PROTOCOL: PreVent-ACaLL

TITLE:	Short-term combined acalabrutinib and venetoclax treatment of newly diagnosed patients with CLL at high risk of infection and/or early treatment, who do not fulfil IWCLL treatment criteria for treatment. A randomized study with extensive immune phenotyping.
STUDY DRUGS:	Acalabrutinib Venetoclax (Venclyxto)
EUDRACT NUMBER:	2019-000270-29
Clinicaltrials.gov number	NCT03868722
FUNDER NUMBER:	ESR-18-14283
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Signature Local Site Investigator **Date**

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STUDY SYNOPSIS

Study Title:	Short-term combined acalabrutinib and venetoclax treatment of newly diagnosed patients with CLL at high risk of infection and/or early treatment, who do not fulfil IWCLL treatment criteria. A randomized study with extensive immune phenotyping. PreVent-ACaLL
Study Drug:	ACALABRUTINIB (ACP-196) VENETOCLAX (ABT-199)
Phase:	Phase 2 with option for extension to phase 3 upon analysis of phase 2 part and agreement between study groups and companies
Comparator:	Observation according to IWCLL criteria
Study Centers:	Subjects will be enrolled in approximately 7 centers in Denmark, Sweden and the Netherlands.
Study Objectives:	The aim of the study is to evaluate whether short-term, pre-emptive combination treatment with venetoclax plus acalabrutinib in patients with high risk of infection and/or CLL treatment can change the natural history of immune dysfunction in CLL in terms of the long-term risk of serious infections and diminish the need for cytotoxic chemotherapy. (Without an increased risk of infection during treatment in that group)
	Primary Objective phase 2 part: • Grade ≥3-Infection-free survival in the treatment arm compared to the observation arm 12 weeks after finishing treatment, (24 weeks after treatment initiation). This is a non-inferiority analysis as detailed in the statistical analysis plan to assure safety of the combination treatment in this preemptive trial population. Primary Objective optional phase 3 part: • Grade ≥3-infection free and CLL-treatment-free survival 2 years after enrollment

	Secondary Objective(s): • Grade ≥3-infection free and CLL-treatment-free survival at end of treatment, 1 year and 2 years after enrollment • Rate of overall survival (OS) and cause of death • Treatment free survival • Rate and CTCAE V5.0 grade of infections • Response rate and duration according to IWCLL criteria • Treatment related adverse events, type, frequency and severity during and for 2 years after treatment • Immune function as assessed by immune phenotyping, functional TruCulture assays and measurements of cytokine levels Exploratory Objective(s): • MRD levels in bone marrow and peripheral blood • Quality of life during and for 2 years after treatment, QLQC30 and CLL17
Study Design:	Newly diagnosed patients with CLL will be assessed by the CLL-TIM algorithm for high risk of infection and/or early CLL treatment need during the screening period. Only patients identified as at high risk, who do not currently fulfil IWCLL treatment criteria, will enter the trial. Assessment of variables for the risk profile including the baseline characteristics and laboratory result based Machine Learning algorithm CLL-TIM will be performed centrally through a plug & play model, which will be part of the eCRF system. Randomization has to occur within 42 days after the first tests for screening were performed. Patients will be randomly assigned to treatment vs observation through 1:1 randomization process with stratification according to country, TP53 aberration status and IGHV mutational status. Treatment or observation period has to be initiated within 14 days after randomization. Acalabrutinib 100 mg BID from cycle 1 day 1 for 3 cycles of 28 days. Due to the preemptive treatment within the trial of patients not fulfilling IWCLL criteria for treatment of CLL, the tumor burden will be lower than in other trial populations, thus it is assessed safe to start acalabrutinib and venetoclax treatment simultaneously. Venetoclax, ramp up during the first five weeks starting cycle 1 day 1, 7 days treatment on each dose level (20-50-100-200-400 mg), thereafter 400 mg once daily for a total of 3 cycles of 28 days counted from cycle 1 day 1. The trial is a phase 2 trial with a non-inferiority analysis for safety.

Efficacy and Safety Parameters:	Due to the pre-emptive treatment of a patient population normally not treated, an independent Data Safety Monitoring Board (DSMB) will assure ongoing assessment of infectious risk and adverse events. The trial may be stopped based on the decision by the independent DSMB if increased infectious risk or AE risk is considered to outweigh the potential benefit during enrollment. For these safety assessments, the treatment period (first 3 cycles of 28 days each) and the follow up period will be assessed independently to improve detection of treatment-related events vs late treatment-related events and CLL-related events. The following early stopping rules will be applied: • Any fatal events or Grade ≥3 infectious events in the treatment arm will be reported immediately (within 7 days of notification) to the DSMB for assessment of whether the event impacts the risk-benefit of the treatment (data on similar events in the observation arm will be made available to the DSMB). All Grade ≥3 infectious events would be considered AESIs to be reported along with SAEs within 24 h in the phase 2 part. • Assuming the incidence of grade ≥3 infectious events is the same in the treatment arm and the observation arm, which based on the predictive model is 10% within the first 6 months, a non-inferiority test can be performed. With a power of 80% and a sample size of 25 patients in each arm, the margin for assessment of non-inferiority will be 21%, i.e. if significantly more infections are seen in the treatment arm than in the observation arm, the treatment arm will be inferior to observation. From a clinical perspective, the margin of error is considered meaningful. The 21% margin of error is acceptable for this high-risk patient population at 24 weeks after initiating treatment/observation, particularly as clinical benefit of treatment is expected to continue for several years. • Any pattern of AEs or individual AEs through the above-mentioned DSMB assessment that indicate that the trial should be stopped. A thorough translational immune phenotype and immune function analysis plan is adjoined to the study. Results of this translational program are included as secondary outcomes. The assessment of the grade ≥3-infection free, CLL-treatment-free survival will be qualified by the results from the translational program. Hereby, the clinical outcome from the study will be significantly strengthened by molecular and functional information about improvements in immune function.

Biomarker Parameters:	• **Immune phenotyping**: A 10-color flow cytometry whole blood panel comprising 10 different tests has been custom-designed (DuraClone, Beckman Coulter) and is currently under validation at the Dept. of Clinical Immunology, Rigshospitalet. The ten different flow tests (I-X) evaluates: I) Major cell lineages in the blood (proportions and counts), II) B cell subsets, III) T cell subsets, IV) T cell receptor (TCR) subsets, V) Regulatory (T_{reg}) and Th_{17} cells, VI) Dendritic cells, VII) Myeloid cells, VIII) Erythrocytes as surrogates for asplenism, IX) Platelets and platelet-leukocyte co-aggregates and X) Neutrophil phagocyte function. The panel captures multiple patterns predictive for cancer patients i.e. deviated maturation, acute/chronic activation, exhaustion, migration/trafficking and expression of immune checkpoint markers (PD-1, PD-1L, CTLA-4, FoxP3, Helios, CD25, TLR2, TLR4, CD38) which will also be extended with assessment of NK cell function and subtypes as well as additional immune activation markers.[1-8] • **Immune function**: Stimulated immune response & platelet function are assessed by TruCulture® (Myriad RBM), which assesses the induced innate and adaptive immune responses to whole blood ligand stimulation. Five different stimuli I-V are applied to screen the response to signaling pathways via Toll Like Receptors (TLRs), critical for the anticancer immune response as TLR ligands may break tolerance to self-antigens and promote immune responses to tumor antigens[9,10]: I) Zymosan; II) Poly I:C; III) Resiquimod; IV) ODN + LPS and V) NegCo. TruCulture® are currently validated at the Dept. of Clinical Immunology, Rigshospitalet and being tested for assessment of immune function in CLL at baseline and during treatment with targeted small molecules. TruCulture® provides an extensive data-readout, with cytokine response data available within days whereas Multiplate®/TEG® provides real-time results also on platelet function and complement cascades. Furthermore, transcriptomics by RNAseq assures in depth analyses of the molecular basis for immune changes upon treatment in stimulated and unstimulated cells.
Sample Size:	A sample size of 25 patients in each arm, 50 patients in total for the phase 2 part.
Inclusion Criteria:	1. CLL diagnosed according to IWCLL criteria within one year prior to randomization 2. High risk of infection and/or progressive treatment within 2 years according to CLL-TIM 3. IWCLL treatment indication not fulfilled 4. Life expectancy > 2 years 5. Age at least 18 years 6. Ability and willingness to provide written informed consent and adhere to study procedures and treatment

	7. Adequate bone marrow function as indicated by platelets above 100 x 10E9, hemoglobin above 10 g/dL and neutrophils above 1 x 10E9 8. Creatinine clearance above 30 mL/min directly measured with 24hr urine collection or calculated according to the modified formula of Cockcroft and Gault 9. Adequate liver function as indicated by a total bilirubin≤ 2 x, AST or ALT ≤ 2.5 x the institutional ULN value, unless directly attributable to the patient's CLL or to Gilbert's Syndrome. 10. Negative serological testing for hepatitis B (HBsAg negative and anti-HBc negative; patients positive for anti-HBc may be included if PCR for HBV DNA is negative and HBV-DNA PCR is performed every month until 12 months after last treatment cycle), negative testing for hepatitis C RNA within 6 weeks prior to registration. 11. Eastern Cooperative Oncology Group Performance Status (ECOG) performance status 0-2. 12. Woman of childbearing potential (WOCBP) who are sexually active must use highly effective methods of contraception during treatment and for 30 days after the last dose of investigational drugs. 13. Willing and able to participate in all required evaluations and procedures in this study protocol including swallowing capsules without difficulty. 14. Ability to understand the purpose and risks of the study and provide signed and dated informed consent and authorization to use protected health information.
Exclusion Criteria:	1. Prior CLL treatment (including monoclonal antibodies, chemotherapy, small molecules, including CD20 antibodies, BTK inhibitors and bcl-2 inhibitors for any indication) 2. Transformation of CLL (Richter's transformation) 3. Previous autoimmune disease as AIHA (autoimmune hemolytic anemia) or ITP (idiopathic thrombocytopenic purpura) treated with immune suppression or uncontrolled AIHA or ITP 4. History of progressive multifocal leukoencephalopathy 5. HIV infection (a negative test required) 6. Known active infection 7. Malignancies other than CLL requiring systemic therapies (except anti-hormonal therapies) or considered to impact survival 8. Requirement of therapy with strong CYP3A4 and CYP3A5 inhibitors/inducers or anticoagulant therapy with vitamin K antagonists 9. History of bleeding disorders or current platelet inhibitors or anticoagulant therapy 10. History of clinically significant cardiovascular disease such as arrhythmias, congestive heart failure, or myocardial infarction within 6 months of screening, or any Class 3 or 4 cardiac disease as defined by the New York Heart Association

	Functional Classification, or corrected QT interval (QTc) > 480 msec at screening. 11. History of stroke or intracranial hemorrhage within 6 months prior to registration. 12. Use of investigational agents which might interfere with the study drug within 28 days prior to registration. 13. Vaccination with live vaccines within 28 days prior to registration. 14. Major surgery less than 30 days before start of treatment. Note: If a subject had major surgery, they must have recovered adequately from any toxicity and/or complications from the intervention before the first dose of study drug. 15. Known hypersensitivity to any active substance or to any of the excipients of one of the drugs used in the trial. 16. Pregnant women and nursing mothers (a negative pregnancy test is required for all women of childbearing potential within 7 days before start of treatment; further pregnancy testing will be performed regularly). 17. Fertile men or women of childbearing potential unless: surgically sterile or ≥ 2 years after the onset of menopause or willing to use two methods of reliable contraception including one highly effective contraceptive method (Pearl Index <1) and one additional effective (barrier) method during study treatment and for 30 days after the end of study treatment. 18. Legal incapacity. 19. Persons who are in dependence to the sponsor or an investigator 20. Persons not considered fit for the trial by the investigator 21. Malabsorption syndrome, disease significantly affecting gastrointestinal function, or resection of the stomach or small bowel that is likely to affect absorption, symptomatic inflammatory bowel disease, partial or complete bowel obstruction, or gastric restrictions and bariatric surgery, such as gastric bypass. 22. Prothrombin time/INR or aPTT (in the absence of Lupus anticoagulant) > 2x ULN. 23. Requires treatment with proton pump inhibitors (e.g., omeprazole, esomeprazole, lansoprazole, dexlansoprazole, rabeprazole, or pantoprazole). Subjects receiving proton pump inhibitors who switch to H2-receptor antagonists or antacids are eligible for enrollment to this study.
Dose Regimen/Route of Administration:	Acalabrutinib is provided as hard gelatin capsules for oral administration approximately every 12 hours. Venetoclax is provided as tablets for oral administration once daily

Concomitant Medications:	**Prohibited Concomitant Therapy** Prohibited (strong CYP3A inhibitors and warfarin/vitamin K antagonists) and cautionary medications are defined as moderate CYP3A inhibitors, and moderate and strong CYP3A inducers.

1. BACKGROUND INFORMATION

1.1 CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

Chronic lymphocytic leukemia (CLL) is a malignancy of antigen reactive, mature B cells involving blood, bone marrow, and lymphoid tissues. It is the most common leukemia in Western countries with approximately 450 new patients diagnosed in Denmark each year. (lymphoma.dk)[11] The overall survival has significantly improved with the introduction of chemoimmunotherapy and targeted treatment, but the clinical course is very heterogeneous. [12] Patients with CLL have an increased risk of infections. Up to 47% suffering from recurrent infections and one-third of all deaths among patients with CLL being due to infection; leading to infections being the major cause of death for treated as well as treatment naïve patients with CLL, while all other causes of death have decreased over the last decades.[13-15]

Figure 1-1. No improvement in infectious deaths despite improvement in overall survival over the last three decades.

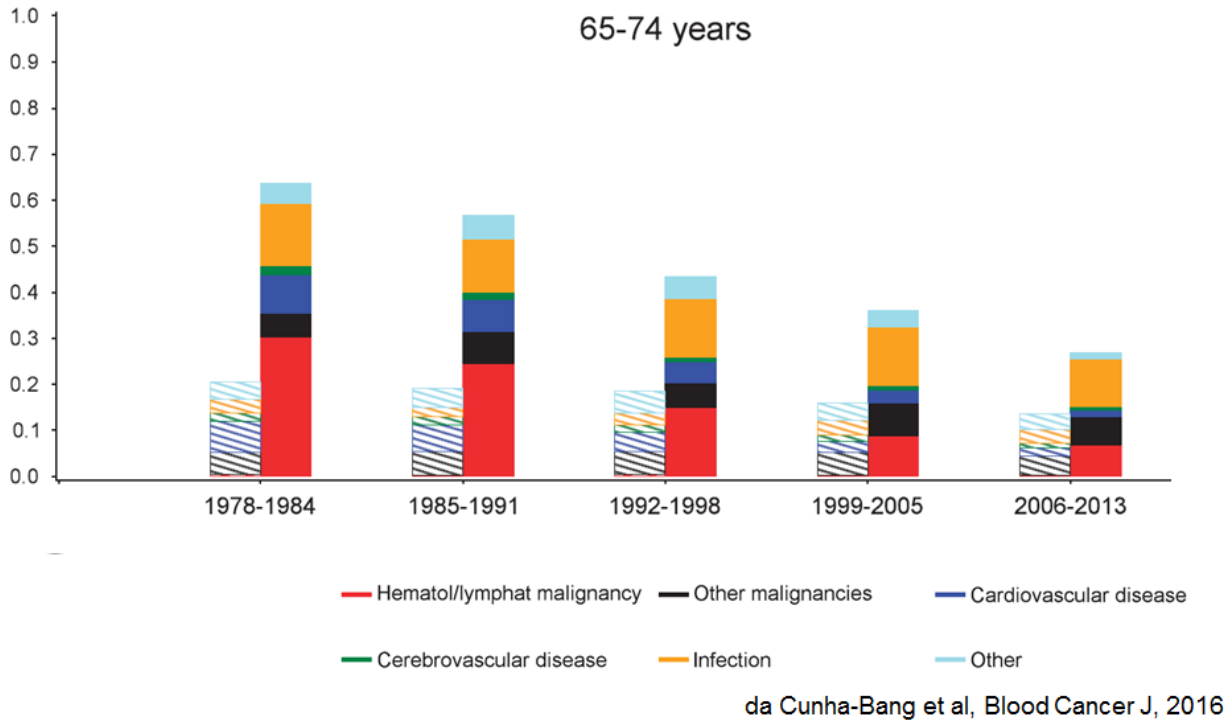
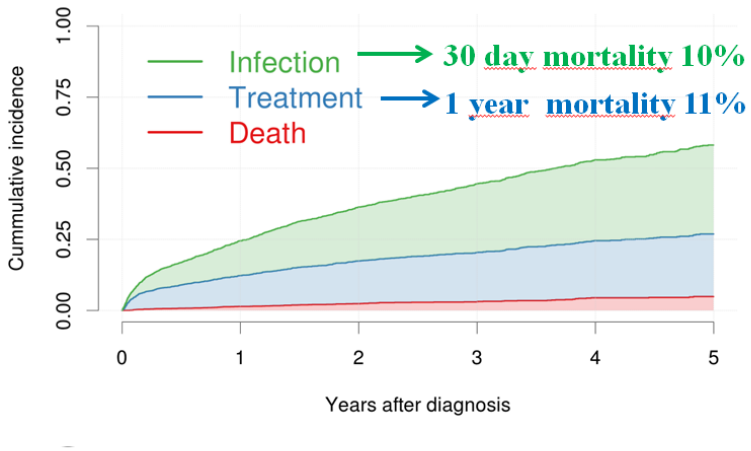


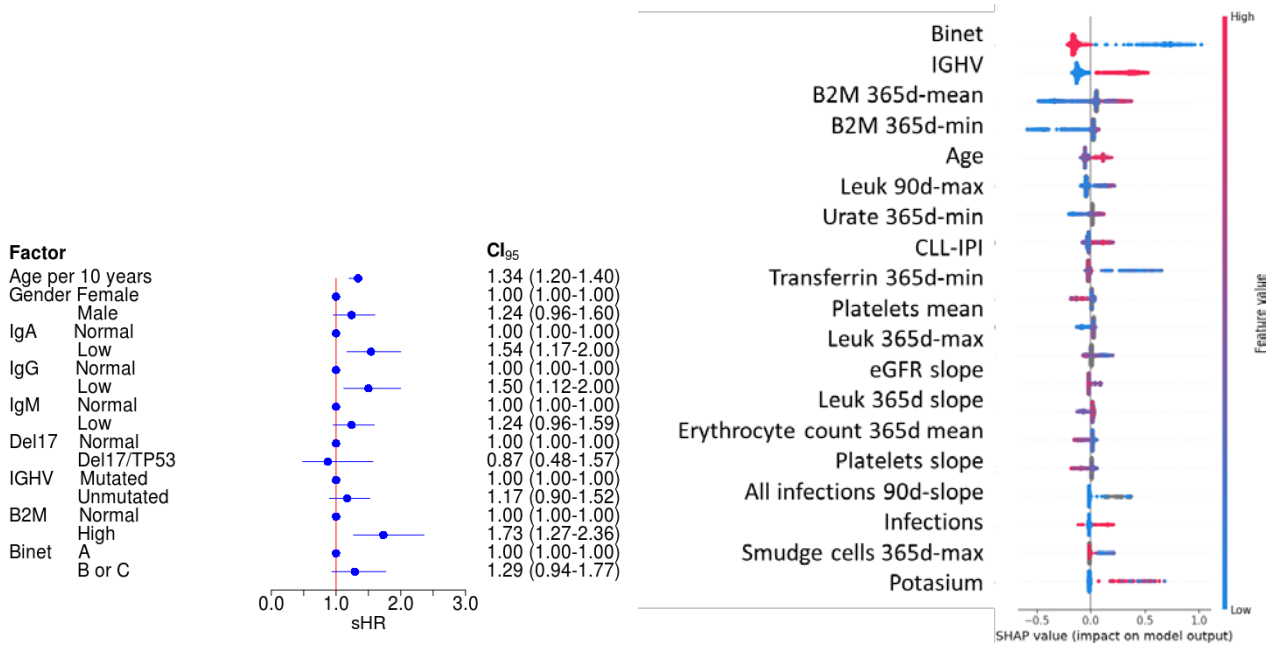
Figure 1-2. Competing risk for either infection, CLL treatment or death as first event from time of diagnosis.



