A randomized phase III study in previously untreated patients with biological high-risk CLL: Fludarabine + cyclophosphamide (FC) versus FC + low-dose alemtuzumab

PROTOCOL

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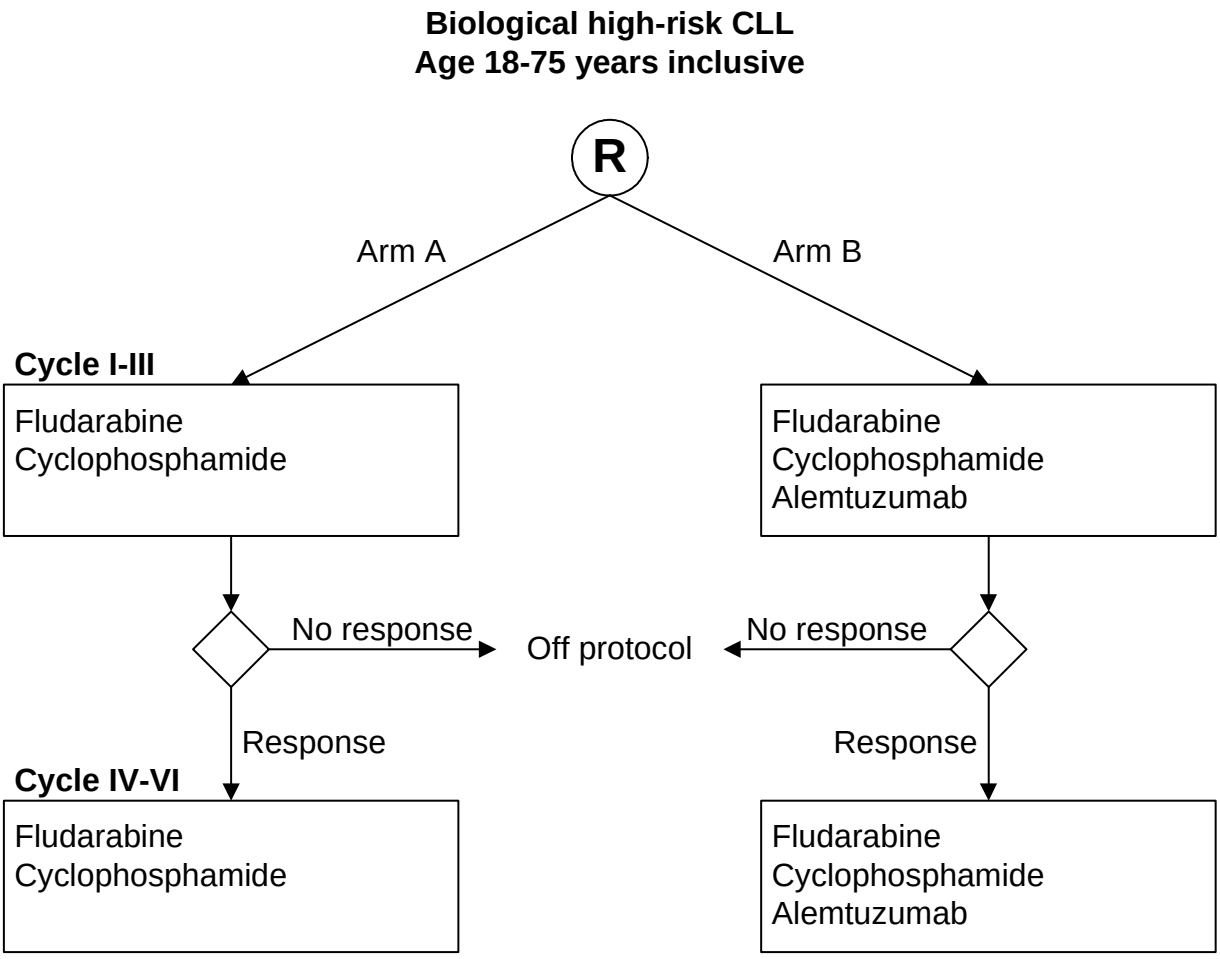
1 Scheme of the study



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3 Synopsis

Study phase	Phase III
Study objectives	Determination of the efficacy and safety of oral fludarabine and cyclophosphamide plus concomitant s.c. alemtuzumab compared to fludarabine and cyclophosphamide alone in terms of progression free survival, event free survival, clinical, flow cytometric and molecular response rates, overall survival and disease free survival.
Patient population	Patients with biological high-risk CLL in symptomatic stage A or B or stage C, irrespective of duration of disease, age 18-75 years inclusive.
Study design	Prospective, multicenter, randomized
Duration of treatment	Expected duration of treatment is 24 weeks.
Number of patients	300 patients registered and randomized
Adverse events	Adverse events will be documented if observed, mentioned during open questioning, or when spontaneously reported.
Planned start and end of recruitment	Start of recruitment: III 2005 End of recruitment: III 2008

4 Investigators and study administrative structure

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4.1 Pathology review

The diagnosis of CLL is established by the hematologist/pathologist and flow cytometry laboratory of each participating center as defined in the WHO classification (Müller-Hermelink et al. 2001). A central review of the diagnosis is performed for each case by one of the study pathologists to confirm the diagnosis of CLL according to the WHO lymphoma classification.

Once a patient is randomized, the local hematologist/pathologist will be asked to send a peripheral blood smear as well as the immunophenotyping results to the review pathologist for his/her respective country. A copy of the results of the review will be sent to the HOVON Data Center. Only in case of doubt will bone marrow specimens be submitted to central review.

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5 Introduction

5.1 Biological risk stratification in CLL

CLL is a B-cell neoplasia, which has recently been dichotomized into two prognostic groups: patients with ≥ 98% homology to germ-line VH gene sequence, i.e. ≤ 2% somatic hypermutations of their VH genes ("unmutated") and patients with < 98% homology to germ-line VH gene sequence, i.e. > 2% somatic hypermutations of their VH genes ("mutated") (Damle et al. 1999, Hamblin et al. 1999). Prognostically, these subtypes differ considerably, as unmutated patients have a median life expectancy of approximately 8 years, while mutated patients have a median life expectancy of approximately 15 years (Damle et al. 1999, Hamblin et al. 1999, Oscier et al. 2002, Kröber et al. 2002), or up to 25 years if CLL unrelated deaths are censored (Hamblin 2003).

Unmutated status may to a certain extent be predicted by the expression of the ZAP-70-gene, which can be measured by gene expression studies, flow cytometry or immunocytochemistry, (Wiestner et al. 2003, Crespo et al. 2003, Rassenti et al. 2004), or by the expression of the surface marker CD38 (Hamblin et al., Chiorazzi et al.). However these "surrogate markers" have not yet been sufficiently validated (ASH2003 abstracts 105-109). Mutated cases with 3H21 gene usage have just as poor a prognosis as unmutated cases irrespective of IgVH gene usage(Tobin et al. 2002, Thorselius et al 2006, Kröber et al 2006).

Further prognostic information – independent of mutational status – is given by cytogenetic abnormalities as measured by FISH technique (Döhner et al. 2000). Patients with 11q deletions and 17p deletions were found to have a significantly shorter life expectancy than patients with other cytogenetic abnormalities or those with normal FISH findings. The time to treatment for patients with 17p deletions, 11q deletions and trisomy 12 was 10, 12 and 36 months respectively, as compared to 48 and 96 months respectively for patients with normal FISH and 13q deletions. This biological risk stratification is independent of clinical stage and mutational status (Kröber et al. 2002, Oscier et al. 2000). A combination of these parameters as well as clinical stage and lymphocyte doubling time may be used to select patients for risk-adapted therapy. Accordingly, high-risk CLL biology may be defined as CLL with unmutated Ig genes and/or FISH-documented 17p deletions or 11q deletions, whereas trisomy 12 may define an intermediary risk group. Biological risk stratification may be the tool for choice of therapy in CLL, and the NCI/IWCLL criteria may still be the tool for the timing of the chosen therapy.

