Allogeneic Stem Cell Transplantation after Reduced Intensity Conditioning for High-risk Relapsed or Refractory CLL

A prospective multi-centre phase II study

PROTOCOL

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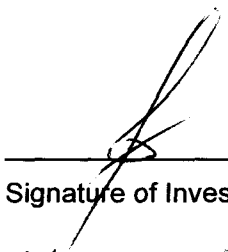

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June 30, 2011

Date

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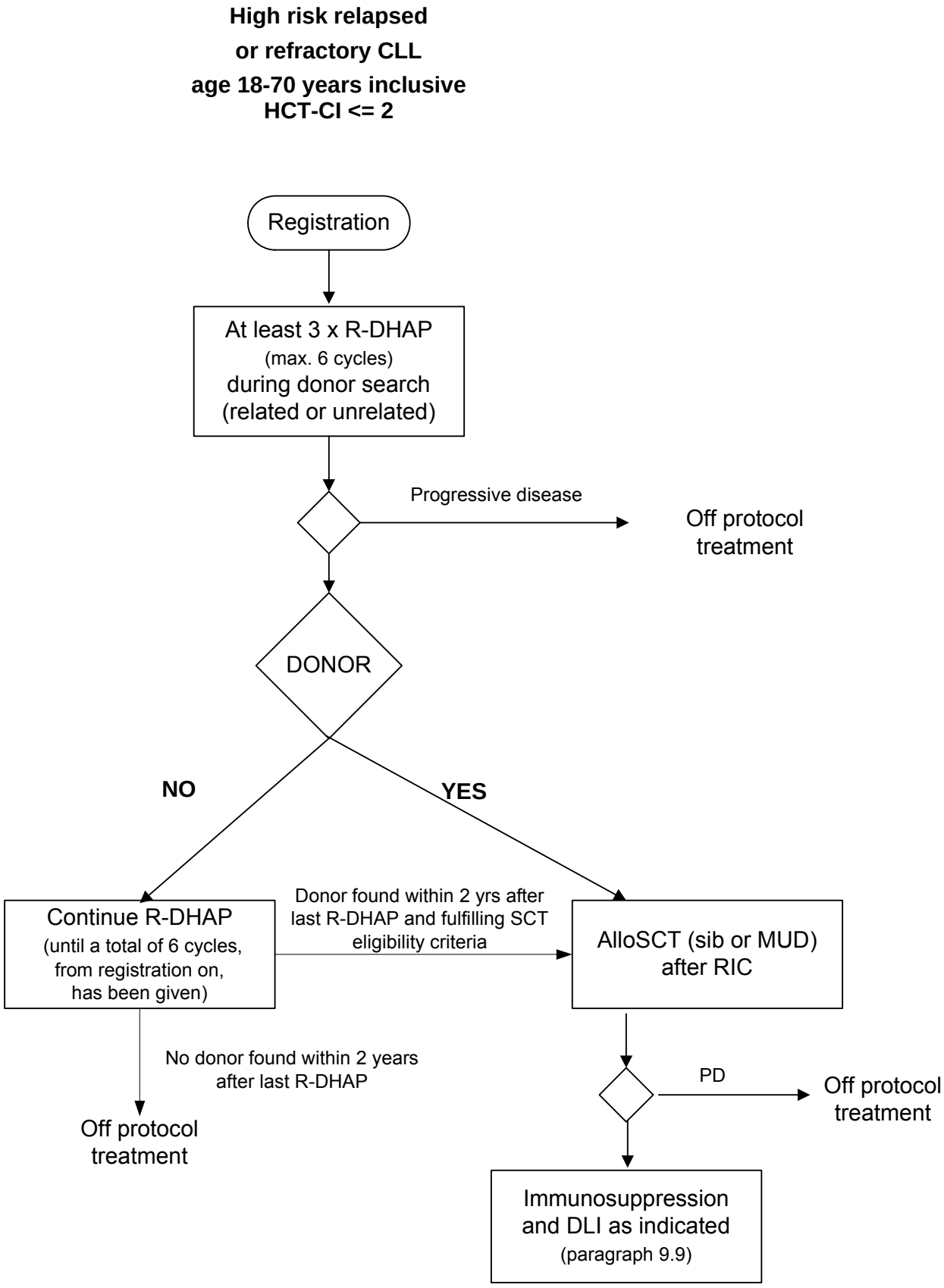
By my signature, I agree to personally supervise the conduct of this study and to ensure its conduct in compliance with the protocol, informed consent, IRB/EC procedures, the Declaration of Helsinki, ICH Good Clinical Practices guideline, the EU directive Good Clinical Practice (2001-20-EG), and local regulations governing the conduct of clinical studies.

1 Scheme of study



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3 Synopsis

Study phase	Phase II
Study objectives	Evaluation of the effect of salvage therapy with R-DHAP followed by reduced-intensity conditioning and allogeneic stem cell transplantation from a sibling or unrelated donor
Patient population	Patients with B-CLL, in need of treatment and either refractory to fludarabine, or relapsed within one year after last fludarabine gift or within two years after fludarabine combined with monoclonal antibody or refractory /relapsed and having 17p deletion and age 18-70 years and hematopoeitic stem cell transplantation co morbidity index ≤2
Study design	Prospective, multicenter, non-randomized
Duration of treatment	Duration of salvage therapy at least three months, depending on donor availability; duration of stem cell transplantation and subsequent period in which immunomodulation may be applied (earlier cessation of immunosuppression or DLI) maximum two years from registration.
End of trial	End of trial will be the date on which of all patients clean data including 1 year follow up (from registration) will be available. Subsequently all patients will be followed until 5 years after registration.
Number of patients	50 registered and treated with R-DHAP salvage therapy
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: II 2008 End of recruitment: IV 2012

4 Investigators and study administrative structure

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5 Introduction

5.1 Chronic lymphocytic leukemia

Chronic lymphocytic leukemia (CLL) is a heterogeneous disease. Some patients survive for decades without treatment while others develop progressive, ultimately lethal disease. Among the latter are those having advanced disease at presentation or short doubling time of lymphocytes or lymph nodes.[1] Patients with the highest stages (10% of the newly diagnosed patients) in the staging systems of Rai and Binet have a median survival of 1.5 to 2 years,[2-4] while patients with doubling times of less than 12 months have median survival of 36 to 66 months.[5;6] Recently, cytogenetic and molecular biological prognostic factors have been identified, which allow differentiation between populations with an indolent and aggressive course (reviewed by Shanafelt et al.[7]). The highest risk of short survival is found in patients with 17p13 deletion (median OS from diagnosis 32 months), followed by patients with mutated Ig VH3-21 genes (median OS 63 months), [8] 11q23- (median OS 79 months), and nonmutated IgVH genes (median OS 79-145 months).
CLL is also a disease of the elderly. Only 20% of the patients are diagnosed when less than 55 years of age[1;9]. There is no difference in both age groups neither in the proportion of patients who need treatment nor in their response. The overall actuarial median survival from diagnosis for both groups is 10 years, which is significantly lower compared with the expected survival probability of the age- and sex-matched population. CLL-related deaths account for 98% of deaths in the younger group and for 72% in the older group.