Andersen et al, Haematologica, 2018

In recent work from our group, assessing the disease course for 2905 patients diagnosed with CLL, the cumulative incidence of severe infection prior to treatment was 31.6 % within 5 years of infection and 9.8 % died within one month after the infection.[16] Based on machine learning modeling, we can now identify patients with a >65% risk of infection and/or treatment within 2 years in newly diagnosed patients with CLL. (Agius et al, EHA 2018, abstract PS-1103, manuscript prepared for journal submission).

Figure 1-3. Factors with impact on risk of infection based on regular multiple regression (left) and Machine Learning (right, incomplete list, not completed).



Chemoimmunotherapy is the standard of care in first-line treatment of CLL patients without del17p or TP53 mutation; physically fit patients are treated with fludarabine, cyclophosphamide and rituximab (FCR) [17] even though recent conference proceedings have indicated that subgroups of these patients may better be treated with ibrutinib.[18] Due to the high risk of severe neutropenia and infections upon FCR treatment, bendamustine and rituximab (BR) is considered the best treatment option in patients aged >65 years.[19] However, these conventional chemoimmunotherapies are associated with side effects caused by the cytotoxic effect of chemotherapy. Therefore, there is an urgent need for alternatives, especially chemotherapy-free regimens. In the clinical setting, Danish real-world evidence demonstrates a 50% 1-year risk of severe infection after chemoimmunotherapy in CLL, with a 3-year infectious risk of 75% - thus emphasizing the impact of chemoimmunotherapy combined with the immune dysfunction of CLL.

1.2 BRUTON TYROSINE KINASE INHIBITION AND BCL-2 INHIBITION IN CLL

The B-cell receptor (BCR) pathway has recently been the core for development of new therapeutics for patients with CLL. Several molecules in the BCR pathway have been clinically targeted in CLL, with the BTK inhibitors ibrutinib and acalabrutinib and the PI3Kδ inhibitor idelalisib approved for clinical use. Acalabrutinib has shown excellent responses and an acceptable safety/toxicity profile.[20,21] Similarly, the significance of the anti-apoptotic peptide bcl-2 in the pathogenesis of CLL has led to test of bcl-2 inhibitors. The bcl-2 antagonist venetoclax has proven highly effective with tumor lysis syndrome as dose limiting toxicity,[22,23] in patients with relapsed and refractory (RR) CLL. Venetoclax has been approved for CLL patients and even been shown to achieve MRD negativity.[24]

We and others have previously demonstrated that the BTK inhibitor ibrutinib used in clinical trials of CLL can reverse immune dysfunction and improve IgA levels along with a decreased risk of infection and improvement in immune phenotype. [6-8,25] Translational data for patients treated with BTK inhibitors [6,25,26] and venetoclax [27] indicate that immune function can be at least partly restored on treatment with decreasing incidence of infections over time on treatment and improved immune parameters with lowered proportion of Treg cells and decreased PD1 expression allowing for reversal of the pseudoexhausted phenotype of T cells in CLL. The combination of BTK inhibitors and venetoclax has demonstrated synergy in primary CLL cells and in several DLBCL cell lines. [28-30] Preliminary data from clinical trials combining BTK inhibitors and venetoclax did not show any new safety signals of specific importance, and no signals indicating increased risk of infection has been identified (confidential communication within the GAIA/CLL13 and VISION trials, for which the applicants are founding members). Furthermore, translational studies indicate that the protective role of microenvironmental interactions in CLL can be disrupted and impact the synergy of venetoclax in combination with BTK inhibitors.[31,32]

Based on former clinical trials testing early treatment at diagnosis of CLL vs treatment only at the time of symptomatic disease according to IWCLL criteria,[33] it is a dogma that only symptomatic CLL patients should be treated. However, the significant morbidity and mortality due to infections in patients with CLL prior to treatment warrants clinical trials addressing this unmet need for targeted CLL treatment that can change the natural history of CLL.

1.3 ACALABRUTINIB (ACP-196)

Acalabrutinib is an imidazopyrazine analogue with a molecular weight of 465.5 g/mol. The compound has 1 stereogenic center and acalabrutinib is the S-enantiomer. Acalabrutinib is orally bioavailable in humans and is suitable for formulating in capsules. Acalabrutinib is approved in the US for the treatment of adult patients with MCL who have received at least 1 prior therapy. It is also being evaluated for the treatment of patients with other B-cell malignancies.

1.3.1 Mechanism of Action

Acalabrutinib is a potent inhibitor of BTK in vitro and in vivo. Pharmacology models have been used to define kinase selectivity of acalabrutinib in comparison to other BTK inhibitors, and to investigate functional effects of on-target and off-target activities. Acalabrutinib shows improved selectivity for BTK compared with ibrutinib.[34] Functional inhibition of non-target cells (e.g., T cells, NK cells, platelets) was not observed for acalabrutinib at clinically relevant concentrations.

1.3.2 Safety Pharmacology

In vitro and in vivo safety pharmacology studies with acalabrutinib have demonstrated a favorable nonclinical safety profile; for detailed information on the safety pharmacology of acalabrutinib, refer to the Investigator Brochure.

1.3.3 Drug-drug Interaction Potential

For more detailed information on drug-drug interaction potential for acalabrutinib, refer to the Investigator Brochure.

Please refer to Concomitant Therapy section for guidance on drugs that may cause drug-drug interactions.

1.4 CLINICAL EXPERIENCE – ACALABRUTINIB

For more detailed information on the clinical experience for acalabrutinib, please refer to the Investigator Brochure.

1.4.1 Adverse Events – acalabrutinib

ADRs observed in clinical studies with 614 subjects receiving acalabrutinib monotherapy in hematological malignancies are summarized as follows (from the IB version 7.1, percentages of all grade adverse events, grade ≥3 in parentheses):

Diarrhea 40% (2.3%), Nausea 24% (1.6%), Constipation 16% (0), Vomiting 15% (1.3%), Abdominal Pain 13% (2.0%), Bruising 41% (0), Rash 22% (0.6%), Headache 42% (1.3%), Hemorrhage 12% (1.5%).

The median duration of exposure to acalabrutinib treatment across the pooled dataset was 21.9 months (range, 0 to 42.4 months). A case of a drug-induced Grade 3 tumor lysis syndrome has been reported in a CLL patient with a bulky disease in Study CL-309 (not included in the INT population).

1.4.2 Warnings and Precautions

1.4.2.1 Hemorrhage

Serious hemorrhagic events, including central nervous system, respiratory, and gastrointestinal hemorrhage, have been reported in clinical trials with acalabrutinib; some of these bleeding events resulted in fatal outcomes. Grade 3 or higher bleeding events, including gastrointestinal, intracranial, and epistaxis have been reported in 2% of patients. Overall, bleeding events including bruising and petechiae of any grade occurred in approximately 50% of patients with hematological malignancies.

The mechanism for hemorrhage is not well understood. Acalabrutinib may further increase the risk of hemorrhage in patients receiving antiplatelet or anticoagulant therapies and patients should be monitored for signs of bleeding. Acalabrutinib should be withheld for 3-7 days pre- and post-surgery depending on the type of surgery and the risk of bleeding.

1.4.2.2 Infection

Serious infections (bacterial, viral or fungal), including fatal events and opportunistic infections, have been reported in clinical studies with acalabrutinib. The most frequently reported Grade 3 or 4 infection was pneumonia. Across the acalabrutinib clinical development program (including subjects treated with acalabrutinib in combination with other drugs), cases of hepatitis B virus (HBV) reactivation (resulting in liver failure and death in 1 case) and cases of progressive multifocal leukoencephalopathy have occurred in subjects with hematologic malignancies. Monitor patients for signs and symptoms of infection and treat as medically appropriate.

1.4.2.3 Cytopenias

Treatment-emergent Grade 3 or 4 cytopenias including neutropenia, anemia, and thrombocytopenia have occurred in clinical studies with acalabrutinib. Subjects should be closely monitored as appropriate.

1.4.2.4 Second Primary Malignancies

Events of second primary malignancies, including non-skin carcinomas, have been reported in clinical studies with acalabrutinib. The most frequently reported second primary malignancy was skin cancer. Advise protection from sun exposure.

1.4.2.5 Atrial Fibrillation

Events of atrial fibrillation/flutter have been reported in clinical studies with acalabrutinib, particularly in subjects with cardiac risk factors, hypertension, diabetes mellitus, acute infections, and a previous history of atrial fibrillation.

The mechanism for atrial fibrillation is not well understood.

1.5 VENETOCLAX (ABT-199)

Venetoclax is approved for treatment of subgroups of patients with CLL. It is co-developed for the treatment of hematological and immunological diseases by the pharmaceutical companies F. Hoffmann-La Roche LTD and AbbVie Inc. For detailed information on venetoclax please see the current version of the Summary of Product Characteristics and the Investigator Brochure.

1.5.1 Mechanism of Action

Venetoclax is a bcl-2-antagonist that is specific for bcl-2 and induces death in bcl-2 dependent tumor cells. Venetoclax has been shown to be potent in cell-killing and displaying anti-tumor effects in CLL cells.

1.5.2 Safety Pharmacology

The overall safety profile of Venclyxto is based on data from 546 patients with CLL treated in clinical trials with venetoclax in combination with rituximab or as monotherapy. The safety analysis included patients from one phase 3 study (MURANO), two phase 2 studies (M13-982 and M14-032), and one phase 1 study (M12-175). MURANO was a randomized, controlled trial in which 194 patients with previously treated CLL received venetoclax in combination with rituximab. In the phase 2 and phase 1 studies, 352 patients with previously treated CLL, which included 212 patients with 17p deletion and 146 patients who had failed a B cell receptor pathway inhibitor were treated with venetoclax monotherapy.

Additional safety and efficacy data are described in detail in the Summary of Product Characteristics.

1.5.3 Drug-drug Interaction Potential

For more detailed information on drug-drug interaction potential for venetoclax, refer to the Summary of Product Characteristics.

Please refer to Concomitant Therapy section for guidance on drugs that may cause drug-drug interactions.

1.6 CLINICAL EXPERIENCE – VENETOCLAX

Please see the Summary of Product Characteristics and the Investigator Brochure for further details.

1.6.1 Clinical Efficacy – Venetoclax

Venetoclax in combination with rituximab for the treatment of patients with CLL who have received at least one prior therapy – study GO28667 (MURANO).

A randomized (1:1), multicenter, open-label phase 3 study evaluated the efficacy and safety of Venclyxto + rituximab versus BR in patients with previously treated CLL. Patients in the Venclyxto + rituximab arm completed the Venclyxto 5-week dose-titration schedule and then received 400 mg once daily for 24 months from Cycle 1 Day 1 of rituximab in the absence of disease progression or unacceptable toxicity. Rituximab was initiated after the 5-week dose-titration schedule at 375 mg/m^2 for Cycle 1 and 500 mg/m^2 for Cycles 2-6. Each cycle was 28 days. Patients randomized to BR received bendamustine at 70 mg/m^2 on Days 1 and 2 for 6 cycles and rituximab as described above.

Median age was 65 years (range: 22 to 85); 74% were male, and 97% were white. Median time since diagnosis was 6.7 years (range: 0.3 to 29.5). Median prior lines of therapy was 1 (range: 1 to 5); and included alkylating agents (94%), anti-CD20 antibodies (77%), B-cell receptor pathway inhibitors (2%) and prior purine analogs (81%, including 55% FCR). At baseline, 46.6% of patients had one or more nodes ≥5 cm, and 67.6% had ALC ≥25 x 10^9/l. A 17p deletion was detected in 26.9% of patients, *TP53* mutations in 26.3%, 11q deletion in 36.5%, and unmutated *IgVH* gene in 68.3%. Median follow-up time for primary analysis was 23.8 months (range: 0.0 to 37.4 months).

Investigator-assessed progression-free survival in patients with previously treated CLL in MURANO.

	Venetoclax + rituximab N = 194	Bendamustine + rituximab N = 195
Number of events (%)	32 (16.5)	114 (58.5)
Disease progression	21	98
Death events	11	16
Median, months (95% CI)	NR	17.0 (15.5, 21.6)
Hazard ratio (95% CI)	0.17 (0.11, 0.25)	
P-value[a]	<0.0001	
12-month PFS estimate (95% CI)	92.7 (89.1, 96.4)	72.5 (65.9, 79.1)
24-month PFS estimate (95% CI)	84.9 (79.1, 90.6)	36.3 (28.5, 44.0)

CI = confidence interval; NR = not reached
[a]Stratified P-value.

At an updated efficacy analysis with all patients off treatment (data cut-off date 8 May 2018 and median follow-up of 36 months) the 36-month PFS estimate in the venetoclax + rituximab arm was 71.4% [95% CI: 64.8, 78.1] and in the bendamustine + rituximab arm was 15.2% [95% CI: 9.1, 21].

In total, 130 patients in the venetoclax + rituximab arm completed 2 years of venetoclax treatment without progression. Of the 130 patients, 92 patients completed the 6-month post treatment follow-up visit. The estimated PFS rate at 6 months post treatment was 92%.

Efficacy results for the pre-specified primary analysis (data cut-off date 8 May 2017) were also assessed by an Independent Review Committee (IRC) demonstrating a statistically significant 81% reduction in the risk of progression or death for patients treated with venetoclax + rituximab (hazard ratio: 0.19 [95% CI: 0.13, 0.28]; P<0.0001).

Median DOR was not reached with median follow up of approximately 23.8 months.

Results of subgroup analyses:

The observed PFS benefit of venetoclax + rituximab compared with bendamustine + rituximab was consistently observed across all subgroups of patients evaluated, including age (< 65, ≥ 65 years and < 75, ≥ 75 years), prior lines of therapy (1, >1), bulky disease (< 5 cm, ≥ 5 cm), 17p deletion, 11q deletion, *TP53* mutation*, IgVH* mutation, and refractory versus relapse to most recent therapy.

Venetoclax as monotherapy for the treatment of patients with CLL harboring 17p deletion or TP53 mutation – study M13-982.

The safety and efficacy of venetoclax in 107 patients with previously treated CLL with 17p deletion were evaluated in a single arm, open-label, multi-center study (M13-982). Patients followed a 4- to 5-week dose-titration schedule starting at 20 mg and increasing to 50 mg, 100 mg, 200 mg and finally 400 mg once daily. Patients continued to receive venetoclax 400 mg once daily until disease progression or unacceptable toxicity was observed. The median age was 67 years (range: 37 to 85 years); 65% were male, and 97% were white. The median time since diagnosis was 6.8 years (range: 0.1 to 32 years; N=106). The median number of prior anti-CLL treatments was 2 (range: 1 to 10 treatments); 49.5% with a prior nucleoside analogue, 38% with prior rituximab, and 94% with a prior alkylator (including 33% with prior bendamustine). At baseline, 53% of patients had one or more nodes ≥5 cm, and 51% had ALC ≥25 x 10^9/l. Of the patients, 37% (34/91) were fludarabine refractory, 81% (30/37) harbored the unmutated *IgVH* gene, and 72% (60/83) had *TP53* mutation. The median time on treatment at the time of evaluation was 12 months (range: 0 to 22 months).

The primary efficacy endpoint was overall response rate (ORR) as assessed by an Independent Review Committee (IRC) using the IWCLL updated NCI-WG guidelines (2008). Efficacy results are shown in Table 6. Efficacy data are presented for 107 patients with data cutoff date 30 April 2015. An additional 51 patients were enrolled in a safety expansion cohort. Investigator-assessed efficacy are presented for 158 patients with a later data cutoff date 10 June 2016. The median time on treatment for 158 patients was 17 months (range: 0 to 34 months).

Minimal residual disease (MRD) was evaluated using flow cytometry in 93 of 158 patients who achieved complete remission (CR), complete remission with incomplete marrow recovery (CRi), or partial remission (PR) with limited remaining disease with venetoclax treatment. MRD negativity was defined as a result below 0.0001 (<1 CLL cell per 10^4 leukocytes in the sample). Twenty-seven percent (42/158) of patients were MRD negative in the peripheral blood, including 16 patients who were also MRD negative in the bone marrow.

Venetoclax as monotherapy for the treatment of patients with CLL who have failed a B-cell receptor pathway inhibitor – study M14-032.

The efficacy and safety of venetoclax in patients with CLL who had been previously treated with and failed ibrutinib or idelalisib therapy were evaluated in an open-label, multi-center, non-randomized, phase 2 study (M14-032). Patients received venetoclax via a recommended dose-titration schedule. Patients continued to receive venetoclax 400 mg once daily until disease progression or unacceptable toxicity was observed.

At the time of data cut-off (26 July 2017), 127 patients were enrolled and treated with venetoclax. Of these, 91 patients had received prior ibrutinib therapy (Arm A) and 36 had received prior idelalisib therapy (Arm B). The median age was 66 years (range: 28 to 85 years), 70% were male, and 92% were white. The median time since diagnosis was 8.3 years (range: 0.3 to 18.5 years; N=96). Chromosomal aberrations were 11q deletion (34%, 43/127), 17p deletion (40%, 50/126), *TP53* mutation (38%, 26/68) and unmutated *IgVH* (78%, 72/92). At baseline, 41% of patients had one or more nodes ≥5 cm and 31% had ALC ≥25 x 10^9/l. The median number of prior oncology treatments was 4 (range: 1 to 15) in ibrutinib-treated patients and 3 (range: 1 to 11) in idelalisib-treated patients. Overall, 65% of patients received prior nucleoside analogue, 86% rituximab, 39% other monoclonal antibodies, and 72% alkylating agent (including 41% with bendamustine). At the time of evaluation, median duration of treatment with venetoclax was 14.3 months (range: 0.1 to 31.4 months).

The primary efficacy endpoint was ORR according to IWCLL updated NCI-WG guidelines. Response assessments were performed at 8 weeks, 24 weeks, and every 12 weeks thereafter.

The efficacy data were further evaluated by an IRC demonstrating a combined ORR of 70% (Arm A: 70%; Arm B: 69%). One patient (ibrutinib failure) achieved complete remission with incomplete marrow recovery. The ORR for patients with 17p deletion and/or *TP53* mutation was 72% (33/46) (95% CI: 56.5, 84.0) in Arm A and 67% (8/12) (95% CI: 34.9, 90.1) in Arm B. For patients without 17p deletion and/or *TP53* mutation, the ORR was 69% (31/45) (95% CI: 53.4, 81.8) in Arm A and 71% (17/24) (95% CI: 48.9, 87.4) in Arm B.

Median OS and DOR were not reached with median follow-up of approximately 14.3 months for Arm A and 14.7 months for Arm B.

Twenty-five percent (32/127) of patients were MRD negative in the peripheral blood, including 8 patients who were also MRD negative in bone marrow.

1.6.2 Adverse Events – venetoclax

The most commonly occurring adverse reactions (≥20%) of any grade in patients receiving venetoclax in the combination study with rituximab were neutropenia, diarrhea, and upper respiratory tract infection. In the monotherapy studies, the most common adverse reactions were neutropenia/neutrophil count decreased, diarrhea, nausea, anemia, fatigue, and upper respiratory tract infection.

The most frequently reported serious adverse reactions (≥2%) in patients receiving venetoclax in combination with rituximab were pneumonia, febrile neutropenia, and TLS. In the monotherapy studies, the most frequently reported serious adverse reactions (≥2%) were pneumonia and febrile neutropenia.

1.6.3 Warnings and Precautions

1.6.3.1 Tumor Lysis Syndrome (TLS)

Tumor lysis syndrome, including fatal events, has occurred in patients with previously treated CLL with high tumor burden when treated with venetoclax.

Venetoclax can cause rapid reduction in tumor, and thus poses a risk for TLS in the initial 5 weeks dose titration phase. Changes in electrolytes consistent with TLS that require prompt management can occur as early as 6 to 8 hours following the first dose of venetoclax and at each dose increase.

