)

VERSION F DATED 07 May, 2014

1. **Subject: Interim-Analysis**

Reason for change: It was decided to perform Interim Analysis in order to identify trends of response rate and safety in the study.

Section Synopsis

New text:

Interim Analysis
Interim Analysis will be conducted on all data received at database until 30 May, 2014. The parameters that will be analysed will include: Primary endpoint - Overall Response rate (including CR and PR); Secondary endpoints - Progression Free Survival (PFS), Overall survival, rates of treatment related adverse events and assessment of quality of life (FACIT).

Old text:

NA.

Section 9.4 Interim Analysis

New text:

Interim Analysis will be conducted on all data received at database until 30 May, 2014. The following parameters will be examined in the Interim Analysis:

Primary endpoint:

- **Overall Response rate**

Overall response rate will include CR and PR; all other situations (e.g. stable disease, non-response, progressive disease or death from any cause) will be rated as treatment failure and therefore as non-responders.

Secondary endpoints:

- **Progression free survival (PFS) defined as the time elapsed from first day of study drug administration to disease progression as defined in the protocol (Appendix I)**
- **Overall survival**
- **Safety of the study drugs especially in relation to: neutropenic fever, Infection rate and number of hospitalization days, thrombocytopenia ≥grade 3, neutropenia ≥grade 3**
- **Rates of treatment-related adverse effects**
- **Quality of life – by FACIT (Appendix VI)**

Old text:

NA.

F. HOFFMANN-LA ROCHE LTD

CLINICAL STUDY PROTOCOL

PROTOCOL NUMBER ML 25464 (FCR LITE)

A PHASE II, MULTICENTER, SINGLE ARM STUDY TO DETERMINE THE EFFICACY AND SAFETY OF LOW DOSE FLUDARABINE AND CYCLOPHOSPHAMIDE COMBINED WITH STANDARD DOSE RITUXIMAB AS PRIMARY THERAPY IN ELDERLY UNTREATED PATIENTS (≥65 YEARS OLD) WITH CHRONIC LYMPHOCYTIC LEUKEMIA

PROTOCOL APPROVAL

Protocol Number / Version: **ML25464 / Version F**

Medical Affairs Manager : [redacted] Date: 07 May 2014

Project statistician: [redacted] Date: 07 May 2014

This protocol is intended for use in a life-threatening indication: Yes ☑ No ☐

A PHASE II, MULTICENTER, SINGLE ARM STUDY TO DETERMINE THE EFFICACY AND SAFETY OF LOW DOSE FLUDARABINE AND CYCLOPHOSPHAMIDE COMBINED WITH STANDARD DOSE RITUXIMAB AS PRIMARY THERAPY IN ELDERLY UNTREATED PATIENTS (≥65 YEARS OLD) WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AE	Adverse Event
ALT	Alanine Aminotransferase/GPT
ANC	Absolute Neutrophil Count
AST	Aspartate Aminotransferase/SGOT
ALK PHOSP	Alkaline Phosphatase
CBC	Complete Blood Count
CLL	Chronic Lymphatic Leukemia
CR	Complete Remission
CRF	Case Report/Record Form
CRO	Contract Research Organization
CT	Computerized Tomography
CY	Cyclophosphamide
FACT-CTC	Functional Assessment of Cancer Therapy - Common Terminology Criteria for cancer Adverse Events
FACS	Flow Cytometry (Fluorescence-Activated Cell Sorting)
DAT	Direct Coombs Test
FLU	Fludarabine
G-CSF	Granulocyte-Colony Stimulating Factor
Hb	Hemoglobin
HIV	Human Immune deficiency Virus
IEP	Immuno-ellectrophoresis
I.V.	Intra-Venously
Ig	Immunoglobulin
IRB	Institutional Review Board
IRR	Infusion Related Reaction
LDH	Lactic Dehydrogenase
MHO	Medical Health Organization
ORR	Overall Response Rate
PD	Progressive Disease
PET-CT	Positron Emission Tomography- Computerized Tomography
PFS	Progression Free Survival
P.O.	Per Os/ by mouth/orally
PR	Partial Response
QOL	Quality Of Life
SAE	Serious Adverse Event
TLS	Tumor Lysis Syndrome
TNT	Time to Next Treatment
ULN	Upper Limit of Normal

SYNOPSIS

A PHASE II, MULTICENTER, SINGLE ARM STUDY TO DETERMINE THE EFFICACY AND SAFETY OF LOW DOSE FLUDARABINE AND CYCLOPHOSPHAMIDE COMBINED WITH STANDARD DOSE RITUXIMAB AS PRIMARY THERAPY IN ELDERLY UNTREATED PATIENTS (≥65 YEARS OLD) WITH CHRONIC LYMPHOCYTIC LEUKEMIA

PROTOCOL NUMBER:	ML25464
DATE PROTOCOL FINAL:	07 May 2014
STUDY DRUG:	FLUDARABINE, CYCLOPHOSPHAMIDE, RITUXIMAB
INDICATION:	CHRONIC LYMPHOCYTIC LEUKEMIA
STUDY PHASE:	II

Background and rationale Chronic lymphocytic leukemia (CLL) is characteristically diagnosed in the older population with a median age at diagnosis ranging between 65 and 72 years. Because the prevalence of co morbidity increases with age, concomitant disease is often present in this age group. Additional factors which may also adversely affect treatment outcome at standard doses in elderly patients include: altered pharmacokinetics and poorer host tissue tolerance ; changes in the bone marrow hematopoietic reserve and microenvironment leading to increased treatment related myelotoxicity and higher rates of treatment related complications such as infections. As a result, elderly patients are often excluded from clinical trials or even from routine treatment on the basis of their age and / or comorbidity. Furthermore when these patients are treated, the drug doses are often markedly reduced in an attempt to avoid causing toxicity and complications. The challenge in treating elderly CLL patients relates to finding the correct balance between drug efficacy and toxicity and because of this optimal treatment for this group of patients is often hard to achieve.

Study Objectives

The aim of this study is to evaluate the safety and efficacy (response rate) of a combination of low dose Fludarabine (FLU) and Cyclophosphamide (CY) combined with standard dose Rituximab given as primary treatment for elderly patients with chronic lymphocytic leukemia.

Primary end point: -Response rate

Secondary end points:

- Safety - Special issues: incidence of neutropenic fever, hospitalizations (no^{o}- days),
- Thrombocytopenia ≥grade 3, neutropenia ≥grade 3, Infection rate.
- Progression Free Survival (PFS).
- QOL

STUDY DESIGN:

This is a multicenter, phase II, single arm study designed to evaluate the efficacy and safety of the combination of low dose Fludarabine (FLU) and Cyclophosphamide (CY) combined with standard dose Rituximab in elderly previously untreated CLL patients (≥ 65 years of age). Potential study subjects will sign an informed consent prior to undergoing any study related procedure. Number of patients to be enrolled is 40.

Treatment plan: Flu 12.5mg/m^2/d will be given intravenously (I.V.) for days 1-3 every 28 days, for a total of 6 cycles.

CY will be given I.V. at the dose of 150 mg/ m^2/d on days 1-3 every 28 days, for a total of 6 cycles.

Rituximab will be given before chemotherapy. In cycle1: 375 mg/m^2 I.V. on day 0, cycles 2-6: 500 mg/m^2 I.V. on day 1 every 28 days.

Dose reduction or delays are recommended in case of grade ≥3 (NCI-CTC) adverse events.

This study consists of 3 periods for each study subject: Pre-treatment, Treatment and Follow up.

Pre-treatment period: patients will undergo screening for protocol eligibility within 28 days (4 weeks) of recruitment.

Subjects who meet all the inclusion criteria and have signed an approved informed consent will be enrolled.

Treatment period FLU 12.5mg/m^2/d I.V. will be given on days 1-3 every 28 days, for a total of 6 cycles. CY will be given (I.V.) 150 mg/ m^2/d on days 1-3 every 28 days, for a total of 6 cycles. Rituximab given before chemotherapy cycle 1: 375 mg/m^2 intravenously on day 0, cycles 2-6: 500 mg/m^2 I.V. on day 1 every 28 days.

Premedication: antihistamines (e.g. 2 mg clemastin or promethazine 25 mg i.v.) and paracetamol (500-1000 mg orally) 30 min before first infusion of Rituximab, and thereafter only if clinically indicated. Hydrocortisone 100-500 mg I.V. (according with local common practice) only if clinically indicated.

Dose reduction or delay in case of drug induced hematological toxicity grade ≥ 3 level.

During the treatment period, all patients will attend twice a month in the first 2 months and thereafter periodic monthly study center visits in order to assess the safety and efficacy of the treatment.

Complete blood count will be performed on day 1 and 14 of each of the first two cycles, and on day 1 in the other 4 cycles, or more frequently at the investigators discretion.

Bone marrow aspiration and biopsy and CT scan (optional) will be performed within 3-to 6 months after the completion of the treatment period.

Follow up period: All patients will be assessed for progression and survival every 3 months via clinic visits, for a total of 30 months.
Complete blood count and chemistry will be performed every 3 months, or more frequently at the investigators discretion.
In the event of suspected progression or relapse BMB and or CT scan will be performed at the treating physicians' discretion.

Duration of study

The duration of the treatment period is approximately 6 months. This time period is required to complete the therapy, to determine the safety profile of the drug combination and the response rate to therapy. The duration of the follow up period will be 30 months. The occurrence of PD will determine the duration of progression-free survival of each patient.

Recruitment period will be 24 months.

Inclusion criteria:

- Diagnosis of B-CLL is established by the presence of: An absolute lymphocyte count> $5X10^9$/L, typical morphology in the peripheral blood smear as seen by light microscopy, CLL cells must show CD5/CD19/CD23 positivity with light chain restriction as recorded by flow cytometry. Low surface immunoglobulin/CD 79b expression is also expected to be evident on these cells while FMC7 should be negative-are both additional typical features on flow cytometry (optional). Patients with different phenotype than described above can be included, in case the pathologist will give a written approval of B-CLL and with the approval of the sponsor.
- Previously untreated B-CLL patients $\geq$ 65 years old.
- Patients requiring treatment (active Binet stage A and B, all stage C disease).
- Written informed consent

Exclusion criteria:

- Calculated or measured creatinine clearance < 30 ml/min
- Hepatic enzymes or bilirubin > 2X ULN, unless thought to be due to CLL
- Suspected or documented CNS involvement by CLL
- Known HIV positivity
- Active or uncontrolled infections, including opportunistic infection, active hepatitis B / C
- Patients with active hepatitis B or HBV surface antigen positive
- Patients with anti-HBV core antibodies (past infection with HBV) but who are negative for HBVsAg (either anti-HBS Ab positive or negative) and are positive for HBV-DNA by PCR analysis - are not eligible
- Various concomitant diseases requiring chronic steroid administration
- Active Coombs positive hemolytic anemia
- Prior treatment for CLL
- CLL with transformation (Richter syndrome).
- Presence of active second neoplasia (excluding non-melanoma skin cancer, or in situ cervix, breast or prostate carcinoma) < 2 years prior to the study.