5.2 Treatment

Clearly, different treatment approaches are warranted for these two CLL subtypes. In view of this newly established dichotomy, however, the treatment data generated by clinical studies performed over the last decade have now become more or less outdated. The optimal first line treatment of unmutated CLL must

now be based on prospective clinical trials. Preliminary data (British MRC, unpublished) suggest that unmutated patients will soon develop resistance to chlorambucil. In contrast, patients with mutated Ig genes and favorable cytogenetics will either never require any treatment, or may be managed by relatively simple monotherapy, e.g. chlorambucil. For high-risk CLL, other active drugs than chlorambucil are available either as single drug treatment or in combination.

5.2.1 Treatment efficacy

Fludarabine monotherapy as first line treatment in unselected (i.e. without specified mutational status) CLL yields overall response rates of 70-85% and CR rates ranging from 5.8% (Flinn et al. 2004) and 8% (Eichhorst et al. 2003) to as high as 30-40% (Rai et al. 2000, Leporrier et al. 2001).

Fludarabine + cyclophosphamide (FC) as primary treatment of unselected CLL leads to response rates of 70% to nearly 100 % with CR rates ranging from about 20 % (Eichhorst et al. 2003, Flinn et al. 2004) to 40-50%. This holds true for both i.v. administration (Keating et al. 2002: F 75 mg/m^2 + C 750 mg/m^2, Flinn et al. 2004: F 100 mg/m^2 + C 600 mg/m^2) and oral administration (Cazin et al. 2003): F 150 mg/m^2 + C 1000 mg/m^2). I.v. FC resulted in a median event free survival of 28-41 months as compared to 18-22 months after F alone (Eichhorst et al. 2003, Flinn et al. 2004), whereas oral FC led to median progression free survival of almost 3 years and a 3-year overall survival of 84% (Cazin et al. 2003). Based on these results the German CLL Study Group (GCLLSG) (Eichhorst et al. 2003) has defined FC as its standard arm in future trials in younger (<66 years) CLL patients. Following oral administration 60% of fludarabine is absorbed, compared to almost 100% of cyclophosphamide. In consequence, instead of the doses chosen by the French CLL collaborative group (Cazin et al. 2003), we have chosen an oral regimen that will theoretically deliver the same doses as the classical FC regimen given intravenously. Fludarabine 40 mg/m^2 daily for 3 days and cyclophosphamide 250 mg/m^2 daily for 3 days.

Of several antibodies, two with documented effect in CLL are presently available on a routine basis: alemtuzumab and rituximab. Both are known to be active in CLL. Both antibodies act synergistically with fludarabine, and are able to induce clinical and molecular CR in a number of patients.

The combination of fludarabine and rituximab has been studied both as concurrent and sequential treatment and now show similar results (Byrd et al. 2004, Del Poeta et al. 2003, Lammana et al. 2003). Comparing two CALGB studies, Byrd et al. (2005) found better results of fludarabine + rituximab than of fludarabine alone, and also suggested longer overall survival. Interestingly, subgroup analysis of the F + rituximab patients of this study according to biological risk stratification, showed identical CR rates of 43% in high-risk and standard-risk patients, whereas the progression free survival was significantly shorter in high-risk patients: 32 vs. 45 months (Byrd et al 2004). Concurrent fludarabine, cyclophosphamide and rituximab is presently being investigated as first line treatment in a non-randomized study (Wierda et al. 2002, yielding a response rate of 95% and a CR rate of 67%, molecular

remission in 57% of cases tested), a randomized first line study (German CLL5 study) and a randomized second line study (Roche BO 17072).

5.2.2 Alemtuzumab

The anti CD52 monoclonal antibody alemtuzumab (Campath-1H/MabCampath®) is a genetically engineered humanized IgG1 kappa monoclonal antibody specific for 21-28 kD lymphocyte cell surface glycoprotein (CD52) by the insertion of rat anti-CD52 CDR into an human IgG1 molecule. CD52 is expressed primarily on the surface membrane of normal and malignant B- and T-cell lymphocytes, as well as monocytes, thymocytes and macrophages. The mechanisms of action are complement-mediated cytolysis (CMC) and antibody-dependent cell mediated cytotoxicity (ADCC) of CD52 positive cells and a non-caspase (lipid-raft associated) apoptosis (Mone et al. 2004, Stanglmaier et al. 2004). The antigen is found on a small proportion (<5%) of granulocytes, but not on erythrocytes or platelets (MabCampath® product monograph 2001).

Following i.v. infusion the maximum serum concentration and AUC show dose dependence and a median half-life of approximately 23–30 hours. Administered as 30 mg i.v. 3 times weekly to CLL patients, the peak and trough levels approached steady state by approximately week 6. Patients with peripheral lymphocyte counts > 30 x 10^9/l had significantly lower peak and trough levels than patients with lower lymphocyte counts. The pharmacokinetics following s.c. administration are similar to those following i.v. administration of equal doses (Hale et al. 2004), but to secure a trough level of at least 1 µg/ml a higher cumulated dose was necessary. Therefore an initial loading dose is given in this study. However, at the time of the subsequent cycles of FC + alemtuzumab, FC is expected already to have reduced the tumor burden to such an extent that the lower doses alemtuzumab s.c. will yield sufficient alemtuzumab to bind to the remaining CLL cells. Subcutaneous administration of alemtuzumab yields bioavailability and results comparable to those obtained after i.v. administration with less systemic side effects (Lundin et al. 2002). Local side effects at the injection sites tended to decrease during treatment. Hale et al. suggested that s.c. administration of alemtuzumab, especially in previously untreated patients, might lead to the formation of antibodies. To get a good incidence of this problem, sequential sera taken prior to alemtuzumab administration in each cycle will be stored and later tested for anti-alemtuzumab antibodies for all patients in the alemtuzumab arm.

Given subcutaneously as first line treatment in standard doses, alemtuzumab as single agent yielded a CR rate of 19%, PR rate of 68% and a median time to treatment failure not reached after 18 months (Lundin et al. 2002). In fludarabine-resistant patients with p53 mutations, responses were obtained in 40% (Lozanski et al. 2004).

Fludarabine with concurrent standard-dose alemtuzumab-1H (FluCam) has been studied by Elter et al. (2004) in advanced, previously treated CLL, with a CR rate of 29% and RR of 85%. Sequential treatment

with fludarabine followed by standard-dose alemtuzumab (30 mg TIW) has been studied by the GCLLSG (Wendtner et al. 2004) as well as the CALGB (Rai et al. 2003). In the GCLLSG study, patients who responded to first line therapy with F or FC were randomized to either alemtuzumab 30 mg i.v. TIW for 12 weeks or observation. The theoretical cumulated alemtuzumab dose was 1093 mg, but the median cumulative dose actually given was 238 mg, range 213-1033 mg. Alemtuzumab led to significantly longer clinical and molecular progression free survival than observation (not reached vs. 24 months, not reached vs. 17.8 months respectively). This study was stopped, however, because of infections. In the CALGB study, 18 patients who had received F as first line treatment, after a two months rest period, went on to receive alemtuzumab 30 mg s.c. TIW for 6 weeks after usual build-up (theoretical cumulated dose 495 mg). The response rate was 66%, (22 % CR, 44 % PR). Fludarabine followed 8 weeks later by low-dose alemtuzumab s.c. (10 mg TIW for 6 weeks, theoretical cumulated dose 180 mg) in fludarabine responders (both first and second line) was shown by Montillo et al. (2003) to give CR rates of 88 % and RR of 100 %. The molecular response rate was 47%. In a study by the CLL Consortium (O'Brien et al. 2003), 58 CLL patients primarily treated with chemotherapy (not specified) went on to receive s.c. alemtuzumab treatment at two dose levels: 10 mg TIW x 4 weeks (cumulative dose 120 mg) and 30 mg TIW x 4 weeks (360 mg). A trend of higher response rate following the higher dose was observed and 38% achieved molecular remission. In relapsed CLL, CFAR (classical FC + classical alemtuzumab + rituximab 375 mg/m^2 day 2 in each 28-day cycle) produced 23% CR and 35% PR (Wierda et al. 2004).