The risk of TLS is a continuum based on multiple factors, including comorbidities. Patients with high tumor burden (e.g., any lymph node with a diameter ≥5 cm or high ALC ≥25 x 109/l) are at greater risk of TLS when initiating venetoclax. Reduced renal function (CrCl <80 ml/min) further increases the risk. Patients should be assessed for risk and should receive appropriate prophylaxis for TLS, including hydration and anti hyperuricaemics. Blood chemistries should be monitored, and abnormalities managed promptly. Dosing should be interrupted if needed. More intensive measures (intravenous hydration, frequent monitoring, hospitalization) should be employed as overall risk increases. The instructions for “Prevention of tumor lysis syndrome” should be followed.

Concomitant use of this medicinal product with strong or moderate CYP3A inhibitors increases venetoclax exposure and may increase the risk for TLS at initiation and during the dose-titration phase. Also inhibitors of P-gp or BCRP may increase venetoclax exposure.
In the initial Phase 1 dose-finding studies, which had a shorter (2 to 3 week) titration phase and higher starting dose, the incidence of TLS was 13% (10/77; 5 laboratory TLS; 5 clinical TLS), including 2 fatal events and 3 events of acute renal failure, 1 requiring dialysis.

The risk of TLS was reduced after revision of the dosing regimen and modification to prophylaxis and monitoring measures. In venetoclax clinical studies, patients with any measurable lymph node ≥10 cm or those with both an ALC ≥25 x 10^9/l and any measurable lymph node ≥5 cm were hospitalized to enable more intensive hydration and monitoring for the first day of dosing at 20 mg and 50 mg during the titration phase.

In 168 patients with CLL starting with a daily dose of 20 mg and increasing over 5 weeks to a daily dose of 400 mg in studies M13-982 and M14-032, the rate of TLS was 2%. All events were laboratory TLS (laboratory abnormalities that met ≥2 of the following criteria within 24 hours of each other: potassium >6 mmol/l, uric acid >476 µmol/l, calcium <1.75 mmol/l, or phosphorus >1.5 mmol/l; or were reported as TLS events) and occurred in patients who had a lymph node(s) ≥5 cm or ALC ≥25 x 10^9/l. No TLS with clinical consequences such as acute renal failure, cardiac arrhythmias or sudden death and/or seizures was observed in these patients. All patients had CrCl ≥50 ml/min.

In the open-label, randomized phase 3 study (MURANO), the incidence of TLS was 3% (6/194) in patients treated with venetoclax + rituximab. After 77/389 patients were enrolled in the study, the protocol was amended to incorporate the current TLS prophylaxis and monitoring measures described in Posology (see section 4.2). All events of TLS occurred during the venetoclax dose-titration phase and resolved within two days. All six patients completed the dose titration and reached the recommended daily dose of 400 mg of venetoclax. No clinical TLS was observed in patients who followed the current 5-week dose-titration schedule and TLS prophylaxis and monitoring measures (see section 4.2). The rates of grade ≥3 laboratory abnormalities relevant to TLS were hyperkalemia 1%, hyperphosphatemia 1%, and hyperuricemia 1%.

For details regarding risk factors, prophylactic measures and handling of TLS see appendix 6.

1.6.3.2 Neutropenia

Neutropenia is an identified risk with Venclyxto treatment. In the MURANO study, neutropenia was reported in 61% (all grades) of patients on the venetoclax + rituximab arm. Forty-three percent of patients treated with venetoclax + rituximab experienced dose interruption and 3% of patients discontinued venetoclax due to neutropenia. Grade 3 neutropenia was reported in 32% of patients and grade 4 neutropenia in 26% of patients. The median duration of grade 3 or 4 neutropenia was 8 days (range: 1-712 days). With venetoclax + rituximab treatment, febrile neutropenia was reported in 4% of patients, grade ≥3 infections in 18%, and serious infections in 21% of patients.

Grade 3 or 4 neutropenia has been reported in patients treated with venetoclax in the combination study with rituximab (GO28667/MURANO) and in the monotherapy studies. Complete blood counts should be monitored throughout the treatment period. Dose interruptions or reductions are recommended for patients with severe neutropenia. Serious infections including events of sepsis

with fatal outcome have been reported. Supportive measures including antimicrobials for any signs of infection should be considered.

1.6.3.3 CYP3A inducers

Co-administration of CYP3A4 inducers may lead to decreased venetoclax exposure and consequently a risk for lack of efficacy. Concomitant use of venetoclax with strong or moderate CYP3A4 inducers should be avoided

1.6.3.4 Women of childbearing potential

Women of childbearing potential must use a highly effective method of contraception while taking venetoclax.

1.6.3.5 Immunization

The safety and efficacy of immunization with live attenuated vaccines during or following venetoclax therapy have not been studied. Live vaccines should not be administered during treatment and thereafter until B-cell recovery.

2. STUDY OBJECTIVES

2.1 PRIMARY OBJECTIVE PHASE 2 PART:

- Grade ≥3-Infection-free survival in the treatment arm compared to the observation arm 12 weeks after finishing treatment (24 weeks after treatment initiation). This is a non-inferiority analysis as detailed in statistical analysis plan to assure safety of the combination treatment in this preemptive trial population.

2.2 SECONDARY OBJECTIVE(S):

- Grade ≥3-infection free, CLL-treatment-free survival at end of treatment, 1 year and 2 years after enrollment
- Overall survival and cause of death
- Treatment free survival
- Rate and CTCAE grade of infections
- Response rate and duration according to IWCLL criteria
- Treatment related adverse events, type, frequency and severity during and for 2 years after treatment
- Immune function as assessed by immune phenotyping, functional TruCulture assays and measurements of cytokine levels

2.3 EXPLORATORY OBJECTIVE(S)

- MRD levels in bone marrow and peripheral blood
- Quality of life during and for 2 years after treatment by QLQC30 and CLL17

3. STUDY DESIGN

The study aims at improving immune function for newly diagnosed (within 1 year of diagnosis, to avoid lead time bias) patients with CLL, who do not currently fulfil IWCLL treatment criteria, who are identified by the machine learning algorithm CLL-TIM to be at increased risk of infection or early CLL treatment. The aim is to reduce the risk of infections for this patient population by short-term combination treatment with the BTK inhibitor acalabrutinitb and the bcl-2 inhibitor venetoclax. The trial is a randomized phase 2 trial.

3.1 IDENTIFYING CLL PATIENTS AT RISK OF INFECTION/TREATMENT

Based on the machine learning algorithm CLL-TIM, (Agius et al, EHA 2018, abstract PS-1103, manuscript in preparation) patients who do not currently fulfil IWCLL treatment criteria, with high risk of infection and/or early CLL treatment are identified. Patients considered gray zone or approaching IWCLL criteria for treatment will not be included for the trial. Variables included for the model generation are routine laboratory results, microbiology findings including frequency of blood cultures prior to CLL diagnosis, pathology findings, diagnostic codes and CLL-IPI risk factors (age, β-2-microglobulin, IGHV mutational status, clinical stage, TP53 aberration).

The trial set up includes the web-based CLL-TIM model for entering of baseline characteristics and laboratory results for prediction of high or low risk of infection and/or early treatment at time of diagnosis for the individual patient during screening. The web-based app for assessment of individual risk group for patients during the screening period is accessible at cll-tim.org. Data for individual patients are entered with a screening ID and the entered data are linked to the eCRF system. Thus, based on the trial, the CLL-TIM predictive model is validated both for clinical implementation of the web-based set up and for the prediction of low risk and high-risk groups, as also patients predicted as low risk will be followed for infectious and treatment events as well as for overall survival. Patients assessed as high risk will be randomized 1:1 between observation or treatment as detailed in the next section. Approximately 20% of newly diagnosed patients are expected to be predicted as high risk, while the remaining approximately 80% of screened patients will be followed for outcome only.

3.2 STUDY TREATMENT

3.2.1 Treatment Schedule (see figure for outline)

Acalabrutinib 100 mg BID from cycle 1 day 1 for 3 cycles of 28 days.

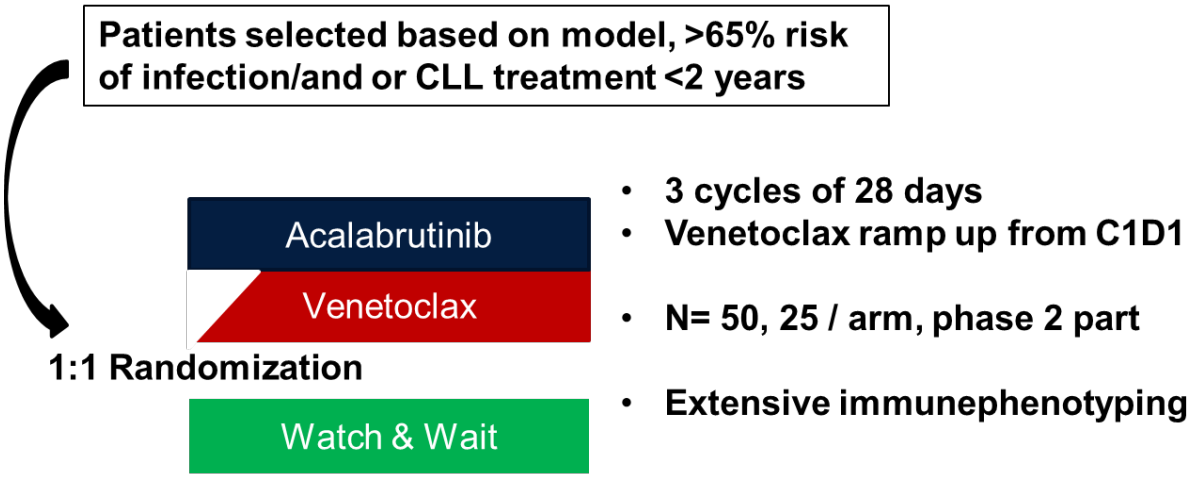
Venetoclax, ramp up during the first five weeks starting cycle 1 day 1, 7 days treatment on each dose level (20-50-100-200-400 mg), thereafter 400 mg once daily until a total of 3 cycles of 28 days counted from cycle 1 day 1.

3.3 STUDY PROCEDURES AND ASSESSMENTS

Please see Appendix 1 for a comprehensive list of study assessments and their timing. The study schema is provided below (Figure 3-1).

Figure 3-1. Study Schema

Phase 2 part:



3.4 STUDY PARAMETERS

3.4.1 Efficacy Parameters

Please see appendix 8 for definitions of efficacy parameters.

3.4.2 Safety Parameters

Due to the pre-emptive treatment of a patient population normally not treated, an independent Data Safety Monitoring Board (DSMB) will assure ongoing assessment of infectious risk and adverse events. The trial may be stopped based on the decision by the independent DSMB if increased infectious risk or AE risk is considered to outweigh the potential benefit during enrollment. Extensive assessment of immune function including phenotyping of immune cells, cytokine profiling and functional assays of immune function will be performed along with assessment of MRD and subclonal development. Infections will be closely monitored for the first 2 years, thus

including the 18-months follow up. Follow up will be aligned for the treatment arm and the observation arm to avoid registration bias.
For consistency of interpretation, AEs and laboratory results will be coded using the Medical Dictionary for Regulatory Activities (MedDRA), and the severity of AEs will be graded using the Common Terminology Criteria for Adverse Events (CTCAE), Version 5. Standard definitions for seriousness will be applied.

3.4.3 Pharmacodynamic and Biomarker Parameters

3.4.3.1 Assessment of Immune Dysfunction

The diagnostic immunological profiling tool is based on novel, standardized techniques revealing in-depth information on immune phenotype and -function, allowing for standardized and robust data readout and ensuing technology transfer. These assessments are based on a collaboration with Sisse Ostrowski, MD, PhD, Rigshospitalet and the Persimune.org project.
These investigations will be performed at a few central laboratories to assure quality and inter-laboratory reproducibility. In combination with MRD results and assessment of subclonal development, immune phenotyping data may indicate optimal treatment length (6 months or 3 months).

- **Immune phenotyping**: A 10-color flow cytometry whole blood panel comprising 10 different tests has been custom-designed (DuraClone, Beckman Coulter) and is currently under validation at the Dept. of Clinical Immunology, Rigshospitalet. The ten different flow tests (I-X) evaluates: I) Major cell lineages in the blood (proportions and counts), II) B cell subsets, III) T cell subsets, IV) T cell receptor (TCR) subsets, V) Regulatory (T_{reg}) and Th_{17} cells, VI) Dendritic cells, VII) Myeloid cells, VIII) Erythrocytes as surrogates for asplenism, IX) Platelets and platelet-leukocyte co-aggregates and X) Neutrophil phagocyte function. The panel captures multiple patterns predictive for cancer patients i.e. deviated maturation, acute/chronic activation, exhaustion, migration/trafficking and expression of immune checkpoint markers (PD-1, PD-1L, CTLA-4, FoxP3, Helios, CD25, TLR2, TLR4, CD38) which will also be extended with assessment of NK cell function and subtypes as well as additional immune activation markers.[1-8]
- **Immune function**: Stimulated immune response & platelet function are assessed by TruCulture® (Myriad RBM), which assesses the induced innate and adaptive immune responses to whole blood ligand stimulation. Five different stimuli I-V are applied to screen the response to signaling pathways via Toll Like Receptors (TLRs), critical for the anticancer immune response as TLR ligands may break tolerance to self-antigens and promote immune responses to tumor antigens[9,10]: I) Zymosan; II) Poly I:C; III) Resiquimod; IV) ODN + LPS and

V) NegCo. TruCulture® are currently validated at the Dept. of Clinical Immunology, Rigshospitalet and being tested for assessment of immune function in CLL at baseline and during treatment with targeted small molecules. TruCulture® provides an extensive data-readout, with cytokine response data available within days whereas Multiplate®/TEG® provides real-time results also on platelet function and complement cascades. Furthermore, transcriptomics by RNAseq assures in depth analyses of the molecular basis for immune changes upon treatment in stimulated and unstimulated cells.

3.4.3.2 MRD Assessment, Analysis of Subclonal Development

Minimal residual disease (MRD) levels will be examined by quantitative highly sensitive flow cytometry (MRD flow) in the peripheral blood and in the bone marrow at: The Department of Hematology, Rigshospitalet, Copenhagen University Hospital, MRD will be quantified by eight-color flow cytometry with a sensitivity of at least 10^{-4} or by DNA based methods.[35] MRD assessments of peripheral blood are to be performed at screening (for baseline characterization of the individual CLL clone), at 3 and 6 months after treatment initiation/start of observation, thereafter once a year for 3 years.

In addition, an NGS capture-based assay for MRD detection based on recurrent mutations and IGHV sequence is under development. DNA is prepared from patient samples at time of MRD assessment by flow cytometry. After full enrollment, these DNA samples will be analyzed by the NGS assay to allow for direct comparison of MRD assessment by the two methods and to allow for assessment of subclonal development during and after treatment. Furthermore, BH3 profiling as developed in collaboration with Assoc. Professor Matthew Davids, Dana Farber Cancer Institute, will be employed for assessment of sensitivity to various targeted therapies of viably frozen primary CLL cells from enrolled patients at baseline. The results of BH3 profiling will be correlated with long term outcome for the patients.

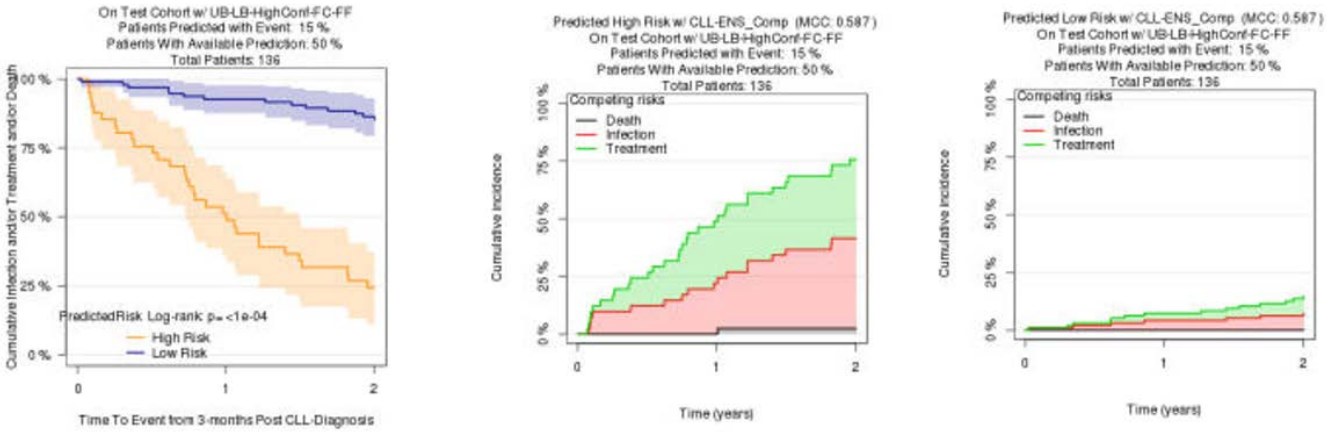
3.5 RATIONALE FOR STUDY DESIGN AND DOSING REGIMEN

Preclinical data supports the combination of BTK inhibitors and the bcl-2 inhibitor venetoclax. The combination of BTK inhibitors and venetoclax showed synergy in a diffuse large B cell lymphoma (DLBCL) cell line model [28] and in primary CLL cells [30] as well as in Mantle Cell Lymphoma (MCL) cell lines.[36] Early clinical trials are currently testing the combination of venetoclax and acalabrutinib for clinical use, with no unexpected toxicities reported so far for combination of BTK and bcl-2 inhibitors through scientific meetings, publications or internal reports from the marketing holders. [37] Translational data for patients treated with BTK inhibitors [6,25,26] and venetoclax [27] indicate that immune function can be at least partly restored on treatment with decreasing incidence of infections over time on treatment and improved immune parameters. These translational data thus

support the hypothesis that short duration (3 months) treatment with the combination of acalabrutinib and venetoclax will improve the immune function for patients with newly diagnosed CLL at high risk of infection and/or treatment within 2 years.

Based on our collaboration with PERSIMUNE.org for multidimensional data-capture, genetic and functional analyses of primary CLL patient samples from our biobank covering samples from +700 patients since 2002 and machine-learning modelling in collaboration with DTU compute, we have developed a novel algorithm for identification of CLL patients at increased risk of infection prior to any CLL specific treatment and/or early CLL treatment, CLL-TIM (see figure 3-2). This model extends on known prognostic markers in CLL as defined based on the CLL-IPI criteria.[38,39] We are thus able to identify a subgroup of newly diagnosed CLL patients without IWCLL treatment indication,[40] who are at high risk of infection and/or treatment by the CLL-TIM. More than 65% of these patients will have a severe infection or CLL treatment within two years.

Figure 3-2. Performance of the CLL-TIM predicting high risk CLL patients for infection and/or treatment at time of diagnosis.



Agius et al, EHA 2018, Abstract PS-1103 and manuscript in preparation

In line with the START trial demonstrating that early treatment is superior to deferred treatment in HIV due to impact on multiple causes of death all related to immune dysfunction,[41] we aim at improving the immune function by early treatment. Consequently, the aim of the current trial is to evaluate whether short-term, pre-emptive combination treatment with venetoclax + acalabrutinib in patients with high risk of infection and/or CLL treatment can change the natural history of immune function in CLL and diminish the need for cytotoxic chemotherapy.

3.6 SELECTION OF STUDY POPULATION

3.6.1 Inclusion Criteria

Eligible subjects will be considered for inclusion in this study if they meet all the following criteria:

1. CLL diagnosed according to IWCLL criteria within one year prior to randomization
2. High risk of infection and/or progressive treatment within 2 years according to CLL-TIM
3. IWCLL treatment indication not fulfilled
4. Life expectancy > 2 years
5. Age at least 18 years
6. Ability and willingness to provide written informed consent and adhere to study procedures and treatment
7. Adequate bone marrow function as indicated by platelets above 100 x 10E9, hemoglobin above 10 g/dL and neutrophils above 1 x 10E9
8. Creatinine clearance above 30 mL/min directly measured with 24hr urine collection or calculated according to the modified formula of Cockcroft and Gault
9. Adequate liver function as indicated by a total bilirubin≤ 2 x, AST or ALT ≤ 2.5 x the institutional ULN value, unless directly attributable to the patient's CLL or to Gilbert's Syndrome.
10. Negative serological testing for hepatitis B (HBsAg negative and anti-HBc negative; patients positive for anti-HBc may be included if PCR for HBV DNA is negative and HBV-DNA PCR is performed every month until 12 months after last treatment cycle), negative testing for hepatitis C RNA within 6 weeks prior to registration.
11. Eastern Cooperative Oncology Group Performance Status (ECOG) performance status 0-2.
12. Woman of childbearing potential (WOCBP) who are sexually active must use highly effective methods of contraception during treatment and for 30 days after the last dose of investigational drugs.
13. Willing and able to participate in all required evaluations and procedures in this study protocol including swallowing capsules without difficulty.
14. Ability to understand the purpose and risks of the study and provide signed and dated informed consent and authorization to use protected health information.