- Use of other investigational agents or participation in any other clinical trials < 30 days prior to the study.
- Low compliance.
- Performance status: ECOG performance status ≥3.
- Patients who mentally or physically are unable to comply with all aspects of the study.

Assessments:

Efficacy

The following evaluations will be done to record the efficacy of the treatment regimen:

- Assessment of disease response (revised International Response Criteria: updated NCI-IWG 2008).[46].
- Date of documentation of disease progression.
- Bone marrow biopsy examination
- Peripheral blood immunophenotyping by FACS.
- Changes in ANC, Hb levels and platelets counts from baseline blood counts and those done during the study period.
- Chest, abdominal and pelvic computerized tomography
- Physical examination for lymphadenopathy and hepato-splenomegaly

Safety

The following evaluations will be recorded to assess the safety of the regimen:

- Complete physical examination including vital signs.
- Clinical laboratory evaluations (hematology, biochemistry)
- Concomitant medication and procedures
- Adverse events
- Health outcomes assessment (any extra medical appointment)

Efficacy analysis:

Primary efficacy will be determined by objective overall response rate. Exact tests for assessing these will be used to compare objective overall response rates. Kaplan Meier procedure will be used to characterize the duration of response for PFS and TNT. Summary statistics (standard deviation, mean, median, minimum and maximum) will be provided for the relevant variables

Safety Analysis:

Data from all subjects who receive any study drug will be included in the safety analyses. The severity of the toxicities will be graded according to the NCI CTC v3 or v2- for cytopenia, whenever possible. In the by-subject analysis, a subject experiencing the same event more than once will be counted only once.

Adverse events will be defined and summarized by the worst NCI CTC grade. Adverse events leading to death or to discontinuation of treatment, events classified as NCI CTC Grade 3 or Grade 4, study-drug-related events, and serious adverse events will be summarized separately. Laboratory data will be graded according to NCI CTC severity grade.

Interim Analysis

Interim Analysis will be conducted on all data received at database until 30 May, 2014. The parameters that will be analysed will include: Primary endpoint - Overall Response rate (including CR and PR); Secondary endpoints – Progression Free Survival (PFS), Overall survival, rates of treatment related adverse events and assessment of quality of life (FACIT).

STUDY DURATION: five years **TOTAL SAMPLE SIZE**: 40

Background Information

Chronic lymphocytic leukemia (CLL) is characteristically diagnosed in older people with a median age at diagnosis ranging between 65 and 72 years. Because the prevalence of co morbidity increases with age, concomitant disease is often evident in this age group. Goede et al [29] reported that increasing numbers of more severe concomitant comorbid illnesses are associated with a shortened survival in patients with CLL. Most recently Thurmes and colleagues [1] have shown that these comorbid conditions are in fact less important than both the age of the patient and the disease stage, in predicting prognosis in untreated patients.

Additional factors which may also adversely affect treatment outcome in elderly patients include: altered pharmacokinetics and poorer host tissue tolerance ; changes in bone marrow hematopoietic reserve and microenvironment which may lead to increased treatment related myelotoxicity and higher rates complications such as infections [21,26]. As a result, elderly patients are often excluded from clinical trials or even from routine treatment at standard doses of drugs, on the basis of their age and / or comorbidity and when treated the drug doses are often markedly reduced when given.

The basic challenge in treating elderly CLL patients appears to be related to finding the correct balance between treatment efficacy and drug toxicity and because of this, optimal treatment for older patients is hard to achieve and remains an open question and topic of debate.

Purine analogues drugs which inhibit DNA synthesis are very effective in CLL and fludarabine, cladribine and pentostatin have all been used effectively in the treatment of CLL [14]. Fludarabine is the most extensively studied purine analogue in CLL and is available in tablet form as well as for intravenous use. When used in combination with cyclophosphamide, fludarabine has been shown to be more effective than when used alone. [13,14,27,28]. In this respect the U.K. MRC LRF CLL4 trial reported in 2007 [11] compared therapy with chlorambucil alone vs fludarabine vs fludarabine (F) alone + cyclophosphamide (FC) in 777 newly diagnosed CLL patients, and showed

that FC achieved superior response rates and better progression-free survival for all CLL patients . Because of this, FC was proposed as the gold standard treatment of all CLL patients irrespective of age [11]. Similar results were published by the German CLL Study Group and the North American Intergroup [14].

However although Fludarabine - based regimes are most efficacious, they also appear to be more toxic, inducing more prolonged myelosuppression with more infections.

Pollizzotto et al [15] investigated the influence of increasing age on the ability to deliver fludarabine based regimens as well as the toxicity obtained thereafter, in patients with indolent lymphoproliferative disorders. They reported a gradual increase in toxicity with advancing age especially in patients age ≥ 70 years but still concluded that regimens were generally well tolerated and could be delivered safely to older patients who had a good performance status (PS). They noted a modestly increased degree of myelosuppression but no increase in the incidence of severe infectious complications or treatment-related mortality.

Table 1

PR	CR	OR	Patients age	Oral Cyclophosphamide dose	Oral Fludarabine dose	Disease status	Journal
NHL-59% CLL-100%	NHL-35% CLL-0%	NHL-94% CLL-100%	61-85 Median 73.5	150mg/m^2 X 4 days Every 28 days , 4 courses	25mg/m^2 (total 40 mg) X 4 days	Mostly relapsed refractory CLPD-28 Patients CLL-9 p	Hematology 2004 (16)
All-44% SLL-4/8	All-40% SLL-3/8	All-84% SLL-7/8	66-85 Med 74	150mg/m^2 X 4 days Every 28 days ; 4 courses	25mg/m^2 (total 40 mg) X 4 days	Untreated 25 patients SLL-8	BJH 2007 (17)

40%	54%	94%	30% above age 70	150mg/m^2 X 5 days Every 28 dX6	24mg/m^2 X5 days	Untreated (196)	CLL4 (11)

2.1 Rituximab

Since the introduction of Rituximab (Rituxan™, IDEC) (FDA Approval Letter Nov. 26, 1997) in the USA and shortly thereafter in Europe in 1998 (MabThera™ (Hoffmann-LaRoche AG) for use in the treatment of CLL and indolent non-Hodgkins' lymphoma (NHL), it has been used extensively in the treatment of indolent and aggressive lymphoma, both as a single agent or more often in combination with chemotherapy. Today Rituximab is already being used to treat a variety of autoimmune disorders and by now has also been accepted as the model monoclonal antibody for the treatment of cancer. Rituximab is a chimeric mouse variable heavy and light chains (IDEC 2B8) with a human IgG_1 constant kappa antibody (C2B8) that reacts specifically with the CD20 antigen found on the surface of malignant and normal B cells and established B-cell lines [31,33]. The antigen is expressed at lower levels on chronic lymphocytic leukemia (CLL) and plasma cells [31]. CD20 is not shed from the surface of CD20+ cells after antibody binding and it is not internalized or downregulated [34]. Compared with its murine counterpart, Rituximab has a longer half-life in humans, interacts with human effector cells [34, 35], and is less immunogenic [36].

Reduced FC doses

There are only a few published reports (including relatively small numbers of CLL/SLL patients which describe the results of an FC regimen in elderly CLL patients-(≥ 70 years). These are listed in Table1.

2.2 Use of Rituximab and Combination of Rituximab and Fludarabine in CLL

Although initial responses to single agent treatment with Rituximab were low and partial responses and not complete remissions, interest continued in the use of this agent in CLL [37,39,40]. The original studies employed 375 mg/m^2 of Rituximab once a week for four weeks. Subsequent studies suggested using this dose three times a week for four weeks for a total of 12 doses instead of 4 doses which resulted in better overall responses [41]. Dose limiting toxicities of Rituximab have not been demonstrated perhaps due to the cost of the drug. In this respect it should be noted that in the initial studies using Rituximab to treat follicular NHL, responses were much higher than in CLL. This was attributed to the lower level of CD20 expression on CLL lymphocytes and the presence of circulating CD20 antigen. These differences were partially overcome when either the dose of Rituximab was increased or the frequency of the standard dose of 375 mg/m2 was increased [41, 44].

After establishing the safety and efficacy of standard dose Rituximab 375 mg/m2 three times a week for four weeks for a total of twelve doses, Byrd et al went on, to compare sequential and concurrent therapy with RF [43]. The concurrent group was therefore treated with 11 doses of Rituximab while the sequential cohort only received four doses. This was a randomized phase II trial of concurrent versus sequential Fludarabine and Rituximab with 51 and 53 patients per arm, respectively. The overall response rate in the concurrent arm was 90%, including CR in 47%while in the sequential arm the overall response rate was 77%, with CR in 28%. The disease-free and overall median survival has yet to be reached yet, but will be in excess of 23 months [44]. Three major side effects were observed: infusion related toxicity, myelosuppression, and infections. Infusion related toxicity were greatest in concurrent treated patients (20%) and almost not existent in the sequentially treated patients. In a phase II study, the German CLL Study Group (GCLLSG) [44] reported the treatment of 20 untreated and 11 relapsed CLL patients. Treatment consisted of Fludarabine administered at standard doses (25mg/m2/d; days 1-5, 29-33, 57-61, 85-89) and Rituximab (375mg/m2/d)

given on days 57, 85. The overall response rate (CR and PR) was 87% with a CR/CRu of 33%. The median duration of response was 75 weeks. Severe anemia (grade III/IV) was observed in 10% of patients, neutropenia in 42%, and thrombocytopenia in 9%, respectively. The most frequent non-hematologic side effects were infections (52%) and infusion-related symptoms. The majority of infections were respiratory and herpes virus infections.

A retrospective comparative analysis of the CALGB 9712 [47] (Rituximab plus Fludarabine) [48] and the CALGB 9011 (Fludarabine only) studies demonstrated that the addition of Rituximab to Fludarabine resulted in a significantly better 2 years progression free survival and overall survival compared to patients treated with Fludarabine alone (67% *Vs* 45% and 93% *Vs* 81%, respectively) and in both treatment approaches the infectious toxicity was similar [45].

The MD Anderson Cancer Center group reported a single arm study that utilized the combination of Rituximab, Fludarabine and Cyclophosphamide (FCR) in untreated and in relapsed and refractory CLL patients (224 and 177 patients, respectively) [24, 25]. In the untreated patients the overall response rate was 95% with a CR rate of 70%, a nodular PR rate of 10% and a PR rate of 15%. Although, during the follow-up period the time to treatment was not reached, a calculated analysis showed that 69% of patients were projected to relapse at 4 years. The major toxicities were myelosupression, predominantly of grade 3 and 4, neutropenia (in 52% of all courses) and infections.