5.3 Rationale of the study

In chronic lymphocytic leukemia (CLL) the recent identification of molecular markers of aggressive disease has made biological risk stratification both possible and necessary. Simultaneously, therapeutic options comprising purine analogs, alkylating agents and monoclonal antibodies have become available. These treatment regimens result in high clinical and molecular response rates. However, their optimal timing and impact on overall survival are at present unclear. Therefore it is now necessary to conduct randomized studies based on this biological risk stratification. A randomized phase III study in previously untreated high-risk CLL is proposed comparing oral fludarabine + cyclophosphamide with oral fludarabine + cyclophosphamide + low-dose s.c. alemtuzumab.

6 Study objectives

- To assess the effect of the addition of alemtuzumab s.c. to 6 courses of oral FC in terms of progression free survival as defined in paragraph 14
- To assess the effect of the addition of alemtuzumab s.c. to 6 courses of oral FC in terms of the secondary endpoints defined in paragraph 14
- To assess the safety of the addition of alemtuzumab s.c. to 6 courses of oral FC with respect to the incidence of severe opportunistic infections (CMV reactivation, CMV disease, Herpes simplex and

Herpes zoster infections, EBV reactivation, deep mycosis, mycobacterial infections, other infections) and infections due to neutropenia

7 Study design

This is an open label, randomized, multicenter, phase III study. Details of all treatments (dose and schedule) are given in section 9. All eligible patients (see section 8) will be randomized on entry between:

Arm A: 6 cycles of oral FC
Arm B: 6 cycles of oral FC combined with s.c. alemtuzumab

All patients will be evaluated for response and toxicity after 3 cycles. Patients who have not attained at least PR after 3 cycles will go off protocol treatment.

8 Study population

8.1 Eligibility for registration

All eligible patients have to be registered and randomized before start of treatment (see section 16). Patients have to meet all of the criteria mentioned below.

8.1.1 Inclusion criteria

- Biological high-risk CLL*
- Patients with symptomatic** stage A, symptomatic** stage B or stage C (see appendix B)
- Age 18-75 years inclusive
- Written informed consent

* Biological high risk is defined as: ≥ 98 % homology of IgV_H genes with germ-line sequences and/or mutated CLL with usage of V_H3-21 and/or FISH with 17p deletions and/or 11q deletions and/or trisomy 12.
** Symptomatic CLL is defined according to the NCI criteria for active disease (Cheson et al. 1996, Hallek et al 2008, see appendix A).

8.1.2 Exclusion criteria

- WHO performance status ≥ 3 (see appendix E), unless related to CLL
- Intolerance of exogenous protein administration

- Severe cardiac dysfunction (NYHA classification III-IV, see appendix F)
- Significant renal dysfunction (serum creatinine ≥ 150 μmol/l or creatinine clearance < 30 ml/min)
- Significant hepatic dysfunction (total bilirubin or transaminases > 2 times ULN), unless related to CLL
- Suspected or documented CNS involvement by CLL
- Known seropositivity of HIV, Hepatitis B and C.
- Active, uncontrolled infections
- Uncontrolled asthma or allergy requiring systemic steroid treatment
- Previously treated with chemotherapy, radiotherapy or immunotherapy for CLL
- History of active cancer during the past 5 years, except non-melanoma skin cancer or stage 0 cervical carcinoma
- Clinically significant auto-immune hemolytic anemia (AIHA)
- Female patients who are pregnant or nursing
- Male and female patients of reproductive potential who are not practicing effective means of contraception, these include oral contraceptives, intrauterine device, depot injection of gestagen, subdermal implantation, hormonal vaginal ring and transdermal depot plaster. These methods must be applied for the entire protocol treatment period, and for patients treated with alemtuzumab until at least 6 months after the end of alemtuzumab administration.

9 Treatments

9.1 Arm A: Fludarabine + cyclophosphamide

Patients randomized to arm A will receive fludarabine p.o. (total 120 mg/m^2 per cycle) and cyclophosphamide p.o. (total 750 mg/m^2 per cycle), repeated every 4 weeks for 6 cycles.

Agent	Dose/day	Route	Days
Fludarabine	40 mg/m^2	p.o.	all cycles: 1, 2, 3
Cyclophosphamide	250 mg/m^2	p.o.	all cycles: 1, 2, 3

9.2 Arm B: Fludarabine + cyclophosphamide + alemtuzumab

Patients randomized to arm B will receive fludarabine p.o. (total 120 mg/m^2 per cycle) and cyclophosphamide p.o. (total 750 mg/m^2 per cycle) combined with alemtuzumab, repeated every 4 weeks for 6 cycles. The loading dose of alemtuzumab is 30 mg/day on day –1, 0 and 1 of cycle 1. During cycle 2-6 30 mg/day alemtuzumab will be given day 1 only (total dose 240 mg in 6 cycles). Alemtuzumab may be given to patients in an outpatient clinic setting or following hospital admission in an inpatient setting.

Agent	Dose/day	Route	Days
Fludarabine	40 mg/m^2	p.o.	all cycles: 1, 2, 3
Cyclophosphamide	250 mg/m^2	p.o.	all cycles: 1, 2, 3
Alemtuzumab	30 mg	s.c.	cycle 1: -1, 0 , 1 cycle 2-6: 1

9.3 Dose modification for hematological toxicity

Dose modifications will not be made during the first cycle. During the next 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, not related to bone marrow infiltration, treatment should be delayed for up to two weeks and given with a 25% dose reduction of fludarabine and cyclophosphamide in the following cycles.
- If after two weeks, the grade ≥ 3 cytopenia, not related to bone marrow infiltration, still prevails, the patient should go off protocol treatment.
- If there is further grade ≥ 3 cytopenia in subsequent cycles despite the first 25% dose reduction treatment should again be delayed for up to two weeks and the dose of fludarabine and cyclophosphamide is further reduced to 50% of the full dose.
- If there is further grade ≥ 3 cytopenia in subsequent cycles despite 50% dose reduction the patient should go off protocol treatment.
- If there is any grade ≥ 3 neutropenia with infection during any cycle, G-CSF should be administered and G-CSF should be given in all subsequent cycles.

The dose of alemtuzumab is not reduced.

9.4 Dose modification for impaired renal function

Fludarabine is partly (40-60%) excreted by the kidneys. If the 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 ≥ 150 μmol/l or creatinine clearance < 30 ml/min) during treatment should go off protocol treatment.

9.5 Dose modification for other non-hematological toxicity

- If other grade ≥ 3 non-hematological, non-renal toxicity occurs during any cycle, treatment should be delayed until recovery to grade ≤ 2, for up to two weeks. All subsequent cycles should be given with a 25% dose reduction of fludarabine and cyclophosphamide.