3.6.2 Exclusion Criteria

Subjects will be ineligible for this study if they meet any of the following criteria:

1. Prior CLL treatment (including monoclonal antibodies, chemotherapy, small molecules, including CD20 antibodies, BTK inhibitors and bcl-2 inhibitors for any indication)
2. Transformation of CLL (Richter's transformation)

3. Previous autoimmune disease as AIHA (autoimmune hemolytic anemia) or ITP (idiopathic thrombocytopenic purpura) treated with immune suppression or uncontrolled AIHA or ITP
4. History of progressive multifocal leukoencephalopathy
5. HIV infection (a negative test required)
6. Known active infection
7. Malignancies other than CLL requiring systemic therapies (except anti-hormonal therapies) or considered to impact survival
8. Requirement of therapy with strong CYP3A4 and CYP3A5 inhibitors/inducers or anticoagulant therapy with vitamin K antagonists
9. History of bleeding disorders or current platelet inhibitors or anticoagulant therapy
10. History of clinically significant cardiovascular disease such as arrhythmias, congestive heart failure, or myocardial infarction within 6 months of screening, or any Class 3 or 4 cardiac disease as defined by the New York Heart Association Functional Classification, or corrected QT interval (QTc) > 480 msec at screening.
11. History of stroke or intracranial hemorrhage within 6 months prior to registration.
12. Use of investigational agents which might interfere with the study drug within 28 days prior to registration.
13. Vaccination with live vaccines within 28 days prior to registration.
14. Major surgery less than 30 days before start of treatment. Note: If a subject had major surgery, they must have recovered adequately from any toxicity and/or complications from the intervention before the first dose of study drug.
15. Known hypersensitivity to any active substance or to any of the excipients of one of the drugs used in the trial.
16. Pregnant women and nursing mothers (a negative pregnancy test is required for all women of childbearing potential within 7 days before start of treatment; further pregnancy testing will be performed regularly).
17. Fertile men or women of childbearing potential unless: surgically sterile or ≥ 2 years after the onset of menopause or willing to use two methods of reliable contraception including one highly effective contraceptive method (Pearl Index <1) and one additional effective (barrier) method during study treatment and for 30 days after the end of study treatment.
18. Legal incapacity.
19. Persons who are in dependence to the sponsor or an investigator
20. Persons not considered fit for the trial by the investigator
21. Malabsorption syndrome, disease significantly affecting gastrointestinal function, or resection of the stomach or small bowel that is likely to affect absorption, symptomatic inflammatory bowel

disease, partial or complete bowel obstruction, or gastric restrictions and bariatric surgery, such as gastric bypass.

22. Prothrombin time/INR or aPTT (in the absence of Lupus anticoagulant) > 2x ULN.
23. Requires treatment with proton pump inhibitors (e.g., omeprazole, esomeprazole, lansoprazole, dexlansoprazole, rabeprazole, or pantoprazole). Subjects receiving proton pump inhibitors who switch to H2-receptor antagonists or antacids are eligible for enrollment to this study.

3.6.3 Screening and Risk Assessment by CLL-TIM

The investigator assumes the responsibility of obtaining written informed consent for each patient for the screening part before any study-specific procedures are performed. Assessment of variables for the risk profile including the baseline characteristics, pathology results, prior medical history and medical history including infectious events along with laboratory results will be performed based on the web-based CLL-TIM algorithm accessed at cll-tim.org. Patients will be assigned a unique screening ID, and the entered data along with the web-based CLL-TIM risk assessment will be part of the eCRF system. To allow for central review of CLL-TIM profile and baseline CLL characteristics, up to 4 weeks of response time is allowed and a screening period up to 6 weeks (42 days) before randomization is allowed. Approval of enrollment by the study office is mandatory before randomization and initiation of study treatment. Additionally, the study office will notify the sites if a patient is potentially at increased risk for development of TLS based on the baseline assessments. Patients not assessed as high risk by CLL-TIM, either assessed as low risk or as low confidence for prediction, will be followed by local medical record reviews and/or registries for 6-monthly assessment of infectious events, treatment initiation and overall survival.

3.6.4 Enrollment and Randomization Procedures

The investigator assumes the responsibility of obtaining written informed consent for each patient for the treatment before any study-specific procedures and randomization is performed. A central medical review of the screening CRF pages and the results of the baseline results in the central laboratories will be performed by study physicians for verification of the eligibility of the patient, especially for confirmation of the CLL diagnosis.

Randomization has to occur within 42 days of the tests for screening, however results included for the CLL-TIM assessment, which were obtained as part of routine care for the patients may be included even prior to randomization. FISH analysis for cytogenetic aberrations and TP53 mutational assessment should be within 6 months of randomization. Patients will be randomly assigned to treatment vs observation through 1:1 randomization process with stratification

according to country, TP53 aberration status and IGHV mutational status. Treatment or observation period has to be initiated within 14 days of randomization.

3.7 STUDY DRUG

3.7.1 Premedications for Patients Randomized for Treatment

Due to risk of tumor lysis, all patients have to initiate allopurinol 300 mg once daily from one day prior to start on treatment until at least start of cycle 3; whether continuing allopurinol until end of cycle 3 is on the discretion of the treating physician. In the case of allergy to allopurinol, probenecid or rasburicase may be used as alternatives on the discretion of the treating physician. Sufficient oral hydration, at least 2 L per day, is required during venetoclax ramp up.

3.7.2 Formulation, Packaging, and Storage, Acalabrutinib

The investigational product, acalabrutinib capsules for oral administration, is supplied as yellow and blue, opaque hard gelatin capsules, with 100 mg of acalabrutinib as the active ingredient. Each capsule also contains compendial inactive ingredients: silicified microcrystalline cellulose, which is composed of microcrystalline cellulose and colloidal silicon dioxide, partially pregelatinized starch, sodium starch glycolate, and magnesium stearate. The capsule shell contains gelatin, titanium dioxide, yellow iron oxide and indigotine (FD&C Blue 2).

Acalabrutinib will be provided in white, high-density polyethylene bottles.

Acalabrutinib will be shipped to trial sites labeled as an Investigational Medicinal Product. Acalabrutinib will be prepared and labeled in compliance with GMP and other applicable regulatory requirements.
Acalabrutinib should be stored and handled in accordance with the instructions in the Investigator's Brochure. The investigational medicinal product should be stored in such a manner that accidental loss or destruction or access by an unauthorized person is prevented.
The sponsor will arrange delivery of Acalabrutinib 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. Acalabrutinib will be dispensed to subjects in bottles at each visit.

Refer to the acalabrutinib Investigator Brochure for additional information regarding the drug product to be used in this trial.

3.7.3 Administration of Study Drug, Acalabrutinib

Acalabrutinib capsule is administered 100 mg BID and taken orally approximately every 12 hours.

The capsules should be swallowed intact with water. Subjects should not attempt to open capsules or dissolve them in water. Acalabrutinib can be taken with or without food.

If a dose is missed, it can be taken up to 3 hours after the scheduled time with a return to the normal schedule with the next dose. If it has been > 3 hours, the dose should not be taken, and the subject should take the next dose at the scheduled time.

Guidance on co-administration of acalabrutinib with agents that affect gastric pH is provided in Section 3.12.2.

3.7.4 Formulation, Packa ging, and Storage, Venetoclax

Venetoclax is available in tablets of 10, 50 or 100 mg.
Venetoclax will be shipped to trial sites labeled as an Investigational Medicinal Product. Venetoclax will be prepared and labeled in compliance with GMP and other applicable regulatory requirements.
Venetoclax should be stored and handled in accordance with the instructions in the Summary of Product Characterization. The investigational medicinal product should be stored in such a manner that accidental loss or destruction or access by an unauthorized person is prevented.
The sponsor will arrange delivery of venetoclax 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. Venetoclax will be dispensed to subjects in blister packs through the dose ramp up period, and in bottles at each subsequent visit.

3.7.5 Administration of Study Drug, Venetoclax

Patients should take venetoclax tablets orally once daily (in the morning) according to the schedule below, please see section 3.9 for management of modifications and tumor lysis risk:

Agent	Dose/day	Route of administration	Cycle	Days
Venetoclax	20 mg	Orally	1	1-7
Venetoclax	50 mg	Orally	1	8-14
Venetoclax	100 mg	Orally	1	15-21
Venetoclax	200 mg	Orally	1	22-28
Venetoclax	400 mg	Orally	2-3	1-28

Patients should be instructed to drink plenty of water daily starting 2 days before and throughout the dose-titration phase. Patients should be particularly instructed to drink 1.5 to 2.0 L of water daily, 2 days prior to and the days of dosing at initiation and each subsequent dose increase. Patients should avoid consumption of foods or beverages containing grapefruit or Seville oranges or starfruit, as these contain certain ingredients that inhibit CYP3A activity.

If the patient misses a dose of venetoclax within 8 hours of the time it is usually taken, the patient should take the missed dose as soon as possible on the same day and resume the normal daily

dosing schedule. If a patient misses a dose by more than 8 hours, the patient should not take the missed dose and should resume the usual dosing schedule the following day.

If vomiting occurs even with all expelled tablets still intact, no replacement dose is to be taken that day. The next dose should be taken at the usual time the following day.

3.7.6 Assuring Subject Compliance

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 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. The investigator should also collect and count remaining medication, empty boxes and blisters of medication to check that the patient has taken the assigned dose.

3.8 STUDY TREATMENT SCHEDULE

Acalabrutinib will be administered orally 100 mg twice daily, every day from cycle 1 day 1 for 3 cycles of 28 days each.
Venetoclax, ramp up during the first five weeks starting cycle 1 day 1, 7 days treatment on each dose level (20-50-100-200-400 mg), thereafter 400 mg once daily for a total of 3 cycles of 28 days counted from cycle 1 day 1.

Please see section 3.9 for management of dosing delays and modifications.

3.9 DURATION OF THERAPY

Duration of treatment with acalabrutinib and venetoclax is for 3 cycles of 28 days; the end of trial is defined as seven years after start of treatment or observation with close follow up for the first two years, while follow up for AESI's, CLL treatment and survival continues until end of trial. The primary endpoint for the phase 2 part is 6 months after start of treatment or observation.

3.10 DOSING DELAYS AND MODIFICATIONS

In the case of AEs attributed to both acalabrutinib and venetoclax, the local investigator should decide whether dose

modifications apply to one or both drugs in collaboration with the sponsor, as no data on pharmacokinetic interaction between acalabrutinib and venetoclax are currently available.

3.10.1 Dosing Delays and Modification for Acalabrutinib

Subjects should be followed closely for AEs or laboratory abnormalities that might indicate acalabrutinib-related toxicity. If a subject experience a treatment-related toxicity or other intolerable AE during the course of therapy, then acalabrutinib should be withheld, as necessary, until the AE resolves or stabilizes to baseline or at least grade 1 toxicity.

Acalabrutinib may further increase the risk of hemorrhage in patients receiving antiplatelet or anticoagulant therapies and patients should be monitored for signs of bleeding. Acalabrutinib should be withheld for 3-7 days pre- and post-surgery depending on the type of surgery and the risk of bleeding.

Dose modifications for the following treatment-emergent toxicities are provided in Table 3-X:

- Grade 4 neutropenia (< 500/μL) for > 7 days (neutrophil growth factors are permitted per American Society of Clinical Oncology (ASCO) guidelines and use must be recorded on the case report form [CRF]).
- Grade 3 thrombocytopenia in presence of significant bleeding.
- Grade 4 thrombocytopenia.
- Grade 3 or 4 nausea, vomiting, or diarrhea, if persistent despite optimal antiemetic and/or anti-diarrheal therapy.
- Any other Grade 4 toxicity or unmanageable Grade 3 toxicity.

Example Table 3-X: Drug Modification Actions for Acalabrutinib

Occurrence	Action
1st – 2nd	Hold acalabrutinib until recovery to Grade ≤ 1 or baseline; may restart at original dose level
3rd	Hold acalabrutinib until recovery to Grade ≤ 1 or baseline; restart at one dose level lower (100 mg QD), may re-escalate to full dose on the discretion of the treating physician as detailed below
4th	Discontinue acalabrutinib if not fully re-escalated 4th occurrence, otherwise as for 3rd occurrence

As appropriate, certain laboratory abnormalities may warrant more frequent monitoring (eg, once per week) until abnormalities have recovered to Grade ≤ 1. If acalabrutinib is reduced for apparent treatment-related toxicity, the dose need not be re-escalated, even if there is minimal or no toxicity with the reduced dose. However, if the subject tolerates a reduced dose of acalabrutinib for ≥ 4 weeks then the dose may be increased to the next higher dose level, at the discretion of the

investigator. Such re-escalation may be particularly warranted if further evaluation reveals that the AE that led to the dose reduction was not treatment-related. The maximum dose of acalabrutinib is 100 mg BID.

Treatment with acalabrutinib should be withheld for any unmanageable, potentially study drug-related toxicity that is Grade ≥ 3 in severity. Any other clinically important events where dose delays may be considered appropriate must be discussed with the Principal Investigator.

3.10.2 Dosing Delays and Modification for Venetoclax

Dose modification for TLS and other toxicities.

Dose at interruption (mg)	**Restart dose (mg[a])**
400	300
300	200
200	100
100	50
50	20
20	10
[a]The modified dose should be continued for 1 week before increasing the dose.	

For patients who have had a dosing interruption lasting more than 1 week during the first 5 weeks of dose titration or more than 2 weeks after completing the dose-titration phase, TLS risk should be reassessed to determine if restarting at a reduced dose is necessary (e.g., all or some levels of the dose titration).

Venetoclax Recommended Dose Modifications for Toxicities

Event	Occurrence	Action
Tumor Lysis Syndrome		
Blood chemistry changes or symptoms suggestive of TLS (Cairo-Bishop grading in Appendix 6)	Any	Withhold the next day's dose. If resolved within 24-48 hours of last dose, resume at the same dose.
		For any blood chemistry changes requiring more than 48 hours to resolve, resume at one dose level reduction
		For any events of clinical TLS, resume at one dose level reduction following resolution
Non-Hematologic Toxicities		
Grade 3 or 4 non-hematologic toxicities	1st occurrence	Interrupt venetoclax Once the toxicity has resolved to Grade 1 or baseline level, venetoclax therapy may be

Event	Occurrence	Action
		resumed at the same dose. No dose modification is required.
	2nd and subsequent occurrences	Interrupt venetoclax. Restart at one dose level reduction when resuming treatment with venetoclax after resolution. A larger dose reduction may occur at the discretion of the investigator.
Hematologic Toxicities		
Grade 3 or 4 neutropenia with infection or fever; or Grade 4 hematologic toxicities (except lymphopenia)	1st occurrence	Interrupt venetoclax. To reduce the infection risks associated with neutropenia, granulocyte-colony stimulating factor (G-CSF) may be administered with venetoclax if clinically indicated. Once the toxicity has resolved to Grade 1 or baseline level, venetoclax therapy may be resumed at the same dose.
	2nd and subsequent occurrence	Interrupt venetoclax. Consider using G-CSF as clinically indicated. Restart at one dose level reduction when resuming treatment with venetoclax after resolution. Additional dose reductions may occur at the discretion of the physician. Re-escalation on the discretion of the treating physician

Patients who require dose reductions to less than 100 mg for more than 4 weeks should stop treatment.

Management of Neutropenia

Nonclinical and clinical experience indicates that venetoclax may cause neutropenia. Subjects with a history of neutropenia or with significant bone marrow involvement may be at a particularly high risk.

Grade 3 or 4 neutropenia has been reported in patients treated with venetoclax. Complete blood counts should be monitored throughout the treatment period. Dose interruptions or dose reductions are recommended for patients with severe neutropenia. Supportive measures including antimicrobials for any signs of infection should be considered and prophylactic use of growth factors (e.g. G-CSF) is mandatory for patients with neutrophil counts below $1x10^9$/L.

Management of Hematologic Toxicities Other Than Neutropenia or Lymphopenia

Venetoclax treatment should be withheld for any Grade 4 hematologic toxicity. Once the toxicity has resolved to Grade 1 or baseline level (recovery), venetoclax may be re-started at the same dose administered prior to holding the drug. If the toxicity recurs, the dose reduction guidelines in

table I1 should be followed when resuming study treatment following resolution. Additional dose reductions may occur at the discretion of the physician.

Management of Non-Hematologic Toxicity

Venetoclax treatment should be withheld for any clinically relevant ≥ Grade 3 non-hematologic toxicity. Once the toxicity has resolved to Grade 1 or baseline level (recovery), venetoclax may be re-started at the same dose administered prior to holding the drug. If the toxicity recurs, the dose reduction guidelines in table I1 should be followed when resuming study treatment following resolution. Additional dose reductions may occur at the discretion of the physician.

3.11 CONCOMITANT THERAPY

3.11.1 Permitted Concomitant Therapy

Steroid treatment and moderate CYP3A inhibitors are permitted with the restrictions detailed below.

3.11.2 Prohibited or Restricted Concomitant Therapy

Patients who require the use of any of the prohibited therapies listed below will be discontinued from study treatment.

Use of the following therapies is prohibited during the study:

- Cytotoxic chemotherapy
- Radiotherapy
- Immunotherapy
- Any therapies intended for the treatment of leukemia whether FDA-approved or experimental (outside of this study)
- Anti-retroviral medications
- Warfarin and other vitamin K antagonists.

Please note: Patients being treated with Direct anti-coagulants (DOACs), **acetylsalicylic acid** and non-steroid anti-inflammatory drugs (NSAIDs) can be included but must be properly informed about the potential risk of bleeding under treatment with acalabrutinib.

Live-virus vaccines should not be given within 28 days prior to the initiation of study treatment, at any time during study treatment, or following study treatment until B-cell levels have returned to normal and any neutropenia have resolved.

Use of the following concomitant medications is prohibited:

- Steroid therapy for anti-neoplastic intent, with the exception of inhaled steroids for asthma, topical steroids, steroids up to 25 mg of prednisolone daily to control autoimmune phenomenon's, or replacement/stress corticosteroids.
- Strong CYP3A inhibitors (see appendix 3 for examples).

Concomitant medications that fall into the categories below could potentially lead to adverse reaction(s) and should be considered cautionary (except where noted). If a potential study patient is taking any of the medications in the categories described below, the investigator will assess and document the use of medications known or suspected to fall in the following medication categories:

- Moderate CYP3A inhibitors. Consider alternative agents with less CYP3A inhibition. If a moderate CYP3A inhibitor must be used, reduce the acalabrutinib dose to 100 mg once daily. At initiation and during the dose-titration phase, concomitant use of venetoclax with moderate CYP3A inhibitors should be avoided. Alternative treatments should be considered. If a moderate CYP3A inhibitor must be used, the initiation dose of venetoclax and the doses for the titration phase should be reduced by at least 50%. Patients should be monitored more closely for signs and symptoms of TLS. For patients who have completed the dose titration phase and are on a steady daily dose of venetoclax, the venetoclax dose should be reduced by 50% when used concomitantly with moderate CYP3A. Patients should be monitored more closely for signs of toxicities and the dose may need to be further adjusted. The venetoclax dose that was used prior to initiating the CYP3A inhibitor should be resumed 2 to 3 days after discontinuation of the inhibitor.
- Moderate and strong CYP3A inducers. Consider alternative treatments with less CYP3A induction.
- Weak CYP3A inhibitors and inducers
- P-gp substrates
- BCRP substrates
- OATP1B1/1B3 substrates
- P-gp inhibitors
- BCRP inhibitors
- OATP1B1/B3 inhibitors

A sample list of prohibited and cautionary medications that fall into these categories is provided in Appendix 3. It is not possible to provide a complete list of medications that fall into these categories, so if in question, please refer to the appropriate product label.