In the relapsed and refractory patients the overall response was 73% with 25% CR, 16% nodular PR and 32% PR and a third of the complete responders achieved molecular remission. The median time to progression in responding patients was 28 months with an overall median survival time of 42 months. About 46% of the patients completed all six intended courses and myelosupression was the major cause for discontinuing treatment (66% and 18% grade 3-4 neutropenia and thrombocytopenia, respectively) with 16 % of the patients having major infection.

Recently, two pivotal phase III studies (CLL8 and REACH) [49,50] evaluated 6 cycles of rituximab (375mg/m^2 on cycle-1 and 500mg/m^2 on cycle 2-6) plus

fludarabine (25mg/m^2 d1-3) and cyclophosphamide (250mg/m^2 d1-3) chemotherapy compared with 6 cycles of fludarabine and cyclophosphamide alone. The primary endpoint of both studies was progression-free survival. Secondary endpoints for both studies included overall survival, event-free survival, duration of response, response rate and complete response. CLL8 involved 817 patients with previously untreated (first-line) CLL and good physical fitness. With a median observation time of 37.7 months, statistically significant differences were observed in OS between the two treatment arms. The OS rate was 84.1% in the FCR arm versus 79 % in the FC arm (p=0.01). In both arms, the median OS has still not been reached. Patients who received rituximab together with chemotherapy had a median progression free survival (PFS) of 51.8 months compared to 32.3 months for those who received chemotherapy alone. (hazard ratio 0.56; p < 0.001). The overall response rate in patients receiving rituximab plus chemotherapy as first-line therapy was 95.1% vs. 88.4% in those receiving chemotherapy alone and with more complete remissions in the FCR arm (44.1 vs 21.8%; p<0.001). Severe (grade 3 or greater) adverse events that occurred more often in the rituximab arm included haematologic toxicity (56% vs. 39%), neutropenia (34% vs. 21%) and leukocytopenia (24% vs. 12%).

The REACH study[49] involved 552 patients with previously treated CLL (second line therapy). Patients who received rituximab plus chemotherapy had a median PFS of 30.6 months compared to 20.6 months for those who received chemotherapy alone (hazard ratio 0.65; p=0.0001). The overall response rate in the Rituximab-containing arm was 70% vs. 58% in those receiving chemotherapy alone without Rituximab. Grade 3 or greater events such as neutropenia (42% vs. 40%), febrile neutropenia (12% vs. 12%) and neoplasms (7% vs. 3%) occurred more often in the rituximab plus chemotherapy arm. Based on the CLL8 and REACH results the FDA approved the use of rituximab as first- and second-line treatment of CLL. In Europe, Rituximab is also approved for treatment of first and relapsing CLL patients.

In the light of the above literature, the combined use of Rituximab and Fludarabine and cyclophosphamide (FCR) is currently regarded as the gold standard and backbone treatment for physically fit patients with CLL.

2.3 Fludarabine Cyclophosphamide Rituximab (FCR) regimens for elderly patients with CLL

Due to major concerns of myleotoxicity and infection complications, this chemo-immunotherapy combination regimen cannot be applied safely to all elderly CLL patients. To address this topic efforts are being made to establish novel FCR-based protocol that will be useful for the average elderly CLL patient. In this regard, Foon and colleagues [50] reported a phase II single arm study of reduced dosages of Fludarabine and Cyclophosphamide (FCR-lite). Fifty untreated CLL patients were treated with 6 cycles of Fludarabine ($20mg/m^2$) and Cyclophosphamide ($150mg/mg^2$) combined with high–dose Rituximab ($500mg/m^2$) given every other week during induction and later as maintenance every 3 months for two years. The OR and CR rates were 100% and 79%, respectively. Median duration of complete response was 22.3 months (range, 5.2 to 42.5 months) and none of the complete responders have relapsed. Grade 3/4 neutropenia was noted in 13% of the cycles of therapy. Another approach to reduce toxicity was to use sequential therapy with fludarabine (25 mg/m^2 on days 1 through 5 for 6 cycles) followed by consolidation with Cyclophosphamide (3,000 mg/m^2 administered every 3 weeks for three cycles) and then by Rituximab 375 mg/m^2 for four cycles (F→C→R). [51] In 36 previously untreated CLL patients an overall response of 89% was achieved including 61% CRs. 33% achieved a molecular CR (PCR negative) and achieving molecular CRs had an excellent prognosis with a plateau in the response duration curve, and 90% remain in clinical CR at 5 years. For the entire group, 5-year survival rate is 71% compared with a rate of 48% with the prior F→C regimen (not significant $P = .10$).

2. Trial Objectives and Purpose

The aim of this study is to evaluate the safety and efficacy of a combination of low dose Fludarabine (FLU) and Cyclophosphamide (CY) combined with standard dose Rituximab given as primary treatment for elderly patients with chronic lymphocytic leukemia.

The purpose of the study is to determine whether a lower dose FC combined with standard dose Rituximab regimen in elderly CLL patients is as effective as the standard FCR dose, given in most studies until now and at the same time to assess if it is indeed less toxic.

3.1 Primary Objective

To evaluate the efficacy (Response Rate) of the combination of lower dose Fludarabine 12.5 mg m^2/d given intravenously for three days and Cyclophosphamide 150 mg/d given intravenously for three days every 28 days, combined with standard dose Rituximab (375mg/ m^2 in cycle 1 and 500 mg/m^2 in cycles 2-6) for a total of 6 cycles.

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3.2 Secondary Objectives

1- Safety of the combination drugs given in the study population, especially in relation to: neutropenic fever, Infection rate, number of hospitalization days, thrombocytopenia ≥grade 3, neutropenia ≥ grade 3.

2- Progression Free Survival (PFS).

3- Quality Of Life (FACIT)

3. Trial Design

4.1 Primary and secondary end points

Primary End Point

Overall response rate (ORR) (CR and PR) as defined in the protocol (see appendix I)

4.1.1 Secondary End Points

1- Safety of the study drugs especially in relation to: neutropenic fever, Infection rate and number of hospitalization days,

thrombocytopenia ≥grade 3, neutropenia ≥grade 3.

2- Progression free survival (PFS) defined as the time elapsed from first day of study drug administration to disease progression as defined in the protocol (see appendix I)

3- QOL(see appendix VI)

4.2 Overall study design

This is a national, multicenter, phase II, single arm (not controlled), open label clinical trial to evaluate the efficacy and safety of the combination of low dose Flu + Cy combined with standard dose Rituximab Flu dosage will be given at 12.5mg/m^2/d given I.V. together with CY150 mg/m^2/d given I.V. for three days every 28 days. Rituximab will be given before chemotherapy
cycle 1: 375 mg/m^2 I.V. on day 0 and in cycles 2-6: 500 mg/m^2 I.V. on day 1 in previously untreated CLL patients who are more than 65 years of age.
Please note: All medication dosages will be rounded up to the nearest dosage according to the medical center routine administration procedures.

Patients will be treated every 28 days for up to 6 cycles. Potential study subjects will sign an informed consent form prior to undergoing any study related procedure. In general, study medications should be continued until disease progression or drug intolerance but no more than 6 cycles. Treatment choices after completion of the study are at the investigators' discretion.
Up to 40 patients are planned to be enrolled during a two year period.

This study consists of 3 periods for each study subject: A pre-treatment period, the treatment period and a follow up period.

Pre-treatment period:

Patients will undergo screening for protocol eligibility within 28 days (4 weeks) of recruitment.

Subjects who meet all the inclusion criteria and who have signed an approved informed consent will be enrolled.

Written informed consent must be obtained before any study - specific medical procedures are performed which include laboratory screening assessments and physical examination as well as documentation of vital signs. If the hematology and/or biochemistry evaluations have been performed within 14 days of the first dose of study drug as part of the screening evaluation, they need not be repeated, and the data may be used as the Day 1 values. Bone marrow biopsy (FISH whenever possible) must have been performed within 12 months of starting the first dose of the study drugs.

CT scan when done, must be performed within 12 weeks prior to starting the first dose of study drugs.

Treatment period

Includes 6 months of combination treatment with low dose Fludarabine and Cyclophosphamide combined with standard dose of Rituximab. Flu dosage will be given at 12.5mg/m^2/d given I.V. together with CY150 mg/m^2/d I.V. for three days every 28 days. Rituximab given before chemotherapy cycle 1: 375 mg/m^2 I.V. on day 0 and in cycles 2-6: 500 mg/m^2 intravenously on day 1 every 28 days.

Premedication: antihistamines (e.g. 2mg clemastin or promethazine 25mg I.V.) and paracetamol (500-1,000 mg orally) 30 minutes before first infusion of Rituximab, and thereafter only if clinically indicated. Hydrocortisone 100 - 500mg I.V.(according with local common practice only if clinically indicated.

In the event of grade ≥ 3 hematological toxicity which in the investigators' opinion, is study drugs related, the administration of the next cycle should be delayed but not for more than 3 weeks and drug dose reduction of 25% of

both CY and F is recommended. Two dose reductions are permitted (FLU and CY Dose modification page 29). In cases where the delay period has been terminated but grade 3-4 toxicity persists, the patient should be removed from the protocol with no further study drug administration.

During the treatment period, all patients will attend periodic twice a month in the first 2 months and thereafter monthly study- center visits in order to assess safety and efficacy of the treatment.

Complete blood counts will be performed on day 1 and 14 of the first two cycles. In the next 4 cycles- on day 1 of each cycle, or more frequently at the investigators' discretion.

Bone marrow biopsy and aspiration examination will be performed before the start of treatment, for response documentation (3 to 6 months after the completion of treatment), for evaluation of cytopenias (disease or treatment related) and for any other clinical indication at the discretion of the investigators.

Supportive care including blood transfusions or administration of growth factors G-CSF or erythropoietin is permitted at the investigators' discretion throughout all study periods.

Follow up period

During the follow up period, of 30 months after the completion of the study drugs administration, all patients will be assessed every 3 months for evidence of progression and documentation of survival at clinic visits.

Complete blood count will be performed every 3 months, or more frequently at the investigators' discretion.

Bone marrow examination, peripheral blood immunophenotyping (FACS), abdominal, pelvic and chest CT will be performed 3 to 6 months after the completion of the treatment regimen, at clinical progression and at any other time according to the investigators' discretion.

In the event of no response (no CR nor PR) in > 5 of the initial 10 subjects enrolled into the study, Fludarabine dosage will be increased for all the next

subjects enrolled into the study and will be given at the dose of 15mg/m^2/d given I.V.

In the event of no response after the above increment in > 5 of the next 10 subjects who received the increased dosages, the study will be terminated and declared inadequate and inefficient. If response is evident in ≥ 5 of the 10 patients, the same dosages will be continued throughout the study until its completion.

All further treatment decisions after completion of the study are at the discretion of the investigators.

4.2.1 Visit schedule and assessments

Visit schedule

Patient must be followed at the study center according to the visit schedule and assessments outlined in Figure 1.

All routine assessments must be performed within a time window of ± 7 days of the day indicated on the visit schedule, except visits 2-5 in which time window will be of ± 3 days.