At present, although several conventional chemotherapy regimens are used to treat CLL patients, cure is not achieved although progression can be significantly postponed by recently introduced more intensive first-line treatments.[10]
Some patient groups are especially at high risk of dying at short term of their disease. Patients with 17p13 deletion show quite short progression-free survival after treatment and have a high probability for developing alkylator- and fludarabine-refractory disease, which is associated with short survival.[11-16] Survival of fludarabine-refractory patients is also quite limited.[17-20] Some may benefit from combined chemo(immune)therapeutic regimens, but even those have 2-year PFS < 10-20%. The combination of fludarabine and cyclophosphamide (FC) results in 30-40% ORR (n=50 patients).[21-23] Combining FC with rituximab might be more effective (ORR 58% in 33 patients).[17] Alemtuzumab monotherapy results in 33% ORR,[18] while the combination with rituximab looks more promising with an ORR of about 65% in 32 patients, of whom 75% had fludarabine refractory disease;[19] responses were more achieved in the blood than in lymph nodes and bone marrow. When mentioned, two-year PFS of fludarabine refractory patients in these studies ranged from <10-20%,[17;18] and median survival was 11-16 months.[18;19] Treatment with high dose methylprednisolone

induced remissions in 12/13 fludarabine-refractory patients (including 2 minimal responses) with a median duration of 12 months, irrespective of the presence or absence of p53 mutation, but infections were frequent.[24] Patients relapsing early after chemoimmunotherapy will likely have a similar short overall survival as those relapsing after high-dose chemotherapy and autologous transplantation, being 27 months for patients having relapsed within 2 years.[25]
Currently, newly diagnosed CLL patients with high-risk cytogenetic or molecular profile or those in second or third relapse will be treated with chemoimmunotherapy (HOVON 68 first-line study, and second-line study in preparation), as this might improve PFS. Ultimately it is expected that far most of these patients still will relapse and become refractory for these treatments; it is currently unknown how these patients are rescued best, especially those relapsing early.

5.2 Allogeneic Stem cell transplantation (SCT) in high risk CLL

Until now no other treatment than allogeneic stem cell transplantation (alloSCT) with myeloablative conditioning has proven to be potentially curative. In the few published series, only a minority of patients relapsed and the survival curves show a plateau on the long run.[26-29] Indications for the existence of a graft-versus-leukemia (GvL) effect comes from the observations that responses have occurred either after cessation of immunosuppression (IS), or after several months after transplant,[30-33] after the administration of donor lymphocyte infusion (DLI)[34;35] and after onset of chronic graft-versus-host disease (cGVHD).[28;30] Unfortunately however, overall survival in most series is less than 60% due to high non-relapse mortality. A further disadvantage for this approach in CLL is the overall median age of patients who suffer from aggressive CLL (65-70 years), which is much higher than the median age of patients in the reported studies (41-47 years). It is a well known fact that treatment-related mortality (TRM) from alloSCT with conventional conditioning increases with age.
Several larger studies show that alloSCT after reduced intensity conditioning (RIC) in fludarabine refractory CLL patients is accompanied with a lower TRM and preserved anti-CLL activity, as summarized in the following table:

FIRST AUTHOR	N =	MEDIAN AGE (YEARS)	% WITH RELATED DONOR	2 YR. TRM	2 YR. PFS	2 YR. OS
Sorror et al.[36]	64[a]	56	44	22%	53%	63%
Schetelig et al.[37]	30[b]	50	15	15%	67%	72%
Khouri et al.[38]	17[a]	54	17	22%	60%	80%
Dreger et al. (EBMT)[39]	73[c]	54	15	18% (1 yr.)	58%	70%

[a] all patients were fludarabine refractory

[b] 50% were fludarabine refractory; no difference in survival with non- refractory patients

[c] 35% were fludarabine refractory.

Conditioning varied between the studies and was either with fludarabine and low-dose TBI,[36] fludarabine, busulfan and ATG,[37] fludarabine, cyclophosphamide +/- rituximab[38] or variable.[39]
Although patients with high disease burden or less responsiveness to the last treatment relapsed more often, it is not yet appropriate to exclude these patients, as some did respond to the procedure quite impressively. It should however be attempted to increase the remission rate by more effective salvage treatment prior to alloSCT. Patients with progressive disease did uniformly bad.
Taken together, these data show that alloSCT after RIC is accompanied by a lower TRM in older CLL patients than after conventional conditioning in younger CLL patients, and suggest that it's efficacious.

5.3 Minimal residual disease (MRD)

Patients in CR may still harbor CLL at very low levels, which is called minimal residual disease (MRD). MRD can be measured by molecular techniques or by flow cytometry. The most sensitive molecular technique is real-time quantitative Allele-Specific Oliginucleotide IgH-PCR (ASO-PCR). The ASO-PCR is very laborious and expensive because for every single patient ASO needs to be defined. Flow cytometry is performed more easily and has sufficient sensitivity for the use in the allogeneic SCT setting (lowest detection level in blood and bone marrow 0.01% B-CLL cells).[42] In that range of detection there is a perfect correlation with the ASO-PCR.[42] The premise for this high sensitivity is a standardized procedure using both a defined set of monoclonal antibodies and analysis procedure.[42]
The detection and, more specifically the increase of MRD after autologous SCT predicts the development of clinical relapse.[29] The detection of MRD after myeloablative allogeneic SCT however is not sufficient for predicting clinical relapse, as MRD most often diminishes over time.[29] The experience with MRD measurement after RIC alloSCT is scarce. However, it seems appropriate to consider an increase of B-CLL cells of 2 log or more predictive for clinical relapse.

5.4 Rationale of the study

Although the phase 2 studies on alloSCT after RIC reveal promising results, the difficulty of translating results of phase 2 studies in daily practice remains the possible bias introduced due to patient selection, as patients were only included in the analyses after the transplantation had been performed. Another point is that nowadays most high-risk patients will have been treated either upfront or after relapse with more intensive fludarabine-containing chemoimmunotherapeutic regimens, unlike the patients in the published studies on RIC alloSCT. It is unknown what percentage of these more heavily pre-treated patients get round to alloSCT when the prerequisite

for alloSCT is at least stable disease on salvage therapy, as at this moment no standard salvage therapy exists for these patients. Furthermore it is unknown what percentage of these more intensive pre-treated patients will ultimately benefit from the RIC alloSCT.

Salvage treatment including alemtuzumab, which eliminates both B- and T-cells, at short-term prior to alloSCT may lead to an increased incidence of opportunistic infections after alloSCT, and seems therefore not suitable. CHOP (the most commonly used first line treatment for aggressive lymphoma) is not indicated. The reason is that COP is equally effective as the alkylating agent chlorambucil and because all patients will be refractory to alkylating agents the putative effect can only be delivered by the added anthracyclin, which is an unrealistic scenario. A combination of high-dose dexamethason, cisplatin and high-dose cytarabine and rituximab (R-DHAP) seems more appropriate, as these patients will not have been treated previously with the first three agents and some not even with rituximab. Rituximab does not affect T cell function and will likely not increase the risk of opportunistic infections. The DHAP regimen, which is routinely used for relapsed large cell lymphoma has also been used in patients with high-risk CLL. Majolino et al. reported on ten high-risk CLL patients, of whom seven were fludarabine-refractory, who were treated with two courses of DHAP after a median of two prior treatment lines.[40] In all ten patients the second course was given as planned one month after the first and no non-hematological toxicity was encountered. Eight responses (at least PR) were observed after two courses, among whom six out of seven fludarabine-refractory patients. Sutton et al. used DHAP combined with etoposide (ESHAP) in 20 fludarabine refractory patients, of whom 13 responded.[56] In both studies similar adverse events, mainly temporary hematological, were seen as seen in the treatment of lymphoma patients.

The addition of rituximab is not expected to increase toxicity, but is expected to increase efficacy as most CLL cells do express CD20. Four series of in total 489 CLL patients treated with rituximab in the same dose as used in this study (i.e. 375 mg/m2 during the first course, and 500 mg/m2 in subsequent courses) revealed no excess grade 3-4 toxicity (1.5%).[45-48] One patient developed grade 3 dyspnoea, one grade 3 fatigue, one grade 3 headache and one grade 4 toxicity of unknown origin; no deaths attributable to rituximab occurred.

In relapsed diffuse large cell lymphoma the addition of rituximab to DHAP-containing salvage therapy resulted in an increase of overall response rate from 49% to 77% without increased toxicity, illustrating the potential of the scheme. [Vellenga, ASH 2006, abstract]

A clinical argument that the addition of rituximab may increase efficacy of chemotherapy in CLL as well comes from two successive phase 2 studies from M.D. Anderson, which showed an ORR of 30-40% after fludarabine-cyclophosphamide without rituximab and of 59% with rituximab in fludarabine-refractory patients.[17,21-23] A single-centre experience with R-DHAP looks very promising (J. Schetelig from Dresden, personal communication).