Management of Potential Acalabrutinib and Venetoclax Interactions with CYP3A Inhibitors

	Venetoclax		Acalabrutinib
Inhibitors	**Initiation and Ramp-Up Phase**	**Steady Daily Dose (After Ramp-Up Phase)**	**At any time**
Strong CYP3A inhibitor	Prohibited		
Moderate CYP3A inhibitor	Avoid inhibitor use, consider alternative agent. If must be used, reduce the venetoclax dose by at least 50%		Avoid inhibitor use, consider alternative agent. If must be used, reduce acalabrutinib to 100 mg once daily

- In addition to these prohibited and cautionary medications, subjects should not consume grapefruit, grapefruit products, Seville oranges (including marmalade containing Seville oranges) or starfruits.

The concomitant use of strong inducers of CYP3A4 (see Appendix 3) should be avoided when possible. The concomitant use of strong inhibitors of CYP3A4 (see Appendix 3) is prohibited.

3.12 PRECAUTIONS

3.12.1 Dietary Restrictions

Acalabrutinib can be taken with or without food.

Patients should take venetoclax tablets orally once daily (in the morning). Each dose of venetoclax will be taken with approximately 240 mL of water during breakfast or first meal of the day. Patients should avoid consumption of foods or beverages containing grapefruit or Seville oranges or starfruit, as these contain certain ingredients that inhibit CYP3A activity.

3.12.2 Drug-Drug Interactions

At the systemic exposure levels expected in this study, acalabrutinib inhibition of CYP metabolism is not anticipated. However, acalabrutinib is metabolized by CYP3A. Concomitant administration with a strong CYP3A and P-glycoprotein (P-gp) inhibitor, itraconazole increased exposure by approximately 5-fold. Conversely, concomitant administration of with a strong CYP3A inducer, rifampin decreases acalabrutinib exposure and could reduce efficacy. Consequently, the concomitant use of strong inhibitors/inducers of CYP3A (see Appendix 3) should be avoided when possible.

Co-administration of 400 mg once daily ketoconazole, a strong CYP3A, P-gp and BCRP inhibitor, for 7 days in 11 previously treated patients with NHL increased venetoclax Cmax by 2.3-fold and AUC∞ by 6.4-fold. Co-administration of 50 mg once daily ritonavir, a strong CYP3A and P-gp

inhibitor, for 14 days in 6 healthy subjects increased venetoclax Cmax by 2.4-fold and AUC by 7.9-fold. Co-administration of venetoclax with other strong CYP3A4 inhibitors is predicted to increase venetoclax AUC by on average 5.8- to 7.8-fold.

Co-administration of a 600 mg single dose of rifampin, a P-gp inhibitor, in 11 healthy subjects increased venetoclax Cmax by 106% and AUC∞ by 78%. Concomitant use of venetoclax with P-gp and BCRP inhibitors at initiation and during the dose-titration phase should be avoided; if a P-gp and BCRP inhibitor must be used, patients should be monitored closely for signs of toxicities.

Co-administration of bile acid sequestrants with venetoclax is not recommended as this may reduce the absorption of venetoclax. If a bile acid sequestrant is to be co-administered with venetoclax, the SmPC for the bile acid sequestrant should be followed to reduce the risk for an interaction, and venetoclax should be administered at least 4-6 hours after the sequestrant.

If medically justified, subjects may be enrolled if such inhibitors or inducers can be discontinued or alternative drugs that do not affect these enzymes can be substituted within 7 days before first dose of study drug. If a subject requires a strong CYP3A4 while on study, the subject should be monitored closely for any potential toxicities.

The effect of agents that reduce gastric acidity (e.g., proton pump inhibitors or antacids) on acalabrutinib absorption was evaluated in a healthy volunteer study (ACE-HV-004). Results from this study indicate that subjects should avoid the use of calcium carbonate-containing drugs or supplements for a period of at least 2 hours before and at least 2 hours after taking acalabrutinib. Use of omeprazole, esomeprazole, lansoprazole or any other proton pump inhibitors while taking acalabrutinib is not recommended due to a potential decrease in study drug exposure. However, the decision to treat with proton-pump inhibitors during the study is at the investigator's discretion, with an understanding of the potential benefit to the subject's gastrointestinal condition and a potential risk of decreased exposure to acalabrutinib.

Although the effect of H2-receptor antagonists (such as famotidine or ranitidine) on acalabrutinib absorption has not been evaluated, if treatment with an H2-receptor antagonist is required, the H2-receptor antagonist should be taken approximately 2 hours after an acalabrutinib dose.

3.12.3 Reproductive Toxicity

The potential for acalabrutinib to be excreted in breast milk of nursing mothers is unknown.
It is unknown whether venetoclax or its metabolites are excreted in human milk.
A risk to the breast-feeding child cannot be excluded.
Breast-feeding should be discontinued during treatment with venetoclax.

For results of acalabrutinib nonclinical reproductive toxicity studies, including definitive embryofetal development studies, please refer to the Investigator Brochure.

Women of childbearing potential (WOCBP) who are sexually active must use highly effective methods of contraception during treatment and for 30 days after the last dose of acalabrutinib. Please refer to the Investigator Brochure for detailed definitions for WOCBP and highly effective methods of contraception.

Women should avoid becoming pregnant while taking venetoclax and for at least 30 days after ending treatment. Therefore, women of childbearing potential must use highly effective contraceptive measures while taking venetoclax and for 30 days after stopping treatment. It is currently unknown whether venetoclax may reduce the effectiveness of hormonal contraceptives, and therefore women using hormonal contraceptives should add a barrier method.

No human data on the effect of venetoclax on fertility are available. Based on testicular toxicity in dogs at clinically relevant exposures, male fertility may be compromised by treatment with venetoclax (see section 5.3). Before starting treatment, counselling on sperm storage may be considered in some male patients.

Subjects should promptly notify the investigator if they, or their partner, become pregnant during this study, or within 30 days after the last dose of acalabrutinib and venetoclax. If a female subject becomes pregnant during the treatment period, she must discontinue acalabrutinib and venetoclax immediately. Pregnancy in a female subject or a male subject's partner must be reported as outlined in Section 6.2.4.

For women of childbearing potential a pregnancy test will be performed at screening. A negative pregnancy test is required for all WOCBP within 7 days before start of study treatment. If WOCBP is randomized to the treatment arm additional pregnancy tests will be performed at the visits C2D1, C3D1 and End of Treatment.

3.12.4 Overdose Instructions

Clinical information relevant to overdose is not available. For results from nonclinical overdose studies in rats and dogs, please refer to the Investigator Brochure.

There is no specific antidote for venetoclax. Patients who experience overdose should be closely monitored and appropriate supportive treatment provided. During dose-titration phase, treatment should be interrupted, and patients should be monitored carefully for signs and symptoms of TLS (fever, chills, nausea, vomiting, confusion, shortness of breath, seizures, irregular heartbeat, dark or cloudy urine, unusual tiredness, muscle or joint pain, abdominal pain and distension) along with

other toxicities (see section 4.2). Based on venetoclax large volume of distribution and extensive protein binding, dialysis is unlikely to result in significant removal of venetoclax.

Study drug overdose is the accidental or intentional use of the drug in an amount higher than the dose being studied. An overdose or incorrect administration of study drug is not an AE unless it results in untoward medical effects.

Any study drug overdose or incorrect administration of study drug should be noted on the appropriate CRF, for the sponsor to report to companies and health authorities according to EU regulations.

All AEs associated with an overdose or incorrect administration of study drug should be recorded on the CRF. If the associated AE fulfills serious criteria, the event should be reported to the companies per contractual guidelines.

In the event of subject ingestion of more than the recommended study drug dosage, observation for any symptomatic side effects should be instituted, and vital signs, biochemical and hematologic parameters should be followed closely (consistent with the protocol or more frequently, as needed). Appropriate supportive management to mitigate adverse effects should be initiated. If the overdose ingestion of acalabrutinib is recent and substantial, and if there are no medical contraindications, use of gastric lavage or induction of emesis may be considered.

3.13 WITHDRAWAL OF SUBJECTS FROM STUDY TREATMENT

The investigator may withdraw any subject from study treatment, if, in the investigator's opinion, it is not in the subject's best interest to continue.

Any subject has the right to withdraw from the study at any time. In addition, subjects may be withdrawn from study treatment for the following reasons:

- Study treatment should be discontinued in the event of a toxicity leading to pausing of any of the investigational drugs > 28 days, unless reviewed and approved by the sponsor.
- Any subject who starts new chemotherapy or chemoimmunotherapy for the treatment of CLL, becomes pregnant or breastfeeding, is significantly noncompliant according to the treating physician's assessment, etc. should be withdrawn from study treatment.

Subjects who discontinue study therapy will continue to be followed on study for follow-up of safety and survival unless they withdraw consent for further follow-up. Thus, all subjects receiving ≥ 1 dose of study drug will be followed during the immediate post-therapy and long-term follow-up assessments unless the subject withdraws consent for such follow-up to be conducted.

3.14 REMOVAL FROM STUDY

Reasons for removal of a subject from the study are:

- Subject's withdrawal of consent from study
- Decision by sponsor to terminate the trial
- Decision by the investigator or sponsor for safety reasons
- Subject lost to follow-up (for more than 2 years)
- Death

3.15 DATA AND SAFETY MONITORING

This trial will be monitored in accordance with the Sponsor's Pharmacovigilance procedures. Adverse events, AESIs, and SAEs will be reviewed internally as part of ongoing safety surveillance and a DSMB will be monitoring the trial as detailed in later sections.

4. STUDY ACTIVITIES AND ASSESSMENTS

The schedule of events is provided in Appendix 1. Descriptions of the scheduled evaluations are outlined below and information on study drug and dosing is provided in section 3.4 and under each study drug description.

Before study entry, throughout the study, and at the follow-up evaluation, various clinical and diagnostic laboratory evaluations are outlined. The purpose of obtaining these detailed measurements is to ensure adequate safety and tolerability assessments. Clinical evaluations and laboratory studies may be repeated more frequently if clinically indicated.

4.1 DESCRIPTION OF PROCEDURES

4.1.1 Informed Consent

The subject must read, understand and sign the ICF approved by the institutional review board or independent ethics committee (IRB/IEC), confirming his or her willingness to participate in this study before initiating any screening activity that is not standard of care. Subjects must also grant permission to use protected health information, if required by local regulations in accordance with GDPR legislation. Subjects must also give permission that biobank material may be analyzed outside the EU.

The ICF will be in two parts, one for the screening part including obtaining information for the screening testing and the usage of data for CLL-TIM algorithm and assessment of risk of infection and/or treatment by the algorithm as well as follow up by medical record review and/or health registries and obtaining blood and other biological specimens for the adjoined research biobank.

The second part of the ICF will only be for patients assessed as high risk by the CLL-TIM algorithm, thus eligible for the clinical study including study procedures and randomization between treatment or observation. Patients assessed as low-risk or low-confidence prediction by CLL-TIM will only be followed by medical record review and/or registries for infections, CLL treatment and survival.

4.1.2 Medical History

Collect and record the subject's complete history through review of medical records and by interview, in particular previous infectious events and medical as well as pathology diagnoses will be collected. Concurrent medical signs and symptoms must be documented to establish baseline severities. A disease history, including the date of initial diagnosis and list of all prior treatment, in particular antimicrobial and anticancer treatments, and responses and duration of response to these treatments, will also be recorded.

4.1.3 Adverse Events

An adverse event (AE) is any untoward medical occurrence in a patient or clinical study subject administered a medicinal product and which 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.

Only information about AE's > grade 1 will be collected, unless the AE qualifies as an AESI or a SUSAR as detailed below.

Adverse event of special interest (AESI)

Atrial fibrillation, ventricular arrhythmias, tumor lysis events, infections, disease progression, treatment for CLL, malignancies and bleeding events are adverse events of special interest in this trial and will be actively requested for.

Serious adverse event (SAE)

A serious adverse event is defined as any untoward medical occurrence or effect that at any dose:

- Results in death
- Is a life-threatening event (i.e. the patient was at immediate risk of death at the time the reaction was observed)
- Requires hospitalization or prolongation of an existing hospitalization
- Results in significant or persistent disability or incapacity

- ♦ Is a congenital anomaly or birth defect
- ♦ Is an *important medical event* (i.e. important adverse events 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 above characteristics/consequences, including suspected transmission of infectious agents by a medicinal product).

Note: Clinical TLS is *an important medical event* in this trial which must be reported as SAE.
SAEs must be reported to the sponsor through the eCRF system, which automatically notifies the sponsor, within 24 hours of awareness. The sponsor must notify within the defined timelines health authorities and the companies providing investigational drugs according to EU regulations.

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 authorized medicinal product).

4.1.4 Severity

Definitions found in the CTCAE version 5 or higher will be used for grading the severity (intensity) of AEs. The CTCAE displays Grades 1 through 5 with unique clinical descriptions of severity for each referenced AE. Should a subject experience any AE not listed in the CTCAE, the following grading system should be used to assess severity:

- Grade 1 (Mild AE) – experiences which are usually transient, requiring no special treatment, and not interfering with the subject's daily activities
- Grade 2 (Moderate AE) – experiences which introduce some level of inconvenience or concern to the subject, and which may interfere with daily activities, but are usually ameliorated by simple therapeutic measures
- Grade 3 (Severe AE) – experiences which are unacceptable or intolerable, significantly interrupt the subject's usual daily activity, and require systemic drug therapy or other treatment

- Grade 4 (Life-threatening or disabling AE) – experiences which cause the subject to be in imminent danger of death
- Grade 5 (Death related to AE) – experiences which result in subject death]

4.1.5 Concomitant Medications and Therapy

Document all concomitant medications and procedures from within 42 days prior to randomization through 2 years after the first dose of study drug.

4.1.6 Confirmation of Eligibility

Subject eligibility for enrollment will be assessed per Section 3.5. All screening procedures, unless otherwise indicated, should be completed within 42 days prior to randomization.

4.1.7 ECOG Performance Status

The ECOG performance index is provided in Appendix 2.

4.1.8 Physical Examination, Vital Signs, Height & Weight

The screening physical examination will include, at a minimum, the general appearance of the subject, height (screening only) and weight, and examination of the skin, eyes, ears, nose, throat, lungs, heart, abdomen, extremities, musculoskeletal system, nervous system, and lymphatic system.

Symptom-directed physical exams will be done during the treatment period and at the safety follow-up (SFU) visits with focus on the lymphatic system and infections.

Vital signs (blood pressure, pulse, oxygen saturation and body temperature) will be assessed after the subject has rested in the sitting position for at least 5 minutes.

4.1.9 Electrocardiogram

Subjects should be in supine position and resting for at least 10 minutes before any study-related ECGs.

4.1.10 Urine or Serum Pregnancy Test

Pregnancy tests will be required only for women of childbearing potential.

4.1.11 Hematology

Hematology studies must include complete blood count (CBC) with differential including hemoglobin, hematocrit, platelet count, ANC, and absolute lymphocyte count (ALC).

4.1.12 Serum Chemistry

Chemistry will include albumin, alkaline phosphatase, ALT, bicarbonate, blood urea nitrogen (BUN), calcium (total or ionized), chloride, creatinine, glucose, lactate dehydrogenase (LDH), magnesium, phosphate/phosphorus, potassium, sodium, total bilirubin, total protein, and uric acid. If an unscheduled ECG is done at any time, then an electrolyte panel (ie, calcium, magnesium, and potassium) must be done to coincide with the ECG testing.

4.1.13 Hepatitis B and C Testing

Hepatitis serology testing must include hepatitis B surface antigen (HBsAg), hepatitis B surface antibody (anti-HBs), anti-HBc, and HCV antibody. In addition, any subjects testing positive for any hepatitis serology must have monthly PCR testing during screening and on treatment, thereafter 3-monthly PCR for the next year.

Subjects who are anti-HBc positive should have quantitative PCR testing for HBV DNA performed during screening and monthly thereafter as per institutional practices. Any subject with a rising viral load (above lower limit of detection) should discontinue study drug and have antiviral therapy instituted and a consultation with a physician with expertise in managing hepatitis B. As IVIG may cause false positive hepatitis serology, monthly PCR testing is not required in subjects who are currently receiving or received prophylactic IVIG within 3 months before study enrollment and have a documented negative anti-HBc test before the initiation of IVIG therapy. PCR testing should be performed when clinically indicated (e.g., in the setting of rising transaminase levels).

Subjects with a known history of hepatitis C or who are hepatitis C antibody positive should be excluded from study.

4.1.14 Immune Phenotyping and Translational Analyses

For the phase 2-part, extensive immune phenotyping and assessment of in vitro full blood response to standardized stimuli will be tested. Part of these test will be performed locally according to the laboratory manual.

4.1.15 Biomarker Studies and Central Biobanking

Blood samples will be used for PD testing including, but not limited to, BTK occupancy, B-cell activation, MDSCs, and T-cell activation, cytokine analysis, and for further characterization of circulating tumor cells, lymphocyte and myeloid cell subsets and cell free DNA as well as immune phenotyping, NGS sequencing for recurrent mutations along with functional and genetic characterization of tumor cells and microenvironmental cells. Bone marrow biopsies/aspirates and tissue sections from archival tumor biopsies and/or any newly obtained biopsies performed during the study will be used for exploratory biomarker studies (including, but not limited to, expression of

BTK, characterization of disease subtype, and evaluations of MDSCs and activated $CD8^+$ cells). Microbiome samples like feces, saliva, skin swabs and buccal swabs will be obtained for assessment of microbiome by shot gun and 16S sequencing.

4.1.16 Pharmacokinetic Analyses

Blood samples will be used for PK testing including but not limited to acalabrutinib and venetoclax concentrations at various time points post-dose on cycle 3 day 1 as detailed in appendix 1.

4.1.17 Tumor Assessments

A pretreatment computerized tomography (CT) scan with contrast (unless contraindicated) is required of the chest, abdomen, and pelvis and any other disease sites within 42 days before randomization.

End of treatment CT scans with contrast (unless contraindicated) of the chest, abdomen, and pelvis and any other disease sites will be done for tumor assessments 8 weeks (+/- 7 days) after last dose of drug (20 weeks after first dose of drug or start of observation). At all other visits, tumor assessments will be done by physical exam and laboratory results.

At suspicion of progression and/or prior to any new CLL treatment, CT scans with contrast (unless contraindicated) of the chest, abdomen, and pelvis and any other disease sites will be done for tumor assessments along with bone marrow biopsy and/or tissue biopsy. Specimens and biobank samples will be sent for central biobanking.

4.2 ASSESSMENT OF RESPONSE TO TREATMENT

Response assessments will be evaluated based on IWCLL 2018 Criteria (Hallek et al., Blood 2018), see appendix 8.

4.3 TREATMENT TERMINATION AND SAFETY FOLLOW-UP VISITS

A treatment termination (TT) visit is required for safety assessments for any subjects who permanently discontinue study drug for any reason, including disease progression. The TT visit should be scheduled within 7 days of the last dose of study drug, if possible, and is not required for subjects who discontinue from the study within 10 days of a scheduled study visit.

Each subject should be followed until the safety follow-up (SFU) visit at 2 years after his or her first dose of study drug or observation to monitor for resolution or progression of AEs and to document the occurrence of any new events, regardless of whether the subject receives a new CLL therapy or demonstrates disease progression within this timeframe. Subjects who withdraw consent for study treatment should still be encouraged to complete the SFU assessments, but these assessments cannot be mandated if subject consent for further study participation is withdrawn

The Schedule of Assessments (Appendix 1) describes the procedures required for the TT and SFU visits.

4.4 FOLLOW-UP FOR INFECTION, PROGRESSION AND SURVIVAL

4.4.1 Discontinuation Follow-up

Each subject should be followed until disease progression or the start of alternative CLL therapy and at least for the first two years after randomization. If neither of these has occurred at the time of the 2-year SFU visit, follow-up visits should occur approximately every 6 months until disease progression or next CLL treatment. During this period, subjects will be followed via out-patient clinic and/or registries per investigator discretion including registration of any infectious events**.**

4.4.2 Long-Term Follow-up

Once subjects progress or start use of alternative CLL therapy—for all subjects who have not withdrawn consent — they will be contacted or followed approximately every 3 months by clinic visit, electronic assessment or telephone, to assess survival, the use of alternative CLL therapy and infections.