Figure 1: Visit schedule and assessments

Week	-4 to 0	1	3	5	7	9	13	17	21	33	45	57	69	81	93-153 every 12 weeks	EOS
Visit No.	**1 Screening**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15-21**	**22**
Study period	**preTx**	**Treatment Period**								**Follow up period**						
Inc. /Excl. criteria	X															
Informed consent	X															
Relevant medical history/conditions	X															
Disease history	X															
BMB [1]	X									X						
Physical exam	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Weight [7]	X	X		X												
CBC	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Blood chemistry	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Beta-2 micro glob	X															
IEP, Ig level,	X												X			X
DAT [3]	X												X			X
FACS [4]	X [4]												X			X
Hepatitis B and C [5]	X															
Imaging - (CT) [2]	X															X
QOL – FACIT	X										X			X		X
Treatment dosage		X		X		X	X	X	X							
Adverse Events	**X [6]**	Ongoing data capture and up to 30 days after completion or discontinuation of the study														
Concomitant medications	X	Ongoing data capture														
Comments	X	Ongoing data capture														

Study completion	X	Complete at any time if study drug is discontinued

1 - Bone marrow examination must be performed within 12 months before start of treatment. BMB will be performed for progression/ lymphoma transformation, cytopenia evaluation, and for treatment response within 3-6 months after completion of treatment. For any other clinical indication it is at the discretion of the investigator whether to perform BMB.

2 - CT scan when done pre-treatment, must be performed within 12 weeks prior to starting the first dose of study drugs. Post therapy a CT scan will be performed for all CLL patients who had an abnormal CT scan prior to treatment or if physical examination is inconclusive - this is an optional assessment.

3 - DAT will be performed at screening and at any clinical suspicion of hemolysis.

4 - FACS results prior to screening are relevant and no further test is necessary at screening. FACS will also be performed at any clinical suspicion of progression/ lymphoma transformation, or for CR confirmation at investigators' discretion.

5 - At screening and at investigator discretion for hepatitis treatment evaluation.

6 - After signature on informed consent, but prior to initiation of study medications, only SAEs caused by a protocol-mandated intervention will be collected. After first study medication, all SAEs must be reported.

7 - Dose of medication will be determined according to weight at screening. If body weight has changed at visit 2 or visit 4 by ± 10% or more, appropriate adjustment in dose of all medications should be performed.

4.3 Trial treatment

4.3.1 Investigational therapy

Fludarabine is supplied to the study investigators by the Medical Health Organization (MHO) as routine first line treatment in all CLL patients. Fludarabine is supplied as 10 mg tablets packaged in blister packs, or as 50 mg/vial, as lyophilized powder that has to be dissolved in 2 Ml of water for injection, than diluted in 100 ml of saline (0/9%) given in 30 minutes.

Cyclophosphamide is supplied to the study investigators by the MHO as a routine first line treatment in CLL patients. Cyclophosphamide (Endoxan) is supplied as 50 mg tablets packed in blister packs or as 500 mg/vial (24 mg/ml) diluted in 100 ml of saline (0/9%) given in 30 minutes.
Rituximab is supplied to the study investigators by the MHO as a routine first line treatment in CLL patients. Rituximab (Mabthera) is supplied as 100mg of rituximab in 10ml (10mg/ml) pack of 2 vials or 500mg of rituximab in 50ml (10mg/ml) pack of 1 vial. The antibody should be diluted into a final volume of normal saline or 5% D-glucose in water to allow for a maximal concentration of 4 mg/ml. Rituximab should be administrated before FC chemotherapy.

First Infusion: Recommended initial rate is 50mg/hr; after the first 30 minutes increase by 50mg/hr every 30 minutes to maximum of 400 mg/hr. *Subsequent Infusions:* Initial rate 100mg/hr; increase by 100mg/hr every 30 minutes to a maximum of 400mg/hr

Use extreme caution and closely monitor first infusion when treating patients with ≥ $25x10^9$/l circulating malignant cells or high tumor burden (higher risk of severe cytokine release syndrome (CRS). It is recommended to administer Hydrocortisone 100-500 mg I.V. (according local common practice) shortly before infusion with Rituximab to decrease the rate and severity of acute IRR/CRS for these patients. Consider reduced rate of Rituximab for first infusion or splitting the dose over two days during the first cycle. In cycle 1 the administration of Rituximab may be delayed up to 7 days after the administration of the Fludarabine and Cyclophosphomide (FC). Rituximab will be given together with FC in cycle 2, 28 days after the administration of FC in cycle 1. Severe CRS: may be associated with some features of tumor lysis

syndrome e.g. hyperuricaemia, hyperkalaemia, hypocalcaemia, hypophosphataemia, acute renal failure, elevated LDH and may be associated with acute respiratory failure and death. If severe CRS manifests stop infusion immediately and start aggressive symptomatic treatment.
If the first dose of rituximab is well tolerated, the starting flow rate for administration of the second and subsequent infusions will be 100 mg/hour and then increased gradually by 100 mg/hour intervals not to exceed 400 mg/hour.
If any grade 1 or 2 infusion related reactions occur during an infusion, the infusion should be slowed or interrupted (at the discretion of the Investigator) and supportive treatment instituted. The infusion rate can be increased or restarted on resolution of the symptoms.
If grade 3 or 4 infusion related reactions occur during an infusion, the infusion has to be discontinued immediately and not restarted until resolution of the symptoms. Treatment can be reinitiated (at half the original infusion rate) at the discretion of the Investigator, but if the same adverse event appears again with the same severity, treatment must be permanently discontinued.
Should bronchospasm or dyspnea occur in the patient during the infusion, the infusion should be stopped immediately. Medication such as anti-histamines or steroids should be given for symptomatic relief. The infusion should not be re-started until symptoms have resolved completely and should be given at half the original infusion rate.
In the event of infusion related reactions to rituximab:
• Stop rituximab infusion, treat symptoms aggressively if necessary
• NaCl 0.9 % i.v., if necessary
• Hydrocortisone 100-500 mg I.V. (according local common practice), if necessary
• Pethidine i.v. if necessary
• Bronchodilators/antihistamines if necessary
• If side effects occur stop Rituximab-infusion, re-try the next day

Table 2: Reduction of Infusion Rate

Drop in systolic blood	Mucosal congestion, edema	Rigors	Fever	Infusion rate
> 30 mm Hg	Mild/moderate	Mild/moderate	> 38.5°C	Decrease to ½ if any of these events are seen

Following the infusion the I.V. line should be kept open for medications as needed. If there are no complications, the line may be discontinued after one hour of observation. If complications occur during the rituximab infusion, the patient should be observed for 2 hours after the completion of the infusion.

Infusion reactions to rituximab tend to be more common and more severe with the first infusion. In patients who were unable to tolerate the first infusion, cautious reintroduction of rituximab may be tried at the second cycle, at the investigator's discretion. The recommended premedication should be given and then the patient carefully re-exposed to a small amount of rituximab (slow infusion of 10 mg I.V.) If a severe reaction occurs, the rituximab must be permanently discontinued. In such case patient will be treated with FC in the next cycles and data will be collected throughout the study period)

If no severe reaction occurs after 30 minutes, the full dose of rituximab infusion should be given.

Labels will comply with the legal requirements in Israel and be printed in Hebrew and English. All study drugs containing infusion bags will be labeled. All infusion bags will be labeled by the study nurse prior to administration and the removable part of the labels will be attached to the drug administration log, signed and dated by the study nurse. Storage conditions for study drug will be described on the medication label. Patients will receive the study drug combination for an exposure period of up to 6 months provided that in the opinion of the investigator the patient is benefiting from treatment with this study drug combination, and in the absence of any safety concerns. Treatment will be withheld only in the case of limiting toxicities (see below).

As administration of cyclophosphamide may cause hemorrhagic cystitis and even urinary bladder malignancy, patients will be instructed to drink at least 10 glasses of fluids on the days they receive cyclophosphamide and urinate at minimal ceasing of bladder distention.

4.3.1.1 DRUG ACCOUNTABILITY

Accountability and patient compliance will be assessed by maintaining adequate "drug administration log" on study medication.

The "drug administration" log for the study medication must be kept current and should contain the following information:

- The identification of the patient (patient number) to whom the study medication was administered.
- Labels of used study drugs - signed and dated by the study nurse.

All accountability administration logs must be available for inspection by the monitor. Infusion bags will be destructed according to local site guidelines.

4.3.1.2 DESTRUCTION OF THE INVESTIGATIONAL MEDICINAL PRODUCT

Used supplies (infusion bags) will be sent for destruction according to the site guidelines. The destruction process in the site should be documented.

4.3.2 Dose modification for hematological toxicity

Dose modifications will not be made during the first cycle of therapy. During the flowing cycles modifications of the treatment schedule will only be made as follows:

• If at day 1 of any cycle, there is a grade ≥ 3 cytopenia (See hematological toxicity section 8), NOT RELATED TO BONE MARROW INFILTRATION DUE TO CLL, treatment should be delayed for up to three weeks and then given with a 25% dose reduction of fludarabine and cyclophosphamide in the following cycles. There is no dose reduction for Rituximab in this study, but drug administration will be delayed for the same time period as for fludarabine and cyclophosphamide.

• If after three weeks, the grade ≥ 3 cytopenia, NOT RELATED TO CLL BONE MARROW INFILTRATION, still prevails, the patient should be removed from the protocol with no further study drug administration.
• If there is further grade ≥ 3 cytopenia in subsequent cycles, despite the first 25% dose reduction treatment should again be delayed for up to three weeks and the dose of fludarabine and cyclophosphamide further reduced to 50% of the full initial dose.
• If there is further grade ≥ 3 cytopenia in subsequent cycles despite the 50% drugs dose reduction the patient should be removed from the protocol with no further treatment.
• If there is any grade ≥ 3 neutropenia with infection during any cycle, G-CSF should be administered and G-CSF should than be given in all subsequent cycles prophylactically.

4.3.3 Dose modification for impaired renal function

Fludarabine and cyclophosphomide are partly (40-60%) excreted by the kidneys. If the calculated (see appendix III) or measured creatinine clearance is at screening or during treatment reduced to 30-70 mL/min, the fludarabine dose should be reduced to 80% and the cyclophosphamide dose will be reduced to 75%. Patients with a creatinine clearance below 30 ml/min are excluded from the study. Patients who develop renal dysfunction (serum creatinine ≥ 1.5 mg/dL or creatinine clearance < 30 ml/min) during treatment should go off protocol treatment.

4.3.4 Dose modification for other non-hematological toxicity (non renal).

• If other grade ≥ 3 non-hematological, non-renal toxicity occurs during any cycle, study drugs treatment should be delayed until recovery to grade ≤ 2, for up to three weeks. In case of grade ≥ 3, non-hematological, non-renal toxicity, which in the investigators' opinion is not study drug related, dose reduction will be performed upon investigator discretion. In case of grade ≥ 3 non-hematological toxicity, which in the investigators' opinion is related to study drugs, all subsequent cycles should then be given with a 25% dose reduction of fludarabine and cyclophosphamide.

