Efficacy and safety of first-line therapy with chlorambucil, rituximab and lenalidomide (Revlimid®) (CR²) in elderly patients and young frail patients with advanced Chronic Lymphocytic Leukemia (CLL): a phase I/II trial

PROTOCOL

Principal Investigator : A.P. Kater

Sponsor : HOVON

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By my signature, I agree to personally supervise the conduct of this study in my affiliation 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

Part I

**Induction 1**
6 cycles, q 28 days

| Level 0 | Chlorambucil: 5 mg/m², days 1-7 | Rituximab cycle I: 375 mg/m², day 1 |
| | | cycle II-VI: 500 mg/m², day 1 |
| | Lenalidomide: cycle I: 2.5 mg, day 9-28 |
| | cycle II: 5 mg*, day 1-28 |
| | cycle III-VI: 10 mg* day 1-28 |

| Level 1 | Chlorambucil: 7 mg/m², days 1-7 | Rituximab cycle I: 375 mg/m², day 1 |
| | | cycle II-VI: 500 mg/m², day 1 |
| | Lenalidomide: cycle I: 2.5 mg, day 9-28 |
| | cycle II: 5 mg*, day 1-28 |
| | cycle III-VI: 10 mg* day 1-28 |

| Level 2 | Chlorambucil: 10 mg/m², days 1-7 | Rituximab cycle I: 375 mg/m², day 1 |
| | | cycle II-VI: 500 mg/m², day 1 |
| | Lenalidomide: cycle I: 2.5 mg, day 9-28 |
| | cycle II: 5 mg**, day 1-28 |
| | cycle III-VI: 10 mg*, day 1-28 |

Part II

**Induction 1**
6 cycles, q 28 days

| Chlorambucil: 7 mg/m², days 1-7 |
| Rituximab cycle I: 375 mg/m², day 1 |
| cycle II-VI: 500 mg/m², day 1 |
| Lenalidomide: cycle I: 2.5 mg, day 9-28 |
| cycle II: 5 mg*, day 1-28 |
| cycle III-VI: 10 mg*, day 1-28 |

* * Dose reduction of Lenalidomide in case of toxicity according to chapter 9.2.2.*
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### 3 Synopsis

**Rationale**
For elderly and young FCR unfit patients, response rates and duration of chlorambucil monotherapy is limited. Addition of rituximab improves ORR, but not CR rate. Lenalidomide is active in CLL mainly by interaction in crosstalk between the microenvironment and leukemic cells. Hypothesis: Addition of lenalidomide to chlorambucil and rituximab will result in better response rates with acceptable toxicity.

**Study objectives**
To investigate the feasibility and efficacy of a maximum of 6 cycles of Chlorambucil and Rituximab plus Lenalidomide at the RDL.

**Study design**
Phase I/II, prospective, multicenter

**Patient population**
Elderly (65 years – 80 years, inclusive) patients and patients 18 - 64 years, inclusive, with CIRS \geq 7 with advanced previously untreated Chronic Lymphocytic Leukemia

**Intervention**
Patients will be treated with 6 cycles of chlorambucil, rituximab, lenalidomide followed by 6 cycles of lenalidomide monotherapy

**Duration of treatment**
Total duration of treatment is 12 months. All patients will be followed until 5 years after registration

**Target number of patients**
Part I: 12
Part II: 50

**Expected duration of accrual**
2 years

**Main study endpoints**
**Part I:**
Dose-limiting toxicity (DLT), maximum tolerated dose (MTD) and recommended part II dose (RDL) of Chlorambucil when combined with Rituximab and Lenalidomide.

**Part II:**
CR+PR rate

**Benefit and nature and extent of the burden and risks associated with participation**
Elderly patients with symptomatic CLL experience severe disease-specific morbidity. Current treatment for this patient group has limited efficacy due to relative low response rates. The hypothesis is that combination treatment will
induce better responses resulting in prolonged disease free survival. Risks for the patient relate to drug specific side-effects, in particular tumor flare reaction, tumor lysis and clinical relevant neutropenia.

Planned interim analysis and DSMB (if applicable)

**Part I:**
After first and second cohort of 6 patients

**Part II:**
After at least 15 patients had at least 3 cycles of therapy.
4 Investigators and study administrative structure

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5 Introduction and rationale

5.1 Chronic lymphocytic leukemia

Chronic Lymphocytic Leukemia (CLL) is a chronic leukemia in which peripheral, clonal B-cells progressively accumulate. The disease is a hematological neoplasm of unknown etiology, characterized by monomorphous small, round B-lymphocytes in the peripheral blood, bone marrow, and lymph nodes that aberrantly co-express T-cell (CD5+) and B-cell (CD19+, CD23+) cell surface markers, with a low expression of CD20. Over the past decade, new information suggests CLL originates from antigen-stimulated mature B-lymphocytes, which either avoid cell death through the effect of external signals, or die by apoptosis, but are replenished by proliferating precursor cells.

CLL follows a variable clinical course with overall survival times ranging from months to decades. Median survival from diagnosis is approximately 10 years in the overall CLL population, but is only 18 months for patients with advanced disease and to 9-13 months for CLL cases refractory to fludarabine.

Traditional clinical staging systems devised by Rai and Binet are the simplest and still best validated means of assessing prognosis for CLL patients, however, there is substantial heterogeneity in the course of the disease within defined stages. In recent years molecular and cellular markers have been correlated with disease aggressiveness. These allow further stratification of subjects into risk groups such as: abnormal cytogenetics, CD38, ZAP-70, beta-2-microglobulin, IgVH mutational status (reviewed by Kay, et al.). So far, unfortunately these parameters have only limited use in determining when and what type of therapy to use. One exception is that p53 deletion was shown to predict for non-response to purine analogues like fludarabine and for poor clinical outcome (reviewed by Kater and Tonino).

The prevalence of CLL rapidly increases with age. The median age at presentation is 65 years, with an incidence of < 1 per 100,000 in persons under 40 years of age, rising to 19 (females) and 36 (males) per 100,000 in persons older than 75 years in the Netherlands. Approximately 50% of patients are initially asymptomatic and are observed for several years before treatment is needed, therefore more than half of patients who finally require therapy are older than 70 years.

5.2 Current treatment for elderly and frail CLL patients

Combination chemotherapy consisting of fludarabine/cyclophosphamide in combination with rituximab has resulted in significant improvement in the outcome of patients with CLL. Recent trials on this combination treatment indicated that the quality of the response highly correlates with disease outcome. Despite this increased efficacy, fludarabine containing regimens generally have an unfavourable toxicity profile in the elderly patients. Keating et al. observed shorter survival of
patients >70 years of age compared with younger patients receiving fludarabine regimens. Shvidel et al. reported on their experience with fludarabine-based chemotherapy in 82 high-risk previously treated CLL patients, including 32 patients >65 years of age. They found that excessive toxicity in elderly patients prevented the completion of the planned treatment in two-thirds of cases. Similar observations were reported with the use of other purine-analogues.

So far, single agent chlorambucil is still widely used as first-line treatment in this patient group. In a head-to-head comparison of chlorambucil and fludarabine in elderly patients progression-free and overall survival was not superior for patients treated with fludarabine. Although treatment with fludarabine resulted in higher response rates, chlorambucil treatment proved to be safer with a lower incidence of therapy-related toxicity than fludarabine.

Despite clear advantages of chlorambucil in the elderly and frail population such as low toxicity, low costs and oral administration, as single agent it is not a highly effective drug in CLL. In most studies the overall response rate (ORR) of chlorambucil is around 50% (31-72%) with virtually no complete remissions (CR) and as a consequence progression-free survival is less than 1.5 years (8.3 – 20 months).

In an attempt to improve these results, the UK CLL study group currently runs a phase II trial on the combination of chlorambucil with rituximab for elderly and unfit untreated patients. First interim analysis (n=47, median age 70 years) indicated that combination treatment results in improved efficacy (ORR of 84%, CR <5%) with acceptable toxicity. Despite still suboptimal responses it seems very likely that in many countries addition of rituximab to chlorambucil will become standard for the elderly patients.

5.3 Lenalidomide

Lenalidomide (REVLIMID®; Celgene Corp., NJ, USA) is a member of a class of pharmaceutical compounds known as immunomodulatory drugs (IMiDs). It offers potential benefit over the first commercially available IMiD, thalidomide, in terms of both safety and efficacy in human subjects.

The key to its therapeutic potential lies in the fact that it has multiple mechanisms of action, which act to produce both anti-inflammatory and anti-tumor effects. These effects are thought to be contextual in that they depend on both the cell type and the triggering stimulus. To date, lenalidomide has been associated with TNF-α inhibitory, T cell co-stimulatory, and anti-angiogenic activities.

Lenalidomide is marketed in the United States and Europe for the treatment of patients with transfusion-dependent anemia due to low- or intermediate-1-risk Myelodysplastic Syndrome (MDS) associated with a deletion 5q cytogenetic abnormality with or without additional cytogenetic abnormalities and in combination for dexamethasone for treatment of multiple myeloma (MM) patients who have received at least one prior therapy. Lenalidomide is also marketed in the European Union.
for use in combination with dexamethasone as a treatment for patients with multiple myeloma who have received at least one prior therapy.

Lenalidomide is being investigated as treatment for various oncologic indications, including multiple myeloma, non-Hodgkin’s lymphoma, and solid tumors. It is also being explored as a treatment for inflammatory conditions, including chronic regional pain syndrome. While many of the studies are ongoing, results from controlled and uncontrolled studies in subjects with MDS and MM are available.

**Clinical studies in MDS**

Lenalidomide has been investigated in subjects with MDS in three Phase II studies. Results from all three studies indicated that lenalidomide is a safe and effective treatment for subjects with lower intermediate-1-risk MDS as well as in MDS with an associated del 5 (q31-33) cytogenetic abnormality. Results from the MDS-003 study (List, 2006), in MDS with an associated del 5 (q31-33) cytogenetic abnormality, showed transfusion independence in 67 % of the subjects. Median duration from the date when red blood cell (RBC) transfusion independence was first declared (the last day of the 56-day RBC transfusion-free period) to the date when an additional transfusion was received after the 56-day transfusion-free period among responders was 44 weeks. Cytogenetic response was achieved in approximately 75 % of subjects with complete cytogenetic response noted in more than 50 % of the cytogenetic responders. Grade 3/4 neutropenia and thrombocytopenia are the most common AEs associated with the use of lenalidomide, but are manageable with dose reductions and/or interruptions.

**Clinical studies in MM**

Two pivotal multi-centre, randomized, placebo-controlled studies comparing lenalidomide plus dexamethasone to dexamethasone in relapsed or refractory MM subjects determined that combination therapy with lenalidomide and high-dose pulse dexamethasone is significantly more effective than high-dose pulse dexamethasone therapy alone as judged by time-to-progression (TTP), progression-free-survival (PFS), time-to-treatment failure (TTF), and the proportion of subjects who responded to therapy. The results also demonstrated that the lenalidomide/dexamethasone combination had a favourable safety profile in subjects with relapsed or refractory multiple myeloma and that the addition of lenalidomide to dexamethasone was accomplished with only a modest increase in toxicity. Overall, the results of this study demonstrated a highly favourable benefit-to-risk ratio for lenalidomide/dexamethasone as treatment for subjects with relapsed or refractory multiple myeloma.
5.3.1 Lenalidomide in Chronic Lymphocytic Leukemia

5.3.1.1 Background

The cellular interplay between the microenvironment and the malignant CLL clone and the decreased sensitivity of CLL cells to apoptotic signal is thought to play an important role in the pathophysiology and development of CLL disease (reviewed by Caligaris-Cappio and Ghia\(^ {27}\)). CLL cells can secrete various pro survival cytokines (TNF-\( \alpha \), IL-6 and VEGF) and modulate their receptor expression in a paracrine/autocrine growth loop manner\(^ {28}\). Lenalidomide has been reported to inhibit TNF-\( \alpha \) production, modulate cytokine production and also to have immunomodulatory properties that are thought to be critical for the activity of this drug in chronic lymphocytic leukemia\(^ {29}\). Lenalidomide co-stimulates T-cells and enhances anti tumor immunity, which is mediated by T-helper-1 type cytokines, such as interferon-\( \gamma \) and interleukin-2 (IL-2); it also enhances other innate immune cells such as natural killer cells, which can increase tumor cell death\(^ {30}\).

Additional experiments performed in vitro confirmed the ability of lenalidomide to increase NK-cell mediated killing of primary CLL tumor cells\(^ {31}\). Co-treatment of CLL cells and NK cells with lenalidomide was shown to enhance rituximab-mediated antibody-dependent cellular cytotoxicity (ADCC) in vitro, however a greater enhancement was observed when CLL cells were not pre-treated with lenalidomide suggesting that alternative sequencing strategies might be also beneficial when combining the two drugs in a clinical setting\(^ {32}\). Interestingly, lenalidomide also synergized with rituximab to promote animal survival in a severe combined immunodeficiency (SCID) mouse-disseminated lymphoma xenograph model. Lenalidomide treatment enhanced rituximab’s NK cell anti tumor activity, and resulted in the in vivo expansion of murine NK cells, suggesting a role for the co-stimulatory properties of this agent in this model\(^ {33}\). The potential ability of lenalidomide to enhance immune cell recognition in CLL was also reported in a recent work by Ramsay et al.\(^ {34}\) demonstrating that lenalidomide can modulate the actin cytoskeleton and improve the defect in CLL cells and T-cells immune synapse formation and function. One potential mechanism for the observed enhancement of immune cell recognition in CLL is the ability of lenalidomide to induce expression of functional CD154 antigen on CLL cells both in vitro and in vivo. This occurs via enhanced CD154 transcription mediated by Nuclear Factor-xB (NF-xB) and also through phosphoinositide-3-kinase pathway-dependent stabilization of CD154 mRNA. As reviewed by Kater, et al.\(^ {35}\) CD154-positive CLL cells up-regulate BID, DR5, and p73, become sensitized to tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-mediated apoptosis, and promote co stimulatory activation of normal B cells to produce antibodies.

Despite these recent observations, the exact mechanism of action of lenalidomide in CLL remains highly uncertain. Additional work to better elucidate lenalidomide’s mechanism of action in CLL and its
ability to modulate the cellular interplay between the microenvironment and the malignant CLL clone will be part of the current study.