4.4.3 Follow-up for Patients Assessed as Low Risk or Low Confidence by CLL-TIM

Patients assessed as low risk or low confidence prediction by CLL-TIM will be followed 6-monthly for assessment of infectious events, CLL treatment and overall survival by medical record review and/or registries. These patients are considered part of the screening cohort for the study, but not part of the clinical trial. Outcome data for this patient population will thus only be followed for assessment of the predictive performance of CLL-TIM and for comparison to the clinical trial population – no study specific procedures will be performed for this population, which will not be considered part of the trial.

4.5 MISSED EVALUATIONS

Missed evaluations should be rescheduled and performed as close to the original scheduled date as possible. An exception is made when rescheduling becomes, in the investigator’s opinion, medically unnecessary because it is too close in time to the next scheduled evaluation. In that case, the missed evaluation should be abandoned.

5. STATISTICAL METHODS OF ANALYSIS

5.1 GENERAL CONSIDERATIONS

The study is designed as a phase 2 study with an optional subsequent extension to a phase 3 study that can be considered if the conditions detailed below are met. Patients enrolled in the

phase 2 study will be included in the optional phase 3 study if the decision is made to extend the study. As the primary outcome for phase 2 is prior to and different from the primary outcome in the optional phase 3 part, this should not affect the power for the phase 3 study. Randomization is between observation and pre-emptive treatment with acalabrutinib and venetoclax for 3 months. Based on the CLL-TIM algorithm, newly diagnosed CLL patients with high (>65%) risk of severe infection and/or treatment within 2 years from diagnosis can be identified. Approximately 20% of newly diagnosed CLL patients will fall into this high-risk group.

5.2 MISSING DATA HANDLING

No imputation of values for missing data will be performed except that missing or partial start and end dates for AEs and concomitant medication will be imputed according to prespecified, conservative imputation rules. Subjects lost to follow-up (or drop out) will be included in statistical analyses to the point of their last evaluation.

5.3 ENDPOINT DATA ANALYSIS

5.3.1 Safety Endpoint(s) for the Phase 2 Part as Primary Endpoint

- Grade ≥3-Infection-free survival 24 weeks after treatment initiation. This is a non-inferiority analysis as detailed in statistical analysis plan to assure safety of the combination treatment in this preemptive trial population.
- Rate and CTCAE grade of infections
- Treatment related adverse events, type, frequency and severity during and for 2 years after treatment

5.3.2 Study Treatment Administration and Compliance

Descriptive information will be provided regarding the number of acalabrutinib and venetoclax doses prescribed, the total number of doses taken, the number of days of treatment, and the number and timing of prescribed dose delay, reductions and interruptions.

For each subject, acalabrutinib and venetoclax compliance will be described in terms of the proportion of study drug actually taken.

5.3.3 Analysis of Primary Endpoint

PRIMARY ENDPOINT, PHASE 2 PART:

Grade ≥3-Infection-free survival in the treatment arm compared to the observation arm 24 weeks after treatment/observation is initiated. The proportion of patients with the primary endpoint will be assessed at 24 weeks after enrollment using a chi-squared test of proportions. - This is a non-

inferiority analysis as detailed in statistical analysis plan to assure safety of the combination treatment in this preemptive trial population.

5.3.4 Secondary Efficacy Endpoint

CLL-treatment-free survival (TFS), is defined as the time from first dose or start of observation to initiation of (another) CLL-treatment or death from any cause.

OS is defined as the time from first dose or start of observation to death from any cause. Data for subjects who are still alive at the time of data cutoff date, lost to follow-up, have discontinued the study (or, if no post-baseline assessment, at the time of first dose plus 1 day). Duration of OS will be estimated using Kaplan-Meier methodology. Approximate 95% CIs for median duration of OS will be computed using the formula proposed by Brookmeyer and Crowley.

5.3.5 Biomarker Analyses including Immune Function and MRD

The diagnostic immunological profiling tool is based on novel, standardized techniques revealing in-depth information on immune phenotype and -function, allowing for standardized and robust data readout and ensuing technology transfer. These assessments are based on a collaboration with Sisse Ostrowski, MD, PhD, Rigshospitalet and the Persimune.org project.
These investigations will be performed at a few central laboratories to assure quality and inter-laboratory reproducibility. In combination with MRD results and assessment of subclonal development, immune phenotyping data may indicate optimal treatment length (6 months or 3 months).

- **Immune phenotyping**: A 10-color flow cytometry whole blood panel comprising 10 different tests has been custom-designed (DuraClone, Beckman Coulter) and is currently under validation at the Dept. of Clinical Immunology, Rigshospitalet. The ten different flow tests (I-X) evaluates: I) Major cell lineages in the blood (proportions and counts), II) B cell subsets, III) T cell subsets, IV) T cell receptor (TCR) subsets, V) Regulatory (T_{reg}) and Th_{17} cells, VI) Dendritic cells, VII) Myeloid cells, VIII) Erythrocytes as surrogates for asplenism, IX) Platelets and platelet-leukocyte co-aggregates and X) Neutrophil phagocyte function. The panel captures multiple patterns predictive for cancer patients i.e. deviated maturation, acute/chronic activation, exhaustion, migration/trafficking and expression of immune checkpoint markers (PD-1, PD-1L, CTLA-4, FoxP3, Helios, CD25, TLR2, TLR4, CD38) which will also be extended with assessment of NK cell function and subtypes as well as additional immune activation markers.[1-8]
- **Immune function**: Stimulated immune response & platelet function are assessed by TruCulture® (Myriad RBM), which assesses the induced innate and adaptive immune

responses to whole blood ligand stimulation. Five different stimuli I-V are applied to screen the response to signaling pathways via Toll Like Receptors (TLRs), critical for the anticancer immune response as TLR ligands may break tolerance to self-antigens and promote immune responses to tumor antigens[9,10]: I) Zymosan; II) Poly I:C; III) Resiquimod; IV) ODN + LPS and V) NegCo. TruCulture® are currently validated at the Dept. of Clinical Immunology, Rigshospitalet and being tested for assessment of immune function in CLL at baseline and during treatment with targeted small molecules. TruCulture® provides an extensive data-readout, with cytokine response data available within days whereas Multiplate®/TEG® provides real-time results also on platelet function and complement cascades. Furthermore, transcriptomics by RNAseq assures in depth analyses of the molecular basis for immune changes upon treatment in stimulated and unstimulated cells.

In addition to the direct assessment of immune dysfunction by this approach at baseline, during and after treatment, infections will be closely monitored during the whole study as a readout for immune dysfunction. Furthermore, microbiomic assessment of the microbiological diversity and changes upon preemptive treatment will be assessed by 16S and shotgun sequencing of skin swaps, saliva and feces samples at baseline and during treatment for an in-depth assessment of immune dysfunction and impact on the microbiome in parallel to microenvironmental changes. By applying the extensive translational program to both the treatment arm and the observation arm, molecular assessment of the immune dysfunction in this high-risk patient group is assured. Minimal residual disease (MRD) levels will be examined by quantitative highly sensitive flow cytometry (MRD flow) in the peripheral blood and in the bone marrow at: The Department of Hematology, Rigshospitalet, Copenhagen University Hospital.

- **MRD assessment**: MRD will be quantified by eight-color flow cytometry with a sensitivity of at least 10^{-4} as previously validated against ASO-primer real-time quantitative IGH-PCR.[35] MRD assessments of peripheral blood are to be performed at screening (for baseline characterization of the individual CLL clone), at 3 and 6 months after treatment initiation/start of observation, thereafter once a year for 2 years.

In addition, an NGS capture-based assay for MRD detection based on recurrent mutations and IGHV sequence is under development. DNA is prepared from patient samples at time of MRD assessment by flow cytometry. After full enrollment, these DNA samples will be analyzed by the NGS assay to allow for direct comparison of MRD assessment by the two methods and to allow for assessment of subclonal development during and after treatment. Furthermore, BH3 profiling as

developed in collaboration with Assoc. Professor Matthew Davids, Dana Farber Cancer Institute, will be employed for assessment of sensitivity to various targeted therapies of viably frozen primary CLL cells from enrolled patients at baseline. The results of BH3 profiling will be correlated with long term outcome for the patients.

5.4 PROCESS FOR DECISION ON THE OPTIONAL EXPANSION TO PHASE 3

When the phase 2 part of the study is fully enrolled, the following assessments will form the basis for the decision on whether to expand the study to the optional phase 3 part with 212 patients in total:

- Non-inferiority for the phase 2 part as stated above should be met
- Immunophenotype analyses as outlined above have been reviewed
- Trend for benefit for patients in the treatment arm should be assessed
- DSMB do not find contraindications or safety reasons against expansion to phase 3
- Assessment by Abbvie, Acerta and the two study groups have been reviewed

The final decision to expand the study to the phase 3 part will be made by the sponsor, taking into account the input from the above-mentioned analyses and assessments.

6. ASSESSMENT OF SAFETY

Due to the pre-emptive treatment of a patient population normally not treated, an independent DSMB assessment of infectious risk and adverse events on an ongoing basis during enrollment is included. The trial may be stopped based on the decision by the independent DSMB if increased infectious risk or AE risk is considered to outweigh the potential benefit. For these safety assessments, the treatment period (first 6 cycles of 28 days each) and the follow up period will be assessed independently to improve detection of treatment-related events vs CLL-related events.
The following early stopping rules will be applied:

- Any fatal events or Grade ≥3 infectious events in the treatment arm will be reported immediately (within 7 days of notification) to the DSMB for assessment of whether the event impacts the risk-benefit of the treatment (data on similar events in the observation arm will be made available to the DSMB). All Grade ≥3 infectious events would be considered AESIs to be reported along with SAEs within 24 h in the phase 2 part.
- Assuming the incidence of grade ≥3 infectious events is the same in the treatment arm and the observation arm, which based on the predictive model is 10% within the first 6 months, a non-inferiority test can be performed. With a power of 80% and a sample size of 25 patients in each arm, the margin for assessment of non-inferiority will be 21%, i.e. if significantly more

infections are seen in the treatment arm than in the observation arm, the treatment arm will be inferior to observation.

- Any pattern of AEs or individual AEs through the above-mentioned DSMB assessment that indicate that the trial should be stopped.
- A thorough translational immune phenotype and immune function analysis plan is adjoined to the study. Results of this translational program are included as secondary outcomes. The assessment of the grade ≥3-infection free, CLL-treatment-free survival will be qualified by the results from the translational program. Hereby, the clinical outcome from the study will be significantly strengthened by molecular and functional information about improvements in immune function.

6.1 DOCUMENTING AND REPORTING OF ADVERSE AND SERIOUS ADVERSE EVENTS

The investigator is responsible for ensuring that all AEs and SAEs that are observed or reported during the study, as outlined in the prior sections, are recorded on the CRF. The eCRF system assures that the sponsor is notified in real time about SAEs and other AEs that need further reporting to health authorities and the companies providing the investigational drugs. This reporting will follow EU regulations and agreements set out in the contract with the companies.

6.1.1 Adverse Event Reporting Period

The AE reporting period for this study begins when the subject receives the first dose of study drug or Cycle 1 Day 1 for the observation arm of the study and ends with the SFU visit.

6.1.2 Assessment of Adverse Events

Investigators will assess the occurrence of AEs and SAEs at all subject evaluation timepoints during the study. All AEs and SAEs whether volunteered by the subject, discovered by study personnel during questioning, or detected through physical examination, or other means, will be recorded in the subject's medical record and on the AE CRF.

Each recorded AE or SAE will be described by its diagnostic term, duration (e.g., start and end dates), severity, regulatory seriousness criteria, if applicable, suspected relationship to the study drug, and any actions taken.

6.1.3 Pregnancy

The Sponsor should report all pregnancies and pregnancies in the partners of subjects to the Regulatory Authorities per institutional and/or regulatory guidelines, and to the companies providing investigational drugs as per contractual guidelines.

Any uncomplicated pregnancy that occurs with the subject or with the partner of a treated subject during this study will be reported. All pregnancies and partner pregnancies that are identified during or after this study, wherein the estimated date of conception is determined to have occurred from the time of consent to 30 days after the last dose of study medication will be reported, followed to conclusion, and the outcome reported.

Subjects should be instructed to immediately notify the investigator of any pregnancies. Any female subjects receiving study drug who become pregnant must immediately discontinue study drug. The investigator should counsel the subject, discussing any risks of continuing the pregnancy and any possible effects on the fetus.

6.1.4 Expedited Reporting Requirements for Serious Adverse Events

The Sponsor should report all SAEs to Regulatory Authorities per EU regulatory guidelines, and to the companies providing investigational drugs per contractual guidelines.

6.1.5 Reporting Adverse Events of Special Interest

Atrial fibrillation, ventricular arrhythmias, tumor lysis events, infections, disease progression, treatment for CLL, malignancies and bleeding events are adverse events of special interest in this trial.

6.1.6 Type and Duration of Follow-up of Subjects after Adverse Events

All AEs and SAEs that are collected during the protocol-specified AE reporting period should be followed to resolution, or until the investigator assesses the subject as stable or remitted to grade 1 or lower, or the subject is lost to follow-up or withdraws consent.

7. STUDY ADMINISTRATION AND INVESTIGATOR OBLIGATIONS

The Sponsor retains the right to terminate the study and remove all study materials from a study site at any time. Specific circumstances that may precipitate such termination are:

- Unsatisfactory subject enrollment with regard to quality or quantity
- Significant or numerous deviations from study protocol requirements, such as failures to perform required evaluations on subjects and maintain adequate study records
- Inaccurate, incomplete and/or late data recording on a recurrent basis
- The incidence and/or severity of AEs in this or other studies indicating a potential health hazard caused by the study treatment

7.1 INSTITUTIONAL REVIEW BOARD AND INDEPENDENT ETHICS COMMITTEE

The investigator will submit this protocol, the informed consent, current Summary of Product Characteristics, and any other relevant supporting information to the appropriate Ethics Committee, Health Authorities and Data Protection Agencies for review and approval before study initiation. Amendments to the protocol must also be approved as appropriate, before the implementation of changes in this study.

This clinical study was designed and will be implemented in accordance with the protocol, the International Conference on Harmonisation (ICH) Harmonised Tripartite Guidelines for Good Clinical Practices, applicable local regulations (including European Directive 2001/20/EC), and the ethical principles laid down in the Declaration of Helsinki.

7.2 INFORMED CONSENT AND PROTECTED SUBJECT HEALTH INFORMATION AUTHORIZATION

The investigator, or designee, must explain to each subject the purpose and nature of the study, the study procedures, the possible adverse effects, and all other elements of consent as defined in national and local regulations governing informed consent form. Each subject must provide a signed and dated informed consent before enrollment into this study. Signed consent forms must remain in each subject's study file and be available for verification by study monitors at any time.

In accordance to individual local and national subject privacy regulations, the investigator or designee **must** explain to each subject that for the evaluation of study results, the subject's protected health information obtained during the study may be shared with regulatory agencies and IRBs/IECs. It is the investigator's or designee's responsibility to obtain written permission to use protected health information from each subject. If a subject withdraws permission to use protected health information, it is the investigator's responsibility to obtain the withdrawal request in writing from the subject **and** to ensure that no further data will be collected from the subject. Any data collected on the subject before withdrawal will be used in the analysis of study results.

7.3 SUBJECT SCREENING LOG

The investigator will keep a record that lists all subjects considered for enrollment (including those who did not undergo screening) in the study. For those subjects subsequently excluded from enrollment, record the reason(s) for exclusion will be kept.

Subjects not assessed as high-risk according to the CLL-TIM algorithm, will not be included for the clinical trial, but still followed outside the trial at 6-monthly intervals through medical record review and/or registries for infectious adverse events, initiation of CLL treatment and survival.

7.4 CASE REPORT FORMS

Authorized study site personnel will complete eCRFs designed for this study according to the completion guidelines that will be provided. The investigator will ensure that the CRFs are accurate, complete, legible, and completed promptly.

7.5 INVESTIGATIONAL STUDY DRUG ACCOUNTABILITY

Investigational drugs must be kept in a limited access cabinet or space. The study drug must not be used outside the context of the protocol.

7.6 RECORD RETENTION

The investigator and other appropriate study staff are responsible for maintaining all documentation relevant to the study. Mandatory documentation includes copies of study protocols and amendments, IRB/IEC approval letters, signed ICFs, drug accountability records, SAE information, subject files (source documentation) that substantiate entries in CRFs, all relevant correspondence and other documents pertaining to the conduct of the study.
The investigator shall retain study records in accordance with institutional and/or national/local regulations, whichever is longer.

8. REFERENCES

1 Zitvogel, L., Kepp, O. & Kroemer, G. Immune parameters affecting the efficacy of chemotherapeutic regimens. *Nature reviews. Clinical oncology* **8**, 151-160, doi:10.1038/nrclinonc.2010.223 (2011).

2 Schreiber, R. D., Old, L. J. & Smyth, M. J. Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. *Science* **331**, 1565-1570, doi:10.1126/science.1203486 (2011).

3 Masucci, G. V. *et al.* Validation of biomarkers to predict response to immunotherapy in cancer: Volume I - pre-analytical and analytical validation. *Journal for immunotherapy of cancer* **4**, 76, doi:10.1186/s40425-016-0178-1 (2016).

4 Galluzzi, L., Buque, A., Kepp, O., Zitvogel, L. & Kroemer, G. Immunological Effects of Conventional Chemotherapy and Targeted Anticancer Agents. *Cancer cell* **28**, 690-714, doi:10.1016/j.ccell.2015.10.012 (2015).

5 Blank, C. U., Haanen, J. B., Ribas, A. & Schumacher, T. N. CANCER IMMUNOLOGY. The "cancer immunogram". *Science* **352**, 658-660, doi:10.1126/science.aaf2834 (2016).

6 Niemann, C. U. *et al.* Disruption of in vivo Chronic Lymphocytic Leukemia Tumor-Microenvironment Interactions by Ibrutinib - Findings from an Investigator-Initiated Phase II Study. *Clin Cancer Res* **22**, 1572-1582, doi:10.1158/1078-0432.CCR-15-1965 (2016).

7 Palma, M. *et al.* Ibrutinib induces rapid down-regulation of inflammatory markers and altered transcription of chronic lymphocytic leukaemia-related genes in blood and lymph nodes. *Br J Haematol* **183**, 212-224, doi:10.1111/bjh.15516 (2018).

8 Palma, M. *et al.* T cells in chronic lymphocytic leukemia display dysregulated expression of immune checkpoints and activation markers. *Haematologica* **102**, 562-572, doi:10.3324/haematol.2016.151100 (2017).

9 Conroy, H., Marshall, N. A. & Mills, K. H. TLR ligand suppression or enhancement of Treg cells? A double-edged sword in immunity to tumours. *Oncogene* **27**, 168-180, doi:10.1038/sj.onc.1210910 (2008).

10 Klein, J. C. *et al.* Combined toll-like receptor 3/7/9 deficiency on host cells results in T-cell-dependent control of tumour growth. *Nature communications* **8**, 14600, doi:10.1038/ncomms14600 (2017).

11 da Cunha-Bang, C. *et al.* The Danish National Chronic Lymphocytic Leukemia Registry. *Clin Epidemiol* **8**, 561-565, doi:10.2147/CLEP.S99486 (2016).

12 Wierda, W. G. *et al.* Prognostic nomogram and index for overall survival in previously untreated patients with chronic lymphocytic leukemia. *Blood* **109**, 4679-4685 (2007).

13 Wadhwa, P. D. & Morrison, V. A. Infectious complications of chronic lymphocytic leukemia. *Semin Oncol* **33**, 240-249, doi:10.1053/j.seminoncol.2005.12.013 (2006).

14 Molica, S. Infections in chronic lymphocytic leukemia: risk factors, and impact on survival, and treatment. *Leuk Lymphoma* **13**, 203-214, doi:10.3109/10428199409056283 (1994).