• If after three weeks, the grade ≥ 3 non-hematological, non-renal toxicity, drug related still persists, the patient should be removed from the protocol.
• If there is further grade ≥ 3 non-hematological, non-renal toxicity, drug related in subsequent cycles despite the first 25% dose reduction treatment should again be delayed for up to three weeks and the dose of fludarabine and cyclophosphamide further reduced to 50% of the initial full dose.
• If there is further grade ≥ 3 non-hematological, non-renal toxicity, drug related in subsequent cycles despite 50% dose reduction the patient should be removed from the protocol treatment and the study.

4.3.5 Dose modification for Weight Changes

Dose of medication will be determined according to weight at screening.
If body weight has changed at visit 2 or visit 4 by ± 10% or more, appropriate adjustment in dose of all medications should be performed.

4.3.6 Treatment assignment

Informed consent must be obtained before any testing for the purpose of determining a patient's eligibility is performed. Each patient will be assigned a unique patient number. Once assigned, numbers for any non-evaluable or discontinued patient will not be reutilized.

4.3.7 Blinding

This is an open label trial.

4.4 Study drug interruption or discontinuation

4.4.1 Discontinuation of study drugs

Events that will be considered as reasons for study drugs discontinuation are described in section 5.3
In addition, patient follow-up information must still be recorded (also in the event of study drug discontinuation) during and at the end of the study period.

4.4.2 Study Discontinuation

Events that will be considered as reasons for study discontinuation are described in section 5.3

4.5 Accountability procedures for the investigational products

Study drug will be prepared and dispensed by the pharmacist at the investigator's institution. Drug accountability & destruction procedures are described in sections 4.3.1.1 & 4.3.1.2, accordingly.

4. Selection and Withdrawal of Subjects

5.1 Inclusion Criteria

1. Diagnosis of B-CLL is established by the presence of: an absolute lymphocyte count > $5X10^9$ L, and typical CLL cell morphology in the peripheral blood smear as seen by light microscopy. CLL cells must show CD5/CD19/CD23 positivity with light chain restriction as recorded by flow cytometry. The levels of surface immunoglobulin and CD 79b are characteristically low and FMC7 is expected to be negative (optional). ZAP 70 and CD38 are optional. Patients with different phenotype than described above can be included, in case the pathologist will give a written approval of B-CLL and with the approval of the sponsor.
2. Previously untreated B-CLL patients ≥ 65 years old.
3. Patients requiring treatment (active Binet classification stage A and B, all stage C disease).(appendixes IV and V)
4. Written informed consent

5.2 Exclusion Criteria

1. Calculated or measured creatinine clearance < 30 ml/min
2. Hepatic enzymes or bilirubin ≥ 2X ULN, unless thought to be due to CLL.
3. Suspected or documented CNS involvement by CLL
4. Known HIV positivity
5. Active or uncontrolled infections, including opportunistic infection and active hepatitis B / C
6. - Patients with active hepatitis B or HBV surface antigen positive.
   - Patients with anti-HBV core antibodies (past infection with HBV) but who are negative for HBVsAg (either anti-HBS Ab positive or negative) and are positive for HBV-DNA by PCR analysis - are not eligible.

7. Various concomitant diseases requiring chronic steroid administration
8. Active Coomb's positive hemolytic anemia.
9. Prior treatment for CLL
10. CLL in transformation (Richter syndrome).
11. Presence of active second neoplasia (excluding non-melanoma skin cancer, or in situ cervix, breast or prostate carcinoma) < 2 years prior to the study.
12. Use of other investigational agents or participation in any other clinical trials < 30 days prior to the study.
13. Low compliance.
14. Performance status: ECOG performance status ≥3. (see appendix II)
15. Patients who are mentally or physically unable to comply with all aspects of the study. Upon investigator discretion, a psycho-geriatric evaluation will be performed for patients ≥ 80 years old.

5.3 Interruption or Discontinuation of Treatment

5.3.1 Discontinuation of study drugs

The following events will be considered as reasons for discontinuation.

- Interruption of study drug/s for longer than 3 consecutive weeks.
- Adverse events, including study drugs induced active autoimmune haemolytic anemia.
- Patient with large cell transformation (Richter syndrome).
- Disease progression as defined at any time during study drug therapy.
- Treatment with other chemotherapeutic or investigational anti-neoplastic drugs
- Intolerable adverse effects that are judged by the investigator to be either physically or psychologically detrimental to the patient. Unresolved or recurrent (more than twice) grade 3 or 4 toxicity.

- The reason for discontinuation should be recorded in the CRF and in the subject's medical records. The sponsor is to be notified of all discontinuations related to study medication.
- In addition, patient follow-up information will be recorded (also in the event of study drug discontinuation) during and at the end of the study period.
- In the case of a severe reaction to Rituximab, Rituximab will be permanently discontinued and the patient's participation in the study will be discontinued.

5.3.2 Interruption of study drugs

In the event of grade 3 or 4 toxicity, or increase of creatinine>1.5 or GFR of less than 30ml/min study drug will be interrupted for no more than 3 weeks (see sections 4.3.2-4.3.4).

5.4 Study Discontinuation

The following events are considered as study discontinuation:

- Death
- Participation in another investigational drug trial
- Loss to follow-up
- Patient withdrawal of consent
- Major violation of the study protocol- such as eligibility criteria. In these cases, no patient follow-up will be recorded.
- Administrative problems
- In the event of no response after dosage increment in > 5 of the next 10 subjects who received the increased dosage.(see chapter 4.2).

The section for "Study Completion" in the CRF must be completed for all patients. The reason for early discontinuation should be given, even if the patient refuses to return for a final visit. Patients who discontinue prematurely due to significant study drug related adverse events (AEs) should continue to be followed until resolution of the AE or up to 30 days after discontinuation. The relevant sections of the CRF should be completed as appropriate.

Patients who discontinue the study drugs for any reason should be scheduled for a visit within 30 days from drug discontinuation at which time all the assessments listed for the final visit should be performed (see figure 1).

Patients lost to follow up should be recorded as such on the CRF. Patients who discontinue study treatment should be followed for tumor assessments as per standard of care, until disease progression or new cancer therapy is initiated. If patients refuse to return for these visits or are unable to do so, the subject should be considered off-study, but a Study Evaluation Completion form should be completed.

6. Treatment of Subjects

6.1 Treatment regimen

Patients will receive FLU , CY and Rituximab combination for an exposure period of up to 6 months- defined as the treatment period. Dosage, dose regimen and route of administration are detailed in 4.3-4.4

6.1.1 Treatment assignment

Informed consent must be obtained before any testing for the purpose of determining a patient's eligibility is performed. Each patient will be assigned a unique patient number. Once assigned, numbers for any non-evaluable or discontinued patient will not be reused.

6.2 Concomitant therapy

In general, concomitant medications and therapies deemed necessary for the supportive care and safety of the patient are permitted in this study, provided their use is documented in the patient records and on the appropriate Case Report Form. The administration of any anticancer agents including chemotherapy and biologic agents is NOT permitted. Similarly, the use of other concurrent investigational drugs is not allowed. The use of blood products (irradiated) transfusions, immunoglobulins or erythroid/myeloid growth factors are permitted at investigator's discretion.

Transfusion-associated graft versus host disease prevention using irradiated blood products is mandatory.

Steroids administration is allowed exclusively for patients with autoimmune thrombocytopenia or as premedication for Rituximab.

Premedication: Any patient considered to be at risk of infusion related reaction (IRR) or TLS (=patients with lymphocytosis > 25,000mm^3) should receive: Appropriate hydration starting 12-24 hours prior to initiating treatment, and thereafter until the risk of IRR/TLS is ruled out. Urine alkalization is at investigator's discretion.

Antihistamines (e.g. 2 mg clemastin or promethazine 25mg i.v.) and paracetamol (500-1,000 mg orally) 30 min before first infusion of Rituximab, and thereafter only if clinically indicated. Hydrocortisone 100-500 mg I.V. (according local common practice) should be given only if clinically indicated (increased risk of IRR).
Pneumocystis carinii pneumonitis prophylaxis with trimethoprim 160mg-sulfamethoxasole 800 mg twice a day, twice a week, for the treatment period and for the next 6 months of follow up are recommended.

Patients with anti-HBV core antibodies (past infection with HBV) but who are negative for HBSAg (either anti-HBVs Ab positive or negative) and had negative baseline for HBV-DNA by PCR analysis will be given anti-viral prophylaxis during and for at least 6 months after the last cycle of treatment. For prevention of hepatitis B reactivation that needs antiviral treatment (prophylaxis treatment) Lamivudine 100mg/day will be administrated for the entire duration of chemotherapy and for the six months afterwards.

Hepatitis B serology will be: HBsAg+, Anti HBcore and anti-HBsAg

Treatment is recommended for: HBsAg+ (carriers), or HBVsAg (-), anti HBcore (+) and anti-HBVsAg (-).

Any use of concomitant medication must be captured in the concomitant medication CRF. Administration of Blood products must also be captured as concomitant medication on CRF.

Steroids, if given (see above) are allowed for no more than 3 consecutive days.

6.3 Treatment compliance

Number of FLU and CY vials administrated I.V to every subject in every study site, will be captured on CRF.
Number of Rituximab containing infusion bags administrated to every subject in every study site, will be captured on CRF

7. Assessment of efficacy

The primary efficacy parameter to be assessed will be response rate.

Response rate include CR and PR; all others (eg. stable disease, non response, progressive disease or death from any cause should be rated as treatment failure.)

An overall objective assessment of all measurable and non-measurable response parameters will be performed according to the Visit Schedules (see Figure 1).

Response rate will be assessed according the updated (2008) NCI-WG guidelines defined in appendix I: [46].

- Peripheral blood immunophenotyping by FACS for absence of clonal lymphocytes**.**
- Physical examination - lymph node examination and measurements for absence of significant (>1.5 cm in diameter), as well as the presence of hepatomegaly and splenomegaly.
- CT scan for CLL patients with a CT found to be abnormal before therapy or alternatively if physical examination is inconclusive.
- Complete blood count for ANC, platelets and hemoglobin levels. Blood counts have to be above the following values: ANC> 1.5 X 10^9/L. platelets > 100X 10^9/L. Hemoglobin > 11.0 g/dL.
- Bone marrow aspiration and biopsy to assess degree of bone marrow involvement by CLL using FACS and immunohistochemistry, should be performed at least 3 months after the last treatment, if the above four clinical and laboratory results support that CR has been achieved. In some cases, it is necessary to postpone BMB until after all the other criteria used to define a CR have been satisfied but, this time interval should not exceed 6 months after the last treatment.
- Patient interview for the presence of constitutional symptoms throughout the study.