Inclusion criteria for the study will be in concordance with the recently published EBMT transplant consensus.[41] They comprise three groups of patients. The first are those refractory to or relapsing within 6 months after treatment with fludarabine. The second are patients with abnormalities of 17p13 needing treatment for progressive disease. The third group are those relapsing within 24 months after either high-dose chemotherapy and autologous SCT or fludarabine containing chemoimmunotherapy.

A uniform conditioning regimen prior to SCT will be applied in all participating centres, to prevent impact of dosage of chemo-radiotherapy on outcome. The Seattle regimen is chosen as all participating centres are familiar with this scheme and because by using this regimen promising results were obtained.[36] In patients with clinical CR, MRD measurement will be performed. We assume that an increase of 2 log or more of MRD predicts clinical relapse. It is then attempted to prevent clinical relapse by either stopping immunosuppression or DLI.

5.5 Risk exposure for patients in case of participation to the study.

For high risk CLL patients fitting to the inclusion criteria for this study RIC alloSCT is considered as best treatment option among alternatives. This view is however based on retrospective studies only. Repetitively it has been shown in the past that outcome of treatments published as retrospective series differ when performed in well-designed prospective trials, underscoring the fact that bias may positively influence results of retrospective trials. In this view it is clear that patients included into this study would have been transplanted anyway, so the risk of the transplant procedure is similar as if not included in the study. Furthermore only patients with low co-morbidity index are eligible. More severe co-morbidity enhances non-relapse mortality, so patients included in the study are expected to have low non-relapse mortality and it therefore harbours no disadvantage for them to participate in the study.

Patients can only be included in the study in the presence of active disease. Active disease in CLL means an immediate treatment indication. RIC alloSCT will never be performed in this situation without debulking, so every patient will be pretreated anyhow. The study aims at evaluating the effect of R-DHAP for this debulking purpose. R-DHAP is routinely used for relapsed lymphoma patients before high-dose chemotherapy and autologous stem cell rescue with a very low incidence of complications. It is expected that R-DHAP will be similarly low toxic for CLL patients, while other current available salvage therapies for CLL patients already have shown to be quite more toxic especially with regard to severe infectious complications. In addition, no alternative standard effective treatment for patients fitting the inclusion criteria exists, as they will have been pretreated with all current available effective-proven therapies. It is therefore legitimate to assess toxicity and efficacy of R-DHAP pretreatment before RIC alloSCT, as it would have been prescribed to the patients anyway. Outside study it would however cost much more time and effort to disclose

unexpected high toxicity. In the study an interim analysis is planned as a safety measure to prevent exposure of additional patients to R-DHAP in the unexpected case of either to low efficacy or to high toxicity (see paragraph 17.3).

6 Study objectives

Primary objective

- To assess, on an intention-to-treat basis, the efficacy and safety of a treatment protocol including salvage chemoimmunotherapy (R-DHAP) followed, in the absence of progression, by RIC alloSCT from sibling or unrelated donors, in high-risk CLL patients as defined in chapter 8.1.1, as measured by the progression free survival as defined in chapter 14.

Secondary objectives

- To assess the safety and toxicity of three courses of R-DHAP
- To assess the response to three courses of R-DHAP.
- To assess PFS and OS after alloSCT.
- ¨To asses PFS and OS after maximally 6 R-DHAP in case no alloSCT was performed
- To assess disease status at two years after SCT.
- To assess the incidence and course of MRD in patients with CR
- To assess overall survival from registration.
- To assess the incidence of complete, partial and no engraftment.
- To evaluate the incidence and severity of acute and chronic GVHD, and of other treatment related toxicity;
- To evaluate the response of progressive disease or increasing MRD to lowering of immunosuppression or DLI.
- To assess the time to next treatment excluding MRD triggered lowering of immunosuppression or DLI.
- To assess the prognostic value of risk factors at entry including IgH mutation status and karyotypic abnormalities with respect to PFS.
- To assess the functionality of mutated p53 at entry and at relapse.

7 Study design

Details of all treatments (dose and schedule) are given in paragraph 9.

All patients will be treated with at least three courses of R-DHAP while a HLA-identical donor is being searched first among siblings and second, if negative, in the world donor bank. Patients with a donor and responsive or stable disease (SD) after at least three courses R-DHAP proceed to RIC

alloSCT. DLI will be given for increasing minimal residual disease (MRD) after cessation of immunosuppression. In case no suitable donor is found, responsive patients are treated with additional courses of R-DHAP until a total of 6 courses from registration on has been administered.

8 Study population

8.1 Eligibility for registration

All eligible patients have to be registered before start of treatment.
Patients have to meet all of the following criteria:

8.1.1 Inclusion criteria

- B-CLL confirmed according to WHO Classification;
- Fludarabine refractory, defined as no response or relapse within 12 months after the last administration of fludarabine monotherapy or fludarabine containing regimen, and needing treatment, **or**
  Refractory or relapsed and needing treatment and having deletion of 17p13 **or**
  Refractory or relapsed within 24 months after the last administration of fludarabine combined with a monoclonal antibody and needing treatment;
- Age 18-70 years inclusive;
- WHO performance status ≤ 2 (see appendix E);
- HCT-CI ≤ 2 (see appendix F);
- Written informed consent.

8.1.2 Exclusion criteria

- Intolerance to exogenous protein administration
- Previously treated with DHAP
- Richter's transformation;
- Suspected or documented CNS involvement by CLL;
- Severe cardiovascular disease (arrhythmias requiring chronic treatment, congestive heart failure or symptomatic ischemic heart disease);
- Severe pulmonary dysfunction (CTCAE grade III-IV, see appendix D);
- Severe neurological or psychiatric disease;
- Significant hepatic dysfunction (serum bilirubin or transaminases ≥ 3 times upper limit of normal) except when caused by leukemic infiltration;

- Significant renal dysfunction (creatinine clearance < 30 ml/min after rehydration);
- History of active malignancy during the past 5 years with the exception of basal carcinoma of the skin or stage 0 cervical carcinoma;
- Active, uncontrolled infections;
- Patient known to be HIV-positive;
- Any psychological, familial, sociological and geographical condition potentially hampering compliance with the study protocol and follow-up schedule;
- Pregnant or breast-feeding female patients. Negative pregnancy test at study is mandatory for female patients of childbearing potential;
- Unwillingness or not capable to use effective means of anticonception (all men and pre-menopausal women).

9 Treatment

9.1 Salvage therapy

9.1.1 Treatment schedule

Following registration patients are treated with at least three courses of R-DHAP q 4 weeks while a donor search is initiated.

Agent	Dose/day	Route of administration	Days
Dexamethasone	40 mg	p.o. or i.v.	1, 2, 3, 4
Cisplatin	100 mg/m^2	24 hrs continuous infusion i.v.	1
Cytarabine	2 g/m^2 q 12 hrs (2 doses)	3 hrs infusion for every administration of 2 g/m^2	2
Rituximab	375 mg/m^2 course 1 only 500 mg/m^2 course 2 - 6	i.v.	1

Cytarabine to be dissolved in 200 ml NaCl 0.9%.

Prophylactic antibacterial antibiotics should be administered during chemotherapy; the prescribed medication is according to local policy.

9.1.2 Special management in conjunction with DHAP

- Prophylactic antibacterial antibiotics should be administered during chemotherapy; the prescribed medication is according to local policy. Hyperhydration during DHAP might

consist of 1000 ml saline 0.9% per 9 hours during 45 hours starting 6 hours prior to the start of cisplatin. The 1 liter saline 0.9% will also contain 15 mmol/l KCl, 15 mg/l magnesium sulfate and 5 mg/l furosemide The fluid balance will be noted every 3 hours. Diuresis should be at least 300 ml every 3 hours. If indicated, diuresis should be enforced employing furosemide.