15 Molica, S., Levato, D. & Levato, L. Infections in chronic lymphocytic leukemia. Analysis of incidence as a function of length of follow-up. *Haematologica* **78**, 374-377 (1993).

16 Andersen, M. A., Brieghel, C., Hellerberg, M., Da Cunha-Bang, C. & Niemann, C. U. in *IWCLL.*

17 Hallek, M. *et al.* Addition of rituximab to fludarabine and cyclophosphamide in patients with chronic lymphocytic leukaemia: a randomised, open-label, phase 3 trial. *Lancet* **376**, 1164-1174, doi:S0140-6736(10)61381-5 [pii] 10.1016/S0140-6736(10)61381-5 (2010).

18 Shanafelt, T. D. *et al.* A Randomized Phase III Study of Ibrutinib (PCI-32765)-Based Therapy Vs. Standard Fludarabine, Cyclophosphamide, and Rituximab (FCR) Chemoimmunotherapy in Untreated Younger Patients with Chronic Lymphocytic Leukemia (CLL): A Trial of the ECOG-ACRIN Cancer Research Group (E1912). *Blood* **132**, LBA-4-LBA-4, doi:10.1182/blood-2018-120779 (2018).

19 Eichhorst, B. *et al.* First-line chemoimmunotherapy with bendamustine and rituximab versus fludarabine, cyclophosphamide, and rituximab in patients with advanced chronic

lymphocytic leukaemia (CLL10): an international, open-label, randomised, phase 3, non-inferiority trial. *The Lancet Oncology*, doi:10.1016/S1470-2045(16)30051-1.

20 O'Brien, S. *et al.* The Bruton's Tyrosine Kinase (BTK) Inhibitor PCI-32765 Induces Durable Responses in Relapsed or Refractory (R/R) Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma (CLL/SLL): Follow-up of a Phase Ib/II Study. *Blood (ASH Annual Meeting Abstracts)* **118** (2011).

21 Byrd, J. C. *et al.* Targeting BTK with ibrutinib in relapsed chronic lymphocytic leukemia. *N Engl J Med* **369**, 32-42, doi:10.1056/NEJMoa1215637 (2013).

22 Seymour, J. ABT-199 for chronic lymphocytic leukemia. *Clin Adv Hematol Oncol* **12**, 698-700 (2014).

23 Stilgenbauer, S. *et al.* Venetoclax in relapsed or refractory chronic lymphocytic leukaemia with 17p deletion: a multicentre, open-label, phase 2 study. *The Lancet Oncology*, doi:10.1016/S1470-2045(16)30019-5.

24 Roberts, A. W. *et al.* Targeting BCL2 with Venetoclax in Relapsed Chronic Lymphocytic Leukemia. *N Engl J Med*, doi:10.1056/NEJMoa1513257 (2015).

25 Sun, C. *et al.* Partial reconstitution of humoral immunity and fewer infections in patients with chronic lymphocytic leukemia treated with ibrutinib. *Blood* **126**, 2213-2219, doi:10.1182/blood-2015-04-639203 (2015).

26 Burger, J. A. & Gribben, J. G. The microenvironment in chronic lymphocytic leukemia (CLL) and other B cell malignancies: insight into disease biology and new targeted therapies. *Semin Cancer Biol* **24**, 71-81, doi:10.1016/j.semcancer.2013.08.011 (2014).

27 de Weerdt, I. *et al.* First Evidence of Restoration of T and NK Cell Compartment after Venetoclax Treatment. *Blood* **132**, 1860-1860, doi:10.1182/blood-2018-99-116283 (2018).

28 Mathews Griner, L. A. *et al.* High-throughput combinatorial screening identifies drugs that cooperate with ibrutinib to kill activated B-cell-like diffuse large B-cell lymphoma cells. *Proc Natl Acad Sci U S A* **111**, 2349-2354, doi:10.1073/pnas.1311846111 (2014).

29 Cervantes-Gomez, F. *et al.* Pharmacological and Protein Profiling Suggests Venetoclax (ABT-199) as Optimal Partner with Ibrutinib in Chronic Lymphocytic Leukemia. *Clin Cancer Res* **21**, 3705-3715, doi:10.1158/1078-0432.CCR-14-2809 (2015).

30 Deng, J. *et al.* Bruton's tyrosine kinase inhibition increases BCL-2 dependence and enhances sensitivity to venetoclax in chronic lymphocytic leukemia. *Leukemia* **31**, 2075-2084, doi:10.1038/leu.2017.32 (2017).

31 Bojarczuk, K. *et al.* BCR signaling inhibitors differ in their ability to overcome Mcl-1-mediated resistance of CLL B cells to ABT-199. *Blood* **127**, 3192-3201, doi:10.1182/blood-2015-10-675009 (2016).

32 Thijssen, R. *et al.* Resistance to ABT-199 induced by microenvironmental signals in chronic lymphocytic leukemia can be counteracted by CD20 antibodies or kinase inhibitors. *Haematologica* **100**, e302-306, doi:10.3324/haematol.2015.124560 (2015).

33 Hallek, M. *et al.* Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia: a report from the International Workshop on Chronic Lymphocytic Leukemia updating the National Cancer Institute-Working Group 1996 guidelines. *Blood* **111**, 5446-5456 (2008).

34 Barf, T. *et al.* Acalabrutinib (ACP-196): A Covalent Bruton Tyrosine Kinase Inhibitor with a Differentiated Selectivity and In Vivo Potency Profile. *J Pharmacol Exp Ther* **363**, 240-252, doi:10.1124/jpet.117.242909 (2017).

35 Rawstron, A. C. *et al.* A complementary role of multiparameter flow cytometry and high-throughput sequencing for minimal residual disease detection in chronic lymphocytic leukemia: an European Research Initiative on CLL study. *Leukemia* **30**, 929-936, doi:10.1038/leu.2015.313 (2016).

36 Portell, C. A. *et al.* Synergistic Cytotoxicity of Ibrutinib and the BCL2 Antagonist, ABT-199(GDC-0199) in Mantle Cell Lymphoma (MCL) and Chronic Lymphocytic Leukemia (CLL): Molecular Analysis Reveals Mechanisms of Target Interactions. *Blood* **124**, 509-509 (2014).

37 Jain, N. *et al.* Combined Ibrutinib and Venetoclax in Patients with Treatment-Naïve High-Risk Chronic Lymphocytic Leukemia (CLL). *Blood* **132**, 696-696, doi:10.1182/blood-2018-186 (2018).
38 da Cunha-Bang, C., Christiansen, I. & Niemann, C. U. The CLL-IPI applied in a population-based cohort. *Blood* **128**, 2181-2183, doi:10.1182/blood-2016-07-724740 (2016).
39 International, C. L. L. I. P. I. w. g. An international prognostic index for patients with chronic lymphocytic leukaemia (CLL-IPI): a meta-analysis of individual patient data. *Lancet Oncol* **17**, 779-790, doi:10.1016/S1470-2045(16)30029-8 (2016).
40 Hallek, M. *et al.* iwCLL guidelines for diagnosis, indications for treatment, response assessment, and supportive management of CLL. *Blood* **131**, 2745-2760, doi:10.1182/blood-2017-09-806398 (2018).
41 Group, I. S. S. *et al.* Initiation of Antiretroviral Therapy in Early Asymptomatic HIV Infection. *N Engl J Med* **373**, 795-807, doi:10.1056/NEJMoa1506816 (2015).

APPENDICES

	Prescreening [1]	At screening [2]	C1D1[2]	C1D8[3,4]	C1D15[1,2]	C1D22[1,2]	C2D1 [2]	C3D1	End of treatment / observation	24 weeks after C1D1	Every 3. Months for two years	1 year after C1D1	2 years after C1D1	Progressive disease
				Only applies to treatment arm for management of TLS risk										
Informed consent	X	X												
Medical history, vaccine usage, immunoglobulins	X	X												
Adverse events	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant	X	X	X				X	X	X	X	X	X	X	X
New CLL treatment														X
Physical examination	X	X	X				X	X	X	X	X	X	X	X
CIRS / CCI	X	X												
Binet stage	X	X												
TLS risk category		X												
(Clinical) response							X	X	X	X		X	X	X
Quality of Life		X					X	X	X	X	X	X	X	
Pregnancy test[5]		X	X[6]				X[7]	X[7]	X[7]					
ECOG Performance	X	X												
ECG		X												
(PET)-CT scan		X								X				X
Lab tests														
Hematology		X	X	X	X	X	X	X	X	X		X	X	X
Blood chemistry		X	X	X	X	X	X	X	X	X		X	X	X
Additional chemistry		X								X		X	X	X
Virology		X								X		X	X	X

[1] No laboratory testing except for standard of care based on the discretion of the local investigator, this visit is for informed consent to assemble data for assessment by the CLL-TIM algorithm
[2] Max 42 days from screening to randomization, however FISH and TP53 mutational results are accepted up to six months prior to randomization, max 14 days from randomization to cycle 1 day 1 (C1D1)
[3] Only for treatment arm.
[4] TLS chemistry the day before, 6 h after ramp up dose of venetoclax and the day after (24 h) before next dose of venetoclax
[5] Only applies for women of child bearing potential
[6] A negative pregnancy test is required for all women of child bearing potential within 7 days before start of study treatment
[7] Only applies for the women of child bearing potential in the treatment arm

Biobank blood		X		X[3]	X[3]		X	X	X	X		X	X	X
Central lab														
IGHV, FISH, Recur		X												X
Flow-MRD		X							X	X		X	X	X
PK samples								X[8]						
Bone marrow														
Biopsy and aspirate		X					X	X	X	X				X
Aspirate biobank		X								X				X[9]
Microbiome samples														
Feces, saliva, skin, buccal swabs		X							X	X				

Appendix 1. Schedule of Assessments (observation and treatment arm)

[8] PK blood samples in terms of 3 mL EDTA blood, kept on ice and centrifuged within 1 h according to laboratory manual to be collected at 2, 4, 6, 8 and 24 h post-dose

[9] Include biopsy from lymph node if available

Appendix 2. Performance Status Scores

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

As published in Am J Clin Oncol:

Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982.

Credit: Eastern Cooperative Oncology Group Chair: Robert Comis, MD

Available at: http://www.ecog.org/general/perf_stat.html. Accessed 23 August 2013.

Appendix 3. List of Prohibited and Cautionary Medications

Prohibited (strong CYP3A inhibitors and warfarin/vitamin K antagonists) and cautionary medications are defined as follows. Refer to sections above for instructions for concomitant use of moderate CYP3A inhibitors, and moderate and strong CYP3A inducers, with acalabrutinib and venetoclax.

INHIBITORS AND INDUCERS		**SUBSTRATES**
Inhibitors of CYP3A (Acalabrutinib and Venetoclax)	**Inducers of CYP3A (Acalabrutinib and Venetoclax)**	
Strong CYP3A inhibitors - PROHIBITED:	**Strong CYP3A inducers:**	**Substrates of P-gp (Acalabrutinib and Venetoclax)**
boceprevir	avasimibe	aliskiren
clarithromycin	carbamazepine	ambrisentan
cobicistat	phenytoin	colchicines
conivaptan	rifabutin	dabigatran etexilate
indinavir	rifampin	digoxin
itraconazole	St. John's Wort	everolimus
ketoconazole	phenobarbital	fexofenadine
lopinavir		lapatinib
mibefradil	**Moderate CYP3A inducers:**	loperamide
nefazodone	bosentan	maraviroc
nelfinavir	efavirenz	nilotinib
posaconazole	etravirine	ranolazine
ritonavir	modafinil	saxagliptin
saquinavir	nafcillin	sirolimus
telaprevir	troglitazone	sitagliptin
telithromycin	oxcarbazepine	talinolol
troleandomycin		tolvaptan
voriconazole*		topotecan
Moderate CYP3A inhibitors:	**Weak inducers:**	**Substrates of BCRP (acalabrutinib and Venetoclax)**
aprepitant	glucocorticoids (e.g. prednisone)	methotrexate
amprenavir	pioglitazone	mitoxantrone
atazanavir	amprenavir	irinotecan
ciprofloxacin	aprepitant,	lapatinib
crizotinib	armodafinil	rosuvastatin
darunavir/ritonavir	clobazamechinacea	sulfasalazine
dronedarone	nevirapine	topotecan
erythromycin	rufinamide	
diltiazem	vemurafenib	**Substrates of OATP1B1/B3 (Venetoclax only)**

fluconazole		atrasentan
fosamprenavir	**Inhibitors of OATP1B1/B3 (Venetoclax only)**	atorvastatin
imatinib	gemfibrozil,	ezetimibe
verapamil	eltrombopag	fluvastatin
	cyclosporine	glyburide
Weak CYP3A inhibitors:	tipranavir	rosuvastatin
alprazolam		simvastatin acid
amiodarone	**Inhibitors of BCRP (Venetoclax only)**	pitavastatin
amlodipine	cyclosporine	pravastatin
atorvastatin	geftinib	repaglinide
bicalutamide		telmisartan
cilostazol	**Inhibitors of P-gp (Venetoclax only)**	valsartan
cimetidine	amiodarone	olmesartan
cyclosporine	azithromycin	
fluvoxamine	captopril	**OTHER**
fluoxetine	carvedilol	**Vitamin K antagonists - PROHIBITED (Acalabrutinib only)**
ginkgo	cyclosporine	warfarin
goldenseal	dronedarone	phenoprocoumon
isoniazid	felodipine	
nilotinib	quercetin	
oral contraceptives	quinidine	
pazopanib	ranolazine	
ranitidine	ticagrelor	
ranolazine		
Suboxone		
tipranavir/ritonavir		
ticagrelor		
zileuton		

Note that this is not an exhaustive list. Further information can be found at the following websites: http://medicine.iupui.edu/clinpharm/ddis/main-table/ and http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm080499.htm.

In addition to the medications listed in this table, subjects should not consume grapefruit, grapefruit products, Seville oranges (including marmalade containing Seville oranges) or starfruits.

Appendix 4. Adverse Event Assessment of Causality

Is there a reasonable possibility that the event may have been caused by study drug? No___ Yes___

The descriptions provided below will help guide the principal investigator in making the decision to choose either “yes” or “no”:

No = There is no reasonable possibility that the event may have been caused by study drug.

The adverse event:

- may be judged to be due to extraneous causes such as disease or environment or toxic factors
- may be judged to be due to the subject’s clinical state or other therapy being administered
- is not biologically plausible
- does not reappear or worsen when study drug is re-administered
- does not follow a temporal sequence from administration of study drug

Yes = There is a reasonable possibility that the event may have been caused by study drug.

The adverse event:

- follows a temporal sequence from administration of study drug
- is a known response to the study drug based on clinical or preclinical data
- could not be explained by the known characteristics of the subject’s clinical state, environmental or toxic factors, or other therapy administered to the subject
- disappears or decreases upon cessation or reduction of dose of study drug
- reappears or worsens when study drug is re-administered

<<Include any applicable response criteria, etc. as separate Appendices>>

Appendix 5. Cumulative illness rating scale (CIRS)

The Modified Cumulative Illness Rating Scale (CIRS)
Please take into account that CLL induced illness or organ damage are not included in this rating scale.

Body system	Score				
1. Cardiac (heart only)	0	1	2	3	4
2. Hypertension (rating is based on severity; organ damage is rated separately)	0	1	2	3	4
3. Vascular (blood, blood vessels and cells, bone marrow, spleen, lymphatics)	0	1	2	3	4
4. Respiratory (lungs, bronchi, trachea below the larynx)	0	1	2	3	4
5. EENT (eye, ear, nose, throat, larynx)	0	1	2	3	4
6. Upper GI (esophagus, stomach, and duodenum; pancreas; do not include diabetes)	0	1	2	3	4
7. Lower GI (intestines, hernias)	0	1	2	3	4
8. Hepatic (liver and biliary tree)	0	1	2	3	4
9. Renal (kidneys only)	0	1	2	3	4
10. Other GU (ureters, bladder, urethra, prostate, genitals)	0	1	2	3	4
11. Muscular-skeletal-integumentary (muscle, bone, skin)	0	1	2	3	4
12. Neurological (brain, spinal cord, nerves, do not include dementia)	0	1	2	3	4
13. Endocrine-Metabolic (includes diabetes, thyroid; breast; systemic infections; toxicity)	0	1	2	3	4
14. Psychiatric/Behavioral (includes dementia, depression, anxiety, agitation/delirium, psychosis)	0	1	2	3	4

RATING SUGGESTIONS (GENERAL PRINCIPLES)

Every single disease must be classified in the appropriate system. If there are several problems in the same system, only the most severe is rated. Example: for a patient suffering from a well-controlled angina (Rated 2) and terminal heart failure (Rated 4), only the higher rated condition would be scored in the Cardiac system (e.g., rating is 4).
The spread of a cancer may lead to rate the condition in more than one category. For example, a lung cancer with bone metastases treated with nonsteroidal anti-inflammatory drugs (NSAIDs) is Rated 4 in Respiratory and 2 in Musculoskeletal.

General rules for severity rating:

0 No problem affecting that system or past problem without clinical relevance.
1 Current mild problem or past significant problem.
2 Moderate disability or morbidity and/or requires first line therapy.
3 Severe problem and/or constant and significant disability and/or hard to control chronic problems (complex therapeutic regimen).
4 Extremely severe problem and/or immediate treatment required and/or organ failure and/or severe functional impairment.

LEVEL 0
No problem or healed minor injuries; past childhood illnesses (chickenpox); minor surgery (carpal tunnel completely healed, caesarean); uncomplicated healed fractures; other past problems healed without sequel, residual or complication (pneumonia).
LEVEL 1
Any current medical problem that causes mild discomfort or disability, or has occasional exacerbations, having only minor impact on morbidity (asthma controlled with bronchodilators, occasional heartburn relieved with antiacids). Medical problems that are not currently active but were significant problems in the past (passage of a kidney stone) or required major surgery (hysterectomy, cholecystectomy, appendectomy).
LEVEL 2
Medical conditions that require daily treatment or first line therapy (asthma controlled with inhaled steroids, gastro-esophageal reflux treated with daily medication, osteoarthritis requiring daily NSAID, etc.) and/or have moderate disability or morbidity.
LEVEL 3
Chronic conditions that are not controlled with first line therapy (asthma needing continuous corticosteroid therapy, symptomatic angina despite medical regimes, heart failure with symptoms or uncontrolled hypertension despite complex therapeutic regimen) and/or constant significant disability, but not severe disability.
LEVEL 4
Any acute condition that requires immediate treatment or hospitalization (unstable angina, acute myocardial infarction, stroke, but also bladder outlet obstruction) and/or extremely severe problems; organ failure (end-stage renal disease needing dialysis, oxygen-dependent chronic obstructive pulmonary disease, terminal heart failure); severe sensory impairment (almost complete blindness or deafness, being wheelchair bound) and/or severely affected quality of life, severe impairment in function; delirium by medical (organic) conditions.

RATING MALIGNANCIES
Consistent scoring of severity ratings for various malignancies is a difficult problem. Each malignancy has its own rating system and prognostic indicators, the complexity of which would quickly exceed the aim of the intended simplicity and ease of use of CIRS.
The following general guidelines are intended to provide a reasonably accurate delineation of medical burden for cancer without excessive complexity.

Level 1: Cancer diagnosed in the remote past without evidence of recurrence or sequel in the past 10 years or skin cancer excised in the past without major sequel (other than melanoma).
Level 2: No evidence of recurrence or sequel in the past 5 years.
Level 3: Required chemotherapy, radiation, hormonal therapy or surgical procedure for cancer in the past 5 years.
Level 4: Recurrent malignancy or metastasis (other than to lymph glands) or palliative treatment stage.

These ratings are to be made in the appropriate organ category for a given malignancy.

ORGAN-SPECIFIC CATEGORIES
The following organ-specific categories will attempt to provide guidelines for consistent rating of comparable severity. Common conditions will be stressed with the focus on the “judgment strategy” that can be applied to other problems not listed.
If there are several problems in the same system, only the most severe is rated.