A single laboratory should perform all evaluations for all patients recruited in the same site. All the above studies must account for disease parameters that

were present at baseline and must use the same techniques as used at baseline. All the above mentioned assessments should be performed within 7 days of the scheduled day of assessment (see Figure 1).

All above mentioned efficacy (response) parameters will be performed at screening and at least 3 months after completing study drug administration for response documentation.

Bone marrow examination can be postponed until after the above clinical and laboratory examinations used to define a CR have been satisfied. However, this time interval should not exceed 6 months after the last treatment.

All known disease (measurable, and non measurable) parameters must be accounted for when assessing objective response rate. Current laboratory and physical examination results will be captured on the response forms in the CRF.

Progression free survival is defined as the interval from the first study drug treatment day to the first sign of disease progression.

Progressive disease (PD) will be assessed according the updated (2008) NCI-WG guidelines defined in appendix I. If needed, BMB will be performed for suspected progression at the investigator discretion. Progression will be captured on the response form CRF.

Time to next treatment is defined as the interval between the first study drug treatment day to the first day of starting the next treatment.

In general, second-line treatment decisions follow the same indications as those used for initiation of first-line treatment.

Re-treatment day will be captured on the treatment form CRF.
QOL questionnaires (FACIT) will be completed by study subjects at: screening visit and at visits number: 11,14 and at the end of study. Questionnaires will be captured on the QOL forms CRF.
CT Scan – In order to prevent contrast nephropathy, patients with GFR < 60ml/min or diabetes mellitus, hydration is recommended and if possible acetylcystein (600 mg in bolus pre CT and 1,200 mg orally twice a day for 48 hours after CT) should be given . Hospitalization for this procedure is recommended.

8. Assessment of Safety

Safety assessments will consist of evaluating adverse events and serious adverse events, laboratory parameters including hematology, chemistry, vital signs, physical examinations, and documentation of all concomitant medications and/or therapies.

8.1 Adverse events

According to the International Conference of Harmonization [ICH], an AE is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign [including an abnormal laboratory finding], symptom, or disease temporally associated with the use of a medicinal [investigational] product, whether or not considered related to the medicinal [investigational] product. Pre-existing conditions which worsen during a study are to be reported as AEs.

Information about all adverse events, whether volunteered by the patient, discovered by investigator questioning, or detected through physical examination, laboratory test or other means, will be collected and recorded on the Adverse Event Case Report Form and followed as appropriate. An adverse event is any undesirable sign, symptom or medical condition occurring after starting study treatment, even if the event is not considered to be treatment-related.

Medical conditions/diseases present before starting study treatment are only considered adverse events if they worsen after starting study treatment. Clinical events occurring before starting study treatment but after signing the informed consent form are recorded on the Medical History/Current Medical Conditions Case Report Form only if the patient receives study treatment. Abnormal laboratory values or test results constitute adverse events only if they induce clinical signs or symptoms or require therapy, when they are recorded on the Adverse Events Case Report Form under the signs, symptoms or diagnosis associated with them.

8.1.2 Grading and reporting of adverse events

All adverse events will be graded according the NCI-CTC scale version 3.0 Anemia and thrombocytopenia should be graded according to NCI-CTC scale version 2.0- the criteria for patients in leukemia studies (see assessment of toxicity below for details), and reported in detail as indicated on the CRF

Adverse events not listed on the CTCAE should be graded as follows:

Definition	Equivalent To:	CTC Grade
Discomfort noticed but no disruption of normal daily activity	Mild	Grade 1
Discomfort sufficient to reduce or affect daily activity; no treatment or medical intervention is indicated although this could improve the overall well-being or symptoms of the subject	Moderate	Grade 2
Inability to work or perform normal daily activity; treatment or medical intervention is indicated in order to improve the overall well-being or symptoms; delaying the onset of treatment is not putting the survival of the subject at direct risk.	Severe	Grade 3
An immediate threat to life or leading to a permanent mental or physical conditions that prevents work or performing normal daily activities; treatment or medical intervention is required in order to maintain survival.	Life threatening/ disabling	Grade 4
AE resulting in death	Death	Grade 5

8.1.3 Assessment of Toxicity

The evaluation of potential treatment-induced toxicity in patients with advanced CLL may be quite difficult requiring careful consideration of both the manifestations of the underlying disease, as well as adverse reactions to the therapy under study. Some of the conventional criteria for toxicity are not applicable especially under circumstances of progressive bone marrow failure from the CLL itself.

8.1.4 Hematological Toxicity

The evaluation of hematological toxicity in patients with CLL must consider the high frequency of hematological compromise already present at the initiation of therapy. Therefore the standard criteria used for solid tumors

cannot be applied directly; many patients would be considered to have grade II to IV hematological toxicity at presentation.

As a consequence, for grading of anemia and thrombocytopenia, we plan to apply the NCI-CTC version 2.0 criteria for patients in leukemia studies, but for grading of neutropenia, the standard NCI-CTC version 3.0 criteria, as given below.

Table 3: NCI-CTC Grading scale for hematological toxicity for patients in this study

ANC mm^3 § (nadir)	Grade	Decrease in platelets*or Hb# from pretreatment value (%).
≥2,000	0	No change-9%
≥1,500 and <2,000	1	10-24%
≥1,000 and <1,500	2	25-49%
≥500 and <1000	3	50-74%
<500	4	≥75%

Grades: 1 = mild, 2 = moderate, 3 = severe, 4 = life-threatening

* if platelets were < 20,000mm^3 [<20 x10^9/L] prior to therapy, the patient is inevaluable for toxicity in platelets

baseline and subsequent Hb determinations must be performed before any given infusion

§ if ANC was < 1,000 prior to therapy, the patient is inevaluable for toxicity in ANC.

Platelets:

If at any level of decrease the platelet count is < 20,000 mm^3, this is considered grade 4 toxicity, unless a severe or life-threatening decrease in the initial platelet count (e.g. < 20,000 mm^3) was present before treatment in which case the patient is inevaluable for toxicity referable to platelet counts.

Hemoglobin:

Baseline and subsequent Hb determinations must be performed before any given infusions.

Fludarabine has been reported to exacerbate or precipitate autoimmune hemolytic anemia and patients should be monitored carefully for this condition. If a rapid decrease of hemoglobin occurs during therapy, the possibility of fludarabine- or autoantibody-induced hemolysis should be considered and appropriate diagnostic tests (LDH, bilirubin, α 2-haptoglobin, reticulocytes, Coombs-test) be performed. In particular, a Coomb's test should be performed for:

- Any grade 3 or 4 anemia (Hb < 8 g/dL)
- A Hb < 10 g/dL sustained for > 2 weeks
- A drop in Hb of 3 g/dL in one week (while pts are receiving weekly blood tests)
- Any patient requiring a blood transfusion

If, in the judgment of the treating physician, there is evidence of hemolytic anemia secondary to fludarabine, study treatment should be promptly withdrawn. Full details of the hemolytic anemia should be recorded on the adverse event pages of the CRF.

Neutrophils:

Absolute neutrophil count (ANC) is used to assess the toxicity of the myeloid lineage. Other decreases in the white blood cell count, or in circulating lymphocytes, are not to be considered, since a decrease in the white blood cell count is a desired therapeutic end point. If the ANC was < 1,000/ mm^3 [<1 $x10^9$/L] prior to therapy, the patient is inevaluable for toxicity referable to the ANC.

Any Adverse Event occurring by the time of study completion (within four weeks of last drug intake) must be recorded on the Adverse Event CRF page.

8.1.5 Drug-Adverse event relationship

The causality relationship of study drug to the adverse event will be assessed by the investigator as either:

Yes or No

If there is a reasonable suspected causal relationship to the study medication, i.e. there are facts (evidence) or arguments to suggest a causal relationship, drug-event relationship should be assessed as **Yes**.

The following criteria should be considered in order to assess the relationship as **Yes:**

- Reasonable temporal association with drug administration
- It may or may not have been produced by the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject.
- Known response pattern to suspected drug
- Disappears or decreases on cessation or reduction in dose
- Reappears on rechallenge

The following criteria should be considered in order to assess the relationship as **No**:

- It does not follow a reasonable temporal sequence from administration of the drug.
- It may readily have been produced by the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject.
- It does not follow a known pattern of response to the suspected drug.
- It does not reappear or worsen when the drug is readministered.

8.2 Serious adverse events

A serious adverse event is any experience that suggests a significant hazard, contraindication, side effect or precaution. It is any Adverse Event that at any dose fulfils at least one of the following criteria:

- is fatal; (results in **death**; NOTE: death is an outcome, not an event)
- is Life-Threatening (NOTE: the term "Life-Threatening" refers to an event in which the subject was at immediate risk of death at the time of the event; it does not refer to an event which could hypothetically have caused a death had it been more severe).
- required in-patient hospitalization or prolongation of existing hospitalization;
- results in persistent or significant disability/incapacity;
- is a congenital anomaly/birth defect;
- is medically significant or requires intervention to prevent one or other of the outcomes listed above

****The term sudden death should be used only when the cause is of a cardiac origin as per standard definition. The terms death and sudden death are clearly distinct and must not be used interchangeably**.

The study will comply with all local regulatory requirements and adhere to the full requirements of the ICH Guideline for Clinical Safety Data Management, Definitions and Standards for Expedited Reporting, Topic E2

Other safety findings may require expedited reporting depending on local legislation, e.g. a major safety finding from a newly completed animal study (such as carcinogenicity).

Progression of underlying malignancy is not reported as an adverse event if it is clearly consistent with the suspected progression of the underlying cancer as defined by RECIST criteria, or other criteria as determined by protocol. Hospitalization due solely to the progression of underlying malignancy should NOT be reported as a serious adverse event. Clinical symptoms of progression may be reported as adverse events if the symptom cannot be determined as exclusively due to the progression of the underlying malignancy, or does not fit the expected pattern of progression for the disease under study.

Symptomatic deterioration may occur in some subjects. In this situation, progression is evident in the subject's clinical symptoms, but is not supported by the tumor measurements. Or, the disease progression is so evident that the investigator may elect not to perform further disease assessments. In such cases, the determination of clinical progression is based on symptomatic deterioration. These determinations should be a rare exception as every effort should be made to document the objective progression of underlying malignancy.

If there is any uncertainty about an adverse event being due only to the disease under study, it should be reported as an AE or SAE.

8.2.1 Reporting of Serious Adverse Events (immediately reportable)

Any clinical adverse event or abnormal laboratory test value that is *serious* and which occurs during the course of the study (as defined in section 8.2 above), occurring from the enrollment visit (start of study screening procedures), including long term follow-up (LTFU) must be reported to Roche *within one* working day of the investigator becoming aware of the event (expedited reporting). The investigator must complete the *SAE Reporting Form* [redacted] and forward it to the SAE Responsible.

After signature on informed consent, but prior to initiation of study medications, only SAEs caused by a protocol-mandated intervention will be collected (e.g., SAEs related to invasive procedures such as biopsies, medication washout, or no treatment run-in). After first study medication, all SAEs must be reported.