- If after two weeks, the grade ≥ 3 non-hematological, non-renal toxicity still prevails, the patient should go off protocol treatment.
- 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 two weeks and the dose of fludarabine and cyclophosphamide is further reduced to 50% of the full dose.
- If there is further grade ≥ 3 non-hematological, non-renal toxicity in subsequent cycles despite 50% dose reduction the patient should go off protocol treatment.

9.6 Special management orders and concomitant medication

- All patients should receive pneumocystis jiroveci pneumonia (PJP) prophylaxis: sulphametoxazol with trimetoprim 400/80 mg daily or 800/160 mg three times a week throughout the study period until at least 6 months after the last treatment day. In case of intolerance to this drug, pentamidine inhalation 300 mg every month, dapsone 100 mg three times a week or any other documented PCP prevention is recommended (Fishman et al. 2001).
- All patients randomized to receive alemtuzumab should receive herpes prophylaxis with either aciclovir 400 mg x 3-4 or valaciclovir 500 mg x 2. For preemptive CMV treatment see appendix G.
- All blood products should be irradiated for one year after treatment to prevent transfusion-related GVHD.
- Patients who receive alemtuzumab should be pretreated 1 hour before the alemtuzumab injection with paracetamol 1g p.o. and antihistaminic according to local routine. Corticosteroids are allowed in patients who experience severe cutaneous reaction, in doses according to local practice, but continuous use of corticosteroids should be avoided, in order to minimize immunosuppression. Following the first 3 alemtuzumab injections (cycle 1) and the alemtuzumab injection of cycle 2, the patient will stay in the out patient clinic for at least 4 hours for observation. If no severe adverse reactions have been observed following the alemtuzumab injection of cycle 2, the post-alemtuzumab observation time may be reduced to 1 hour in the remaining 4 cycles.
- All patients should receive allopurinol 300 mg daily day –2 until day 7 of cycle 1. During the following cycles allopurinol may be given according to local practice. In case of allopurinol allergy, alternative drugs (probenecid) may be used. During cycle 1 all patients will be instructed to secure relevant fluid intake, and will be monitored for tumor lysis syndrome with daily blood tests day 1-5 and day 8.
- In addition to the specifications in 9.3, G-CSF will be used for neutropenia throughout treatment according to the ASCO criteria (35).
- When patients in the experimental arm develop fever, the possibility of a potentially severe opportunistic infection should be kept in mind. Physicians with special experience in opportunistic infections should be involved in the management of such patients, directly or by consultation.

10 End of protocol treatment

Reasons for going off protocol treatment are:

- No response after 3 cycles of FC or FC + alemtuzumab
- Progression / relapse after initial response (i.e. before completion of protocol treatment)
- Excessive toxicity (including toxic death) requiring permanent discontinuation of protocol treatment
- No compliance of the patient (especially refusal to continue treatment)
- Intercurrent death
- Major protocol violation
- Completion of protocol treatment
- Withdrawal by the investigator for clinical reasons not related to protocol treatment

11 Required clinical evaluations

11.1 Time of clinical evaluations

- At entry: at time of randomization, within 14 days prior to start cycle 1
- Prior to each cycle: within 14 days prior to each cycle
- After cycle 3: Prior to start of cycle 4.
- End of protocol: At least 3 months after the last treatment day, see footnote to Table 11.2.
- Follow up: at 7, 8, 9, 12, 15, 18, 21 and 24 months after start treatment, thereafter twice yearly
- At PD or PAPR: within 14 days after progression is diagnosed

Required investigations at entry, during treatment and during follow up

	At entry	Prior to each cycle	After cycle 3	End of protocol	Follow up (< 6 months after end of protocol)	Follow up (> 6 months after end of protocol)	At PD or PAPR
Medical history	X						
Physical examination	X	X	X	X	X	X	X
Blood tests:							
Hematology	X	X[1]	X	X	X	X	X
Blood chemistry	X	X	X	X	X	X	X
Anti-viral antibodies	X						
CMV-PCR		X[2]	X[3]	X	X[4]		
EBV-PCR		X[4]	o.i.	o.i.	o.i.		
Bone marrow biopsy	X		X[5]	X[12]		X[6]	X
Molecular evaluations:							
PB Flow cytometry	X		X[5]	X[12]		X[6]	X
BM Flow cytometry	X[7]		X[5]	X[12]		X[6]	X[7]
PB FISH	X[8]						X
BM FISH	X[7,8]						X[7]

Mutational status	X[9]						
PB MRD studies			X[5]	X[12]		X[6]	
BM MRD studies			X[5]	X[12]		X[6]	
PB storage future studies	X		X[10]	X[10]		X[6,10]	X
BM storage future studies	X		X[10]	X[10]		X[6,10]	X
Specific investigations:							
ECG	X						
CT scan	X		X	X[13]		X[6]	X
Alemtuzumab antibodies[11]	X	X		X		X	
Additional investigations	o.i.	o.i.	o.i.	o.i.	o.i.	o.i.	o.i.

[1] weekly, both treatment arms
[2] weekly, alemtuzumab arm only
[3] every 2nd week, alemtuzumab arm only
[4] monthly for 6 months, alemtuzumab arm only
[5] only as confirmation of CR
[6] at least once annually starting 6 months after end of protocol treatment
[7] only if not done on PB
[8] may be done up to 6 months prior to inclusion
[9] may be done at any time prior to inclusion
[10] any excess material
[11] alemtuzumab arm only
[12] At least 3 months after the last treatment, and only if clinical, laboratory and CT results are compatible with CR[13] at least 3 months after the last treatment.
[o.i.] on indication

11.2.1 Medical history

Standard medical history, including B symptoms and concomitant medications.

11.2.2 Physical examination

Standard physical examination, with special attention to:

- vital signs
- WHO performance status (see appendix E)

11.2.3 Hematology

- Hb
- WBC and differential count
- Platelet count
- Reticulocytes
- DAT (direct antiglobulin test/Coombs test)

11.2.4 Blood chemistry

- Sodium

- Potassium
- Creatinine
- Uric acid
- ASAT
- ALAT
- Alkaline phosphatase
- Bilirubin
- LDH
- Haptoglobin
- C-reactive protein

11.2.5 Additional blood chemistry at entry

- Glucose
- BUN
- Total protein
- Albumin
- IgG*
- IgM*
- IgA*
- β-2 microglobulin

* IgG, IgM and IgA should be repeated after cycle 3, at the end of protocol, every 3 months during the first year in follow up and every 6 months later on.

11.2.6 Anti-viral antibodies

- CMV
- EBV
- HIV
- HBV
- HCV

11.2.7 Bone marrow biopsy

At entry, after cycle 3, at the end of protocol treatment only as confirmation of CR. In CR patients at least annually during follow up starting 6 months after the end of protocol treatment and at progression. For pathology study of immunocytochemistry of persisting nodules (required markers: CD5, CD19, CD20, CD23, CD79b, kappa, lambda, cyclin D1).

11.2.8 Molecular evaluations

Flow cytometry

At entry on PB (on BM if not done on PB), after cycle 3 on PB and BM only as confirmation of CR, at the end of protocol treatment on PB and BM only as confirmation of CR, at least annually on PB and BM during follow up starting 6 months after the end of protocol treatment and at progression on PB (on BM if not done on PB).

- Diagnosis of classical CLL immunophenotype (required markers: CD5/CD19/CD23 triple positive with light chain restriction)
- Documentation of expression of target antigen
- Documentation of a diagnostic phenotype that can be applied for subsequent minimal residual disease analysis by flow cytometry
- Minimal residual disease analysis by flow cytometry
- Expression of potential prognostic markers (e.g. ZAP-70 and CD38) not applied for risk stratification in this study
- Residual normal lymphocyte subpopulations (optional)

FISH

At entry on PB (on BM if not done on PB) and at progression on PB (on BM if not done on PB).