- Especially for the first cycle precautions to prevent tumor-lysis syndrome, including the use of allopurinol, are strongly recommended.
- The dose of cisplatin will be adapted to the creatinine clearance:

Creatinine clearance (ml/min)	Modification cisplatin
> 60	none
40 - 60	25 % reduction
< 40	delete cisplatin

9.1.3 Special management Rituximab administration

Antibody infusions may be given to patients in an outpatient clinic setting or following hospital admission as an inpatient. A peripheral or central intravenous (IV) line will be established. Vital signs (blood pressure, pulse, respiration, temperature) should be monitored every 15 minutes during the first hour or until stable and then hourly until the infusion is discontinued and vital signs are stable. Premedication with paracetamol and/or antihistaminics (e.g. clemastine) is allowed. The initial dose rate should be 25 mg/hr for the first hour in case of detectable circulating tumor cells (>10 x 10^9/l), and 50 mg/hr when less tumor cells circulate. If no adverse event is seen, the dose rate may be escalated in 30 minutes intervals with increment steps of 50 mg/hr, to a maximum of 400 mg/hr. Patients may experience transient fever, rigors, shortness of breath and/or hypotension with infusion of rituximab. When these adverse events are noted, antibody infusion should be temporarily discontinued, the patient should be observed and the severity of the adverse events should be evaluated. The patient should be treated according to the best available local practices and procedures. Following observation, if the patient's symptoms improve, the infusion should be continued, initially, at ½ the previous rate. Following the antibody infusion, the IV line should be kept open for medications. If there are no complications, the IV line may be discontinued after one hour of observation. If complications occur during infusion, the patient should be observed for two hours after the completion of the infusion. If no adverse event is seen with the previous infusion, the infusion rate at the start of following infusions can be increased to 100 mg/hr and if no further adverse event is observed the infusion rate can be increased with 30 minutes intervals with increment steps of 50 mg/hr to a maximum of 400 mg/hr.

9.2 Donor search

Following registration a HLA-identical donor search must be initiated as soon as possible, first among siblings and second in the world donor bank. In order to avoid inappropriate delay in case no suitable sibling is present, high-resolution HLA typing should be performed immediately after registration enabling a more rapid MUD search. A suitable MUD has at least a 7/8 allele match. In case no HLA-identical sibling is available, patients are referred to one of the MUD centres participating in the study as soon as possible.

9.3 Eligibility criteria for transplantation

9.3.1 Inclusion criteria

- Responsive or stable disease after at least three courses R-DHAP, and
- Availability of HLA-identical family donor (except monozygotic twin) or at least 7/8 allele matched unrelated donor, and
- WHO performance status ≤ 2 (see appendix E), and
- HCT-CI ≤ 2 (see appendix F).

9.3.2 Exclusion criteria

- Severe cardiovascular disease (arrhythmias requiring chronic treatment, congestive heart failure or symptomatic ischemic heart disease);
- Severe pulmonary dysfunction (CTCAE grade III-IV, see appendix D);
- Severe neurological or psychiatric disease;
- Significant hepatic dysfunction (serum bilirubin or transaminases ≥ 3 times upper limit of normal) except when caused by leukemic infiltration;
- Significant renal dysfunction (creatinine clearance < 30 ml/min after rehydration);
- Active, uncontrolled infections;
- Any psychological, familial, sociological and geographical condition potentially hampering compliance with the study protocol and follow-up schedule;

9.4 Conditioning

All patients without progression after at least three courses of R-DHAP having a HLA-identical related or matched unrelated donor proceed to transplantation.

Conditioning for alloSCT

Agent	Dose/day	Route	Days
Fludarabine	30 mg/m^2	i.v.	-4, -3, -2
Total Body Irradiation (TBI)*	2.0 Gy at 10-20 cGy/min.		0
PBSC infusion**		i.v.	0

* TBI to be administered between 11.00 and 14.00 hrs to avoid interference with immunosuppressive drugs

** Additional measures: In case of major ABO blood group incompatibility and a high load of red blood cells (>200x10^9), and/or an anti-A or anti-B titer of ≤1/16:

- prehydration 1L NaCl 0.9% over 4 hrs
- antihistamines, e.g. clemastine 2 mg IV before infusion of stem cells
- infuse stem cells slowly starting with 1 ml/min., to be doubled after 30 min. and then further increased to 3 ml/min. if no adverse reactions occur.

9.5 Donor peripheral blood stem cell (PBSC) collection

Day -5: start G-CSF 5 µg/kg s.c. b.i.d.
Day -1 and/or day 0: leucapheresis aimed at harvesting 5x10^6 CD34$^+$ PBSC/kg body weight of the recipient.
If the stem cell harvest provides an insufficient number of PBSC, continue G-CSF b.i.d. and repeat apheresis daily as long as necessary; consider doubling the dose of G-CSF.

9.6 Special management orders during and after RIC allo-SCT

For GVHD prophylaxis standard Cyclopsorin A (starting Day -3) and Mycophenol (MMF or Myfortic, starting Day 0) will be given.
Prophylaxis against bacterial and viral infections, and Pneumocystis carinii pneumonitis will be performed according to local practice. Monitoring of CMV and EBV will be performed following standard procedures and pre-emptive therapeutic intervention will be initiated when appropriate.

9.7 Additional treatment after transplantation

Serial measurements for residual disease or progression should be made as specified under 11. In general, evaluations are at three months intervals. The interval is two months in case of earlier than scheduled withdrawal of immunosuppression or DLI because of progressive clinical progression or MRD.

- no change in planned immunosuppression schedule nor DLI are indicated for insufficient engraftment or mixed chimerism;
- in case of clinical or MRD progression, earlier withdrawal of immunosuppression or DLI is applied in the absence of GVHD, as specified in the following tables. Subsequent DLI are administered after at least two months interval and in the absence of GVHD when appropriate (see following tables).
- The doses of DLI are:
  - first DLI $1x10^7$ $CD3^+$/kg
  - second DLI $5x10^7$ $CD3^+$/kg
  - third DLI $10x10^7$ $CD3^+$/kg

A: protocol treatment at three months post SCT

disease status* at 3 mo post SCT compared with at SCT	**protocol treatment**	**next evaluation at*** **
PD / PAPR*	off protocol@; stop IS recommended	2 months (recommended)@ (C)
no change (in case of previous SD, PR or NPR)*	no change in scheduled IS	3 months (B)
responsive disease	no change in scheduled IS	3 months (B)
CR, ≥2 log increased MRD	stop IS	2 months (C)
CR, <2 log increased MRD	no change in scheduled IS	3 months (B)
CFCR*,**	no change in scheduled IS	3 months (B)

* for abbreviations see appendix C (response criteria for CLL)

** measured in PB (in BM only if PB is negative)

*** for (B) or (C): see table B or C respectively

IS: immunosuppression

@ because data of patients who went off-protocol due to PD / PAPR will also be actively collected during follow-up, it is recommended to do 2-3 monthly evaluations, depending on response after off-protocol treatment, until 2 years after transplantation.

B: Protocol treatment at three-monthly evaluation points from six months post SCT on (until 2 years after transplantation).

disease status to be compared with the best obtained response after SCT*	**protocol treatment**	**next evaluation at*****
PD / PAPR*	off protocol[@]; DLI recommended	2 months (recommended)[@] (C)
no change (in case of previous SD, PR or NPR)*	no treatment	2 months (C)
responsive disease	no treatment	3 months (B)
CR, ≥2 log increased MRD**	DLI	2 months (C)
CR, <2 log increased MRD**	no treatment	3 months (B)
CFCR*,**	no treatment	3 months (B)

* for abbreviations see appendix C (response criteria for CLL)

** measured in PB (in BM only if PB is negative)

*** for (B) or (C): see table B or C respectively

[@] because data of patients who went off-protocol due to PD / PAPR will also be actively collected during follow-up, it is recommended to do 2-3 monthly evaluations, depending on response after off-protocol treatment, until 2 years after transplantation

C: Protocol treatment at two-monthly evaluations after either accelerated cessation of immunosuppression or after DLI (until 2 years after transplantation)

disease status to be compared with the previous evaluation point*	**protocol treatment**	**next evaluation at*****
PD / PAPR*	off protocol[@]; DLI recommended	2 months (recommended)[@] (C)
no change (in case of previous SD, PR or NPR)*	DLI	2 months (C)
responsive disease	no treatment	3 months (C)
CR, <1 log decreased MRD**	DLI	2 months (C)
CR, ≥1 log decreased MRD**	no treatment	3 months (C)
CFCR*,**	no treatment	3 months (B)

* for abbreviations see appendix C (response criteria for CLL)

** measured in PB (in BM only if PB is negative)

*** for (B) or (C): see table B or C respectively

[@] because data of patients who went off-protocol due to PD / PAPR will also be actively collected during follow-up, it is recommended to do 2-3 monthly evaluations depending on response after off-protocol treatment, until 2 years after transplantation

10 End of protocol treatment

Reasons for going off protocol treatment are:

- Completion of protocol treatment (this includes a period of two years after transplantation or 2 years after last R-DHAP in case of no donor available);
- Progression after three courses of R-DHAP;
- Progression or relapse, excluding increase of MRD, after SCT (see *recommended* follow-up treatment);
- Excessive toxicity (including toxic death) requiring permanent discontinuation of treatment;
- No compliance of the patient (especially refusal to continue treatment);
- Death due to any cause;
- Major protocol violation, defined as other CLL treatment given than as described in paragraph 9, or not meeting eligibility criteria for inclusion as described in paragraph 8.1.