5.3.1.2 Single agent lenalidomide

5.3.1.2.1 Relapsed/refractory CLL

Initial clinical studies were conducted with thalidomide, a less potent immune modulator than lenalidomide also capable of inhibiting TNF-α, that has known anti-angiogenic properties. Thalidomide was studied in combination with fludarabine in previously untreated CLL. Although this study investigated the efficacy and safety of the combination regimen of fludarabine and thalidomide, the design of the study called for the administration of thalidomide alone for the first 7 days of therapy, after which fludarabine was added. Anti tumor effects, manifested as reduction on the peripheral blood lymphocyte count, occurred within 7 days of starting thalidomide, prior to institution of fludarabine, suggesting that thalidomide alone has anti-CLL activity. Based on the observations made with thalidomide, single-agent lenalidomide was studied in relapsed/refractory CLL. Chanan-Khan treated patients with relapsed/refractory CLL with lenalidomide (25mg x 21 d q 28 days). Patients with stable disease (SD) or better response were continued on therapy for a maximum of 12 months while those with progressive disease (PD) were to receive monthly rituximab (375mg/m2) added to lenalidomide. Forty-five patients were enrolled. Median age was 64 years (range: 47 to 75 years), and advanced Rai stage (III or IV) disease was noted in 64%. The median number of prior therapies given was three (range: 1 to 10 therapies), with 51% of the patients refractory to fludarabine. Using an intent-to-treat analysis, major responses were seen in 26 patients (57.7%; 95% CI, 32% to 62%) with six patients (13.3%) achieving a CR, including 5 (11%) molecular CRs, and 20 (44.4%) achieving a PR. Anti tumor activity of lenalidomide was evident as early as day 8 of treatment with 24 (70.5%) of 34 patients (34 patients who had day 1 ALC of >5 ×10^9/l and day 8 ALC available) demonstrating a decrease in their peripheral blood absolute lymphocyte count (ALC). Clinical responses (CR or PR) were observed in 40% of the patients with del (11)(q23) adverse cytogenetics and in 40% of patients with bulky disease. The median PFS of all patients enrolled was 19.4 months (range 1.2-31.8).

Fatigue (83%) and flare reaction (tender swelling of lymph nodes and/or rash) (58%) were the most common non-hematological AEs reported. Other important AEs reported were tumor lysis syndrome (5%); Grade 3 and 4 thrombocytopenia (45%), and Grade 3 and 4 neutropenia (70%). Pulmonary embolism was reported in two patients (5%). Ferrajoli treated patients with relapsed/refractory CLL with lenalidomide administered daily for 28 days out of 28 day cycles up to disease progression. All patients received lenalidomide at 10 mg daily for 28 days followed by titration upward by 5 mg increments every 28 days to a maximum dose of 25 mg daily. Forty-four (44) patients have been enrolled and were evaluated for response. The median daily dose of lenalidomide tolerated by the
patients was 10 mg. The median age was 64 years (range: 49 to 86 years), the median number of prior treatments was 5 (range: 1 to 15), and the median β2M at entry was 4.3 mg/dL (1.6 to 10.1). Fifty nine percent of the patients carried unfavourable genomic abnormalities (17p or 11q23 deletions) and 66% had unmutated VH. Twelve patients (27%) were refractory to fludarabine. Responses according to NCI-WG criteria assessed after 3 months of treatment based on an intent-to-treat analysis showed that 14 patients (32%) had achieved a response [3 CR (7%), 1 nodular PR (2%), 10 PR (23%)]. Time to best response was 6 months in 11 patients and 9 months in 3 patients. The median response duration has not been reached. Eleven patients (25%) had stable disease (SD) or clinical improvement and were able to continue on treatment past the third month, and 17 patients (39%) progressed and two patients died of early infectious complication. Thirty two patients (73%) are alive with a median follow up of 14 months. Myelosuppression was the most common toxicity with in the courses, 41% Grade 3 neutropenia and 16% Grade 3 thrombocytopenia. Tumor flare Reaction (TFR) was observed in 12% of the courses (Grade 2 in 10% and Grade 3 in 2%), with a greater incidence (53%) in patients with lymph nodes larger than 5 cm. Grade 3 fatigue and diarrhoea were reported in 1% and 2% of the courses, respectively. TFR was a frequent adverse event reported in both studies and was well managed with non steroidal anti-inflammatory agents (NSAIDs) and/or corticosteroids or in some case narcotic analgesics. In Chanan-Khan’s study 37, out of the 45 patients treated with lenalidomide, no TFR prophylaxis was given to the first 29 patients (Group A). Subsequently, 16 patients received prophylaxis with prednisone 20 mg po qd x 5 days followed by 10 mg po qd x 5 days starting on Day 1 of treatment (Group B). The prophylaxis did not decrease the incidence of TFR (83% [Group A] vs. 81% [Group B]). TFR ≥ grade 2 was seen in 31% of Group A and 6% of Group B. It thus appears that steroid prophylaxis decreases the severity of the reaction, but not the overall incidence. In the group of patients who developed tumor flare, 4 CRs were achieved, all in patients who had experienced ≥ grade 2 tumor flare reaction in comparison to 19 patients with ≤ PR who had experienced a median tumor flare severity grade 1. These preliminary data seem to indicate that patients who develop a more severe flare reaction may also achieve a better remission. However, in Ferrajoli et al. study the occurrence of a tumor flare reaction did not predict for a higher response rate with an overall response of 38% and 34% in patients with and without a TFR).

Based on these initial data in patients with relapsed/refractory CLL, a large multicenter phase 2 study (CLL-001) was initiated to investigate two lenalidomide dose regimens, 10 mg daily and 25 mg x 21 days q 28 days, and confirm the efficacy and safety of lenalidomide in the treatment of relapsed/refractory CLL 38. The study did not include a dose escalation period. After 18 patients were enrolled into the CLL-001 study, four patients developed Tumor Lysis Syndrome (TLS) of varying severity associated with concomitant TFR characterized by severe back and bone pain, with onset during the first 15 days of treatment in the first cycle of treatment. Metabolic abnormalities and/or
renal dysfunction resolved with supportive therapy in 2 patients, however 2 patients died. The CLL-001 protocol was amended into a phase 1/2 study where the safety of several lenalidomide doses was investigated. In this ongoing revised study patients with prior treatment with an alkylating agent and who have failed fludarabine were started on 2.5 mg of lenalidomide daily, followed by slow intra-patient dose escalation to 5 mg after 28 days as tolerated. Doses were then escalated as tolerated by 5 mg every 28 days by initial cohorts of 6 patients, until the maximum tolerated dose escalation level (MTDEL) was defined or a maximum dose of 20 mg daily. Patients were treated until disease progression, and all patients received TLS prophylaxis with hydration starting 3 days prior to lenalidomide treatment and continuing for at least the first 3 cycles. Thirty patients, with a median age of 64 years (range 37–76), have been enrolled on the amended protocol. Patients had a median of 4 prior therapies (range 2–14), with 43% of patients refractory to fludarabine and 20% having had prior alemtuzumab therapy. Among these, 77% of patients had bulky lymphadenopathy. Treatment is continuing in 14 patients, while 16 patients have discontinued treatment (with 7 for lack of therapeutic effect). During therapy, common toxicities included ≥ grade 3 neutropenia [10/30 (33%) at 2.5 mg; 8/19 (42%) at 5 mg; 6/13 (46%) at 10 mg], ≥ grade 3 thrombocytopenia [2/30 (67%) at 2.5 mg daily, 1/19 (5%) at 5 mg; 0/13 (0%) at 10 mg], and tumor flare reaction [TFR; 9/30 (30%) at 2.5 mg; 2/19 (11%) at 5 mg and 1/13 (8%) at 10 mg]. TFR ≥ grade 3 occurred in 3 patients, in two patients at the 2.5 mg dose and in one at the 5 mg dose and was effectively managed with non-steroidal anti-inflammatory drugs, corticosteroids and/or temporary interruption of treatment. On the amended protocol, one case of subclinical TLS (per Cairo-Bishop definition) occurred at the 2.5 mg dose. Study drug was maintained, and electrolyte abnormalities were corrected with adequate oral hydration. The patient was able to continue treatment without TLS recurrence. To date, the median number of days on study is 87 days (range 2–198), the MTDEL has not been reached and escalation up to 15 mg daily dose has been declared tolerable.

5.3.1.2.2 Previously untreated symptomatic CLL

Two studies were conducted in previously untreated symptomatic CLL subjects. Chen treated previously untreated symptomatic CLL subjects with single agent lenalidomide. The starting dose for lenalidomide was initially 10 mg daily with weekly 5 mg dose escalations to the target dose of 25 mg daily x 21 days every 28 day cycle. Toxic events in the first 2 subjects (TLS requiring dialysis; neutropenic sepsis) led to a study halt with DSMB review, and subsequent protocol amendments to reduce both the starting and target doses (2.5 mg and 10 mg, respectively, days 1-21), slow the dose escalation rate (2.5 mg cycle 1, 5 mg cycle 2, 10 mg cycle 3 and thereon), extend allopurinol TLS prophylaxis to a minimum of 3 cycles, and increase frequency of TLS lab monitoring. Deep vein thrombosis (DVT) prophylaxis with low dose aspirin was mandated. Steroids were allowed for management of TFR symptoms as needed. Twenty-five (25) subjects have been enrolled on the
amended protocol. The median age is 60 (range 33-78), 10 subjects (40%) were Rai stage III-IV, bulky nodes were present in 9 subjects (36%), organomegaly in 23 pts (92%). Twenty-three (23) subjects have received at least 1 cycle and are evaluable for toxicity. Ten (10) subjects (43%) developed grade 3-4 neutropenia during at least 1 cycle (at doses 2.5-10 mg) which was the most common cause for dose reductions/interruptions. Six (6) subjects have required intermittent GCSF support (none requiring routine use). Three (3) subjects (13%) developed grade 3-4 thrombocytopenia (without bleeding). The most common non-hematological toxicities were: Fatigue (74%), non-desquamating rash (48%), all were reported as grade 1-2. Infections (43%) were mostly minor non-neutropenic respiratory/sinus/skin infections. TFR occurred in 18 subjects (78%). Although most subjects experienced TFR in the first week on study, many also continued to experience flare symptoms with subsequent cycles (30.6% of all 186 cycles). Most tumor flare symptoms resolved spontaneously but eight subjects required steroids on at least one occasion with prompt resolution. 4 subjects were hospitalized for febrile neutropenia and/or pneumonia. No further TLS has been noted. 17 subjects completed at least 3 cycles and were evaluable for response. 11 subjects (65%) achieved a PR, 6 SD (35%), and none have progressed. Responses were reached at a median of 4 cycles (range 2-15). Although dramatic lymphocyte reductions were seen as early as week 1 (lenalidomide dose 2.5 mg/d), rebound during cycle days 22-28 off-drug were common. Two subjects have withdrawn from the study due to lack of response after 10 cycles (SD) and prolonged grade 3-4 neutropenia and thrombocytopenia, respectively. The median daily tolerated dose is 10 mg with 26% of subjects requiring dose reductions to 5 mg (most due to cytopenias). Ferrajoli 41 used lenalidomide as initial treatment of elderly subjects with CLL. Subjects were eligible for this study if age 65 or older and met requirements for treatment according to the 1996 NCI-WG guidelines. All subjects received lenalidomide at 5 mg daily for the first 56 days. The lenalidomide dose could then be titrated up by 5 mg increments every 28 days to reach a maximum dose of 25 mg daily. Allopurinol 300 mg daily was given from day 1-14 as TLS prophylaxis. Forty-three (43) subjects have been enrolled at time of interim analysis. The median age was 72 years (range 66-85), 18 subjects (42%) had Rai stage III-IV disease and 35 subjects were evaluable for response having received treatment for at least 3 months. 19 subjects (54%) achieved a PR, 14 subjects (40%) had SD and 2 subjects (6%) experienced PD after 4 and 5 months respectively. 39 subjects were evaluable for toxicity having received at least 2 months of treatment. Grade 3-4 neutropenia and/or thrombocytopenia occurred in 10 subjects (26%). Infectious complications were observed in 3 subjects: neutropenic fever in 2 and pneumonia in 1. TFR was observed in 17 subjects (44%) and was limited to grade 1-2 and managed with oral steroids. TLS was not observed. Two (2) subjects discontinued after 6 and 8 months of treatment because of toxicity (rash and fatigue).
5.3.1.3 Lenalidomide combination therapy

To study potential synergy, a phase II study was initiated by the MD Anderson Cancer Center to evaluate the activity of the combination of lenalidomide and rituximab in patients with relapsed CLL, including 27% with fludarabine-refractory disease. Sixty CLL patients who relapsed after purine analogue–based therapy, received rituximab 375 mg/m² I.V. on days 1, 8, 15, and 22 of cycle 1, and then once every 4 weeks during cycles 3-12. Lenalidomide (10 mg/day) was given orally starting on day 9 of cycle 1 and continued daily for 12 cycles. An interim analysis after 6 cycles of treatment showed that 25 patients achieved a response (6 nodular PRs [16%] and 19 PRs [51%]), resulting in an ORR of 68%. Six patients (16%) attained stable disease or clinical improvement and are continuing treatment, and 6 patients (16%) failed to respond, including 1 death that occurred on day 34 as a result of infectious complications. The most common side effect was neutropenia with 31% of infections reported; the occurrence of lenalidomide-associated TFR was limited to grade 1 and 2. Results of this study suggest that the combination of lenalidomide and rituximab is not only superior to single-agent lenalidomide but also reduces lenalidomide related side-effects.

5.4 Rationale of the study

5.4.1 Rationale for addition of lenalidomide to chlorambucil and rituximab

As described above, lenalidomide (Revlimid®) is a promising non-chemotherapeutic agent for the treatment of CLL which acts especially on the protective microenvironment. Although as single agent, lenalidomide is active in relapsed and in untreated patients with CLL, comparable to single agent chlorambucil rates of responses are below 50% (32%-47%) without complete remissions. Besides marrow suppression, toxicity seems disease specific and consists mainly of TFR and TLS. In relapsed patients, effectiveness seems significantly improved by addition of rituximab (ORR 64%, CR 6%)42. Responses (both overall and quality) improved with extended duration of treatment. Interestingly, addition of rituximab not only improved outcome, but also seemed to significantly diminish the incidence of grade 3 and 4 TFR and TLS. Given the key role for the microenvironment in chemo resistance, we hypothesize that lenalidomide will further improve response rates and quality of elderly patients when added to six cycles of chlorambucil and rituximab, resulting in improved clinical outcome. Also, probably due to its mode of action, kinetics of responses of lenalidomide seems to differ from classic chemotherapeutic agents: Responses (both overall and quality of responses) significantly improve with extended duration of treatment. Therefore, in this study we will also explore clinical benefit of an additional six cycles of single agent lenalidomide following 6 cycles of triple therapy. Currently it is not known whether triple therapy (chlorambucil, rituximab, lenalidomide) followed by single agent lenalidomide is feasible in terms of safety and toxicity and effective. Therefore we feel a
non-randomized phase II toxicity trial is mandatory before initiation of a phase III randomized trial on addition of lenalidomide to combination therapy of chlorambucil with rituximab. To guarantee clinical relevant data from the phase III randomized clinical trial that will follow the proposed phase II trial, starting point must be that the comparator is a non-inferior regimen. It can be learned from previously published randomized phase III trials in which chlorambucil monotherapy was the comparator in first-line CLL studies that a total dose of chlorambucil < 800mg results in inferior outcome\textsuperscript{19-22}. Based on these findings we feel that the dosage of chlorambucil in the comparator of triple therapy needs to be 10mg/m\textsuperscript{2}/day days 1-7; 6 cycles. However, at this moment we have no information whether triple therapy will result in increased risk of clinical relevant myelosuppression. To guarantee maximum patient safety and maximum knowledge on toxicity of triple therapy, we therefore choose to start this study with a run-in phase. During this part of the study which will include a maximum of 12 patients, the maximum tolerated dose (MTD) and recommended phase II dose level (RDL) of chlorambucil when combined with rituximab and lenalidomide in a 28-days schedule will be determined. The RDL for the phase II part of the study is defined as the highest dose level with 0 or 1 dose limiting toxicities (DLTs) observed among 6 patients.