- Classification of cytogenetic abberations for risk stratification (required markers: 17p13 deletion, 11q22-23 deletion, trisomy 12 and 13q14 deletion)
- Detection of clonal evolution during protocol treatment

Mutational status and MRD studies

At entry mutational status on PB (on BM if not done on PB), after cycle 3 MRD studies on PB and BM only as confirmation of CR, at the end of protocol treatment MRD studies on PB and BM only as confirmation of CR and at least annual MRD studies on PB and BM during follow up starting 6 months after the end of protocol treatment.

- Immunoglobulin heavy chain sequencing for classification of mutational status for risk stratification
- Identification of a clone specific sequence to be used for subsequent MRD analysis
- MRD analysis

In addition to these investigations, all patients are asked for informed consent to store biological material for future studies. Material for future investigations will be shipped to the core laboratories identified in appendix H. All materials are anonymized and stored for a maximum of 15 years after inclusion has been completed, after which the samples will be destroyed. Any study to be undertaken on these

materials must be approved of by the study coordinators, members of the writing committee and relevant ethical authorities, and will be specified in protocol amendments prior to undertaking the studies.

Guidelines for performance of molecular evaluations will be generated, implemented and, if necessarry, revised by the molecular evaluation committee identified in appendix H. In case of unequivocal results for risk stratification or immunophenotyping, original data or additional material will be distributed to one or more core laboraties in order to review the molecular evaluation. A copy of the results of the review will be sent to the local laboratory and to the HOVON Data Center.

11.2.9 CT scan

At entry, after cycle 3 , at the end of protocol treatment and at least annually during follow up starting 6 months after the end of protocol treatment, including CT of neck, thorax, abdomen and pelvis..

11.2.10 Anti-alemtuzumab antibodies

Serum will be collected before alemtuzumab administration in all cycles and at 3 and 12 months after end of protocol treatment, to be frozen and stored at -70°C for later analysis. This is only applicable to patients treated in centers with the appropriate facilities for storage at -70°C.

11.3 Evaluation of response

Response will be evaluated after cycle 3 and at the end of protocol treatment. Assessment of response is described in appendix C.

12 Toxicities

12.1 Fludarabine + cyclophosphamide

The most frequently reported side-effects are myelosuppression and immunosuppression which may hamper patient adherence to the projected schedule of FC. The known risk of AIHA following fludarabine appears to be reduced in the FC regimen (Catovsky et al. 2004: 2.7%). Oral FC (Cazin et al. 2003) led to 4% grade IV infections and 9% hemolytic anaemia.

12.2 Alemtuzumab

The side effects which follow i.v. administration of alemtuzumab (rigors, fever, urticaria and hypertension) are clearly reduced by the s.c. injection. Local cutaneous reactions are common, but tolerable in the majority of patients. Furthermore, it has been demonstrated that s.c. alemtuzumab does not require dose escalation, but can be initiated as 30 mg injections (Lundin et al 2005). Lundin et al. (2002) administered alemtuzumab s.c. as first-line single drug treatment in standard doses: 30mg TIW to

41 patients for 18 weeks, with a median cumulative dose of 1213 mg. CMV reactivation was encountered in 4 cases (10%) with fever but no pneumonitis. Three responded to ganciclovir, one patient recovered spontaneously. The treatment led to profound lymphopenia of all subsets for more than 9 months (Lundin et al. 2004). In the GCLLSG study (Wendtner et al. 2004) 7 of 11 patients randomized to alemtuzumab following treatment with F or FC experienced grade III-IV opportunistic infections (4 CMV reactivations , one pulmonary tuberculosis, one pulmonary aspergillosis and one H. zoster). All were successfully treated and there was no significant correlation to alemtuzumab cumulated dose. Based on this toxicity, however, the study was stopped in order to redefine the optimal Alemtuzumab dose following fludarabine or FC. In 18 patients treated up-front with fludarabine followed by 6 weeks of standard-dose alemtuzumab s.c. (30 mg TIW with usual build-up, theoretical cumulated dose 540 mg) only 3 (16%) CMV reactivations were observed (Rai et al. 2003). Montillo et al. (2003), using sequential low-dose alemtuzumab s.c. 8 weeks after fludarabine therapy found that half of the patients reactivated CMV but CMV-disease was prevented by prompt treatment with oral ganciclovir. One of 14 FluCam patients developed grade 3-4 infection (fungal). O'Brien et al. (2003) administered alemtuzumab s.c. (two dose-levels) to 58 patients who had achieved minimal residual disease state following unspecified chemotherapy. The main infection reported was CMV reactivation, but also 3 cases (5%) of EBV-positive large-cell lymphoma were encountered.

Toxicities will be scored according to the NCI Common Terminology Criteria for Adverse Events, version 3.0 (appendix D).

13 Reporting serious adverse events

An Adverse Event (AE) is any untoward medical occurrence or experience in a patient or clinical investigation subject which occurs during or following treatment regardless of the causal relationship. This can include any unfavorable and unintended signs (such as rash or enlarged liver), or symptoms (such as nausea or chest pain), an abnormal laboratory finding (including blood tests, x-rays or scans) or a disease temporarily associated with the treatment. In this study only grade ≥ 2 AE's will be reported.

Adverse Reactions (AR) are those AE's of which a reasonable causal relationship between any dose administered of the investigational medicinal product and the event is suspected.

Serious Adverse Events (SAE) are defined as any undesirable experience occurring to a patient, whether or not considered related to the treatment. Adverse events which are considered as serious are those which result in:

- death

- a life-threatening event (i.e. the patient was at immediate risk of death at the time the reaction was observed)
- hospitalization or prolongation of hospitalization
- severe/permanent disability
- a congenital anomaly

Note that any death, whether due to side effects of the treatment or due to progressive disease or due to other causes is considered as a serious adverse event.

Unexpected Serious Adverse Events are those SAE's of which the nature or severity is not consistent with information in the relevant source documents. For a medicinal product not yet approved for marketing in a country, a company's Investigator's Brochure will serve as a source document in that country.

Suspected Unexpected Serious Adverse Reactions (SUSAR) are all suspected AR's which occur in the trial and that are both unexpected and serious.

The **protocol treatment period** is defined as the period from registration till 30 days after discontinuation of the protocol treatment.

Reporting Serious Adverse Events

During protocol treatment all deaths, all SAE's that are life threatening and any unexpected SAE must be reported to the HOVON Data Center by fax **within 48 hours of the initial observation of the event**. All details should be documented on the **Serious Adverse Event and Death Report**. In circumstances where it is not possible to submit a complete report an initial report may be made giving only the mandatory information. Initial reports must be followed-up by a complete report within a further 14 calendar days and sent to the HOVON Data Center. All SAE Reports must be dated and signed by the responsible investigator or one of his/her authorized staff members.

At any time after the protocol treatment period, unexpected Serious Adverse Events that are considered to be at least suspected to be related to protocol treatment must also be reported to the HOVON Data Center using the same procedure**, within 48 hours after the SAE or death was known to the investigator**.