11 Required clinical evaluations

11.1 Time of clinical evaluations

Disease specific:

- at entry;
- after 3 courses of R-DHAP;
- at 3 months after SCT and thereafter at 3 or 2 months intervals until 24 months, depending on disease status (see 9.9);
- in case no donor was found: after the last R-DHAP and then at 3, 6, 9, 12 and 24 months or until progression.

Chimerism:

- at 1, 2, 3, 6, 12 and 24 months after SCT

Follow-up:

Follow-up will be planned every 6 months after off protocol until 5 years after registration.

11.2 Required investigations

Required investigations at entry, during treatment and follow up (in case of progression) until 2 years after SCT or 2 years after last R-DHAP in case of no donor available.

				post SCT or after last R_DHAP in case of no donor available (months)					
	At entry	During 1st R-DHAP	After 3 R-DHAP and prior to SCT	1†	2†	3	at 3 (or 2) months intervals§	24	F.U.
Medical history	X		X	X	X	X	X§	X	X
Physical examination	X		X	X	X	X	X§	X	X
Blood tests									
Hematology	X		X	X	X	X	X§	X	X
DAGT	X		X[8]			X[8]	X[8]	X[8]	
Flow cytometry (B-CLL diagnosis)	X[1]								
Flow cytometry (follow-up)			X[3]			X[3]	X[3 §]	X[3]	X[3]
Blood chemistry	X		X	X	X	X	X§	X	X
Tumor lysis parameters		X							
Anti-CMV antibodies	X								
HCT-CI (see appendix F)	X		X						
Bone marrow									
Aspirate (% of lymphocytes)			X[4]			X[4]	X[4 §]	X[4]	
Flow cytometry	X[2]		X[3,4]			X[3,4]	X[3,4 §]	X[3,4]	
Biopsy			X[10]			X[10]	X[10]	X[10]	
Mutational status	X[9]								
FISH (PB or BM)									
17p13 deletion, 11q22-23 deletion, trisomy12, 13q14 deletion	X[1]								
17p13 deletion	X[5]								
PB (or BM) cryopreservation[6]	X		X	X	X	X	X	X	
CT neck, thorax, abdomen and pelvis	X		X			X[7]	X[7 §]	X	X[7]
Chimerism (PB)				X†	X†	X†	X¥†	X†	
ECG	X								

[1] Flow cytometry performed at any time before inclusion to confirm the diagnosis B-CLL according to WHO classification **incorporating at least** CD19, CD5, CD23, kappa and lambda, and preferably also CD3, CD4 and CD8 (for procedure see HOVON website, open studies, HOVON 68, Molecular Guidelines, chapter 3: Diagnostic Flowcytometry); it is obligatory to test the expression of CD20, CD22, CD38,CD79b, CD43 and CD81, to detect possible aberrant expression of one or more antigens to be used in future measurement of MRD.

[2] only if not done on PB

[3] using the MoAb combinations CD19/3/45/14 and CD19/5/kappa/lambda, and in case of CR also CD19/5/20/38, CD19/5/79b/43 and CD19/5/81/22 according to appendix H

[4] only if PB is MRD negative

[5] only in case no 17p13 deletion was detected earlier in the course of the disease, because 17p13 deletions do not necessarily need to be present at diagnosis but can arise during the course of the disease

[6] Viable ficoll separated cells ($1x10^7$/ml in standard freezing medium (RPMI1640 with 30% FCS and 10% DMSO), 1ml per vial) should be procured from blood and bone marrow at diagnosis, and stored in liquid nitrogen for future studies. It is recommended even to store dry pellets ($1x10^7$ cells per vial) in liquid nitrogen. If DNA, RNA or protein has been isolated, excess material should be stored.

[7] in the absence of lymphadenopathy at entry, evaluation with CT may be scheduled at six months intervals from alloSCT on

[8] needs only be repeated if positive at entry, until tested negative once

[9] only if not performed earlier in the disease course; cells can be stored as mentioned under 6 and tested at a later time point

[10] only performed when CR is presumed and MRD is present, to be able to assess the possibility of NPR

[§] in case of progression, stable disease or in case of increasing MRD from day SCT on, these investigations should be performed at two months intervals until MRD negative complete remission, and then at three months intervals

[¥] chimerism is at 6,9, 12, 18 months

[†] only to be done after alloSCT

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)
- Size of enlarged spleen and liver

11.2.3 Hematology

- Hb
- WBC and differential count
- Platelet count
- Reticulocytes
- Direct Antiglobulin test

11.2.4 Blood chemistry

- creatinine
- LDH

11.2.5 Tumor lysis parameters

During the first week of the first course of R-DHAP serial blood samples are tested for:

- creatinine
- potassium
- uric acid
- LDH

11.2.6 Anti-viral antibodies

- CMV

11.2.7 HCT-CI

See appendix F

11.2.8 Chimerism

Chimerism is determined on blood leukocytes by standard methods used in the different centers. If possible, chimerism is also determined on red blood cells.

11.3 Evaluation of response

Evaluation of response is after 3 courses of R-DHAP, at 3 months after transplantation and thereafter at 3 or 2 months intervals until 24 months, depending on disease status (see 9.9). In case patients are transplanted after more than 3 R-DHAP courses due to delayed donor search, evaluation should be performed after the third and after the last R-DHAP course. Assessment of response is described in appendix C.

12 Toxicities

12.1 R-DHAP

All the chemotherapeutic agents used in the protocol cause pancytopenia and can induce septic or hemorrhagic complications. Every center has decennial experience with DHAP in the treatment of relapsed aggressive lymphoma. The treatment may cause tumor lysis in CLL; prophylaxis is strongly recommended and monitoring during the first week is demanded.
Prophylactic antibacterial antibiotics should be administered during chemotherapy; the prescribed medication is according to local policy

12.2 RIC alloSCT

12.2.1 Fludarabine

Fludarabine is a drug used to treat CLL and lymphomas. It has been used in stem cell transplants to reduce the risk of graft rejection. Its main side effects include lowering of blood counts and infections. In early studies some patients who received high doses experienced nerve damage, but in doses used in this study this side effect would not be expected. Hemolytic anemia has occurred in some patients with CLL who received fludarabine, but this has not been reported when used in pre-SCT conditioning for this disease.

12.2.2 Total body irradiation

The dose of TBI used in this protocol is approximately one-sixth of that used in conventional transplant protocols, and severe side effects from the TBI are not expected. TBI may induce non-infectious fever within 12 hours after administration and is self-limiting. TBI has been associated with causing sterility and there is a risk of major genetic damage to any children produced soon after transplantation.

12.2.3 Peripheral blood stem cell transplant (PBSCT)

Side effects include low blood count, infections, bleeding, and failure of the donor stem cells to grow. Supportive care with red cell and platelet transfusions and antibiotic therapy may be necessary. Graft-versus-host disease (inflammation of skin, liver and gastrointestinal system), may also occur and require treatment with immune suppressing drugs. In addition, organ damage may occur as a result of radiation or the treatment with immune suppressing drugs. There is a risk that the patient will reject the donor's PBSC and that donor cells will not be detected after transplant. The

dose of chemotherapy and radiation used directly before PBSCT is not expected to cause permanent marrow suppression in the event that graft rejection occurs.