5.4.2 Rationale of correlative ex vivo studies

Apoptosis and microenvironment

Although clinically active in CLL as described above the exact mechanism of action of lenalidomide is uncertain. Even at concentrations that are not clinically achievable (i.e., 200 μM), \textit{in vitro} lenalidomide had no direct cytotoxic effect on CLL cells\textsuperscript{43}, pointing to alternative mechanisms of action. Next to its possible role on cytokine production by bystander cells\textsuperscript{43}, recent \textit{in vitro} findings support a model where lenalidomide alters the immunogenicity of CLL cells thereby promoting cellular and innate immune activation: lenalidomide \textit{in vitro} increases co-stimulatory molecules like CD40, CD80, CD86 on CLL cells\textsuperscript{32,44} and co-culture of CLL cells and autologous T cells with lenalidomide reverses the T cell immune synapse defect present in this disease\textsuperscript{34}. One possible mechanism for these effects of lenalidomide is its ability to induce NF-κB dependent CD154, the co-stimulatory ligand for CD40 which is normally expressed on activated T-cells\textsuperscript{45}. As we previously have shown, activation of CD40 on CLL cells results not only in increased expression of co-stimulatory molecules but also in induction of pro-apoptotic Bid, and expression of death receptors DR5, and p73, making the leukemia cells more susceptible to T-cell mediated killing\textsuperscript{46-50} (and reviewed in\textsuperscript{35}). These \textit{in vitro} findings prompt to important clinical questions for application of lenalidomide in the treatment of CLL. First, despite activation of T-cells and NK-cells, it also has been reported that lenalidomide down modulates CD20 expression\textsuperscript{32} and the net effect of lenalidomide on rituximab mediated Activation Dependent Cellular Cytotoxicity (ADCC) is controversial\textsuperscript{31,45}. Second, we and others have shown that CD40-receptor signalling dependent NF-κB activation in CLL cells results in increased expression of anti-apoptotic
proteins, like Bcl-xL, Bfl-1 and Mcl-1 which renders CLL cells resistant to chemotherapy induced apoptosis \[^{46,51-53}\]. To what extent these in vitro findings on the mechanism of lenalidomide in CLL apply to the clinical setting is unknown.

For future development of rational clinical protocols (both in CLL and other hematological malignancies) several key questions still need to be answered:

1. What is the impact of lenalidomide therapy on T-cell subsets?
2. What is the impact of lenalidomide therapy on the interaction between leukemia cells and bystander cells within the lymph node micro-environment?
3. What is the effect of lenalidomide treatment on the regulation of expression of apoptosis regulators?

We plan to address these issues by combining ex vivo data from samples obtained at different time-points (see 10.4) with clinical data (CT-scans, outcome, toxicity, etc).

6 Study objectives

The aim of this study is to assess the feasibility (part I) and efficacy (part II) of a combination of Chlorambucil, Rituximab and Lenalidomide (CR\(^2\)) as first-line treatment for young and elderly frail patients with advanced previously untreated CLL.

6.1 Part I

Primary objective:
- To determine the maximum tolerated dose (MTD) and recommended part II dose level (RDL) of Chlorambucil when combined with Rituximab and Lenalidomide in a 28-days schedule.

Secondary objectives:
- To evaluate toxicity, especially tumor lysis syndrome (TLS), tumor flare reaction (TFR) and clinically relevant hematologic toxicity

6.2 Part II

Primary objective:
- To investigate the efficacy of a maximum of 6 cycles of Chlorambucil with Rituximab plus Lenalidomide at the RDL, as determined by the CR+PR rate

Secondary objectives:
- To evaluate the efficacy of Lenalidomide monotherapy in patients without progressive disease after 6 cycles of CR\(^2\)
- To evaluate toxicity, especially tumor lysis syndrome (TLS), tumor flare reaction (TFR) and clinically relevant hematologic toxicity
- To evaluate progression free survival
- To evaluate event-free survival
- To evaluate overall survival

7 Study design

This is a prospective, open label, phase I/II study. During part I of the study, the MTD and RDL of chlorambucil when combined with rituximab and lenalidomide will be determined according to the dose-escalation scheme as illustrated in the figure below. In fact it is similar to a '3+3' scheme, but without a temporary discontinuation of accrual while waiting for the DLT information of the first 3 patients. This modification is chosen based on the expected low frequency of serious toxicity due to a series of precautionary measures like frequency of visits, stepwise dose-escalation of the lenalidomide and tumor lysis prophylaxis. A maximum of 2 dose levels will be evaluated: Dose level 1, followed by either dose level 2 (after 0-1 DLT) or dose level 0 (after more than 1 DLT).

![Study design diagram]

(1) L* should be read as '0', '1', or '2', whichever applicable
Enrolment at each dose level will consist of (a maximum of) 6 evaluable patients. When after inclusion of 6 patients at dose level 1 a decision to escalate to a higher dose level can be made (eg. if the first 5 patients are evaluable for DLT and no DLT occurred in these patients), the study will continue at the next dose level. If not, inclusion will be discontinued until a decision can be made. When 6 patients have been entered at the second dose level, inclusion will be discontinued until an interim analysis has been performed and a decision of the DSMB on continuation of part II of the trial has been received.

The dose level stops as soon as at least two patients in a dose level experience a DLT. Before opening the lower dose level (level 0), all DLT’s of dose level 1 will be reviewed.

Patients, who die of CLL within 28 days after start of cycle II, but without a DLT, will be considered not evaluable, and will be replaced by another patient.

Although dose level 2 was feasible according to these decision rules, it was decided to continue part II of the trial with the Recommended Dose Level 1 of chlorambucil: 7 mg/m².

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

8 Study population

8.1 Eligibility for registration/randomization

All patients must be registered/ randomized before start of treatment and must meet all of the following eligibility criteria.

8.1.1 Inclusion criteria

- Diagnosis of CLL without prior treatment;
- Patients with symptomatic (according to IWCLL guidelines⁵⁶) Binet stage A / Rai stage 0 or Binet stage B or C / Rai I, II, III or IV (appendix A);
- Age 65 - 80 years, inclusive, at the time of signing the informed consent form, or age 18 – 64, inclusive, and CIRS ≥ 7⁵⁷ (appendix E);
- Able to adhere to the study visit schedule and other protocol requirements;
- WHO performance status of ≤ 2;
- Laboratory test results within these ranges: absolute neutrophil count ≥ 1.0 x 10⁹/l, platelet count ≥ 30 x 10⁹/l, creatinine clearance ≥ 60 ml/min, total bilirubin ≤ 25 µmol/L, AST & ALT ≤ 2 x ULN; in case the estimated creatinine clearance is too low (≥40, <60 ml/min) one may
determine the Glomular Filtration Ratio (GFR) from creatinine by 24 hours urine collection. This should be ≥ 60 ml/min,

- Females of childbearing potential must have a negative serum or urine pregnancy test within 10 - 14 days prior to and again within 24 hours of starting lenalidomide;
- Patients who are willing and capable to use adequate contraception during the therapy (all men, all women of childbearing potential). Patients must be able to adhere to the requirements of the Lenalidomide Pregnancy Prevention Risk Management Plan;
- Written informed consent.

8.1.2 Exclusion criteria

- Patients that are unable or unwilling to adhere to the requirements of the Lenalidomide Pregnancy Prevention Risk Management Plan;
- Intolerance of exogenous protein administration;
- Hepatitis B Ag positive, Hepatitis C positive and/or HIV positive patients;
- Patients with uncontrolled Autoimmune Hemolytic Anemia (AIHA) or autoimmune thrombocytopenia (ITP);
- Active fungal, bacterial, and/or viral infection;
- Pregnant or breast-feeding females (lactating females must agree not to breast feed while taking lenalidomide);
- Use of any other experimental drug or therapy within 28 days of baseline;
- Known hypersensitivity and/or serious adverse reactions to lenalidomide or similar drugs;
- Any prior use of lenalidomide;
- Concurrent use of other anti-cancer agents or treatments;
- Uncontrolled hyperthyroidism or hypothyroidism;
- Patients with history of idiopathic deep venous thrombus and/or pulmonary embolism within last three years;
- Neuropathy ≥ grade 2;
- History of active malignancy during the past 5 years with the exception of basal carcinoma of the skin; squamous cell carcinoma of the skin, carcinoma in situ of the cervix, carcinoma in situ of the breast, prostate cancer (TNM stage of T1a or T1b)
- Current inclusion in other clinical trials;
- Any psychological, familial, sociological and geographical condition potentially hampering compliance with the study protocol and follow-up schedule.
9 Treatment

9.1 Part I

9.1.1 Treatment schedule

Patient will be treated with 6 cycles (q 28 days) of a combination of chlorambucil, rituximab and lenalidomide. Subsequently patients will be treated with 6 cycles (q 28 days) of lenalidomide. During treatment it is important to check serum chemistry (especially tumor lysis lab) as well as hematology as described in chapter 10.

Treatment will be administered according to the schedule below:

The patient will be treated at the dose level assigned at registration.

Induction I: cycle I – VI

Dose level 0

<table>
<thead>
<tr>
<th>Agent</th>
<th>Dose/day</th>
<th>Route of administration</th>
<th>Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorambucil</td>
<td>Cycle I-VI: 5 mg/m²</td>
<td>p.o.</td>
<td>1-7</td>
</tr>
<tr>
<td>Rituximab</td>
<td>Cycle I: 375 mg/m², Cycle II-VI: 500 mg/m²</td>
<td>i.v.</td>
<td>1</td>
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</tbody>
</table>

Dose level 1

<table>
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<th>Agent</th>
<th>Dose/day</th>
<th>Route of administration</th>
<th>Days</th>
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<tbody>
<tr>
<td>Chlorambucil</td>
<td>Cycle I-VI: 7 mg/m²</td>
<td>p.o.</td>
<td>1-7</td>
</tr>
<tr>
<td>Rituximab</td>
<td>Cycle I: 375 mg/m², Cycle II-VI: 500 mg/m²</td>
<td>i.v.</td>
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</table>
**Dose level 2**

<table>
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<th>Days</th>
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<td>Chlorambucil</td>
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<td>p.o.</td>
<td>1-7</td>
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<tr>
<td>Rituximab</td>
<td>Cycle I: 375 mg/m²</td>
<td>i.v.</td>
<td>1</td>
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<tr>
<td></td>
<td>Cycle II-VI: 500 mg/m²</td>
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<tr>
<td>Lenalidomide</td>
<td>Cycle I: 2.5 mg*</td>
<td>p.o.</td>
<td>Cycle I: 9-28</td>
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<tr>
<td></td>
<td>Cycle II: 5 mg*</td>
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<tr>
<td></td>
<td>Cycle III-VI: 10 mg*</td>
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</table>

*Dose escalation/reduction of lenalidomide strictly according to chapter 9.2.2.

**Induction II: cycle VII – XII**

<table>
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<th>Dose/day</th>
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<th>Days</th>
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</thead>
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<tr>
<td>Lenalidomide</td>
<td>10 mg*</td>
<td>p.o.</td>
<td>1-28</td>
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</tbody>
</table>

*Dose escalation/reduction of lenalidomide strictly according to chapter 9.2.2.

The administration of Rituximab will be according to local practice.
Patients with progressive disease after cycle III or VI will go off protocol treatment.

9.1.2 Dose modifications during Part I

For patients who develop DLT during cycles I-II (as defined in section 13.1) in dose level 1 or 2, dose reduction of chlorambucil to a lower dose level is permitted for the next cycles. If a patient develops DLT in dose level 0 the patient is allowed to receive one more cycle. If again a DLT occurs, the patient will go off protocol treatment.

Guidelines for managing lenalidomide dose modification can be found in 9.2.2 and in appendix F

9.2 Part II

9.2.1 Treatment schedule

Data from the phase I as well as data from large international trials resulted in the choice for chlorambucil dose-level of 7mg/m² for the second part of the study.
Patient will be treated with 6 cycles (q 28 days) of a combination of chlorambucil, rituximab and lenalidomide and subsequently 6 cycles (q 28 days) of lenalidomide.
Treatment will be administered according to the schedule below.

**Induction I: cycle I - VI**

<table>
<thead>
<tr>
<th>Agent</th>
<th>Dose/day</th>
<th>Route of administration</th>
<th>Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorambucil</td>
<td>Cycle I-VI: 7 mg/m²</td>
<td>p.o.</td>
<td>1-7</td>
</tr>
<tr>
<td>Rituximab</td>
<td>Cycle I: 375 mg/m²</td>
<td>i.v.</td>
<td>1</td>
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<tr>
<td></td>
<td>Cycle II-VI: 500 mg/m²</td>
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</tr>
<tr>
<td>Lenalidomide</td>
<td>Cycle I: 2.5 mg*</td>
<td>p.o.</td>
<td>Cycle I: 9-28</td>
</tr>
<tr>
<td></td>
<td>Cycle II: 5 mg*</td>
<td></td>
<td>Cycle II-VI: 1-28</td>
</tr>
<tr>
<td></td>
<td>Cycle III-VI: 10 mg*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Dose escalation/reduction of lenalidomide strictly according to chapter 9.2.2.

Patients with progressive disease after cycle III or VI will go off protocol treatment.

**9.2.2 Dose modifications during Part II**

**Chlorambucil**

Dose modification of chlorambucil is not allowed during part II

**Lenalidomide dose escalation**

All patients will start Lenalidomide at 2.5 mg daily from day 9-28 of cycle I. Prior to dose escalation to 5 mg on day 1 of cycle II the subject will be evaluated for toxicity. If there is no grade 3 or greater toxicity and/or if the subject has not required a dose reduction (or holding of therapy during cycle I) the subject will receive lenalidomide 5 mg daily for days 1-28.

If the subject has not required a dose reduction or holding of therapy during cycle II and all toxicity has resolved, starting with cycle III the dose of lenalidomide should be increased by one dose level from 2.5 mg to 5 mg or 5 mg to 10 mg daily on days 1-28. Again, if the subject has not required a dose reduction or holding of therapy in a next cycle, the dose of lenalidomide should be increased by one dose level for the next cycle until the maximum tolerated dose (to a maximum of 10 mg daily) has been reached. This dose will be maintained until cycle XII. If the subject did not tolerate the lowest
dose of lenalidomide during cycle I but did tolerate 2.5 mg in the second cycle, dose should be escalated as described above until the 4th cycle.