Related Serious Adverse Events ***MUST*** be collected and reported regardless of the time elapsed from the last study drug administration, even if the study has been closed. Suspected Unexpected Serious Adverse Reactions (SUSARs) are reported to investigators at each site and associated IRB/IEC when the following conditions occur:

- The event must be a SAE.
- There must be a certain degree of probability that the event is an adverse reaction from the administered drug.
- The adverse reaction must be unexpected, that is to say, not foreseen in the SPC text (Summary of Product Characteristics (for an authorized medicinal product) or the Investigator's Brochure (for an unauthorized medicinal product).

Unrelated Serious Adverse Events must be collected and reported during the study and for up to 30 days after the last dose of study medication.

This study adheres to the definition and reporting requirements of **ICH Guideline for Clinical Safety Data Management, Definitions and Standards for Expedited Reporting, Topic E2.**

8.2.2 Treatment and Follow-up of AEs

During the study period and up to 30 days after completion or discontinuation of the study, continue to follow up AEs as follows:

Related AEs: Follow until one of the following occurs:

- Resolved or improved to baseline
- Relationship is reassessed as unrelated
- Death
- Start of new anti-cancer regimen
- Investigator confirms that no further improvement can be expected
- Clinical or safety data will no longer be collected, or final database closure

Unrelated severe or life threatening AEs: Follow until one of the following occurs:

- Resolved or improved to baseline
- Severity improved to Grade 2
- Death
- Start of new anti-cancer regimen
- Investigator confirms that no further improvement can be expected
- Clinical or safety data will no longer be collected, or final database closure

Unrelated Grade 1 or Grade 2 AEs: Follow up to 30 days after completion or discontinuation of the study.

The final outcome of each adverse event must be recorded on the CRF

8.2.3 Laboratory evaluations

Laboratory test results will be recorded on the laboratory results form of the CRF, or appear on electronically produced laboratory reports submitted directly from the central laboratory, if applicable.

Any laboratory result abnormality fulfilling the criteria for a serious adverse event (SAE) should be reported as such, in addition to being recorded as an AE in the CRF.

Any treatment-emergent abnormal laboratory result which is clinically significant, i.e., meeting one or more of the following conditions, should be recorded as a single diagnosis on the adverse event page in the CRF:

- Accompanied by clinical symptoms
- Leading to a change in study medication (e.g. dose modification, interruption or permanent discontinuation)
- Requiring a change in concomitant therapy (e.g. addition of, interruption of, discontinuation of, or any other change in a concomitant medication, therapy or treatment)

No special laboratory or tests are required to be performed for the purpose of the study. Measurement of Complete Blood Counts, chemistry and immunological tests such as: serum Ig level, serum immunoellectrophoresis, DAT, peripheral and bone marrow FACS and hepatitis B and C serology are in fact regarded as current clinical practice for CLL patients, and should therefore be collected according to the Visit Schedules (see Figure 1). In case of suspected hemolysis - DAT, reticulocyte count, serum haptoglobin, bilirubin and LDH are recommended.

The institution will perform laboratory analyses according to the Visit Schedules (see Figure 1). The sponsor must be provided with a copy of the laboratory's certification, and a tabulation of the normal ranges for each parameter required. Additionally, if at any time a patient has laboratory parameters obtained from a different outside laboratory, the sponsor must be provided with a copy of the certification and a tabulation of the normal ranges for that laboratory.

At any time during the study, abnormal laboratory parameters which are clinically relevant (e.g. require dose modification and/or interruption of study drug, lead to clinical symptoms or signs or require therapeutic intervention), whether specifically requested in the protocol or not, must be recorded on the

appropriate Comment CRF page in addition to the appropriate laboratory CRF page. When abnormal laboratory values or test results constitute an adverse event (i.e., induces clinical signs/symptoms or requires therapy) they must be recorded on the Adverse Events CRF.

When, in the opinion of the investigator, other clinical laboratory evaluations may be relevant for assessing the patient's status, they will be completed and entered into the database as appropriate.

8.3 Special tests

PET-CT will be performed in case of suspected Richter transformation.

Lymph node biopsy is recommended for definitive diagnosis of Richter transformation and for any rapidly progressive disease or unusual phenomena which may be suspicious of alteration in the normal behavior of the disease or suspicious for another disease or a different type of lymphoma.

8.4 Physical examinations/vital signs

A physical examination including vital signs will be performed according to the Visit Schedules (see Figure 1). Information about the physical examination and vital signs must be present in the source documentation at the study site. Significant findings present prior to the start of study drug must be included in the Relevant Medical History/Current Medical Conditions CRF. Significant findings made after the start of study drug which meet the definition of an adverse event must be recorded on the Adverse Event Case Report Form. There are no case report forms to capture routine normal findings from physical examinations and vital signs assessments.

8.5 Laboratory examinations

8.5.1 Hematology

Hematological evaluations (CBC) are regarded as clinical practice for CLL patients and should therefore be collected according to the Visit Schedules (see Figure 1).

8.5.2 Blood chemistry

The following blood chemistry results are regarded as clinical practice for CLL patients and should therefore be collected according to the Visit Schedules (see Figure 1).

Serum creatinine, ALT, AST, alkaline Phosphatase, LDH, bilirubin. globulin, serum immunoellectrophoresis and beta-2 microglobulin.

8.6 Physical examinations/vital signs

A physical examination including vital signs will be performed according to the Visit Schedules (see Figure 1). Information about the physical examination and vital signs must be present in the source documentation at the study site. Significant findings present prior to the start of study drug must be included in the Relevant Medical History/Current Medical Conditions CRF. Significant findings made after the start of study drug which meet the definition of an adverse event must be recorded on the Adverse Event Case Report Form. There are no Case Report Forms to capture routine normal findings from physical examinations and vital signs assessments.

8.6.1 Warning and Precautions

No evidence available at the time of the approval of this study protocol indicated that special warnings or precautions were appropriate regarding Rituximab, other than those noted in the Investigators' Brochure.

Safety information regarding Fludarabine and Cyclophosphamide can be found in the respective local MoH approved product label.

9. Statistics

Subjects' age, weight, height and other continuous demographic and baseline variables will be summarized using descriptive statistics (mean, standard deviation, minimum and maximum), while performance status, gender, and other categorical variables will be summarized with frequency tabulations.

9.1 Sample size

This is a single arm phase II study. The number of subjects planed to be enrolled into the study is 40. Such a low number of patients as in most phase II studies) constitute an underpowered study for revealing any statistical significance, but is indeed clinically meaningful. So, the sample size chosen for this study was based on clinical considerations.

Those patients achieving PR or CR by the study medication will be regarded as responders. All the others (stable disease, progressive disease or death from any cause) will be regarded as non responders. Determining the ratio of responders versus non responders, even if not statistically significant, will still be clinically meaningful.

9.1.1 Data analysis

Categorical data will be presented as frequencies and percentages. For continuous data, mean, standard deviation, median, 25^{th} and 75^{th} percentiles, minimum, and maximum will be presented.

9.2 Efficacy and Safety evaluation

9.2.1 Efficacy analysis:

All analyses will be according to the intention to treat principle.

9.2.2 Response rate

The primary efficacy parameter to be assessed will be response rate determined by objective overall response rate.

The primary endpoint is a binary variable where each patient is classified as a responder or not responder.

95% Confidence Interval (CI) will be calculated to the proportions of responders.

Response rate include CR and PR; all other situations (eg. stable disease, non response, progressive disease or death from any cause) will be rated as treatment failure and therefore as non responders.

Response rate will be assessed according the updated (2008) NCI-WG guidelines defined in appendix 1:

- Peripheral blood FACS for lymphocytes clonality.
- Physical examination for lymph node examination and measurements, and documentation of the presence of hepatomegaly and splenomegaly.
- Blood count for ANC, platelets and hemoglobin levels.
- Bone marrow aspiration and biopsy for bone marrow involvement by CLL cells using FACS immunophenotyping or immunohistochemistry.
- Patient interview for the presence of constitutional symptoms throughout the study.

All above mentioned efficacy (response) parameters will be performed at screening and at least 3 months after completing study drug administration for response documentation.

All known disease (measurable, and non measurable) parameters must be accounted for when assessing objective response rate.

Progression free survival is defined as the interval between the first treatment day to the first sign of disease progression.

Progressive disease (PD) will be assessed according the updated (2008) NCI-WG guidelines defined in appendix I. If needed, BMB will be performed for suspected progression at the investigator's discretion. Progression will be captured on the response form CRF.

A Kaplan-Meier method will be used to estimate median PFS and 90% confidence interval.

QOL questionnaires will be completed by study subjects at: screening visit and visits number: 11, 14, and end of study. Questionnaires will be captured on the QOL forms CRF.

Summary statistics (standard deviation, median, minimum and maximum) will be provided for the relevant variables**.** The Paired T-test or Signed Rand Test for paired observations (as is appropriate) will be applied for analysing the changes in the components and total score of the FACIT QOL assessment.

The data will be analyzed using the SAS ® version 9.1.3 (SAS Institute, Cary North Carolina).

9.3 Safety Analysis

Data from all subjects who receive any study drug will be included in the safety analyses. The severity of the toxicities will be graded according to the NCI CTCAE v 3.0
http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcaev3.pdf
or v 2.0 for cytopenia, whenever possible.

All adverse events will be coded according to coding dictionaries (MedDRA version 12.1) and presented in tables by system organ class and preferred term.

In the by-subject analysis, a subject experiencing the same event more than once will be counted only once. Adverse events will be defined and summarized according to the worst NCI CTC grade. Adverse events leading to death or to discontinuation of treatment, events classified as NCI CTCAE Grade 3 or Grade 4, study-drug-related events, and serious adverse events will be summarized separately. Laboratory data will be graded according to NCI CTCAE severity grade.

9.4 **Interim Analysis**

Interim Analysis will be conducted on all data received at database until 30 May, 2014. The following parameters will be examined in the Interim Analysis:

Primary endpoint:

- **Overall Response rate**

Overall response rate will include CR and PR; all other situations (e.g. stable disease, non-response, progressive disease or death from any cause) will be rated as treatment failure and therefore as non-responders.

Secondary endpoints:

- **Progression free survival (PFS) defined as the time elapsed from first day of study drug administration to disease progression as defined in the protocol (Appendix I)**
- **Overall survival**
- **Safety of the study drugs especially in relation to: neutropenic fever, Infection rate and number of hospitalization days, thrombocytopenia ≥grade 3, neutropenia ≥grade 3**
- **Rates of treatment-related adverse effects**
- **Quality of life – by FACIT (Appendix VI)**

10. Quality Control and Quality Assurance

Data from the CRFs are entered into the study database by Contract Research Organization staff following their own internal standard operating procedures that have been reviewed and approved by the sponsor.