12.2.4 Graft-versus-Host Disease (GVHD)

The major toxicity associated with infusion of donor PBMC is GVHD. GVHD has occurred in > 50% of patients. Diagnosis of GVHD: Skin involvement will be assessed by biopsy with percentage of body surface area involved recorded. GI symptoms suspicious for GVHD will be evaluated by biopsy as indicated. Acute GVHD and chronic GVHD will be graded according to established criteria (Appendix G).

12.2.5 Cyclosporine

The immediate effects of this drug may include nausea or vomiting when given orally. Other side effects include the possibility of developing high blood pressure (hypertension), shaking of the hands (tremor), increased hair growth and possibly an effect on mental function. These effects are generally reversible upon decreasing the dose of the drug. An occasional patient has had a seizure but it is unclear whether cyclosporine, other drugs, or a combination of drugs was responsible. Some patients given intravenous cyclosporine for the treatment of GVHD experienced painful sensation in hands or feet or both. The pain subsided with the improvement of the GVHD or when the cyclosporine was switched from the intravenous to the oral form. Patients may experience a change of liver or kidney function, in which case, the dose may be reduced or possibly even stopped for a while. This effect on kidneys seems to increase when other drugs which might cause kidney problems are given at the same time, especially certain antibiotics. Occasionally the kidney damage is severe enough to require the use of an artificial kidney machine (hemodialysis). During treatment cyclosporine blood levels will be monitored to determine if there are increased risks of side effects that warrant changing the dose.

12.2.6 Mycophenolate

Mycophenolate can be administered as mofetil (MMF) or sodium (Myfortic). MMF is routinely used for suppressing the immune system and after stem cell transplantation. This drug is reasonably well tolerated. There are a small number of patients who had received solid organ transplants and had reversible fall in their red cell or white cell count while receiving MMF. The blood counts will be watched closely and, if significant decrease is noted, dose adjustments or stopping MMF may be indicated. Other uncommon side effects include nausea, vomiting, diarrhoea, and abdominal discomfort. Cases of intestinal bleeding have also been reported. The abdominal side-effects are

less pronounced with use of Mycophenolate sodium, which showed equal activity in solid organ transplanted patients,[52-55] and is therefore presumed to have equal activity in SCT patients as well.

12.3 Toxicity assessment

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

13 Reporting serious adverse events and SUSARS

13.1 Definitions

Adverse event (AE)

An adverse event (AE) is any untoward medical occurrence in a patient or clinical study subject during protocol treatment. An AE does not necessarily have a causal relationship with the 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 related to the medicinal (investigational) product.

Adverse reaction (AR)

Adverse reactions (AR) are those AEs of which a reasonable causal relationship to any dose administered of the investigational medicinal product and the event is suspected.

Serious adverse event (SAE)

A serious adverse event is defined as any untoward medical occurrence that at any dose results 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
- significant / persistent disability
- a congenital anomaly / birth defect
- any other medically important condition (i.e. important adverse reactions that are not immediately life threatening or do not result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed above)

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 SAE

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 reaction (SUSAR)

All suspected ARs which occur in the trial and that are both unexpected and serious.

Protocol treatment period

The protocol treatment period is defined as the period from registration until 30 days after going off protocol treatment (see chapter 10)

13.2 Reporting of (serious) adverse events

Adverse event

AEs of CTCAE grade >= 2, with the exception of hematotoxicity and GVHD, have to be reported on the CRF and sent to the HOVON Data Center as soon as possible.
All adverse events, with the exception of disease progression, will be reported from the first study-related procedure until 30 days after going off protocol treatment or until the start of subsequent systemic anti-CLL therapy, if earlier. Adverse events occurring after 30 days should also be reported if considered related to study drug.

SAE and Unexpected serious adverse event

During protocol treatment all SAEs must be reported to the HOVON Data Center by fax **within 24 hours of the initial observation of the event**, except hospitalizations for:

- a standard procedure for protocol therapy administration. Hospitalization or prolonged hospitalization for a complication of therapy administration will be reported as a Serious Adverse Event.
- the administration of blood or platelet transfusion. Hospitalization or prolonged hospitalization for a complication of such transfusion remains a reportable serious adverse event.
- a procedure for protocol/disease-related investigations (e.g., surgery, scans, endoscopy, sampling for laboratory tests, bone marrow sampling). Hospitalization or prolonged hospitalization for a complication of such procedures remains a reportable serious adverse event.

- prolonged hospitalization for technical, practical, or social reasons, in absence of an adverse event.
- a procedure that is planned (i.e., planned prior to starting of treatment on study; must be documented in the source document and the CRF). Prolonged hospitalization for a complication considered to be at least possibly related to the study drug remains a reportable serious adverse event.

All details should be documented on the Serious Adverse Event 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 2 working 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 24 hours after the SAE 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 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 judgment of the causal relationship.

13.3 Processing of serious adverse event reports

The HOVON Data Center will forward all reports within 24 hours of receipt to the principal investigator and the study central data manager. The reporting of an SAE will be the signal for the central data manager to ask the investigator or the responsible local data manager to complete and send as soon as possible all relevant CRFs for the involved patient with details of treatment and outcome.

Any suspected unexpected serious adverse reactions (SUSARs), from any source, will be reported by HOVON Data Center to the investigators, the Ethics Committee that approved the study, and to all applicable Health Authorities within required timelines.

Furthermore HOVON will submit an annual safety report to the regulatory authorities and the Ethics Committee.

14 Endpoints

14.1 Primary endpoint

- progression-free survival from registration with progression defined as time to:
  a. death due to any cause, or
  b. progression or relapse excluding progressive MRD triggering cessation of immunosuppression or DLI whichever comes first

14.2 Secondary endpoints

- incidence and severity of tumor lysis during first course of R-DHAP
- response to three courses of R-DHAP including SD;
- percentage of successful donor searches,
- percentage of patients who received alloSCT
- best response on protocol
- engraftment after alloSCT;
- incidence and severity of acute and chronic GVHD;
- toxicity;
- overall survival (OS) from registration;
- response of MRD to immunomodulation (either accelerated cessation of immunosuppression or DLI)
- response of PD to recommended off-protocol immunomodulation (either accelerated cessation of immunosuppression or DLI)
- disease status at two years after registration;
- PFS and OS after alloSCT

15 Registration

15.1 Regulatory Documentation

The following documents must be provided to the HOVON Data Center before enrollment of the first patient.

By the principal investigator or coordinating investigator for all sites within their country:

- name and address of the (central) Ethical Committee including a current list of the members and their function;
- any other documentation required by local regulations.

By the local investigator for each investigational site:

- HDC Hospital Registration Form, signed and dated by the local investigator;
- a copy of the dated and signed (central) Ethical Committee approval of the protocol, any amendments and informed consent form for the investigational site. This approval must clearly identify the specific protocol by title, number and version date and must be signed by the chairman or authorized designee. The approval must also clearly identify the site(s) the approval applies to;
- a copy of the approved local version of the Patient Information and Informed Consent form;
- approval of participation by site's Board of Directors, if required by local regulations;
- CV of local investigator (dated and signed if not recently provided);
- signed local investigator signature page ;
- local lab accreditation and list of local lab normal values (if not recently provided);
- any other documentation required by local regulations.

15.2 Registration

Eligible patients should be registered before start of treatment. Patients need to be registered at the HOVON Data Center of the Erasmus MC Rotterdam – location Daniel via the Internet via TOP (Trial Online Process; https://www.hdc.hovon.nl/top) or by phone call: +31.10.7041560 or fax +31.10.7041028 Monday through Friday, from 09:00 to 17:00 CET. A logon to TOP can be requested at the HOVON Data Center for participants.

The following information will be requested at registration:
Protocol number
Institution name
Name of caller/responsible investigator
Patient's initials or code
Patient's hospital record number (not obligatory)
Sex
Date of birth
Date written informed consent
Eligibility criteria

All eligibility criteria will be checked with a checklist. Each patient will be given a unique patient study number. Patient study number will be given immediately by TOP or phone and confirmed by fax or email.