**Lenalidomide dose reduction**

Lenalidomide intolerance and/or toxicity should be managed as indicated in appendix F. Non-hematological toxicity will be graded using the NCI Common Terminology Criteria for Adverse Events, CTCAE version 4.0. (appendix D) except for TLS and TFR which should be graded according to appendix H and appendix I. A modified hematologic toxicity grading scale will be used for grading hematologic toxicity (appendix G).

**Instruction for restarting Lenalidomide**

A new cycle of therapy may begin if the following criteria are met:

- The ANC is $\geq 0.5 \times 10^9/\text{l}$;
- The platelet count is $\geq 30 \times 10^9/\text{l}$ (unless thrombocytopenia is due to marrow infiltration);
- Any lenalidomide-related allergic reaction/hypersensitivity/rash or sinus bradycardia/other cardiac arrhythmia adverse event that may have occurred has resolved to $\leq$ grade 1 severity;
- Any other lenalidomide-related adverse event that may have occurred has resolved to $\leq$ grade 2 severity.
- Pregnancy test is confirmed negative for women of child bearing potential (test max. 28 days old)
- Treatment is not held for more than 28 days (if treatment is held for more than 2 weeks clinically appropriate biochemical monitoring for TLS should be employed). If treatment is required to be held for toxicity $> 28$ days the subject will go off protocol treatment;

If these conditions are not met the subject will be evaluated weekly and lenalidomide will not be initiated until the toxicity has resolved as described above. If therapy was held in the previous cycle, the new cycle will begin with a one step dose reduction. If the dose was reduced during the previous cycle and did not require further delay, the new cycle will begin with that dose. If the new cycle is held due to toxicity newly encountered on day one, the next cycle will begin with a one step dose reduction. If no toxicity occurs, dose can be increased in the following cycle. Patients who experience hematologic toxicity on lenalidomide 2.5 mg may be continued following recovery from toxicity if investigator feels that the subject is otherwise benefiting from therapy within 2 months.

**Handling of neutropenia during lenalidomide monotherapy (cycles VII-XII)**

Following 6 cycles of combination treatment, patient will continue with lenalidomide monotherapy continuously at the MTD that was reached during combination treatment for 6 more cycles.
• In case of neutropenia (<0.5x10^9/l) without clinical symptoms (including fever), hold lenalidomide and measure neutrophil count weekly for a maximum of 2 weeks.
• In case neutrophil count recovers (at least ≥0.5x10^9/l), continue same dose level.
• In case neutrophil count drops again, see bullet below

• In case of sustained neutropenia (<0.5x10^9/l) following a maximum of two weeks without study drug start G-CSF and follow lab weekly.

• In case neutrophil count recovers (at least ≥0.5x10^9/l), continue with G-CSF and restart lenalidomide at same dose level
• Continue G-CSF support for at least one cycle; One might try to stop G-CSF at the following cycle but if neutropenia occurs, continue with G-CSF and maintain dose-level
• In case of recurrence of neutropenia (<0.5x10^9/l) during lenalidomide despite G-CSF support, stop lenalidomide, wait for a maximum of another 2 weeks while on G-CSF support and in case neutrophil count recovers (at least ≥0.5x10^9/l) restart lenalidomide with 1 dose level reduction
• In case of sustained neutropenia (<0.5x10^9/l), stop study medication and plan end of treatment evaluation (including CT-scan and central lab for MRD

Lab should be monitored weekly during neutropenic phase (<0.5x10^9/l) and once every two weeks in case neutropenia has recovered while on of G-CSF support.

9.3 Co-intervention

9.3.1 Primary prophylaxis

• TLS: Especially during the first cycle, there is major risk of TLS following initiation of lenalidomide. Therefore, the following precautions are mandatory:
  - Assure appropriate hydration several days before start of lenalidomide (attention: in case of chlorambucil-induced nausea consider IV hydration)
  - Allopurinol 300 mg orally should be administered on a daily basis at day 1 until day 21 during cycle 1. Additional use of allopurinol is permitted at the investigator’s discretion and may be administered continuously until the subject is deemed no longer at risk for tumor lysis.
• Before each rituximab dose patient should receive prednisone 25 mg i.v., clemastine 2 mg i.v. or p.o., and acetaminophen 1000 mg orally or according to local policy;
• (Venous) Thromboembolism: all subjects should receive anti-thrombotic prophylaxis during cycles 1-6, unless at the investigator’s discretion a medical justification exists why
thromboprophylaxis should be withheld. Such reasons for withholding thromboprophylaxis should be clearly documented. The recommended prophylaxis is low-dose aspirin [ASA] 100 mg daily. The used measure of thromboprophylaxis in each cycle should be registered on the CRF.

9.3.2 Secondary prophylaxis

- Tumor-flare reaction > grade 2 should be treated as follows: prednisone; day 1-3 25 mg, day 4-6 20 mg, day 7-10 10 mg, day 11-14 5 mg, then stop
  If TFR ≤ grade 2 consider administration of NSAIDs.
- In case of neutrophils < 1.0x10^9/l G-CSF should be administered to prevent dose-reduction and febrile neutropenia aiming at > 0.5x10^9/l neutrophils.
  For handling neutropenia during monotherapy (cycle VII-XII), see paragraph 9.2.2
- All subjects should receive antibiotic prophylaxis for prevention of neutropenic fever (consisting of a fluoroquinolone and a triazole) as soon as neutrophils < 1.0x10^9/l until neutropenia is resolved.

9.4 Chlorambucil

9.4.1 Summary of known and potential risks

The very common (≥ 1/10) and common (≥1/100 - < 1/10) side effects of chlorambucil are:

Very common:

- Leukopenia, neutropenia, thrombocytopenia
  Although bone marrow suppression frequently occurs, it is usually reversible if chlorambucil is withdrawn

Common:

- Anemia
- Gastro-intestinal disturbances such as nausea and vomiting, diarrhoea and oral ulceration
- Acute secondary hematologic malignancies, particularly after long term treatment

For a complete list of side effects please see the Summary of Product Characteristics

9.4.2 Preparation and labeling

Chlorambucil with commercial labeling and packaging will be used.
9.4.3 Storage and handling

Chlorambucil should be stored and handled in accordance with the instructions in the summary of product characteristics or package insert.

9.4.4 Study drug supply

The investigator should use commercially available chlorambucil. If applicable national laws and regulations do not allow this, the sponsor will arrange delivery of chlorambucil to trial sites. No investigational medicinal product will be shipped until the sponsor has verified that all regulatory required documents and approvals for the site are available.

9.4.5 Drug accountability

If chlorambucil will be used from commercial stock, no drug accountability is required other than regular pharmacy procedures.

If applicable national laws and regulations request the sponsor to arrange delivery of chlorambucil to trial sites, drug accountability records should be maintained.

9.5 Rituximab

9.5.1 Summary of known and potential risks

When used to treat non-Hodgkin’s lymphoma or CLL, the most common side effects with rituximab (seen in more than 1 patient in 10) are reactions related to the infusion (mainly fever, chills and shivering).

For a complete list of side effects please see the Summary of Product Characteristics

9.5.2 Preparation and labeling

Rituximab with commercial labeling and packaging will be used.

9.5.3 Storage and handling

Rituximab should be stored and handled in accordance with the instructions in the Summary of Product Characteristics or package insert.
9.5.4 Study drug supply

The investigator should use commercially available rituximab. If applicable national laws and regulations do not allow this, the sponsor will arrange delivery of rituximab to trial sites. No investigational medicinal product will be shipped until the sponsor has verified that all regulatory required documents and approvals for the site are available.

9.5.5 Drug accountability

If rituximab will be used from commercial stock, no drug accountability is required other than regular pharmacy procedures.

If applicable national laws and regulations request the sponsor to arrange delivery of rituximab to trial sites, drug accountability records should be maintained.

9.6 Lenalidomide

9.6.1 Summary of known and potential risks

The most common side effects with lenalidomide (seen in more than 1 patient in 10) are neutropenia, fatigue, asthenia, constipation, muscle cramp, thrombocytopenia (low platelet counts), anemia, diarrhoea and rash. For the full list of all side effects reported with lenalidomide, see the Summary of Product Characteristics.

Lenalidomide is expected to be harmful to the unborn child. Therefore, lenalidomide must not be used in women who are pregnant. It must also not be used in women who could become pregnant, unless they take all of the necessary steps to ensure that they are not pregnant before treatment and that they do not become pregnant during or soon after treatment.

9.6.2 Preparation and labeling

Lenalidomide will be shipped to trial sites in containers labeled as an Investigational Medicinal Product. Lenalidomide will be prepared and labeled in compliance with GMP and other applicable regulatory requirements.

9.6.3 Storage and handling

The investigational medicinal product should be stored in such a manner that accidental loss or destruction or access by an unauthorized person is prevented.
9.6.4 Study drug supply

The sponsor will arrange delivery of Lenalidomide to trial sites. No investigational medicinal product will be shipped until the sponsor has verified that all regulatory required documents and approvals for the site are available.

9.6.5 Drug accountability

The investigator, or a pharmacist or other appropriate individual who is designated by the investigator, should maintain records of the product’s delivery to the trial site, the inventory at the site, the use/return by each patient, and the return to the sponsor or alternative disposition of unused product(s). These records should include dates, quantities, batch/serial numbers, expiration dates (if applicable), and the unique code numbers assigned to the investigational product(s) and trial patients (if applicable). Investigators should maintain records that document adequately that the patients were provided the doses specified by the protocol and reconcile all investigational product(s) received from the sponsor.

9.6.6 Study drug return and destruction

Partially used investigational medicinal product should not be redispensed to either the same or another patient after it has been returned.

The trial site should destroy used or partially used study drug containers after drug accountability records have been completed. Destruction should be documented.

Unused investigational medicinal product: should be destroyed by the trial site. Destruction should be documented. Documentation of destruction should be send to HOVON Data Center.

10 Study procedures

10.1 Time of clinical evaluations

♦ At entry; within 14 days prior to start cycle I
♦ Weekly during cycle I and cycle II
♦ Every 2 weeks during cycle III-VI
♦ Prior to each cycle
♦ At day 7 following the first cycle of lenalidomide dose escalation
♦ End of protocol
♦ Follow up: every 3 months until 3 years after start treatment and every 6 months thereafter up to 5 years after registration.
♦ At progressive disease
### 10.2 Required investigations

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<tr>
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<th>At entry</th>
<th>Weekly cycle I and II</th>
<th>Prior to each cycle</th>
<th>Day 7 of lenalidomide dose escalation</th>
<th>After cycle VI</th>
<th>End of protocol</th>
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1. Laboratory tests should be performed within 2 weeks prior to start of treatment; flow, BM and CT can be maximally 4 weeks old, mutational status not when earlier performed
2. During part I: cycle III-VI every 2 weeks lab must be done
3. Only as confirmation of CR (if CR is reached after cycle VI BM does not have to be repeated at end of protocol)
4. At least once annually starting 6 months after end of protocol treatment
5. Only if not done on PB
6. Blood should be sampled prior to study, prior to first administration of lenalidomide (cycle I, day 8), after second week of lenalidomide, prior to cycle IV, prior to cycle VII, prior to the 4th cycle of Lenalidomide (cycle X), at end of treatment, and after three months of follow-up as specified in lab manual. For the coagulation side study in part II of the study blood should be sampled prior to study and on day 1 of cycles I, III, VII, VIII and IX.
7. In all women of childbearing potential: as defined in the Lenalidomide Pregnancy Prevention Risk Management Plan (also see below)
8. The CT scan at progression is optional
9. o.i. on indication
Medical history
Standard medical history, including B symptoms, concomitant diseases and concomitant medications. CIRS score at study entry

Physical examination
Standard physical examination, with special attention to:
- Vital signs
- WHO performance status (see appendix C)
- Tumor flare reaction
- Adverse Events
- Palpable lymphnodes, spleen and liver sizes

Hematology
Hematology should be checked:
- Weekly during cycle I and II;
- Every 2 weeks during cycle III-VI,
- At day 7 following any dose escalation of lenalidomide;
- Prior to each cycle (max. day -7)
- End of treatment
- Each Follow up visit
- At progression

Lab should include:
Hb, WBC and differential count, platelet count.
DAT (direct antiglobulin test/Coombs test) -at entry and on indication-

Blood chemistry
Serum chemistry (especially tumor lysis lab) should be checked:
- Weekly during cycle I and II;
- Every 2 weeks during cycle III-VI,
- At day 7 following any dose escalation of lenalidomide;
- Prior to each cycle (max. day -7)
- End of treatment
- Each Follow up visit
- At progression
Lab should include:
- Potassium
- Sodium
- Calcium
- Phosphate
- Creatinine
- Bicarbonate
- Uric acid
- ASAT
- ALAT
- Alkaline phosphatase
- Bilirubin
- LDH
- Haptoglobin

Special attention to tumor lysis lab: creatinine, calcium, phosphate, uric acid, potassium, LDH

Additional blood chemistry at entry
- Glucose
- BUN
- Total protein
- Albumin
- IgG
- IgM
- IgA
- β-2 microglobulin

Pregnancy testing
In all women of childbearing potential a pregnancy test should be performed as defined in the Lenalidomide Pregnancy prevention Risk management plan:
- at entry
- in women with regular or no menstrual cycles: weekly for the first 28 days of lenalidomide treatment and then every 28 days while on lenalidomide treatment, at lenalidomide discontinuation and at day 28 following lenalidomide discontinuation.
- If menstrual cycles are irregular, the pregnancy testing must occur weekly for the first 28 days and then every 14 days while on lenalidomide treatment, at lenalidomide discontinuation, and at days 14 and 28 following lenalidomide discontinuation.
Bone marrow biopsy/aspirate
Pathology studies include morphology and at baseline or if nodules are found in case of CR immunohistochemistry (required markers at on study: CD5, CD19, CD20, CD23, CD79b, kappa, lambda, cyclin D1; as confirmation of CR: CD19, CD20, kappa, lambda). Biopsy is indicated at:
- At entry
- After cycle VI, only as confirmation of CR
- At the end of protocol treatment only as confirmation of CR (at least 2 months after the final treatment and if not performed after cycle VI).
- In CR patients at least annually during follow up starting 6 months after the end of protocol treatment
- At progression, if necessary to define progressive disease (see Response criteria in Appendix B)

Flow cytometry (minimal required markers: CD5/CD19/CD23 triple positive with light chain restriction)
- At entry on PB or BM if not done on PB
- At progression on PB or BM if available and if not done on PB

FISH (required markers: 17p13 deletion, 11q22-23 deletion, trisomy 12 and 13q14 deletion)
At entry on PB (on BM if not done on PB)
At progression on PB (on BM if not done on PB)

Mutational status (Immunoglobulin heavy chain sequencing)
At entry

Storage for future studies
In addition to these investigations, all patients will be asked for informed consent to store biological material for future studies. Material for future investigations will be shipped to the Hematology laboratory of the Academic Medical Center. More details can be found in the study lab manual. All materials are anonymized and stored for a maximum of 15 years after end of study, after which the samples will be destroyed. Any study to be undertaken on these materials must be approved of by the principal investigator and co-investigators, and members of the writing committee and relevant ethical authorities, and will be specified in protocol amendments prior to undertaking the studies.