The investigator will decide whether the serious adverse event is related to the treatment (i.e. unrelated, unlikely, possible, probable, definitely and not assessable) and the decision will be recorded on the Serious Adverse Event and Death Form. The assessment of causality is made by the investigator using the following:

RELATIONSHIP	DESCRIPTION
UNRELATED	There is no evidence of any causal relationship
UNLIKELY	There is little evidence to suggest there is a causal relationship (e.g. the event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (e.g. the patient's clinical condition, other concomitant treatments).
POSSIBLE	There is some evidence to suggest a causal relationship (e.g. because the event occurs within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (e.g. the patient's clinical condition, other concomitant treatments).
PROBABLE	There is evidence to suggest a causal relationship and the influence of other factors is unlikely.
DEFINITELY	There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.
NOT ASSESSABLE	There is insufficient or incomplete evidence to make a clinical judgement of the causal relationship.

The HOVON Data Center will forward all reports within one working day of receipt to the study coordinator and the study central datamanager. The report of an SAE will be the signal for the central datamanager to ask the investigator or the responsible local datamanager to complete and send as soon as possible all relevant CRF's for the involved patient with details of treatment and outcome. It is of utmost importance that all SAE's (including all deaths due to any cause) are reported in a timely fashion. Patients without a report of an SAE are implicitly considered alive without SAE. This information will be used in monitoring the incidence of SAE's, the estimation of overall survival and safety monitoring.

The study coordinator or manufacturer will notify the HOVON Data Center of any new information, which becomes available during the course of the study, which may affect the overall safety profile of Alemtuzumab. Any SUSAR's, from any source, which are considered by the study coordinator or manufacturer to be reportable to investigators, Health Authorities and Ethics Committees will be sent to the HOVON Data Center within 12 calendar days of the study coordinator becoming aware of such events, or 5 calendar days for fatal or life-threatening reports. The study coordinator has responsibility for reporting such events to the Ethics Committee, which approved the study within the required timelines. Additionally, the HOVON Data Center will report all such events within one working day to co-

investigators (for multi-centre studies). Co-investigators will report all such events to their Ethic Committees, where required. The study coordinator will report to all applicable Health Authorities within required timelines. All events will also be reported to Schering AG in parallel to reporting to Ethics Committees and Health Authorities, according to agreement.

14 Endpoints

Primary:

- Progression free survival (i.e. time from registration to disease progression, relapse or death due to CLL whichever occurs first)

Secondary :

- Event free survival (i.e. time from registration to induction failure, progression, relapse or death whichever occurs first); the time to failure of patients with induction failure is set at one day
- Clinical, flow cytometric and molecular response rate
- Overall survival
- Disease free survival (i.e. time from CR to relapse)
- Toxicity

15 Data collection

Data will be collected on Case Report Forms (CRF) to document eligibility, safety and efficacy parameters, compliance to treatment schedules and parameters necessary to evaluate the study endpoints. Data collected on the CRF are derived from the protocol and will include at least:

- inclusion and exclusion criteria;
- baseline status of patient including medical history and stage of disease;
- timing and dosage of protocol treatment;
- adverse events;
- parameters for response evaluation;
- any other parameters necessary to evaluate the study endpoints;
- survival status of patient;
- reason for end of protocol treatment.

Each CRF page will be identified by a pre-printed trialnumber, and a unique combination of patient study number (assigned at registration), hospital and patient namecode (as documented at registration) to be filled out before completing the form.

The CRF will be completed on site by the local investigator or an authorized staff member. Each page must be dated and signed by the local investigator upon completion. All CRF entries must be based on

source documents. The CRF and written instructions for completing the CRF will be provided by the HOVON Data Center.
Copies of the CRF will be kept on site. The original CRF pages must be sent to the HOVON Data Center at the requested timepoints. How and when to send in forms is described in detail in the CRF header and the CRF instructions.
All data from the CRF will be entered into the study database by the HOVON Data Center.

16 Randomization

The patient should be randomized after diagnosis and before the start of treatment. Patients need to be randomized at the HOVON Data Center of the Erasmus MC – Daniel den Hoed by phone call: +31.10.4391568 or fax +31.10.4391028 Monday through Friday, from 09:00 to 17:00 or via the Internet via TOP (Trial Online Process; https://www.hdc.hovon.nl/top). A logon to TOP can be requested at the HOVON Data Center for participants.

The following information will be requested at randomization:

1. Protocol number
2. Institution name
3. Name of caller/responsible investigator
4. Patient's initials or code
5. Patient's hospital record number (optional)
6. Sex
7. Date of birth
8. Date of diagnosis of CLL
9. Eligibility criteria

All eligibility criteria will be checked with a checklist.
Patients will be randomized, stratified by center, 17p deletion confirmed by FISH and time from diagnosis (≤ 2 years or > 2 years) with a minimization procedure, ensuring balance within each stratum and overall balance. The randomization result will be given immediately by TOP or phone and confirmed by fax or email.

17 Statistical considerations

17.1 Patient numbers and power considerations

For the calculation of the required number of patients to be entered in the study, the progression free survival (PFS), as defined in chapter 14, will be considered as primary endpoint. Based on the results of Eichhorst et al. (2003), Flinn et al. (2004) and Byrd et al. (2004) it is estimated that the median PFS in

the FC arm will be 28 months. It is expected that the addition of alemtuzumab will increase the median PFS to 42 months. This corresponds with a relative hazard rate of 0.67 for the experimental arm. The hypotheses of difference between the two arms will be tested at a 5% significance level (two-sided test). The number of events needed to detect the stated difference with a power of 80% is 196. This number of events is expected to be reached with the inclusion of 300 patients in three years with an additional follow up of three years.

17.2 Statistical analysis

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

17.2.1 Efficacy analysis

The main endpoint for the comparison of the two treatment arms will be PFS as defined in chapter 14. Formal test for the difference in PFS between the treatment arms will be done with a multivariate Cox regression analysis with adjustment for the stratification factor time from diagnosis and prognostic factors (mutational status, 17p13 deletion, 11q22-23 deletion, trisomy 12 and 13q14 deletion). Secondary survival endpoints will be tested similarly to the main endpoint. The test for the difference in CR rate between the two treatment arms will be done with multivariate logistic regression with adjustment for the factors mentioned above.

17.2.2 Toxicity analysis

The analysis of treatment toxicity will be done primarily by tabulation of the incidence of adverse events with CTCAE grade 2 or more by treatment arm and cycle.

17.2.3 Additional analyses

Additional analyses involve a univariate and multivariate evaluation of other known prognostic factors, especially ZAP-70 and CLLU1 with respect to PFS, OS, DFS and CR rate. Cox regression and logistic regression will be used for this purpose.

17.3 Data and safety monitoring board

An independent data and safety monitoring board (DSMB) will be installed before start of the study.

Results of both planned interim analyses stated in paragraph 17.4 and eventually other unplanned interim analyses, will be presented confidentially to the DSMB. Only if the DSMB recommends that the study should be stopped or modified the results will become available to the principal investigators for further decisions.

The presented reports include by treatment arm the number of entered patients and at that time evaluable patients, treatment given, the number of failures, type of failures and incidence of SAE's and other adverse events (CTCAE grade).

17.4 Interim analysis

An early interim analysis is planned when the first 20 patients in each arm have available information about the complete induction treatment. The main endpoint for this interim analysis is the toxicity rate on induction treatment, with special emphasis on opportunistic infections due to immunosuppression by the addition of alemtuzumab to FC. Stopping or modification of the trial should be considered when an excess of five or more SAE's are reported with at least probable causal relationship with the treatment in one of the treatment arms compared to the other treatment arm.

The second interim analysis is focused on efficacy and is planned, primarily to guard against unfavorable results in the experimental arm (FC + alemtuzumab). This analysis is planned after 200 patients have been included and is expected to take place 2 years after the start of the trial.