16 Data collection

16.1 CRF's

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 trial number, and a unique combination of patient study number (assigned at registration), hospital and patient name code (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 time points. 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.

17 Statistical considerations

17.1 Patient numbers and power considerations

The aim of this study is to assess in adults with CLL aged 18-70 years inclusive, the feasibility and efficacy of reinduction chemotherapy with at least three courses of R-DHAP, followed by RIC alloSCT if no progression has occurred and if a suitable donor is available.
For the sample size calculation, the following assumptions have been made:

- Salvage treatment without an alloSCT is expected to results in 2-year PFS (PFS_{2y}) of about 25% [17,18,41];
- After salvage chemotherapy with R-DHAP at least 80% of the patients will probably have stable disease (SD) or any response;
- For 85-90% of the patients with at least SD after R-DHAP, a suitable donor will be found (30% chance for HLA-identical sibling, 84% chance for MUD[43]);
- RIC alloSCT might improve PFS of patients who actually receive a stem cell transplantation to 60% at 1¾ years from date SCT (= 2 years from start R-DHAP)

An overall 2-year PFS from registration of at least 45% would therefore be expected, which would be a clinically relevant improvement and warrants further investigation in this patient population.
We assume uniform accrual over time, no loss to follow up, and exponentially distributed failure times. In order to reject the null hypothesis H_0: $PFS_{2y} = 0.25$ in favour of the alternative hypothesis H_1: $PFS_{2y} = 0.45$ with power $1 - \beta = 0.80$ (2-sided significance level $\alpha = 0.05$), 41 eligible patients are required.[50] In order to overcome possible dropout, 50 patients will be registered.
With an expected accrual of about 20-30 patients per year, the required number of patients would be achieved in about 2 years. The final analysis will not be performed until a minimum follow up of 1 year is available for all patients.

17.2 Statistical analysis

All main analyses will be according the intention to treat principle, restricted to eligible patients.

The estimated PFS at 2 years from registration along with a 95% confidence interval (CI) will be calculated for all patients using the actuarial method of Kaplan and Meier.[51] In addition, actuarial probabilities of PFS, progression, and death without progression with corresponding standard errors will be calculated using the competing risk method.

The proportion of patients with a suitable donor will be calculated.

The proportion of patients who actually receive an alloSCT will be calculated.

The best response will be determined, during/after R-DHAP as well as after RIC-allo SCT.

The actuarial curves for PFS and OS will be computed using the Kaplan-Meier method and 95% CIs will be constructed. This will be performed for all patients (measured from registration), as well as for the patients who actually receive a RIC allo-SCT (calculated from transplantation).

Acute and chronic graft-versus-host-disease (GVHD) after RIC allo-SCT will be summarized by the maximum grade observed, as well as using actuarial methods to evaluate the time to acute GVHD grade 2-4, time to limited/extensive chronic GVHD, and time to extensive chronic GVHD.

The analysis of toxicity will be done primarily by tabulation of the incidence of side effects and infections with CTCAE grade 2 or more (appendix D), after each R-DHAP separately, for the collective R-DHAP cycles per patient, and after RIC-allo.
Engraftment data will be summarized by calculating the proportion of patients with a donor chimerism (> 90%), a mixed chimerism, and a patient chimerism, in all patients who received a RICallo SCT.
Chimerism will be summarized at specific dates. Samples will be assigned to day 28 if chimerism was determined between days 14-42, to day 56 (43-70), day 84 (71-132), day 180 (133-270) or day 365 (271-455). Per patient only the sample nearest to the specific endpoint will be included. Samples obtained after progression or relapse occurred, will be excluded from the analysis.

17.3 Interim analysis

A formal interim analysis will not be performed, as it is expected that accrual has already been completed when the first patient has actually reached 2-year PFS.
Nevertheless the study will be reconsidered if of the first 20 patients, only 7 or fewer patients (35%) have been transplanted, which is half of the numbers expected, due to any cause, also with emphasis to hematological toxicity of the three R-DHAP courses. The study will be closely monitored, including monitoring of unexpected early mortality after R-DHAP or alloSCT. Excessive mortality may also be a reason to discontinue the trial. Monitoring will be based on the reported SAEs, which are not subject to data delay

17.4 Comparison with German CLL-X2 protocol

The German CLL-X2 protocol studies with an intention-to-treat design the effect of salvage therapy and RIC alloSCT in a similar group of CLL patients. As the inclusion criteria are similar, we (M van Gelder and P Dreger) agreed on the intention to compare the results of both studies with regard to toxicity and efficacy.

18 Ethics

18.1 Accredited ethics committee or Institutional review board

The study protocol and any substantial amendment will be approved by an accredited Ethics Committee or Institutional Review Board. The principal investigator will inform the subjects and the reviewing accredited METC if anything occurs, on the basis of which it appears that the disadvantages of participation may be significantly greater than was foreseen in the research proposal. The study will be suspended pending further review by the accredited METC, except insofar as suspension would jeopardize the subject's health. The investigator will take care that all subjects are kept informed.

18.2 Ethical conduct of the study

The study will be conducted in accordance with the ethical principles of the Declaration of Helsinki (Tokyo 2004), the ICH-GCP Guidelines, the EU directive for Good Clinical Practice (2001/20/EG), and applicable regulatory requirements. The local investigator is responsible for the proper conduct of the study.

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.

20 Publication policy

The final publication of the trial results will be written by the Principal Investigator and 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 and all co-authors for review. After revision by the Data Center, the other co-authors, the manuscript will be sent to a peer reviewed scientific journal.

Authors of the manuscript will include the study coordinator(s), investigators who have included more than 10% of the evaluable patients in the trial (by order of inclusion), the statistician(s) and the HOVON data manager in charge of the trial, and others who have made significant scientific contributions.
The results of the trial will be published in compliance with the CCMO statement on publication policy.

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 Principal Investigator and Study Coordinator(s). This is applicable to any individual patient 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.

Glossary of abbreviations
(in alphabetical order)

AE	Adverse Event
ANC	Absolute Neutrophil Count
ATG	Anti-thymocyteglobulin
ALLO-SCT	Allogeneic Stem Cell Transplantation
ASO-PCR	Allele-specific oligonucleotide Polymerase Chain Reaction
BM	Bone Marrow
CFCR	Complete Flowcytometric Remission
CKTO	Commissie voor Klinisch Toegepast Onderzoek'
CLL	Chronic Lymphocytic Leukemia
CMV	Cytomegalovirus
CNS	Central Nervous System
CR	Complete Remission
CRF	Case Report Form
CSA	Cyclosporine A
CTCAE	Common Terminology Criteria for Adverse Events
DLI	Donor Lymphocyte Infusion
EBMT	European Group for Blood and Marrow Transplantation
EBV	Epstein-Barr virus
ECG	Electrocardiogram
FISH	Fluorescence In Situ Hybridisation
GCP	Good Clinical Practice
G-CSF	Granulocyte-Colony Stimulating Factor
GI	Gastro-intestinal
GVHD	Graft versus Host Disease
GVL	Graft versus Leukemia
HB	Hemoglobin
HCT-CI	Hematopoietic cell transplantation-specific co morbidity index
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte histocompatibility Antigen
HOVON	Dutch-Belgian Hematology-Oncology Cooperative Group
ICH	International Conference on Harmonization of technical requirements for registration of pharmaceuticals for human use
IS	Immunosuppression

ITT	Intention To Treat
IU	International Units
IV	Intravenous
KCl	Potassium chloride
LDH	Lactate Dehydrogenase
METC	Medical Ethical Review Committee
MMF	mycophenolate mofetil
MRD	Minimal Residual Disease
MUD	Matched Unrelated donor
NaCl	Sodium Chloride
NCI	National Cancer Institute
NPR	Nodular Partial remission
ORR	Overall Response Rate
OS	Overall Survival
PAPR	Progression After Previous Remission
PB	Peripheral Blood
PBSC	Peripheral Blood Stem Cell
PD	Progressive Disease
PFS	Progression Free Survival
PO	Per Os
PR	Partial Reponse
R-DHAP	Rituximab- Dexamethason Cytarabin (High dose Ara-C) Cisplatin (Platinol)
RIC	Reduced Intensity Conditioning
SAE	Serious Adverse Event
SC	Subcutaneous
SCT	Stem Cell Transplantation
SD	Stable Disease
TBI	Total Body Irridiation
TRM	Treatment Related Mortality
ULN	Upper Limit of Normal
WHO	World Health Organization
WMO	Wet Medisch-Wetenschappelijk Onderzoek met mensen

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A. NCI criteria for active CLL[49]

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. Massive (i.e., > 6 cm below the left costal margin) or progressive splenomegaly
4. Massive nodes or clusters (i.e., > 10 cm in longest diameter) or progressive lymphadenopathy
5. 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.