Virology
- Including test (PCR or serology) for hepatitis B and C.
CT scan
A CT scan of neck, thorax, abdomen and pelvis must be done:
- At entry within 4 weeks before registration
- After cycle VI
- At the end of protocol treatment
- Optionally, during follow up in case of expected progression

10.3 Response evaluation
Response will be evaluated:
- After cycle III (clinical response)
- After cycle VI
- At the end of protocol treatment
- Follow-up

See the following subsections and appendix B for assessment of response.

10.3.1 Definition of Response
Response will be determined according to the definitions of response in the IWCLL updated NCI-WG guidelines [Hallek et al., 2008] and documented at visits outlined in the time and events table. For a tabular summary of all criteria of response definition in CLL patients see Appendix B.

10.4 Side studies
We intend to obtain blood samples prior to study, prior to first administration of lenalidomide (cycle I, day 8/9), after second week of Lenalidomide, following third cycle, following sixth cycle of combination therapy, prior to fourth cycle of lenalidomide monotherapy (cycle X), at end of treatment and after three months of follow-up.
In addition to these investigations, in at least 10 patients enrolled in Academic Medical Center with palpable enlarged lymph nodes who have given informed consent for the fine needle aspiration (FNA)/core biopsy side study the following will be performed: Prior to start of treatment, prior to first administration of lenalidomide (cycle I, day 8/9; only FNA no biopsy) and prior to cycle II ultrasound-guided FNA and core biopsies of an involved lymph node. More details can be found in the study lab manual.
Finally, sequential plasma citrate samples and DNA samples for the coagulation side study will be obtained from all patients in part II of the study prior to study and on day 1 of cycles I, III, VII, VIII and IX. More details can be found in the laboratory manual.

Studies will include (PB=peripheral blood; FNA=fine needle aspirate, CB=core biopsy):

1. Impact on and T-cell subsets
   - To monitor relative and absolute numbers of T cell subsets (CD4, CD8, T-regs, naïve, memory, effector memory both during first six cycles of therapy (chlorambucil, rituximab, lenalidomide (PB and FNA) and during Lenalidomide monotherapy (PB);
   - To assess functional studies (proliferation, cytokine production, MLR response capacity) on T-cell subsets on samples obtained during the trial (PB);

2. Impact on interaction within the lymph node micro-environment?
   - To determine differences in bystander cells (e.g. follicular dendritic cells, CD14+ nurse like cells) within the microenvironment during the course of the study (FNA);
   - To determine in vivo effects of lenalidomide on expression of membrane-bound molecules on the leukemia cells (adhesion-, co stimulatory-, TNF-receptor-, death receptor molecules) (PB and FNA);

3. Impact of Lenalidomide therapy on apoptosis regulation
   - To determine in vivo effects of lenalidomide on the gene expression profile of apoptosis regulators of CLL cells by RT-MLPA analysis (PB, CB)\textsuperscript{46,54}.

4. Minimal Residual Disease measurement: on PB in case of CR

5. Impact of lenalidomide therapy on coagulation activity in CLL
   - To determine the presence and incidence of thrombophilic mutations in patients with CLL;
   - To determine in vivo effects of lenalidomide in combination with chlorambucil, rituximab on the plasmatic coagulation system in CLL by sequential (PB) samples;
   - To determine in vivo effects of lenalidomide monotherapy on the plasmatic coagulation system in CLL by sequential (PB) samples;
   - To prospectively determine the incidence of (venous) thromboembolism in all included patients with CLL treated with lenalidomide, by central registration (CRF) of thrombotic events of all grades.
11 Withdrawal of patients or premature termination of the study

11.1 Specific criteria for withdrawal of individual patients

Patients can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a patient from the study for urgent medical reasons. Specific criteria for withdrawal are:

- Death
- Excessive toxicity
- No compliance of the patient
- Refusal to continue protocol treatment
- Progression/relapse during treatment

11.2 Follow up of patients withdrawn from treatment

Patients who are withdrawn from protocol treatment for other reasons than death will be followed as described in 10.2 for follow up.

For patients who are withdrawn from treatment because in hindsight they did not fulfil the eligibility criteria (see 8.1) at time of enrolment, data will be collected until 30 days after the last protocol treatment given. SAE information will be collected as described in 12.3.

No further information will be collected for patients who have withdrawn their consent. If a patient withdraws consent please consult HOVON Data Center.

Patients who are withdrawn from protocol treatment will receive medical care according to local practice.

11.3 Premature termination of the study

The sponsor may decide to terminate the study prematurely based on the following criteria:

- There is evidence of an unacceptable risk for study patients (i.e. safety issue);
- There is reason to conclude that it will not be possible to collect the data necessary to reach the study objectives and it is therefore not ethical to continue enrolment of more patients; for example insufficient enrolment that cannot be improved.
- The DSMB recommends to end the trial based on viable arguments other than described above

The sponsor will promptly notify all concerned investigators, the Ethics Committee(s) and the Regulatory Authorities of the decision to terminate the study. The sponsor will provide information
regarding the time lines of study termination and instructions regarding treatment and data collection of enrolled patients.

12 Safety

12.1 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.

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, including suspected transmission of infectious agents by a medicinal product).

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.

Suspected unexpected serious adverse reaction (SUSAR)
All suspected Adverse Reactions which occur in the trial and that are both unexpected and serious. Suspected 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. Unexpected adverse reactions are adverse reactions, of which the nature, or severity, is not consistent with the applicable product information (e.g. Investigator’s Brochure for an unapproved IMP or Summary of Product Characteristics (SPC) for an authorised medicinal product).
12.2 Adverse event

12.2.1 Reporting of adverse events

Adverse events will be reported from the first study-related procedure until 30 days following the last dose of any drug from the protocol treatment schedule or until the start of subsequent systemic therapy for the disease under study, if earlier.

Adverse events occurring after 30 days should also be reported if considered at least possibly related to the investigational medicinal product by the investigator.

Adverse Events have to be reported on the Adverse Events CRF. Adverse Events will be scored according to the NCI Common Terminology Criteria for Adverse Events, version 4 (see appendix D), except for TLS and TFR which should be graded according to appendix H and appendix I.

Pre-existing conditions will be collected on the baseline concomitant diseases CRF, i.e. active (symptomatic) diseases of CTCAE grade ≥ 1, diseases under treatment, chronic diseases and long term effects of past events as present at the time of baseline assessment.

It is important to consider low blood counts at the initiation of therapy when evaluating hematologic toxicity of CLL subjects. Due to this, standard solid tumor toxicity criteria cannot be used or subjects would exhibit grade II to IV toxicity at study onset. For this reason, severity of AEs/SAEs that are hematologic toxicities (platelets, hemoglobin and neutrophils) will be scored according to an adaptation of the IWCLL Grading Scale for Hematological Toxicity in CLL Studies 55 (Appendix G).

All Adverse Events have to be reported, with the exception of:

- A pre-existing condition that does not increase in severity; the pre-existing condition should be reported on the baseline concomitant diseases CRF
- AE’s of CTCAE grade 1. However TLS, TFR and thromboembolic events MUST be reported in case of CTCAE grade ≥ 1.
- Abnormal laboratory values that have been recorded as being not clinically significant by the investigator in the source documents
- Progression of the disease under study; complaints and complications as a result of disease progression remain reportable Adverse Events
- B cell depletion, IgG below LLN, low CD19+ count, and hypogammaglobulinemia
12.2.2 Follow up of adverse events

All adverse events will be followed clinically until they have been resolved, or until a stable situation
has been reached. Depending on the event, follow up may require additional tests or medical
procedures as indicated, and/or referral to the general physician or a medical specialist.
Any ongoing adverse event that increases in severity is to be reported as a new adverse event on the
CRF. Other follow up information is not collected on the CRF.

12.3 Serious adverse events

12.3.1 Reporting of serious adverse events

Serious Adverse Events (SAEs) will be reported from the first study-related procedure until 30 days
following the last dose of any drug from the protocol treatment schedule or until the start of
subsequent systemic therapy for the disease under study, if earlier.
Serious Adverse events occurring after 30 days should also be reported if considered at least possibly
related to the investigational medicinal product by the investigator.

SAEs must be reported to the HOVON Data Center by fax within 24 hours after the event was
known to the investigator, using the SAE report form provided. This initial report should contain a
minimum amount of information regarding the event, associated treatment and patient identification,
as described in the detail in the instructions for the SAE report form. Complete detailed information
should be provided in a follow-up report within a further 2 business days, if necessary.
The following events are not considered to be a serious adverse event:

♦ Hospitalization for protocol therapy administration. Hospitalization or prolonged
  hospitalization for a complication of therapy administration will be reported as a Serious
  Adverse Event.
  Infusion related AEs may lead to a prolonged infusion time. Overnight stay at the hospital
due to slow infusion rate is not to be reported as an SAE

♦ Hospitalization for diagnostic investigations (e.g., scans, endoscopy, sampling for laboratory
tests, bone marrow sampling) that are not related to an adverse event. 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.

♦ Hospitalization for a procedure that was planned prior to study participation (i.e. prior to
  registration or randomization). This should be recorded in the source documents. Prolonged
hospitalization for a complication of such procedures remains a reportable serious adverse event.

12.3.2 Causality assessment of Serious Adverse Events

The investigator will decide whether the serious adverse event is related to trial medication, i.e. any of the products from the protocol treatment schedule. The decision will be recorded on the serious adverse event report. The assessment of causality is made by the investigator using the following:

<table>
<thead>
<tr>
<th>RELATIONSHIP</th>
<th>DESCRIPTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>UNRELATED</td>
<td>There is no evidence of any causal relationship</td>
</tr>
<tr>
<td>UNLIKELY</td>
<td>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).</td>
</tr>
<tr>
<td>POSSIBLE</td>
<td>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).</td>
</tr>
<tr>
<td>PROBABLE</td>
<td>There is evidence to suggest a causal relationship and the influence of other factors is unlikely.</td>
</tr>
<tr>
<td>DEFINITELY</td>
<td>There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.</td>
</tr>
<tr>
<td>NOT ASSESSABLE</td>
<td>There is insufficient or incomplete evidence to make a clinical judgment of the causal relationship.</td>
</tr>
</tbody>
</table>

12.3.3 Follow up of serious adverse events

All serious adverse events will be followed clinically until they are resolved or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist. Follow up information on SAE’s should be reported monthly until recovery or until a stable situation has been reached. The final outcome of the SAE should be reported on a final SAE report.
12.3.4 Processing of serious adverse event reports

The HOVON Data Center will forward all SAE reports within 24 hours of receipt to the Principal Investigator, Celgene and Roche.
The HDC safety desk will evaluate if the SAE qualifies as a suspected unexpected serious adverse reaction (SUSAR).
The IB/SmPC will be used as a reference document for expectedness assessment.
The HOVON Data Center will ensure that a six-monthly line listing of all reported SAE’s is provided to the Ethics Committee(s) if this is required by national laws or regulations or by the procedures of the Ethics Committee.

12.4 Reporting suspected unexpected serious adverse reactions

The HDC Safety Desk, on behalf of the sponsor, will ensure the reporting of any SUSARs to the Ethics Committees (EC), the Competent Authorities (CA), Celgene and the investigators in compliance with applicable laws and regulations, and in accordance with any trial specific agreements between the sponsor and a co-sponsor or Celgene

Expedited reporting of SUSARs will occur no later than 15 days after the HOVON Data Center had first knowledge of the serious adverse event. For fatal or life-threatening cases this will be no later than 7 days for a preliminary report, with another 8 days for a complete report.
The manner of SUSAR reporting will be in compliance with the procedures of the Ethics Committees and Health Authorities involved.

12.5 Pregnancies

In order to prevent pregnancies during the use of lenalidomide, patient information, patient registration and patient counseling will occur as defined in the Risk Management Program.

Pregnancies and suspected pregnancies (including a positive pregnancy test regardless of age or disease state) of a female subject or the female partner of a male subject, occurring while the subject is on protocol treatment or within 30 days following the last dose of any drug from the protocol treatment schedule, should be reported to the sponsor. Pregnancies and suspected pregnancies must be reported to the HOVON Data Center by fax within 24 hours after the event was known to the investigator, using the pregnancy report form provided.

The investigator will follow the female subject until completion of the pregnancy, and must notify the sponsor of the outcome of the pregnancy within 5 days or as specified below. The investigator will provide this information as a follow-up to the initial pregnancy report. If the outcome of the pregnancy
meets the criteria for classification as a SAE (i.e., spontaneous or therapeutic abortion, stillbirth, neonatal death, or congenital anomaly - including that in an aborted fetus), the investigator should follow the procedures for reporting SAEs. In the case of a live “normal” birth, the sponsor should be informed as soon as the information is available. All neonatal deaths that occur within 30 days of birth should be reported, without regard to causality, as SAEs. In addition, any infant death after 30 days that the investigator suspects is related to the in utero exposure to the investigational medicinal product(s) should also be reported.

The investigator is encouraged to provide outcome information of the pregnancy of the female partner of a male subject, if this information is available to the investigator and the female partner gives her permission.

HOVON Data Center will forward any information regarding (suspected) pregnancies to Celgene immediately by phone then by email or by fax.

12.6 Second Primary Malignancies

Second primary malignancies (SPM) will be monitored as events of interest and must be reported as serious adverse events. This includes any second primary malignancy, regardless of causal relationship to any study drug, occurring at any time for the duration of the study, from the time of signing informed consent until 5 years after registration in the trial.

Events of second primary malignancy are to be reported using the SAE report form and must be considered an “Important Medical Event” even if no other serious criteria apply. Documentation on the diagnosis of the second primary malignancy must be provided at the time of reporting as a serious adverse event (e.g. pathology report).

The incidence of second primary malignancies is also monitored via a separate form (Second Primary Malignancy Report Form). This form should be filled out, dated and signed by the responsible investigator and returned to the HOVON Data Center by fax within 24 hours after establishment of a second primary malignancy.

SPM must also be documented in the other appropriate page(s) of the CRF (e.g. Adverse Event Form and Follow up Form).

For each case of SPM occurring during treatment, contact the Principal Investigator to discuss if treatment needs to be discontinued.
12.7 Reporting of safety issues

The sponsor will promptly notify all concerned investigators, the Ethics Committee(s) and the regulatory authorities of findings that could affect adversely the safety of patients, impact the conduct of the trial, increase the risk of participation or otherwise alter the EC’s approval to continue the trial. In the occurrence of such an event the sponsor and the investigators will take appropriate urgent safety measures to protect the patients against any immediate hazard. The accredited Ethics Committee will suspend the study pending further review, except insofar as suspension would jeopardize the patient’s health. The local investigator will inform the patients.