The DSMB is free in its recommendations to the study coordinators, but the following guidelines apply:

- A worse PFS in the experimental arm with a P-value < 0.1 (logrank-test) is a good reason to recommend the stopping of the trial or recommendations for modifications.
- A better PFS in the experimental arm is in general no reason to recommend early stopping of the study, unless the associated P-value is very extreme (P < 0.001, logrank).

The study will be closely and sequentially monitored before the second interim analysis. Monitoring will be based on the reported SAE's which are not subjected to data delay. The difference in the number of patients with an SAE in both arms and the difference in the number of deaths in both arms are tested using the binomial test. It will be repeatedly tested whether those incidences in the experimental arm are higher at a significance level of 0.05, adjusted for multiple testing (adjustment based on simulation). If one of both incidences is significantly higher in the experimental arm an early report will be presented to the DSMB.

18 Ethics

18.1 Independent ethics committee or Institutional review board

The study protocol and any amendment that is not solely of an administrative nature will be approved by an Independent Ethics Committee or Institutional Review Board.

18.2 Ethical conduct of the study

The study will be conducted in accordance with the ethical principles of the Declaration of Helsinki and the ICH-GCP Guidelines. The local investigator is responsible for ensuring that the study will be conducted in accordance with the protocol, the ethical principles of the Declaration of Helsinki, current ICH guidelines on Good Clinical Practice (GCP), and applicable regulatory requirements.

18.3 Patient information and consent

Written Informed consent of patients is required before randomization. The procedure and the risks and the opinions for induction therapy in CLL will be explained to the patient.

19 Trial insurance

The HOVON insurance program covers all patients from participating centers in the Netherlands according to Dutch law (WMO). The WMO insurance statement can be viewed on the HOVON Web site www.hovon.nl.

The writing committee will ensure that risk insurance of patients from centers from outside the Netherlands is in place according to all applicable laws and regulations.

19.1 Intergroup studies

The HOVON insurance program does not cover the risk insurance of patients from centers participating within another cooperative group taking part in an intergroup study. The other participating groups will cover the insurance of patients registered/randomized through their offices.

20 Publication policy

The final publication of the trial results will be written by the Study Coordinator(s) on the basis of the statistical analysis performed at the HOVON Data Center. A draft manuscript will be submitted to the Data Center, all co-authors and Schering for review. After revision by the Data Center, the other co-authors and Schering, the manuscript will be sent to a peer reviewed scientific journal.

Authors of the manuscript will include the study coordinator(s), the lead investigators of the major groups (in case of intergroup studies), investigators who have included more than 5% of the evaluable patients in the trial (by order of number of patients included), the statistician(s) and the HOVON datamanager in charge of the trial, and others who have made significant scientific contributions.

Interim publications or presentations of the study may include demographic data, overall results and prognostic factor analyses, but no comparisons between randomized treatment arms may be made publicly available before the recruitment is discontinued.

Any publication, abstract or presentation based on patients included in this study must be approved by the study coordinator(s). This is applicable to any individual patient registered/randomized in the trial, or any subgroup of the trial patients. Such a publication cannot include any comparisons between randomized treatment arms nor an analysis of any of the study end-points unless the final results of the trial have already been published.

21 Glossary of abbreviations

(in alphabetical order)

ADCC	Antibody-Dependent Cell mediated Cytotoxicity
AE	Adverse Event
AIHA	Autoimmune Hemolytic Anemia
ALAT	Alanine Amino Transferase
ANC	Absolute Neutrophil Count
AR	Adverse Reaction
ASAT	Aspartate Animo Transferase
ASCO	American Society of Clinical Oncology
BM	Bone Marrow
BUN	Blood urea nitrogen
C	Cyclophosphamide
CALGB	Cancer and Leukemia Group B
CDR3	Complementarity Determining Region 3
CLL	Chronic Lymphocytic Leukemia
CLLU1	CLL-upregulated Gene 1
CMC	Complement Mediated Cytolysis
CMV	Cytomegalovirus
CNS	Central Nervous System
CR	Complete Remission/response
CRF	Case Report Form
CT	Computerized Tomography
CTCAE	Common Terminology Criteria for Adverse Events
DAT	Direct Antiglobulin Test
DFS	Disease Free Survival
DSMB	Data and Safety Monitoring Board
EBV	Epstein-Barr virus
ECOG	Eastern Cooperative Oncology Group
EFS	Event Free Survival
F	Fludarabine
FC	Fludarabine + Cyclophosphamide
FCLLSF	French CLL study group
Gamma-GT	Gamma Glutamyl Transferase
GCLLSG	German CLL study group
GCP	Good Clinical Practice
G-CSF	Granulocyte Colony Stimulating Factor
GVHD	Graft Versus Host Disease
Hb	Hemoglobin
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus

HIV	Human Immunodeficiency Virus
HOVON	*Hemato-Oncologie voor Volwassenen Nederland* (Dutch-Belgian Hemato-Oncology Group)
HSV	Herpes Simplex Virus
HZV	Herpes Zoster Virus
ICH	International Conference on Harmonization of technical requirements for registration of pharmaceuticals for human use
IV	Intravenous
IWCLL	International Workshop on CLL
LDH	Lactate Dehydrogenase
LVEF	Left Ventricular Ejection Fraction
MRC	Medical Research Council
MRD	Minimal Residual Disease
NCI	National Cancer Institute
nPR	Nodular PR
NYHA	New York Heart Association
OI	On Indication
OS	Overall Survival
PA	Pathology
PAPR	Progression After Previous Response
PB	Peripheral Blood
PCR	Polymerase-chain reaction
PD	Progressive Disease
PO	Per Os
PR	Partial Response
RBC	Red Blood Cells
RR	Response rate
SAE	Serious Adverse Event
SC	Subcutaneous
SD	Stable disease
SUSAR	Suspected Unexpected Serious Adverse Reaction
TIW	Three Times a Week
TOP	Trial Online Process
VZV	Varicella Zoster Virus
WBC	White Blood Count
WHO	World Health Organization
WMO	*Wet Medisch-Wetenschappelijk Onderzoek met Mensen*
ZAP-70	Zeta-associated Protein 70

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A. NCI criteria for active CLL

For active CLL at least one of the following criteria should be met:

1. At least one of the following disease-related symptoms must be present:
   a. Weight loss ≥ 10% within the previous 6 months
   b. Extreme fatigue (i.e., WHO performance status ≥ 2)
   c. Fevers ≥ 38.6 °C for ≥ 2 weeks without evidence of infection
   d. Night sweats without evidence of infection
2. Evidence of progressive marrow failure as manifested by the development of, or worsening of anemia and/or thrombocytopenia
3. Autoimmune anemia and/or thrombocytopenia poorly responsive to corticosteroid therapy
4. Massive (i.e., > 6 cm below the left costal margin) or progressive splenomegaly
5. Massive nodes or clusters (i.e., > 10 cm in longest diameter) or progressive lymphadenopathy
6. Progressive lymphocytosis with an increase of > 50% over a 2-month period, or an anticipated doubling time of less than 6 months

Marked hypogammaglobulinemia or the development of a monoclonal protein in the absence of any of the above criteria is not sufficient for protocol therapy.

B. Binet classification system (Binet et al. 1981)

Stage A: Lymphocytosis and lympadenopathy/organomegaly involving < 3 areas*

Stage B: Lymphocytosis and lympadenopathy/organomegaly involving ≥ 3 areas*

Stage C: Lymphocytosis and Hb < 6,2 mmol/l (< 10 g/dl) or platelet count < 100 x 10^9/l

* An involved area is either:

- cervical (head and neck, including Waldeyers ring, involvement of more than one group of nodes counts as one area)
- axillary (involvement of both axillae counts as one area)
- inguinal lymphadenopathy (including superficial femorals, involvement of both groins counts as one area)
- splenomegaly
- hepatomegaly

C. Response criteria for CLL (IWCLL criteria with adjustments (Hallek et al 2008))

Complete molecular remission (CMR)

CMR requires all of the following for at least 3 months:

- all the criteria for CFCR are met (see below);
- undetectable patient specific clonal CDRIII rearrangement in blood and bone marrow samples by PCR with allele specific oligonucleotides.