The following adjustment is made for this trial: clinical autoimmune cytopenia 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 femora's, involvement of both groins counts as one area)
- splenomegaly
- hepatomegaly

C. Response criterial for CLL

NCI criteria[49] with adjustments conform new international guidelines in preparation

Complete flowcytometric remission (CFCR) (adjustment)

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

- all the criteria for CR are met (see below);
- undetectable (<0.01%) B-CLL cells by flowcytometry in blood and bone marrow samples (see appendices G and H).

Complete remission (CR)

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

- absence of lymphadenopathy;
- absence of hepatomegaly;
- absence of splenomegaly;
- absence of constitutional symptoms (see appendix A criteria 1a, 1b, 1c and 1d);
- normal blood counts defined as:
  - polymorphonuclear leukocyte count ≥ 1.5 x 10^9/l;
  - platelet count > 100 x 10^9/l;
  - untransfused hemoglobin > 6.8 mmol/l (> 11 g/dl);
  - bone marrow < 30 % lymphocytes.

CR does not require the absence of B-CLL cells detectable by flowcytometry.

Nodular partial remission (NPR)

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

- all the criteria for CR are met (see above);
- but with persisting nodules in bone marrow biopsy.

Partial response (PR)

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

- ≥ 50 % decrease in peripheral blood lymphocyte count;
- one or more of the following criteria:
  - ≥ 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);
- one or more of the following criteria:
  - 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 > 6.8 mmol/l (> 11 g/dl) (or 50 % increase compared to baseline).

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/protocolDevelopment/electronic_applications/ctc.htm
http://www.hovon.nl (under Studies > General information about studies)

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. Hematopoietic cell transplantation-specific co morbidity index (HCT-CI)

Definitions of comorbidities included in the new HCT-CI and HCT-CI scores[57]

Co morbidity	**Definitions of co morbidities included in the new HCT-CI**	**HCT-CI weighted scores**
Arrhythmia	Atrial fibrillation or flutter, sick sinus syndrome, or ventricular arrhythmias	1
Cardiac	Coronary artery disease, congestive heart failure, myocardial infarction, or EF ≤50%	1
Inflammatory bowel disease	Crohn disease or ulcerative colitis	1
Diabetes	Requiring treatment with insulin or oral hypoglycemics but not diet alone	1
Cerebrovascular disease	Transient ischemic attack or cerebrovascular accident	1
Psychiatric disturbance	Depression or anxiety requiring psychiatric consult or treatment	1
Hepatic, mild	Chronic hepatitis, bilirubin > ULN to 1.5 x ULN, or AST/ALT > ULN to 2.5 x ULN	1
Obesity	Patients with a body mass index > 35 kg/m^2	1
Infection	Requiring continuation of antimicrobial treatment after day 0	1
Rheumatologic	SLE, RA, polymyositis, mixed CTD, or polymyalgia rheumatica	2
Peptic ulcer	Requiring treatment	2
Moderate/severe renal	Serum creatinine > 175 μmol/l, on dialysis, or prior renal transplantation	2
Moderate pulmonary	DLco and/or FEV_1 66%-80% or dyspnea on slight activity	2
Prior solid tumor	Treated at any time point in the patient's past history, excluding nonmelanoma skin cancer	3

Heart valve disease	Except mitral valve prolapse	3
Severe pulmonary	DLco and/or FEV_1 ≤65% or dyspnea at rest or requiring oxygen	3
Moderate/severe hepatic	Liver cirrhosis, bilirubin > 1.5 x ULN, or AST/ALT > 2.5 x ULN	3

EF: ejection fraction; ULN: upper limit of normal; SLE: systemic lupus erythmatosis;
RA: rheumatoid arthritis; CTD: connective tissue disease; Dlco: diffusion capacity of carbon monoxide.

§ One or more vessel-coronary artery stenosis requiring medical treatment, stent, or bypass graft.

G. Grading of GVHD

Acute GVHD

Severity of organ involvement

Skin +1 maculopapular eruption involving less than 25% of the body surface
+2 maculopapular eruption involving 25-50% of the body surface
+3 generalized erythroderma
+4 generalized erythroderma with bullous formation and often with desquamation

Liver +1 moderate increase in AST* (150-170 IU) and bilirubin (20-40 µmol/l)
+2 bilirubin rise 40-75 µmol/l with or without an increase in AST
+3 bilirubin rise 75-200 µmol/l with or without an increase in AST
+4 bilirubin rise to > 200 µmol/l with or without an increase in AST

GI
Diarrhea, nausea and vomiting graded +1 to +4 in severity
The severity of GI involvement is assigned to the most severe involvement noted
Diarrhea
+1 > 500 ml of stool/day
+2 > 1000 ml of stool/day
+3 > 1500 ml of stool/day
+4 > 2000 ml of stool/day

* increases in AST temporally related to either the onset or worsening of the skin rash

Severity of acute GVHD

Grade I +1 to +2 skin rash
no GI involvement
no more than +1 liver involvement
no decrease in performance

Grade II +1 to +3 skin rash
+1 to +2 GI involvement and/or
+1 to +2 liver involvement
mild decrease in performance

Grade III +2 to +4 skin rash and
+2 to +4 GI involvement with or without +2 to +4 liver involvement
marked decrease in performance with or without fever

Grade IV pattern and severity of GVHD similar to grade III with extreme constitutional symptoms

Chronic GVHD

Limited	Localized skin involvement and/or liver function abnormalities
Extensive	Generalized skin involvement, or localized skin involvement and/or liver function abnormalities + other organ involvements

H. Minimal Residual Disease Measurement by flowcytometry of blood and/or bone marrow

MRD is only measured in patients in CR.

Before antibodies are added, PBNMC are ammonium chloride red cell lysed (10 fold excess) for 10 minutes, centrifuged 5 minutes at 300g and then washed twice in 15ml buffered saline solution. After antibodies incubation cells are treated with FACSlyse according to manufacturer's protocol (BDIS, www.bdbiosciences.com)

At least $1x10^6$ leukocytes are incubated with antibodies; at least 500.000 leukocytes are acquired to optimally achieve a limit of detection of 0.01%.[42]

ANTIBODY COMBINATIONS (TUBE NUMBERS)

tube no.	FL1	FL2	FL3	FL4
1	**CD45** (2D1)	**CD14**	**CD19** PerCP-Cy5.5, or PE-Cy5.5[€])	**CD3** (SK7)
2	**kappa** (TB28-2)	**lambda** (1-155-2)	**CD19**	**CD5** (L17F12)
3	**CD20** (B9E9*, or L27)	**CD38** (HB7)	**CD19**	**CD5**
4	**CD81** (JS-81)[§]	**CD22** (S-HCL-1, or HIB22[§])	**CD19**	**CD5**
5	**CD43** (L10[€], 1G10[§])	**CD79b** (CB3-1[&], SN8[€])	**CD19**	**CD5**

clones in brackets; most antibodies are from BDIS (BD Immunocytometry Systems), some from Immunotech (*), from BD Pharmingen (§), from Caltag Laboratories (€) or from Beckman Coulter/Immunotech (&)

MRD is absent when the percentage of CD19+ cells is < 0.01%
In all other cases MRD must be enumerated. Gating and analysis should be performed as described in the SOP MRD measurement and analysis (see HOVON website) to obtain the lowest possible inter-laboratory variability and false-postivity rate.[42]