12.8 Annual safety report

The sponsor will submit, once a year throughout the clinical trial, a safety report to the Ethics Committees and Competent Authorities of the concerned Member States. The content of the annual safety report will be according to the EU guidance document ‘Detailed guidance on the collection, verification and presentation of adverse reaction reports arising from clinical trials on medicinal products for human use’.

12.9 Data Safety and Monitoring Board

The Data and Safety Monitoring Board will advise the chair of the HOVON working group, the Principal Investigator and the Co-investigator(s) about the continuation of the study. The DSMB will evaluate the general progress and the feasibility of the study, the quality and completeness of the data, and side effects and safety.

The DSMB consists of at least 3 members, among whom (at least) one statistician and minimally two physicians. The members of the DSMB are invited on personal title on the basis of their expert knowledge of the disease involved or the research methodology. Members of the DSMB will have ample experience with randomized clinical trials.

The members of the DSMB will not be involved in the study, work at the HOVON Data Center, be a member of the HOVON board, or work in a hospital department participating in the study. The members will not have a conflict of interest due to ties with a company involved in the study.

The DSMB reports their written recommendations to the trial statistician. The report may consist of a confidential and a public part, where the confidential part contains references to unblinded data. The trial statistician forwards the public part of the DSMB recommendation to the Principal Investigator, the Co-investigator(s) and the chair of the HOVON working group involved. The DSMB recommendations are not binding.
The DSMB will receive at least the following reports from the trial statistician for review:

- Interim analysis reports (as described in 14.3)
- Annual safety data listing the incidence of (serious) adverse events, (serious) adverse reactions and SUSARs
- Annual progress data listing the number of enrolled patients and the status of data collection

13 Endpoints

13.1 Definitions

<table>
<thead>
<tr>
<th>Term</th>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>Dose-limiting toxicity</td>
<td>DLT</td>
<td>Adverse event of severity or consequence that may limit dose escalation.</td>
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<tr>
<td></td>
<td></td>
<td>In this trial, DLT is defined as:</td>
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<tr>
<td></td>
<td></td>
<td>- Cairo-Bishop grade III or IV tumor lysis syndrome (TLS), defined as (appendix H)</td>
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<td></td>
<td></td>
<td>- Presence of laboratory tumor lysis syndrome (LTLS)*</td>
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<td></td>
<td></td>
<td>- Creatinine &gt;3 X ULN and at least one of the following:</td>
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<tr>
<td></td>
<td></td>
<td>- Cardiac arrhythmia: symptomatic and incompletely controlled medically or controlled with device (e.g. defibrillator), or life threatening</td>
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<tr>
<td></td>
<td></td>
<td>- Seizure in which consciousness is altered</td>
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<td></td>
<td></td>
<td>- Seizure of any kind which are prolonged, repetitive or difficult to control (e.g. status epilepticus, intractable epilepsy)</td>
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<tr>
<td></td>
<td></td>
<td>- Tumor flare grade IV, defined as</td>
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<tr>
<td></td>
<td></td>
<td>Painful enlargement of lymph nodes and/or spleen with associated low-grade fever and rash, resulting in disabling disease.(appendix I)</td>
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<tr>
<td></td>
<td></td>
<td>- Neutropenic sepsis defined as grade IV sepsis (Life-threatening consequences; urgent intervention indicated)</td>
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<tr>
<td></td>
<td></td>
<td>Please note: Grade III infections (infections which require intravenous antibiotics, do not count as DLT)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Death not due to CLL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>occurring between start cycle I and 28 days after start of cycle II</td>
</tr>
</tbody>
</table>
Maximum MTD  Maximum safe dose, i.e. the maximum dose at which only 0/1 of tolerant dose 6 patients exhibit DLT
Recommended RDL  Dose (level) recommended for further study in part II of the part II dose study.

### 13.2 Part I

Primary endpoint:
- Dose-limiting toxicity (DLT), maximum tolerated dose (MTD) and recommended part II dose (RDL) of chlorambucil when combined with rituximab and lenalidomide

Secondary endpoints:
- Toxicity, especially tumor lysis syndrome (TLS), tumor flare reaction (TFR) and neutropenic sepsis

### 13.3 Part II

Primary endpoint:
- CR+PR rate. In order for patients to be considered a success for the primary endpoint, a PR or CR must be documented according to criteria in Appendix B. All other patients will be considered as not having achieved at least a PR. In this analysis we will consider the best response obtained during induction 1 therapy (cycles I-VI).

Secondary endpoints:
- Improvement of response due to lenalidomide monotherapy
- Toxicity, especially tumor lysis syndrome (TLS), tumor flare reaction (TFR) and neutropenic sepsis
- Progression free survival (PFS; i.e. time from registration to progression or death from any cause, whichever comes first)
- Event-free survival (EFS; i.e. time from registration to induction failure, progression or death from any cause, whichever comes first. Induction failure is defined a not having achieved at least a PR during/after a maximum of 12 cycles. EFS for patients with induction failure will be set at 1 day)
- Overall survival (OS) measured from registration. Patients still alive or lost to follow up are censored at the date they were last known to be alive
- PFS calculated from start of Lenalidomide monotherapy
- OS calculated from start of Lenalidomide monotherapy
14 Statistical considerations

14.1 Patient numbers and power considerations

14.1.1 Part I
Due to the dose-escalation scheme, a maximum of 12 evaluable patients will be entered in part I. Patients who die of CLL within 28 days after start cycle II without having obtained a DLT, will be considered as not-evaluable for DLT, and will be replaced.

14.1.2 Part II
Part II is designed to determine whether induction treatment with chlorambucil at the RDL in combination with rituximab and lenalidomide warrants further investigation in clinical trials. The CR+PR rate will be considered as primary endpoint for the sample size calculation.
- Let $P_0$ be the largest CR+PR rate which, if true, implies that the therapeutic activity is too low and therefore does not warrant further investigation. In the present trial, $P_0$ has been taken as 60%.
- Let $P_1$ be the smallest CR+PR rate which, if true, implies that the therapeutic activity is sufficiently high and therefore the RDL warrants further investigation in clinical trials. In the present trial, $P_1$ has been taken as 80%.

In order to reject the null hypothesis $H_0: P = P_0$ in favor of the alternative hypothesis $H_1: P = P_1$ with power $1 - \beta = 0.80$ (2-sided significance level $\alpha = 0.05$), 43 eligible patients are required. However, in order to overcome dropout due to ineligibility, 50 patients will be included in part II of the trial.

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

14.2.1 Efficacy analysis
The main endpoint of part II is the proportion of patients who obtain a CR or PR during induction chemotherapy. A 95% confidence interval (CI) will be constructed, and the null hypothesis $H_0: P = P_0$ will be rejected in favor of the alternative hypothesis $H_1: P = P_1$ if the lower bound of the 95% CI is larger than 0.60.
Secondary efficacy endpoints concern (improvement of) response, and survival endpoints. Response rates will be described as percentages with 95% CI. Actuarial survival curves for all time-to-event endpoints will be computed using the Kaplan-Meier method and 95% CI will be constructed.

14.2.2 Toxicity analysis

The analysis of treatment toxicity will be done primarily by tabulation of the incidence of adverse events and infections (Appendix D, G, H and I) by treatment cohort and cycle.

The incidence of the adverse events defined as DLT will be reported by treatment cohort and cycle.

14.2.3 Additional analyses

Additional analyses may involve the analysis of prognostic factors (especially abnormal cytogenetics, CD38, beta-2-microglobulin and IgVH mutational status) with respect to response rate, PFS, EFS, and OS from registration. Logistic and Cox regression analysis could be used for this purpose.

Before any additional analysis will be performed, a separate analysis plan will be discussed with the principal investigator. Any such analysis should, however, be considered as exploratory, i.e. hypothesis generating, and not confirmatory.

14.3 Interim analysis

14.3.1 Part I

Up to 2 formal interim analyses are planned during part I of the study when the decision rules indicate to change the current dose level. So, the first interim analysis will be performed after inclusion of (a maximum of) 6 evaluable patients at the first dose level and a second one after inclusion of (a maximum of) 6 evaluable patients at the second dose level. The interim analyses report (send to the DSMB) will primarily contain listings on DLTs and SAEs.

14.3.2 Part II

During part II of the study a safety interim analysis is planned after 15 patients have received at least 3 cycles of therapy. The interim analysis report includes the number of entered patients and given treatment cycles, information on SAEs and adverse events. The interim analysis report will be send to the DSMB for advice.
15  Registration

15.1  Regulatory Documentation

Required regulatory and administrative documents must be provided to the HOVON Data Center before shipment of study drug and before enrolment of the first patient. This will always include an Ethics Committee approval for the investigational site. The HOVON Data Center will provide each investigator with an overview of the required documents. Each investigational site will be notified when all requirements are met and enrolment can start.

15.2  Registration

Eligible patients should be registered before start of treatment. Patients need to be registered at the HOVON Data Center by one of the following options:

- Trial Online Process (TOP, https://www.hdc.hovon.nl/top). A logon to TOP can be requested at the HOVON Data Center for participants.
- By faxing the completed registration CRF +31.10.7041028 Monday through Friday, from 09:00 to 17:00 CET
- By phone +31.10.7041560 Monday through Friday, from 09:00 to 17:00 CET

*Patients for part I of the trial can only be registered by phone of by fax.*

The following information will be requested at registration:

- Protocol number
- Institution name
- Name of caller/responsible investigator
- Local patient code (optional)
- 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 (a sequence number by order of enrolment in the trial). Patient study number will be given immediately by TOP or phone and confirmed by fax or email.
Local Patient Code is a code assigned to the patient by the investigational site for local administrative purposes. The code may be up to 8 characters long (letters and numbers allowed). The code should be in compliance with privacy regulations. It should not contain identifying data, such as patient initials or the complete hospital record number. The local code will be visible in the confirmation messages sent by TOP to local participants after registration of the patient. The key to this local patient code should only be accessible by the local investigator and the local trial staff. Using or entering a local patient code is not obligatory.

16 Data collection and quality assurance

16.1 Case Report Forms

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;
- Baseline concomitant diseases and 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 trial number, and a combination of patient study number (assigned at registration) and hospital identification. 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. The CRF pages must be made available to the HOVON Data Center at the requested time points as specified in the CRF instructions. All data will be collected in the study database by the HOVON Data Center.

16.1.1 DLT data collection

To monitor the incidence of dose limiting toxicity (DLT) among the patients in part I a separate CRF (DLT-form) will be used. This DLT-form must be filled out for every patient, during cycle I and II. The
form should be dated, signed by the responsible investigator and returned to the HOVON Data Center by fax within 24 hours after DLT-occurrence, or if no DLT occurred, weekly between start cycle I and 28 days after start cycle II, or until start cycle III. DLTs should be reported until day 28 after start cycle II or until start next treatment. Duration of myelosuppression must be reported on the DLT form until ANC recovery or until start next treatment (if not yet recovered). Local investigators will weekly receive a reminder for sending in a new DLT form.

16.1.2 Rapid reporting

In this trial the occurrence of TLS of grade $\geq 3$ and TFR of grade 4 (graded according to appendix H and I) during induction treatment are considered of special interest. During cycle I and II of part I these events will be reported on the DLT form as described above. To monitor the incidence of these events during cycles III-XII in part I and among the patients in part II a separate CRF (Rapid Reporting Form) will be used. The form should be filled out, dated and signed by the responsible investigator and returned to the HOVON Data Center by fax within 24 hours after occurrence of these events or, if these events did not occur, at day 28 of all cycles. Investigators will receive a reminder to fill out a Rapid Reporting Form weekly in cycles I and II and after cycles III-XII. If TLS or TFR reoccurs after it had been resolved, it should be reported again as a new event.

16.2 Data quality assurance

Steps to be taken to ensure the accuracy and reliability of data include the selection of qualified investigators and appropriate study centers, review of protocol procedures with the investigator before the study, and site visits by the sponsor. Data collected on the CRF will be verified for accuracy. If necessary, queries will be sent to the investigational site to clarify the data on the CRF. The investigator should answer data queries within the specified time line.

16.3 Monitoring

This trial is part of the HOVON Site Evaluation Visit program. Site evaluation visits are performed for HOVON studies to review the quality of overall trial conduct on a participating site and not the quality of one specific trial. The purpose is to collect quality data and facilitate improvement of the participating site. Data cleaning is not the goal of the site evaluation visits. Site evaluation visits will be performed according to the site evaluation visit plan. A fundamental ingredient of the site evaluation visit is the interview with an investigator regarding the site’s organization and trial procedures. The site documents from a randomly selected HOVON trial
will serve as a guide to review the results of these procedures: the rights and well-being of patients are protected, the reported trial data are accurate, complete, and verifiable from source documents and the conduct of the trial is in compliance with the currently approved protocol/amendment(s), with GCP, and with the applicable regulatory requirement(s).

The HOVON site evaluation visit plan applies to sites in the Netherlands and Belgium only. Monitoring of the quality of trial conduct in participating sites from other countries will be organized by the coordinating investigator or co-sponsor. The frequency and content of site visits will be equal to the specifications of the site evaluation visit plan.

Direct access to source documentation (medical records) must be allowed for the purpose of verifying that the data recorded in the CRF are consistent with the original source data. The sponsor expects that during site evaluation visits the relevant investigational staff will be available, the source documentation will be available and a suitable environment will be provided for review of study-related documents.

16.4 Audits and inspections

The investigator will permit site-visits to carry out an audit of the study in compliance with regulatory guidelines. These audits will require access to all study records, including source documents, for inspection and comparison with the CRFs. Patient privacy must, however, be respected.

Similar auditing procedures may also be conducted by agents of any regulatory body reviewing the results of this study. The investigator should immediately notify the sponsor if they have been contacted by a regulatory agency concerning an upcoming inspection.

17 Ethics

17.1 Accredited ethics committee or Institutional Review Board

An accredited Ethics Committee or Institutional Review Board will approve the study protocol and any substantial amendment.

17.2 Ethical conduct of the study

The study will be conducted in accordance with the ethical principles of the Declaration of Helsinki, the ICH-GCP Guidelines, the EU Clinical Trial Directive (2001/20/EG), and applicable regulatory requirements. The local investigator is responsible for the proper conduct of the study at the study site.
17.3 Patient information and consent

Written informed consent of patients is required before enrolment in the trial and before any study related procedure takes place.
The investigator will follow ICH-GCP and other applicable regulations in informing the patient and obtaining consent. Before informed consent may be obtained, the investigator should provide the patient ample time and opportunity to inquire about details of the trial and to decide whether or not to participate in the trial. All questions about the trial should be answered to the satisfaction of the patient.
There is no set time limit for the patient to make a decision. The investigator should inform each patient if there is a specific reason why he/she must decide within a limited time frame, for example if patients condition necessitates start of treatment or if the trial is scheduled to close for enrolment.