Complete flow cytometric remission (CFCR)

CFCR requires all of the following for at least 3 months:

- all the criteria for CR are met (see below);
- undetectable CLL cells by flow cytometry in blood and bone marrow samples.

Complete remission (CR)

CR requires all of the following for at least 3 months:

- Absence of clonal lymphocytes in the peripheral blood;
- absence of lymphadenopathy;
- absence of hepatomegaly;
- absence of splenomegaly;
- absence of constitutional symptoms (see appendix A criteria 1a, 1b, 1c and 1d);
- Blood counts above the following levels:
  - polymorphonuclear leukocyte count $\geq 1.5 \times 10^9$/l;
  - platelet count $> 100 \times 10^9$/l;
  - untransfused hemoglobin > 6.8 mmol/l (> 11 g/dl);
- bone marrow without CLL cells by flow cytometry or immunochemistry.

CR with incomplete bone marrow recovery (CRi)

Patients who fulfill all the criteria for CR but who have persistent anemia or or thrombocytopenia or neutropenia apparently unrelated to CLL, but to drug toxicity.

Partial response (PR)

PR requires all of the following for at least 2 months:

- ≥ 50 % decrease in peripheral blood lymphocyte count;

  - ≥ 50 % reduction in the sum of the products of at least two lymph node diameters on two consecutive examinations;
  - ≥ 50 % reduction in total size of liver (if abnormal prior to therapy);
  - ≥ 50 % reduction in total size of spleen (if abnormal prior to therapy);

The blood counts should show one or more of the following results*:

- polymorphonuclear leukocyte count ≥ 1.5 x 10^9/l (or 50 % increase compared to baseline);
- platelet count > 100 x 10^9/l (or 50 % increase compared to baseline);
- untransfused hemoglobin > 11 g/dl (> 6.8 mmol/l) (or 50 % increase compared to baseline).

Patients otherwise in CR but with persising B-lymphoid nodules in the bone marrow are designated PR

) Following cycle 3, response score based on clinical parameters only is allowed.

Stable disease (SD)

SD requires that the criteria for PR are not met and that none of the criteria for PD are met.

Progression after previous response (PAPR)

PAPR must be reported if after a previous response any of the following criteria are met:

- ≥ 50 % increase in the sum of the products of at least two lymph node diameters on two consecutive examinations (at least one node must be ≥ 2 cm) compared to nadir or the appearance of new palpable lymph nodes;
- ≥ 50 % increase in peripheral blood lymphocyte count (to at least 5 x 10^9/l) compared to nadir;
- ≥ 50 % increase in size of liver below costal margin compared to nadir;
- ≥ 50 % increase in size of spleen below costal margin compared to nadir;
- transformation to a more aggressive histology (Richter's syndrome or PLL with > 55 % prolymphocytes).

Progressive disease (PD)

PD must be reported if any of the following criteria are met:

- ≥ 50 % increase in the sum of the products of at least two lymph node diameters on two consecutive examinations (at least one node must be ≥ 2 cm) or the appearance of new palpable lymph nodes;
- ≥ 50 % increase in peripheral blood lymphocyte count (to at least 5 x 10^9/l);
- ≥ 50 % increase in size of liver below costal margin;
- ≥ 50 % increase in size of spleen below costal margin;
- transformation to a more aggressive histology (Richter's syndrome or PLL with > 55 % prolymphocytes).

D. Toxicity criteria

The grading of toxicity and adverse events will be done using the NCI Common Terminology Criteria for Adverse Events, CTCAE version 3.0, published December 12, 2003. A complete document (72 pages) may be downloaded from the following sites:

http://ctep.info.nih.gov/reporting/ctc.html
http://www.hovon.nl (under Studies > Documents)

A hardcopy may be obtained from the HOVON Data Center on request.

E. ZUBROD-ECOG-WHO Performance Status Scale

0 Normal activity

1 Symptoms, but nearly ambulatory

2 Some bed time, but to be in bed less than 50% of normal daytime

3 Needs to be in bed more than 50% of normal daytime

4 Unable to get out of bed

F. NYHA* scoring list

Grade 1	No breathlessness
Grade 2	Breathlessness on severe exertion
Grade 3	Breathlessness on mild exertion
Grade 4	Breathlessness at rest

The *New York Heart Association functional and therapeutic classification applied to dyspnoea

G. Guidelines for CMV monitoring and treatment

All patients are tested before the initiation of protocol treatment for antibody-response status (IgG and IgM) against CMV antigens and CMV-specific quantitative polymerase chain reaction (PCR) for CMV in peripheral blood.

In the alemtuzumab arm CMV-PCR will be performed every week during the first three cycles and every other week thereafter during the treatment phase.

Asymptomatic patient

In case of positivity by PCR (significant copies according to the lab method) immediate confirmation is recommended.

Patients with low number of copies (according to the lab method) will be followed closely clinically until a significant rise in CMV transcripts is detected by weekly PCR.
If confirmation of a rising number of copies the patient will be treated with oral valganciclovir (900 mg x 2 or equivalent), while protocol treatment is continued.

If an asymptomatic patient with CMV reactivation starts showing clinical manifestations of CMV infection, therapy should be changed to ganciclovir intravenously and the protocol treatment (FC or FC + alemtuzumab) will be held (see below symptomatic patient).

Symptomatic patient

In a PCR-positive patient with clinical symptoms of apparent CMV infection the study therapy will be held and ganciclovir will be administered either as ganciclovir intravenously 10mg/kg/day divided into two doses during a sufficient time to get the patient asymptomatic, or oral valganciclovir (900 mg x 2 or equivalent).

In the case of manifest CMV infection, protocol treatment will be held for a maximum of four weeks. If CMV infection is not resolved after four weeks patients will go off protocol treatment.

Duration of valganciclovir treatment

Symptomatic: Duration of valgancyclovir treatment 14-21 days until resolution of symptoms and negative test result or until 2 negative test results. Protocol treatment is held the same period. Asymptomatic: 7-14 days or until 2 consecutive negative test results. Protocol treatment not held.

- Neutropenia is a common side-effect of ganciclovir and valganciclovir. Monitoring of neutrophil counts is an effective and sensitive safety parameter during treatment.

- Regular monitoring of creatinine clearance for dose adaptation of ganciclovir (valganciclovir) in case of renal insufficiency is essential.

- Monitoring of neutrophil counts is essential also after continuation of protocol treatment (especially with FC + alemtuzumab).

- After study completion CMV testing will be continued monthly during at least three months.

H. Molecular evaluations

Materials for mutational status and MRD studies are to be sent to a member of the molecular evaluation committee at one of the core laboratories:

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I. Estimates of annual accrual per country

	Incidence	Age ≤ 75 years (~60 %)	High risk (~42 %)*	Symptomatic (~75%)	Realistically
Czech Republic	unknown	unknown	unknown	Unknown	6-10
Denmark	250	150	63	47	9
Finland	150	90	37	28	6
The Netherlands	600	360	150	112	28
Norway	150	90	37	28	14
Poland	1100	660	275	206	15-25
Sweden	350	210	88	66	20-40
Total	>2600	>1560	>650	>487	98-132

* (~75% stage A * ~33% high risk) + (~25% stage B and C * ~67% high risk)