Subsequently, the entered data are systematically checked by Data Management staff, using error messages printed from validation programs and database listings. Obvious errors are corrected by Data Management personnel. Other errors or omissions are entered on Data Query Forms, which are returned to the investigational site for resolution. The signed original and resolved Data Query Forms are kept with the CRFs at the investigator site, and a copy is sent to the sponsor so the resolutions can be entered into the database. Quality control audits of all key safety and efficacy data in the database are made prior to locking the database.

11. Direct Access to Source Data/Documents

11.1 Study monitoring

The monitoring visits will enable the sponsor or the monitor acting on his behalf, to assess the study's progress status, to verify data accuracy and degree of completeness of the CRFs, to ensure the protocol as well as local regulations are being followed, that the investigator is fulfilling his obligations and to correct errors in the CRFs compared to the source documents. The investigators' will authorize the monitor, at a mutually convenient time during the study to periodically review all of the CRFs and the related parts of administrative, medical and laboratory files of each participant in the study. The CRFs must be filled out before the monitoring visits.

11.2 Audits

During the study, people belonging to the sponsor quality assurance group may visit the investigator site in order to audit the study. The purpose of this visit is to check that the study is carried out according to Good Clinical Practices. Before conducting an audit, the monitor will contact the investigator in order to agree upon a mutually suitable date. The investigator and his team are required to cooperate with the auditors and to grant them access to the patients' medical files and to the study documents (CRFs and investigator's binders).

11.3 IRB and regulatory inspections

A regulatory authority may also wish to conduct an inspection (during the study or even after its completion). If an inspection is requested by a regulatory authority, the investigator must inform the sponsor immediately that this request has been made.

12. Ethics

12.1 Institutional Review Board

Before implementing this study, the protocol, the proposed informed consent form and other information to subjects, must be reviewed by a properly constituted Institutional Review Board (IRB). A signed and dated statement that the protocol and informed consent have been approved by the IRB must be given to the sponsor before study initiation. The name and occupation of the chairman and the members of the IRB must be supplied to the sponsor. Any amendments to the protocol, other than administrative ones, must be approved by this committee.

12.2 Informed consent

The investigator must explain to each subject (or legally authorized representative) the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits involved and any discomfort it may entail. Each subject must be informed that participation in the study is voluntary and that he/she may withdraw from the study at any time and that withdrawal of consent will not affect his/her subsequent medical treatment or relationship with the treating physician.

This informed consent should be given by means of a standard written statement, written in non-technical language. The subject should read and consider the statement before signing and dating it, and should be given a copy of the signed document. If the subject cannot read or sign the documents, oral presentation may be made or signature given by the subject's legally appointed representative, if witnessed by a person not involved in the study, mentioning that the patient could not read or sign the documents. No patient can enter the study before his/her informed consent has been obtained.

The informed consent form is considered to be part of the protocol, and must be submitted by the investigator with it for IRB approval.

12.3 Declaration of Helsinki

The investigator must conduct the trial in accordance with the principles of the Declaration of Helsinki which can be accessed via the website of the World Medical Association at http://www.wma.net/e/policy/17-c_e.html.

13. Data Handling and Record Keeping

The information obtained from the execution of the protocol will be listed on the CRFs provided by the sponsor.
The CRFs must be filled out on a timely basis, signed and dated by the investigator or a co-investigator named by the former and clinically responsible for the patient during the study.

The completed CRFs will be collected by the sponsor. The investigator must remit one CRF for every participant in his center for a time period of 15 years.

14. Financing and Insurance

Financing and insurance will be addressed in the future in a separate agreement.

15.Publication Policy

Any formal presentation or publication of data from this trial will be considered as a joint publication by the investigator(s) and appropriate sponsor's personnel. Authorship will be determined by mutual agreement taking in consideration that the group of PI's will prepare the manuscript and write it initially and decide which member of steering committee and which other active investigators who provided cases will be involved.

It is a multicenter study, and it is mandatory that the first publication is based on data from all centers, analyzed as stipulated in the protocol, by the sponsor statisticians, and not by the investigators. Investigators participating in multicenter studies agree not to present data gathered from one center or a small group of centers before the full publication, unless formally agreed to by all other investigators and the PI's and steering committee.

The investigator may be required to sign the clinical study report, if it is to be used in a registration submission to the health authorities of some countries. For multicenter studies only the coordinating (principle) investigator nominated by the sponsor and the steering committee at the start of the trial would provide any needed signature.

References

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16. Supplements

Appendix I Response definitions

CR (complete remission) = requires **all** of the following criteria as assessed at least 3 months after completion of therapy:

Absence of clonal lymphocytes in peripheral blood.

Absence of significant lymphadenopathy. (lymph nodes > 1.5 cm in diameter) by physical examination. CT is desirable for not palpable lymph nodes.

No hepatomegaly or splenomegaly by physical examination. (Abdominal, pelvix and thorax CT for subjects found to be abnormal before therapy or if physical examination is inconclusive.

Absence of constitutional symptoms.

Blood counts above the following values: ANC> 1.5 x 10^9/L . Platelets > 100 x10^9/L . Hemoglobin > 11.0 g/dL; untransfused).

Bone marrow aspirate and biopsy should be performed at least 3 months after the last treatment and if the above 5 clinical and laboratory results demonstrate that CR has been achieved.
Bone marrow should be analyzed by FACS/or immunohistochemistry to demonstrate marrow free of B-CLL cells.
In some cases, it is necessary to postpone BMB until after all the other criteria to define a CR have been satisfied. However, this time interval should not exceed 6 months after the last treatment.

Parameter	CR	PR	PD	SD
Group A				
Lymphadenopathy*	None more than 1.5 cm	Decrease ≥ 50%	Increase ≥ 50%	Change of −49% to +49%
Liver and/or spleen size	Normal size	Decrease ≥ 50%	Increase ≥ 50%	Change of −49% to +49%
Constitutional symptoms	None	Any	Any	Any
Polymorphonuclear leukocytes	>1500/μL	>1500/μL or > 50% improvement over baseline	Any	Any
Circulating clonal B lymphocytes	None	Decrease ≥ 50% from baseline	Increase ≥ 50% over baseline	Change of −49% to +49%
Group B				
Platelet count	>100 000/μL	>100 000/μL or increase ≥ 50% over baseline	Decrease of ≥ 50% from baseline secondary to CLL	Change of −49 to +49%
Hemoglobin	>11.0 g/dL (untransfused and without erythropoietin)	>11 g/dL or increase ≥ 50% over baseline	Decrease of >2 g/dL from baseline secondary to CLL	Increase ≤11.0 g/dL or <50' over baseline, or decrease <2 g/dL
Marrow	Normocellular, <30% lymphocytes, no B-lymphoid nodules; hypocellular marrow defines CRi	≥ 30% lymphocytes, or B-lymphoid nodules, or not done	Increase of lymphocytes to more than 30% from normal	No change in marrow infiltrat

Values in SI units for leukocytes are 1.5 × 10^9/L; for platelets 100 × 10^9/L; and for hemoglobin 110 g/L and 20 g/L.

CR indicates complete remission (all of the criteria have to be met); PR, partial remission (at least one of the criteria of group A and one of the criteria of group B have to be met); PD, progressive disease (at least one of the criteria of group A or one of the criteria of group B have to be met); SD, stable disease (all of the above criteria have to be met).

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

Relapse = a patient who has previously achieved CR or PR but after a period of 6 or more months, demonstrates evidence of disease progression

Refractory disease = defined as treatment failure or disease progression within 6 months from the last antileukemic therapy.

Progression = see above table under PD.

Progression free survival (PFS) = defined as the time from start of treatment to progression.

Time to next treatment (TNT) = defined as the time from start of treatment to start of second-line treatment

Appendix II ECOG Performance status

ECOG	Grade
Fully active, able to carry on all pre-disease performance without restriction	0
Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work	1
Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours	2
Capable of only limited self-care, confined to bed or chair more than 50% of waking hours	3
Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair	4
Dead	5

Appendix III calculated creatinine clearance

$$\text{GFR (mL/min)} = \frac{(140\text{-age}) \text{ X weight (Kg)}}{\text{Serum cr (mg/dL) X } 72} \text{ X } 0.85 \text{ (for women)}$$

Appendix IV Active disease

At least one of the following criteria should be met:

1. Evidence of progressive marrow failure as manifested by the development of, or worsening of, anemia and/or thrombocytopenia
2. Massive (ie, > 6 cm below the left costal margin) or progressive or symptomatic splenomegaly.
3. Massive nodes (ie, > 10 cm in longest diameter) or progressive or symptomatic lymphadenopathy.
4. Progressive lymphocytosis with an increase of more than 50% over a 2-month period or lymphocyte doubling time of less than 6 months; patients with initial blood lymphocyte counts of less than 30X109/L (30 000/µL) may require a longer observation period to determine the lymphocyte doubling time. In addition, factors contributing to lymphocytosis or lymphadenopathy other than CLL (eg, infections) should be excluded.
5. Autoimmune anemia and/or thrombocytopenia poorly responsive to corticosteroides or other standard therapy.
6. A minimum of any of the following disease-related symptoms must be present: a- Unintentional weight loss more than or equal to 10% within the previous 6 months. b- Significant fatigue (ie, ECOG PS 2 or worse; cannot work or unable to perform usual activities). c- Fevers of greater than 38.00C for 2 or more weeks without other evidence of infection. d- Night sweats for more than 1 month without evidence of infection.

Appendix V Binet staging system

The areas of involvement considered for staging are as follows: 1- Head and neck, including the Waldeyer ring. 2- Axillae 3- Groins 4- Palpable spleen 5- Palpable liver.

Stage A. Hb 10 g/dL or more and platelets 100X 10^9/L or more and up to 2 of the above areas involved.

Stage B. Hb 10 g/dL or more and platelets 100X 10^9/L or more and organomegaly greater than that defined for stage A (3 or more areas).

Stage C. All patients who have Hb less than 10 g/dL and/or a platelets count less than 100X 10^9/L, irrespective of organomegaly.

Appendix VI FACIT-QOL

FACIT − Fatigue Scale (Version 4)

Below is a list of statements that other people with your illness have said are important. **By circling one (1) number per line, please indicate how true each statement has been for you during the past 7 days.**

		Not At all	A little bit	Some what	Quite a bit	Very much
HI7	I feel fatigued	0	1	2	3	4
HI12	I feel weak all over	0	1	2	3	4
An1	I feel listless ("washed out") 0	1	2	3	4	
An2	I feel tired	0	1	2	3	4
An3	I have trouble starting things because I am tired 0	1	2	3	4	
An4	I have trouble finishing things because I am tired 0	1	2	3	4	
An5	I have energy	0	1	2	3	4
An7	I am able to do my usual activities 0	1	2	3	4	
An8	I need to sleep during the day 0	1	2	3	4	
An12	I am too tired to eat	0	1	2	3	4
An14	I need help doing my usual activities	0	1	2	3	4
An15	I am frustrated by being too tired to do the things I want to do	0	1	2	3	4
An16	I have to limit my social activity because I am tired 0	1	2	3	4	