The content of the patient information letter, informed consent form and any other written information to be provided to patients will be in compliance with ICH-GCP and other applicable regulations and should be approved by the Ethics Committee in advance of use.
The patient information letter, informed consent form and any other written information to be provided to patients will be revised whenever important new information becomes available that may be relevant to the patient’s consent. Any revised informed consent form and written information should be approved by the Ethics Committee in advance of use. The patient should be informed in a timely manner if new information becomes available that might be relevant to the patient’s willingness to continue participation in the trial. The communication of this information should be documented.

17.4 Benefits and risks assessment

Elderly patients with symptomatic CLL experience severe disease-specific morbidity. Current treatment for this patient group has limited efficacy due to relative low response rates. The hypothesis is that combination treatment will induce better responses resulting in prolonged disease free survival.
Risks for the patient relate to drug specific side-effects, in particular tumor flare reaction, tumor lysis and clinical relevant neutropenia.

17.5 Trial insurance

Prior to the start of the trial, the sponsor will ensure that adequate insurance for patients is in place covering losses due to death or injury resulting from the trial, in accordance with applicable laws and regulations in each country where the trial is conducted. The sponsor will take out an insurance policy or delegate this responsibility to a national co-sponsor. Proof of insurance will be submitted to the Ethics Committee.
In addition, the sponsor will ensure that adequate insurance is in place for both investigator(s) and sponsor to cover liability pertaining to death or injury resulting from the trial.

18 Administrative aspects and publication

18.1 Handling and storage of data and documents

18.1.1 Patient confidentiality

Each patient is assigned a unique patient study number at enrolment. In trial documents the patient’s identity is coded by patient study number as assigned at enrolment. In some cases date of birth is also listed.

The local investigator will keep a subject enrolment and identification log that contains the key to the code, i.e. a record of the personal identification data linked to each patient study number. This record is filed at the investigational site and should only be accessed by the investigator and the supporting site staff, and by representatives of the sponsor or a regulatory agency for the purpose of monitoring visits or audits and inspections.

18.1.2 Filing of essential documents

Essential Documents are those documents that permit evaluation of the conduct of a trial and the quality of the data produced. The essential documents may be subject to, and should be available for, audit by the sponsor’s auditor and inspection by the Regulatory Authority(ies)

The investigator should file all essential documents relevant to the conduct of the trial on site. The sponsor will file all essential documents relevant to the overall conduct of the trial. Essential documents should be filed in such a manner that they are protected from accidental loss and can be easily retrieved for review.

18.1.3 Record retention

Essential documents should be retained for 15 years after the end of the trial. They should be destroyed after this time.

Source documents (i.e. medical records) of patients should be retained for at least 15 years after the end of the trial. Record retention and destruction after this time is subject to the site’s guidelines regarding medical records.
18.1.4 Storage of samples

Biological samples should only be stored for the purpose of additional research if the patient has given consent. If no informed consent was obtained, samples should be destroyed after the patient has completed all protocol treatment and procedures.

Storage of biological samples on site is subject to the site’s guidelines; samples may be labeled with the patients identifying information (e.g. name, hospital record number).

Samples that are shipped to another facility (e.g. a central laboratory) for a purpose as described in this protocol or for additional scientific research, should be stripped from any identifying information and labeled with a code (trial name or number and patient study number as assigned at enrolment).

18.2 Amendments

A ‘substantial amendment’ is defined as an amendment to the terms of the Ethics Committee application, or to the protocol or any other supporting documentation, that is likely to affect to a significant degree:

- the safety or physical or mental integrity of the patients of the trial;
- the scientific value of the trial;
- the conduct or management of the trial; or
- the quality or safety of any intervention used in the trial.

All substantial amendments will be submitted to the Ethics Committee and to the Competent Authority.

Non-substantial amendments will not be submitted, but will be recorded and filed by the sponsor.

18.3 Annual progress report

The sponsor will submit a summary of the progress of the trial to the accredited Ethics Committee once a year. Information will be provided on the date of inclusion of the first patient, numbers of patients included and numbers of patients that have completed the trial, serious adverse events/serious adverse reactions, other problems, and amendments.

18.4 End of study report

The sponsor will notify the accredited Ethics Committee and the Competent Authority of the end of the study within a period of 90 days. The end of the study is defined as the last patient’s last visit.
In case the study is ended prematurely, the sponsor will notify the accredited Ethics Committee and the competent authority within 15 days, including the reasons for the premature termination.

Within one year after the end of the study, the sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited Ethics Committee and the Competent Authority.

18.5 Publication policy

*Final publication of trial results*

Trial results will always be submitted for publication in a peer reviewed scientific journal regardless of the outcome of the trial – unless the trial was terminated prematurely and did not yield sufficient data for a publication.

The final publication of the trial results will be written by the Principal Investigator, the Co-investigators and the trial statistician on the basis of the statistical analysis performed by the trial statistician. A draft manuscript will be submitted for review to:

♦ All co-authors
♦ The chair of the relevant HOVON working group, who is entitled to share and discuss the manuscript with working group members
♦ An industry partner if so agreed in the contract between HOVON and company

After revision the final manuscript is submitted to the HOVON secretary for review of compliance with this policy. After approval by the HOVON board the manuscript will be sent to a peer reviewed scientific journal.
**Authorship**

Authors of the main manuscript will include the Principal Investigator, the Co-investigators, investigators who have included more than 5% of the evaluable patients in the trial (by order of inclusion rate), the trial statistician and the trial manager. If a substantial part of the publication is based on centrally reviewed data (e.g. cytogenetics or pathology), the central reviewer will be included as author. Others who have made a significant contribution to the trial may also be included as author, or otherwise will be included in the acknowledgement.

Authors of correlative manuscripts (e.g. results of side studies) will include the Principal Investigator, the Co-investigators, and those persons who have made a significant contribution to the published results.

The Principal Investigator should discuss and decide on the matter of authorship of the main manuscript prior to the start of the trial – with the exception of authors included on account of inclusion rate. The Principal Investigator is urged to use the maximum number of authors allowed by the journal to the full extent.

**Interim and partial publications**

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

Investigators participating in the trial have a right to publish results from data they collected for the study. The Principal Investigator, the Co-investigator(s) and the trial statistician must approve any such publication, abstract or presentation based on patients included in this study. 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 endpoints unless the final results of the trial have already been published.

**Abstracts and presentations**

Abstracts and presentations at public meetings will represent the trial as a project under HOVON affiliation. The abstract or presentation should not be represented under affiliation of the working group or a specific hospital.
Slides will be designed using the HOVON style template and any other presentation materials will show the HOVON logo.

If the trial is conducted in partnership with a co-sponsor (e.g. intergroup trial), the abstract and presentation should represent the co-sponsor contribution and slides may show the co-sponsor logo in addition to the HOVON logo.

Prior to its public use, the abstract or presentation is submitted to the HOVON secretary for review of compliance with this policy.
Glossary of abbreviations
(in alphabetical order)

AE Adverse Event
ANC Absolute Neutrophil Count
BM Bone Marrow
Ca Calcium
CA Competent Authority
CKS Commissie voor Klinisch Studies
CLL Chronic Lymphocytic Leukemia
CR Complete Remission
CR² Chlorambucil Rituximab Revlimid
CRi Complete Remission with incomplete blood count recovery
CRF Case Report Form
CTCAE Common Terminology Criteria for Adverse Events
DFS Disease Free Survival
DSMB Data Safety and Monitoring Board
ECG Electrocardiogram
EFS Event Free Survival
FFS Failure Free Survival
FISH Fluorescence In Situ Hybridisation
GCP Good Clinical Practice
G-CSF Granulocyte-Colony Stimulating Factor
Hb Hemoglobin
HIV Human Immunodeficiency Virus
HOVON Dutch-Belgian Hematology-Oncology Cooperative Group
ICH International Conference on Harmonization of technical requirements for registration of
pharmaceuticals for human use
IMP Investigational Medicinal Product
ITT Intention To Treat
IU International Units
KCl Potassium Chloride
LDH Lactate Dehydrogenase
METC Medical Ethical Review Committee
MTD Maximum Tolerated Dose
NaCl Sodium Chloride
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>OS</td>
<td>Overall Survival</td>
</tr>
<tr>
<td>PB</td>
<td>Peripheral Blood</td>
</tr>
<tr>
<td>PD</td>
<td>Progressive Disease</td>
</tr>
<tr>
<td>PFS</td>
<td>Progression Free Survival</td>
</tr>
<tr>
<td>PML</td>
<td>Progressive Multifocal Leukoencephalopathy</td>
</tr>
<tr>
<td>PO</td>
<td>Per Os</td>
</tr>
<tr>
<td>PR</td>
<td>Partial Response</td>
</tr>
<tr>
<td>RDL</td>
<td>Recommended Dose Level</td>
</tr>
<tr>
<td>SAE</td>
<td>Serious Adverse Event</td>
</tr>
<tr>
<td>SC</td>
<td>Subcutaneous</td>
</tr>
<tr>
<td>SD</td>
<td>Stable Disease</td>
</tr>
<tr>
<td>SPM</td>
<td>Second primary malignancy</td>
</tr>
<tr>
<td>SUSAR</td>
<td>Suspected Unexpected Serious Adverse Reaction</td>
</tr>
<tr>
<td>TFR</td>
<td>Tumor Flare Reaction</td>
</tr>
<tr>
<td>TLS</td>
<td>Tumor Lysis Syndrome</td>
</tr>
<tr>
<td>ULN</td>
<td>Upper Limit of Normal</td>
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<tr>
<td>VTE</td>
<td>Venous thromboembolism</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>WMO</td>
<td>Wet Medisch-Wetenschappelijk Onderzoek met mensen</td>
</tr>
</tbody>
</table>
19 References

13. Hallek M, Fingerle-Rowson G, Fink A et al. Immunochemotherapy with Fludarabine (F), Cyclophosphamide (C), and Rituximab (R) (FCR) Versus Fludarabine and Cyclophosphamide (FC) Improves Response Rates and Progression-Free Survival (PFS) of Previously Untreated Patients (pts) with Advanced Chronic Lymphocytic Leukemia (CLL). ASH Annual Meeting Abstracts 2008;112:325.


39. Wendtner CM, Mahadevan D, Stilgenbauer S et al. Preliminary Results of a Phase 1/2, Multi-Center, Open-Label Study (CLL-001) Investigating a Stepwise Dose-Escalation Schedule of


A. Criteria for diagnosis and staging

A. IWCLL criteria for symptomatic CLL

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

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

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

B. Binet classification system

Stage A: Lymphocytosis and lymphadenopathy/organomegaly involving < 3 areas*
Stage B: Lymphocytosis and lymphadenopathy/organomegaly involving ≥ 3 areas*
Stage C: Lymphocytosis and Hb < 6.2 mmol/l (< 10 g/dl) or platelet count < 100 x 10⁹/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. Rai classification system

Stage 0: Lymphocytosis in blood or bone marrow
Stage I: Lymphocytosis and lymphadenopathy
Stage II: Lymphocytosis and hepato- or splenomegaly
Stage III: Lymphocytosis with anemia
Stage IV: Lymphocytosis with anemia and thrombocytopenia
B. Response criteria

Response definition summary\textsuperscript{55}

**Complete Remission (CR)**

CR requires all of the following criteria:

1. Peripheral blood lymphocytes (evaluated by blood and differential count) below $4 \times 10^9/l$
2. Absence of significant lymphadenopathy (e.g. lymph nodes > 1.5 cm diameter) (evaluated by CT-scan).
3. No hepatomegaly or splenomegaly (evaluated by CT-scan).
4. Absence of disease related symptoms
5. Blood counts above the following values:
   - Neutrophils > $1.5 \times 10^9/l$\textsuperscript{*}
   - Platelets > $100 \times 10^9/l$\textsuperscript{*}
   - Hemoglobin > 6.8 mmol/l\textsuperscript{**}

\textsuperscript{*}without need for exogenous growth factors
\textsuperscript{**}without red blood cell transfusion or need for exogenous erythropoietin

In case of CR (see above) either at response assessment after cycle 6 or at end of treatment a bone marrow aspirate and biopsy should be performed in order to confirm the CR.

In case CR is reached after cycle 6 the aspirate and biopsy need to be performed prior to cycle 7. If CR is reached at end of treatment, aspirate and biopsy should be performed 2 months after the last treatment has been finished.

To confirm a CR, the marrow sample must be at least normocellular for age, with less than 30% of nucleated cells being lymphocytes. Lymphoid nodules should be absent. In some cases, lymphoid nodules can be found, which often reflect residual disease. Immunohistochemistry should be performed to define whether these nodules are composed primarily of T cells or lymphocytes other than CLL cells or of CLL cells. If the marrow is hypocellular, a repeat determination should be performed after 4 weeks, or until peripheral blood counts have recovered. However, this time interval should not exceed 6 months after the last treatment. A marrow biopsy should be compared with that of pretreatment marrow.

**Partial Remission (PR)**

To define a PR parameters 1, 2, 3 as well as one or more of the features listed in 5 need to be documented for a minimal duration of 2 months. Constitutional symptoms persisting for more than 1 month should also be documented, but are not considered in the evaluation of PR.
1. A decrease in the number of peripheral blood lymphocytes by 50% or more from the value prior to therapy;

2. Reduction in lymphadenopathy as defined by: a decreased lymph node size by below 50% or more, either in the sum products of up to 6 lymph nodes or in the largest diameter of one of the enlarged lymph node(s) detected prior to therapy and no increase in any lymph node and no new lymph nodes. In small lymph nodes (< 2 cm), an increase of < 25% is not considered to be significant.

3. A decrease in the noted pre treatment enlargement of liver or spleen by 50% or more

4. Constitutional symptoms: not applicable

5. The blood count should show at least one of the following results:
   - Neutrophils > 1.5 x 10^9/l *
   - Platelet counts > 100 x 10^9/l or 50% improvement over baseline*
   - Hemoglobin > 6.8 mmol/l or 50% improvement over baseline **

   *without need for exogenous growth factors
   ** without red blood cell transfusion or need for exogenous erythropoietin.

**Progressive Disease (PD)**

PD during or after therapy is characterized by at least one of the following:

1. An increase by 50% or more in the numbers of blood lymphocytes with at least 5000 B-lymphocytes per microliter (5.0 x 10^9/l).

2. Lymphadenopathy. Progression of lymphadenopathy, if one of the following is observed:
   - Appearance of new lesion such as enlarged lymph nodes (>1.5 cm),
   - or an increase by 50% or more in largest determined diameter of any previous site.

3. An increase by 50% or more in the previously noted enlargement of the liver or spleen or de novo appearance of hepatomegaly or splenomegaly

4. Transformation to a more aggressive histology (e.g. Richter’s transformation).

5. Occurrence of cytopenia (neutropenia, anemia or thrombocytopenia) attributable to CLL

During therapy: Cytopenias cannot be used to define disease progression.

After treatment: The progression of any cytopenia (unrelated to autoimmune cytopenia), as documented by a decrease of Hb levels by more than 20 g/l (2 g/dl) or to less than 100 g/l (10 g/dl), or by a decrease of platelet counts by more than 50% or to less than 100 x 10^9/l, which occurs at least 3 months after treatment, defines disease progression, if the marrow biopsy demonstrates an infiltrate of clonal CLL cells.
Stable Disease (SD)

SD is defined as absence of progressive disease (PD) and failure to achieve at least a PR. Subjects who have changed from a CR or a PR, but who have not exhibited PD, will be considered to have SD.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CR</th>
<th>PR</th>
<th>PD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Response definition:</td>
<td>All criteria to be met</td>
<td>At least 2 of criteria 1, 2, 3 plus one criteria of 5a-c to be met (minimum duration of 2 months)</td>
<td>At least one criterium to be met</td>
</tr>
<tr>
<td>1 Blood lymphocytes</td>
<td>&lt; $4 \times 10^9$/l</td>
<td>$\geq 50%$ decrease from BL</td>
<td>$\geq 50%$ increase over BL ($\geq 5.0 \times 10^9$/l-B-cells)</td>
</tr>
<tr>
<td>2 Lymphadenopathy$^1$</td>
<td>absent (none &gt; 1.5cm)</td>
<td>$\geq 50%$ decrease from BL, no increase or new</td>
<td>$\geq 50%$ increase or new (&gt;1.5cm)</td>
</tr>
<tr>
<td>3 Hepato/spleno megaly</td>
<td>Absent</td>
<td>$\geq 50%$ decrease from BL</td>
<td>Increase $\geq 50%$ or new</td>
</tr>
<tr>
<td>4 Constitutional symptoms</td>
<td>Absent</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>5a Neutrophils</td>
<td>$&gt;1.5 \times 10^9$/l</td>
<td>$&gt;1.5 \times 10^9$/l</td>
<td>n/a</td>
</tr>
<tr>
<td>5b Platelet count</td>
<td>$&gt;100 \times 10^9$/l</td>
<td>$&gt;100 \times 10^9$/l or $\geq 50%$ increase over BL</td>
<td>$\geq 50%$ decrease from BL or to $&gt;100 \times 10^9$/l second. to CLL$^2$</td>
</tr>
<tr>
<td>5c Hemoglobin</td>
<td>$&gt;6.8 \mu$mol/l</td>
<td>$&gt;6.8 \mu$mol/l or increase $\geq 50%$ over BL</td>
<td>Decrease of $&gt;1.3 \mu$mol/l from BL or to $&lt;6.2 \mu$mol/l second to CLL$^2$</td>
</tr>
<tr>
<td>6 Marrow</td>
<td>Normocellular, no B-lymphoid nodules, $&lt;30%$ lymphocytes</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>7 other</td>
<td>n/a</td>
<td>n/a</td>
<td>CLL- transformation, cytopenia after treatment</td>
</tr>
</tbody>
</table>

1. in greatest largest determined diameter of any previous site
2. Occurrence of cytopenia attributable to CLL at least 3 months after treatment defines disease progression, if the marrow biopsy demonstrates an infiltrate of clonal CLL cells

BL=baseline
### C. ZUBROD-ECOG-WHO Performance Status Scale

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Normal activity</td>
</tr>
<tr>
<td>1</td>
<td>Symptoms, but nearly ambulatory</td>
</tr>
<tr>
<td>2</td>
<td>Some bed time, but to be in bed less than 50% of normal daytime</td>
</tr>
<tr>
<td>3</td>
<td>Needs to be in bed more than 50% of normal daytime</td>
</tr>
<tr>
<td>4</td>
<td>Unable to get out of bed</td>
</tr>
</tbody>
</table>
D. Common Terminology Criteria for Adverse Events

The grading of adverse events will be done using the NCI Common Terminology Criteria for Adverse Events, CTCAE version 4. A complete document may be downloaded from the HOVON website:

http://www.hovon.nl (under Trials > General information about studies)
E. Cumulative Illness Rating Scale (CIRS)

### Rating Strategy of Comorbidity

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No problem, organ system not compromised.</td>
</tr>
<tr>
<td>1</td>
<td>Mild, illness/impairment with or without requirement of therapy, excellent prognosis, patient with normal activity.</td>
</tr>
<tr>
<td>2</td>
<td>Moderate, illness/impairment requiring therapy, good prognosis, comprised activity of patient.</td>
</tr>
<tr>
<td>3</td>
<td>Severe, illness/impairment with urgent requirement of therapy, prognosis unclear, marked restriction in activity.</td>
</tr>
<tr>
<td>4</td>
<td>Extremely severe, life threatening illness/impairment, emergency case of therapy, adverse prognosis.</td>
</tr>
</tbody>
</table>

Please take into account that CLL induced illness or organ damage are not included in this rating scale. The goal of this rating scale is to assess comorbidity other than CLL in the patient. If there are two or more illnesses/impairments of one organ system, the illness/impairment with the highest severity should be evaluated.

<table>
<thead>
<tr>
<th>Organ System</th>
<th>A) If illness/impairment present, please specify</th>
<th>B) Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Heart</td>
<td></td>
<td></td>
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<tr>
<td>2. Blood pressure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Vascular</td>
<td></td>
<td></td>
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<tr>
<td>4. Respiratory</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Ear/nose/throat</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. Upper gastrointestinal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. Lower gastrointestinal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8. Liver</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9. Renal</td>
<td></td>
<td></td>
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<tr>
<td>10. Genitourinary</td>
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</tr>
<tr>
<td>11. Musculoskeletal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12. Endocrine/metabolic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13. Neurological</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14. Psychiatric</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>15. Score:</th>
<th>Total</th>
</tr>
</thead>
</table>

From: Miller MD, Paradis CF, Houck PR et al.\textsuperscript{53}
## F. Managing lenalidomide dosing according to toxicity

### Hematologic Toxicity

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>Management</th>
</tr>
</thead>
</table>
| Handling neutropenia during combination treatment (cycle I-VI) : ANC<1x10^9/l. | 1. In case of neutropenic fever or neutropenia associated with other toxicity: start G-CSF support, hold (interrupt dose) until ANC ≥ 0.5x10^9/l. Restart with one dose step reduction. Follow CBC weekly.  
2. If neutropenia is the only toxicity (including no fever) for which a dose reduction is required, G-CSF should be used and the dose should be maintained. |
| For handling neutropenia during monotherapy (cycle VII-XII), see paragraph 9.2.2 |                                                                                                  |
| Thrombocytopenia platelet count < 30x10^9/l unless due to marrow infiltration | Hold (interrupt dose) If thrombocytopenia resolves to ≥ 30x10^9/l restart at one step dose reduction and follow CBC weekly. |

### Non-Hematologic Toxicity

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>Management</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-blistering rash Grade 3</td>
<td>If Grade 3 hold (interrupt) dose, follow weekly. If the toxicity resolves to ≤ grade 1 can restart with one step dose reduction.</td>
</tr>
<tr>
<td>Grade 4</td>
<td>If Grade 4 rash- discontinue lenalidomide study drug.</td>
</tr>
<tr>
<td>Desquamatiting (blistering) rash- any Grade</td>
<td>Discontinue lenalidomide therapy</td>
</tr>
<tr>
<td>Erythema multiforme ≥ Grade 3</td>
<td>Discontinue lenalidomide study drug</td>
</tr>
<tr>
<td>Sinus bradyardia/ other cardiac arrhythmia Grade 2</td>
<td>Hold (interrupt dose), if the toxicity resolves to ≤ grade 1 restart at one step dose reduction.</td>
</tr>
<tr>
<td>≥ Grade 3</td>
<td>If ≥ Grade 3 discontinue lenalidomide study drug.</td>
</tr>
<tr>
<td>Allergic reaction or hypersensitivity Grade 2</td>
<td>Hold (interrupt dose) If the toxicity resolves to ≤ grade 1 option to restart at one step dose reduction.</td>
</tr>
<tr>
<td>Grade 3-4</td>
<td>If Grade 3-4 discontinue lenalidomide study drug.</td>
</tr>
<tr>
<td>Venous thrombosis/embolism ≥ Grade 3</td>
<td>Hold (interrupt dose) and start anticoagulation (management as clinically indicated) and discontinue Lenalidomide therapy.</td>
</tr>
<tr>
<td>Other non-hematologic toxicity assessed as Lenalidomide-related ≥ Grade 3</td>
<td>Hold (interrupt dose), follow at least weekly. If the toxicity resolves to ≤ grade 2 restart at one step dose reduction.</td>
</tr>
<tr>
<td>Laboratory Tumor Lysis Syndrome</td>
<td>If there is evidence of laboratory tumor lysis syndrome (LTLS) by Cairo-Bishop criteria without clinical tumor lysis Lenalidomide should be held and oral or IV hydration administered until laboratory abnormalities resolve at which time Lenalidomide can be restarted with continued monitoring every 2-3 days for at least one week for LTLS. If LTLS occurs on rechallenge, after laboratory abnormalities return to baseline Lenalidomide can be restarted with a one step dose reduction.</td>
</tr>
<tr>
<td>Hyperthyroidism or hypothyroidism</td>
<td>Hold lenalidomide (interrupt dose), evaluate etiology, and initiate appropriate therapy. Restart lenalidomide next cycle (decrease dose by one dose step reduction)</td>
</tr>
</tbody>
</table>

‡Patients that are not able to tolerate the lowest dose (2.5 mg) for any reason except for hematologic toxicity will be discontinued from therapy. Patients who experience hematologic toxicity on lenalidomide 2.5 mg may be continued following recovery from toxicity if investigator feels that the subject is otherwise benefiting from therapy.
G. Grading scale for hematological toxicity


This grading must be used instead of CTCAE version 4.0

<table>
<thead>
<tr>
<th>Grade</th>
<th>Decrease in Platelets^2 or Hb^3 (nadir) from pretreatment value (%)</th>
<th>Absolute Neutrophil Count/l^4 (nadir)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11%-24%</td>
<td>≥1.5 and &lt;2.0</td>
</tr>
<tr>
<td>2</td>
<td>25%-49%</td>
<td>≥1.0 and &lt;1.5</td>
</tr>
<tr>
<td>3</td>
<td>50%-74%</td>
<td>≥0.5 and &lt;1.0</td>
</tr>
<tr>
<td>4</td>
<td>≥75%</td>
<td>&lt; 0.5</td>
</tr>
</tbody>
</table>

1. Grades: 1-mild; 2-moderate; 3-severe; 4-life-threatening; 5-fatal. Death occurring as a result of toxicity at any level of decrease from pretreatment will be recorded as grade 5.

2. Platelet counts must be below normal levels for grades 1-4. If, at any level of decrease the platelet count is <20x10^9/l, this will be considered grade 4 toxicity, unless a severe or life threatening decrease in the initial platelet count (e.g., 20x10^9/l) was present pretreatment, in which case the subject is not evaluable for toxicity referable to platelet counts.

3. Hb levels must be below normal levels for grades 1-4. Baseline and subsequent Hb determinations must be performed before any given transfusions. The use of erythropoietin is irrelevant for the grading of toxicity, but should be documented.

4. If the absolute neutrophil count (ANC) reaches less than 1.0x10^9/l, it should be judged to be grade 3 toxicity. Other decreases in the white blood cell count, or in circulating granulocytes, are not to be considered, since a decrease in the white blood cell count is a desired therapeutic endpoint. A gradual decrease in granulocytes is not a reliable index in CLL for stepwise grading of toxicity. If the ANC was less than 1.0x10^9/l prior to therapy, the subject is not evaluable for toxicity referable to the ANC. The use of G-CSF is irrelevant for the grading of toxicity, but should be documented.
### Cairo-Bishop grading classification of tumor lysis syndrome

<table>
<thead>
<tr>
<th>LTLS</th>
<th>Grade 0*</th>
<th>Grade I</th>
<th>Grade II</th>
<th>Grade III</th>
<th>Grade IV</th>
<th>Grade V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine</td>
<td>≤1.5×ULN</td>
<td>&gt;1.5×ULN</td>
<td>&gt;3.0×1.5×ULN</td>
<td>&gt;6.0×60×ULN</td>
<td>&gt;6.0×ULN</td>
<td>Death§</td>
</tr>
<tr>
<td>Cardiac arrhythmia</td>
<td>None</td>
<td>Non-urgent medical intervention indicated</td>
<td>Symptomatic and incompletely controlled medically or controlled with device (e.g. defibrillator)</td>
<td>Death§</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seizure</td>
<td>None</td>
<td>One brief generalised seizure; seizure(s) well controlled by anti-convulsants or infrequent focal motor seizures not interfering with ADL</td>
<td>Seizure in which consciousness is altered; poorly controlled seizure disorder; with breakthrough generalized seizures despite medical intervention</td>
<td>Seizure of any kind which are prolonged, repetitive or difficult to control (e.g. status epilepticus, intractable epilepsy)</td>
<td>Death§</td>
<td></td>
</tr>
</tbody>
</table>

Clinical tumor lysis syndrome (CTLS) requires one or more clinical manifestations along with criteria for laboratory tumor lysis syndrome (LTLS). Maximal CTLS manifestation (renal, cardiac, neuro) defines the grade.

*No laboratory tumor lysis syndrome (LTLS).
†Creatinine levels patients will be considered to have elevated creatinine if their serum creatinine is 1.5 times greater than the institutional upper limit of normal (ULN) below age/gender defined ULN. If not specified by an institution, age/gender ULN creatinine may be defined as: > 1 < 12 years, both male and female: 61.0 μmol/l; ≥ 12 < 16 years, both male and female: 88 μmol/l; 16 years, female: 105 μmol/l; 216 years, male: 114 μmol/l.
‡Not directly or probably attributable to a therapeutic agent (e.g. rise in creatinine after anthracycin administration).
§Attributable probably or definitely to CTLS.

# Laboratory tumor lysis syndrome (LTLS) is defined as either a 25% change or level above or below normal, as defined above, for any two or more serum values of uric acid, potassium, phosphorus, and calcium within 3 days before or 7 days after the initiation of chemotherapy. This assessment assumes that a patient has or will receive adequate hydration (± alkalinization) and a hypouricosuric agent(s).

From: Cairo MS, Bishop M. Tumor lysis syndrome: new therapeutic strategies and classification⁵⁰
I. Grading of tumor flare syndrome

According to CTCAE version 3.0

<table>
<thead>
<tr>
<th>Grade</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor flare</td>
<td>Mild pain not interfering with function</td>
<td>Moderate pain; pain or analgesics interfering with function, but not interfering with ADL</td>
<td>Severe pain; pain or analgesics interfering with function and interfering with ADL</td>
<td>Disabling</td>
<td>Death</td>
</tr>
</tbody>
</table>

REMARK: Tumor flare is characterized by a constellation of signs and symptoms in direct relation to initiation of therapy (e.g., anti-estrogens/androgens or additional hormones). The symptoms/signs include tumor pain, inflammation of visible tumor, hypercalcemia, diffuse bone pain, and other electrolyte disturbances. ALSO CONSIDER: Calcium, serum-high (hypercalcemia).