HEART

In this category, only heart and coronary disease have to be considered (not vascular): coronary arteries disease, heart failure, valvular heart diseases, heart disease secondary to hypertension, endocarditis, myocarditis, pericarditis, arrhythmias (extrasystoles, bundle-branch blocks, atrial fibrillation, pacemaker placement), heart malignancies. Functional impact must be considered too (e.g., NYHA II heart failure has different value between dependent and independent persons).

0 No problems

1 Remote myocardial infarction (MI) (> 5 years ago); occasional [exertion] angina; asymptomatic valvular disease

2 Chronic heart failure (CHF) compensated with meds (NYHA I-II); daily anti-angina meds; left ventricular hypertrophy; atrial fibrillation, bundle branch block, daily anti-arrhythmic drugs (even for prophylaxis); PMK placement for asymptomatic bradycardia (relieved by Holter ECG monitoring); valvular disease requiring medical treatment

3 Previous MI (< 5 years ago); abnormal stress test; status post (previous) percutaneous coronary angioplasty, coronary artery bypass graft surgery or other cardiac surgery (valve replacement); moderate CHF (NYHA II–III) or complex medical treatment; bifascicular block; PMK placement for cardiogenic syncope; pericardial effusion or pericarditis

4 Acute coronary syndrome, unstable angina or acute MI; intractable CHF (NYHA III–IV acute or chronic); marked restriction to the normal activity of daily living secondary to cardiac status

HYPERTENSION

Consider only hypertension severity; organ damage (complications) should be considered into the respective categories.

0 Normotension

1 Borderline hypertension; hypertension compensated with salt restriction and weight loss, drug free (when drug therapy is indicated, but the patient does not take meds, the score is at least 2)

2 Daily antihypertensive meds: hypertension controlled by 1 pill therapy (even fixed doses combinations)

3 Hypertension requiring two or more pills for control

4 Malignant hypertension, or hypertension non-controlled by complex therapeutic regimen

VASCULAR-HEMATOPOIETIC

Artery disease: carotid atherosclerosis, peripheral arteries disease (PAD), aneurysms (every site);
Venous disease: venous insufficiency, varices, deep venous thrombosis (DVT), pulmonary embolism, primary pulmonary hypertension;
Immunologic disease: systemic lupus erythematosus, systemic sclerosis (scleroderma), sarcoidosis, hypersensitivity

0 No problem

1 Venous insufficiency, varices, lymphedema; carotid stenosis < 70%; hemoglobin 10–12 g/dL (in females), 12–14 g/dL (in males); anemia of chronic “inflammatory” disease

2 Previous DVT; one symptom of atherosclerosis disease (claudication, bruit, amaurosis fugax, absent pedal pulses) or daily meds (e.g., anti-platelets drugs); PAD IIa–IIb by Fontaine; carotid stenosis > 70%; aortic aneurysm < 4 cm; hemoglobin 8–10 g/dL (in females), 10–12 g/dL (in males); anemia secondary to iron, B12 vitamin or folate deficiency, or to chronic renal failure; total white blood cell (WBC) 2000–4000/mmc; mild thrombocytopenia (50000–150000/mmc)

3 DVT or recent DVT (< 6 months ago); two or more symptoms of atherosclerosis (see above); PAD Fontaine III or recent/previous angioplasty (with or without stenting); hemoglobin < 8g/dL (in females), <10 g/dL (in males); dyserythropoietic anemia; WBC < 2000/mmc; severe thrombocytopenia (< 50000/mmc)

4 Pulmonary embolism (acute or recent/previous); atherosclerosis requiring surgical intervention (e.g., aortic aneurysm > 4 cm, symptomatic carotid stenosis > 70%, PAD Fontaine IV or amputation for vascular causes, etc.); recent/previous vascular surgery; any hematological or vascular malignancy (including multiple myeloma)

In case of immunological disease, score should be assigned by considering blood abnormalities, stadium of organ damage and/or functional disability (2: symptoms controlled by daily meds; 3: symptoms not well controlled; 4: symptoms impossible to be controlled or short time poor prognosis).

RESPIRATORY

In this category COPD, asthma, emphysema, restrictive pulmonary interstitial lung diseases, malignancies of lung and pleura, pneumonia, and smoking status are considered.

0 No problem

1 Recurrent episodes of acute bronchitis; currently treated asthma with prn inhalers when required; cigarette smoker > 10 but < 20 pack years

2 Instrumental diagnosis of COPD or pulmonary interstitial disease (X-ray, CT, spirometry); daily prn inhalers (≤ 2 pharmacological classes); two or more episodes of pneumonia in the last 5 years; cigarette smoker > 20 but < 40 pack-years

3 Exertion dyspnea secondary to limited respiratory capacity, not well controlled by daily meds; required oral steroids for lung disease; daily prn inhalers (3 pharmacological classes); acute pneumonia treated as an outpatient

4 Chronic supplementation of oxygen; respiratory failure requiring assisted ventilation, or previous (at least one episode); any lung or pleural neoplasm; acute pneumonia requiring hospitalization

Smoking is an important respiratory and cardiovascular risk, so it is considered as a disease, and it is rated according to ***lifetime pack-years***:

Number of cigarette packs smoked per day × Number of years smoked in their lifetime
e.g., 1 pack-year = 20 cigarettes/day (1 pack) × 1 year

Ex-smokers should be rated too, but those who have been smoke-free for the most recent 20 years would merit a lower rating than those who are currently smoking.

Examples:

a) Patient smoking 20 cig/day (1 pack) for 25 years = 25 pack-years – CIRS score: 2

b) Patient smoking 40 cig/day (2 packs) for 25 years = 50 pack-years – CIRS score: 3

c) Ex-smoker of 20 cig/day (1 pack) for 25 years, he stopped 5 years ago – CIRS score: 2

d) Ex-smoker of 20 cig/day (1 pack) for 25 years, he stopped 20 years ago – CIRS score: 1

Classification of COPD could be more specific when instrumental data (objective evidence) are available: blood gases, forced expiratory volume in 1 second (FEV1), etc.

EYES, EARS, NOSE & THROAT, and LARYNX

To simplify the potential complexity of this category it was decided to score according to the severity of the disability created by sensory diseases (degree of limited autonomy and communication), and avoid rating each type of pathology. Sensory impairments should be rated after instrumental correction (corrective lenses, hearing aid, etc.).

Eyes: glaucoma, cataracts, macular degeneration (diabetic/hypertensive retinopathy), any other pathology
Ears: otitis, dizziness, any cause of hearing impairment
Nose & Throat: rhinitis, pharyngitis, nasal polyps, sinusitis, malignancies
Larynx: dysphonia, acute and chronic laryngitis, malignancies

0 No problems
1 Corrected vision with glasses; mild hearing loss; chronic sinusitis
2 Difficulty in reading newspaper or drive although glasses; required hearing aid; chronic sinonasal complaints requiring medication; vertigo/dizziness requiring daily meds
3 Severe low vision, partially blind (required an escort to venture out, unable to read newspaper); severe ear impairment (conversational heading still impaired with hearing aid); laryngeal dysphonia (not neurological dysarthria)
4 Functional blindness/deafness: unable to read, recognize a familiar face, unable to conversational hearing, even if "organically" he is not completely blind or deaf; laryngectomy (every cause, especially malignancies); required surgical intervention for vertigo; aphonia secondary to laryngeal impairment.

UPPER GASTROINTESTINAL SYSTEM

This category is comprehensive of the intestinal tract from esophagus to duodenum, and pancreatic trees: dysphagia, gastroesophageal reflux disease (GERD), hiatal hernia, esophageal diverticula, any type of gastritis (consider also *H. Pylori* eradication or not), gastric/duodenal ulcer, acute or chronic pancreatitis, malignancies (comprehensive of gastric lymphoma).

Ensure that type 1 diabetes is rated under "metabolic."

0 No problem
1 Hiatal hernia, GERD or gastritis requiring meds; previous ulcer (> 5 years ago); previous *H. Pylori* eradication therapy (> 5 years ago)
2 Daily proton pump inhibitor/anti-acid meds; documented gastric or duodenal ulcer or *H. Pylori* eradication therapy within 5 years
3 Active gastric or duodenal ulcer; positive fecal occult blood test; any swallowing disorder or dysphagia; chronic pancreatitis requiring supplemental pancreatic enzymes for digestion; previous episode of acute pancreatitis
4 Any type of malignancies (see "Rating Malignancies"); previous gastric surgery because of cancer; history of perforated ulcer (gastric surgery not because of cancer, ulcorrhaphy); melena/heavy bleeding from upper GI source; acute pancreatitis

LOWER GASTROINTESTINAL SYSTEM

Comprehensive of the rest of the GI system, from small bowel to anus: Whipple's disease, diverticulosis, irritable bowel, malignancies. Constipation is rated, too, by type and frequency of laxatives required, or by history of impaction.

0 No problems, previous appendectomy, previous hernia repair (without complications)

1 Constipation managed with meds; active hemorrhoids; intestinal hernia requiring surgery; previous hernia repair with complications (intestinal adherences, laparocele, etc.); irritable bowel syndrome (few symptoms)
2 Constipation requiring daily bulk laxatives (psyllium, policarbophil, sterculia, guar gum, etc.), or stool softeners; diverticulosis (previous diverticulitis); inflammatory bowel disease in remission with meds (> 5 years ago)
3 Bowel impaction/diverticulitis within the last year; daily use of stimulant (irritant) or osmotic laxatives (bisacodyl, senna, glycerol, sodium docusate; lactulose, polyethylene glycol) or enemas; chronic bowel inflammation in remission with meds (< 5 years ago)
4 Diverticulitis flare up; active inflammatory disease; current impaction; hematochezia/active bleeding from lower GI source; bowel carcinoma

LIVER AND BILIARY TREES
Comprehensive of liver, gallbladder, biliary trees, portal system: acute and chronic hepatitis (viral, alcoholic, toxic, autoimmune, idiopathic), cirrhosis, portal hypertension, hemochromatosis, primary biliary cirrhosis, cholelithiasis, cholangitis, primary malignancies. As the hepato-biliary system is difficult to assess through the physical examination, therefore, laboratory results must be used.

0 No problem
1 History of hepatitis (actually normal values of transaminases); cholecystectomy
2 Cholelithiasis; chronic hepatitis or previous hepatitis (< 5 years ago) or any other liver disease (hemochromatosis, primary biliary cirrhosis) with mildly elevated transaminases (within 3-times normal values); heavy alcohol use within 5 years (to rate in “psychiatric”, too)
3 Chronic hepatitis or any other liver disease with marked elevation of transaminases (> 3-times normal values); elevated bilirubin
4 Acute cholecystitis; any biliary obstruction; active hepatitis/liver cirrhosis; any liver or biliary tree carcinoma

RENAL
This category is exclusive of kidney: kidney stones, acute/chronic renal failure, glomerulonephritis; nephrosic/nephritic syndrome; active/chronic pyelonephritis, diabetic or hypertensive nephropathy (albuminuria/proteinuria), renal carcinoma. Bence-Jones proteinuria in multiple myeloma should not be considered.

0 No problem
1 Asymptomatic kidney stone; kidney stone passage within the last ten years; pyelonephritis within 5 years; kidney cysts without hematuria
2 Serum creatinine > 1.5 but < 3 mg/dL without diuretic or antihypertensive medication (particularly ACE-inhibitors or SRAA blockers); kidney calculi requiring daily meds
3 Serum creatinine > 3 mg/dL or < 1.5 mg/dL in conjunction with diuretics, antihypertensive, or bicarbonate therapy; active pyelonephritis; nephrosic syndrome; colic symptoms treated as an outpatient
4 Required dialysis; renal carcinoma; colic symptoms requiring hospitalization

GENITO-URINARY
Ureters, bladder, urethra. Genitals, prostate, testicles, penis, seminal vesicles.
Uterus, ovaries. *Mammary gland is rated under “metabolic”.*
This category is comprehensive of all GU tract impairments: ureteral or bladder stones, benign prostate hypertrophy (BPH), urinary tract infections (UTI’s), prolapses, etc. Urinary incontinence and indwelling catheter should also be considered.

0 No problem
1 Stress incontinence; BPH without urinary symptoms; hysterectomy or ovariectomy (uterine fibroma, benign neoplasm)
2 Pathological pap smear (or 2 consecutives abnormal); frequent UTIs (3 or more in the past year) in female or current UTIs; urinary incontinence (not stress) in females; BPH with urinary symptoms (frequency, urgency, hesitancy); status post TURP; any urinary diversion procedure; indwelling catheter; bladder calculi
3 Prostatic cancer in situ (e.g., incidentally found during TURP); vaginal bleeding; cervical carcinoma in situ; hematuria (any cause); urinary incontinence (not stress) in males; bladder polyps
4 Acute urinary retention; current urosepsis; any GU malignancies except as above

MUSCULOSKELETAL/INTEGUMENT

This is a very broad category, including: osteoarthritis, osteoporosis, any bone fracture; primary neoplasm (bone, muscle, connective tissue, skin), distinguishing melanoma from other localized skin cancers; rheumatoid arthritis and polymyalgia rheumatica; muscular injuries (rotator cuff, long head of the biceps); pressure sores; any dermatological disease.

The scores of this category are strictly correlated to the disability they cause; for the evaluation of the level of disability, refer to basic activities of daily living (BADL) and instrumental activities of daily living (IADL).

NOTICE: Score the severity of each illness according to the level of disability caused by the same illness in this category, without considering the disability caused by other diseases. For example: a patient affected both by osteoarthritis and hemiplegia from a previous stroke has a high level of disability, but you have to score 2 for disability by osteoarthritis (in this category) and 4 for disability by stroke (in the neurological category); for a patient with both a deforming rheumatoid arthritis and a previous stroke without remaining outcomes you have to score 4 for disability from arthritis (in this category) and 2 for disability from stroke (in the neurological category).

0 No problem
1 Requires meds for osteoarthritis (NSAID) or has mildly limited IADL from joint pathology; excised skin cancers (except melanoma); skin infections requiring antibiotics within a year
2 Daily anti-osteoarthritis meds (NSAID) or use of assistive devices or little limitation in ADL (previous arthroprosthesis or treated fracture with a low level of remaining disability); osteoporosis without vertebral fractures; daily meds for chronic skin diseases (even local, as psoriasis or pressure sores); non-metastatic melanoma; daily meds for rheumatoid arthritis (except steroids) with a low level of disability
3 Osteoarthritis with a moderate level of disability in ADL; requires chronic treatment with steroids for arthritic conditions or joints' deformities or severely impaired; osteoporosis with vertebral compression fractures
4 Wheelchair bound for osteomuscular disease; severe joint deformities or severely impaired usage; osteomyelitis; any bone or muscle or connective tissue neoplasm (see "Rating Malignancies"); metastatic melanoma.

Fractures and/or arthroprosthesis (both recent and old) have to be scored according to the level of disability they cause (considering outcomes too), in order to avoid confusion about possible classifications of different fractures or joints. The same is true for muscular diseases.

CENTRAL AND PERIPHERAL NERVOUS SYSTEM

This category includes the "somatic" pathologies of the central and peripheral nervous system: any kind of stroke, neurodegenerative diseases (Parkinson's disease and parkinsonism, multiple sclerosis, amyotrophic lateral sclerosis, etc.), myelopathies, traumas with neurological outcomes, primary or secondary epilepsy, neuropathies (diabetic, alcoholic, any other etiology), primary tumors, chronic headaches (migraine), insomnia, etc. It must carefully estimate the severity and prognosis of the illness but also the functional impairment that the illness causes.

0 No problem (or fewer convulsions in childhood)

1 Frequent headache requiring meds without impairment in Advanced ADL; previous TIA (one event); previous epilepsy, actually not treated, without crisis since more than 10 years ago.

2 Chronic headache requiring daily meds (even for prophylaxis) or with regularly functional impairment in Advanced ADL (bed rest, job withdrawal, etc.); actual TIA or more than one previous TIA; previous stroke without significant residual; mild severity neurodegenerative diseases (see above), treated and well controlled; epilepsy controlled with drugs.

3 Previous stroke with mild residual dysfunction (hemiparesis, dysarthria); any neurosurgical procedure; moderate severity neurodegenerative diseases (see above), not well controlled by meds; epilepsy in treatment but with periodic crisis.

4 Acute stroke or previous stroke with severe residual dysfunction (hemiplegia, aphasia, severe vascular dementia) or more than one previous stroke (multi-infarct encephalopathy); severe neurodegenerative diseases (see above) causing disability in ADL; neurological coma.

Alzheimer's disease and dementia should not be rated into this category (Psychiatric and behavioral diseases): Alzheimer's disease should be listed only under psychiatric disorders; if dementia stems from vascular and/or mixed dementia and/or other neurological condition (e.g., Parkinson's Disease), both "neurologic" and "psychiatric" categories should be endorsed at the appropriate level for severity, considering in this category the stroke and the multi-infarct encephalopathy responsible for the cognitive impairment (score 3 for stroke with remaining outcomes, score 4 for multi-infarct encephalopathy).

ENDOCRINE-METABOLIC SYSTEM AND BREAST (systemic infections and poisonings)

Type 1 and Type 2 diabetes (organ damage should be considered into the respective categories, like for hypertension), obesity and dyslipidemia (hypercholesterolemia) represent the core of this category; it includes also hypo- and hyper-thyroidism, hypo- and hyper-parathyroidism, adrenal pathologies (Cushing' or Addison' disease), hypogonadism, hypopituitarism, etc. Malignancies of these glands, both benignant (like thyroid nodules) and malignant (like thyroid or adrenal cancer, vipoma, etc.) are included too.

Even if it is an exocrine gland, breast was included in this category because the authors did not find a more appropriate one; so, it also includes breast cancer.

Moreover, it includes electrolyte disorders, sepsis, systemic infections (like tuberculosis, syphilis, AIDS) scored according to their severity and the functional impairment they cause (see general indications) and poisonings (chronic by metals or acute by pesticides or carbon monoxide).

0 No problem

1 Diabetes and/or dyslipidemia compensated with diet; mild obesity (BMI 30-35 kg/m2); hypothyroidism in replacement therapy (L-thyroxin); hyperthyroidism caused by Plummer' adenoma surgically treated.

2 Diabetes compensated with oral hypoglycemic drugs or insulin (hemoglobin $A_{1c} < 7\%$); dyslipidemia well controlled by daily meds (c-LDL lower than the recommended target according to the individual global cardiovascular risk); moderate obesity (BMI 35–45 kg/m^2);

hyperthyroidism in pharmacologic treatment; asymptomatic or surgically treated hyperparathyroidism; fibrocystic breast disease.

3 Diabetes not well compensated by therapy (hemoglobin A_{1c} 7–8.5%, presence of complications); dyslipidemia not well controlled (c-LDL higher than the recommended target according to the individual global cardiovascular risk; for instance, c-LDL > 100 mg/dL in patients with previous myocardial infarction or stroke); severe obesity (BMI > 45 kg/m^2); symptomatic hyperparathyroidism (e.g., hypercalcaemia); replacement therapy for adrenal failure; any electrolytes disorder requiring hospitalization.

4 Uncontrolled diabetes (hemoglobin A_{1c} > 8.5%) or one diabetic ketoacidosis or nonketotic hyperosmolar coma during the past year; genetic uncontrolled dyslipidemia; acute adrenal failure during hormonal replacement therapy; any neoplasm of thyroid, breast, adrenal gland (see “Rating Malignancies”).

NOTICE: when the patient is not treated with drug therapy for diabetes or dyslipidemia but he should be for the optimal control of the pathology (for instance, hemoglobin A_{1c} > 7%, total cholesterol > 250 mg/dL), score the pathology according to the laboratory values, which really define its severity.

PSYCHIATRIC AND BEHAVIORAL DISEASES

This category includes both dementia and related behavioral disorders (psychosis, anxiety, depression, agitation) and all the pre-existing and/or not related to dementia psychiatric disorders. Since this is the only item analyzing patient’s mental status (all the others refer to physical status), it is very important to evaluate it carefully considering further information derived from the Comprehensive Geriatric Assessment (MMSE; Geriatric Depression Scale, Neuro-Psychiatric Inventory if available).

0 No psychiatric problem or history thereof

1 Minor psychiatric condition or history thereof: previous (occasional) psychiatric treatment without hospitalization; major depressive event and/or use of antidepressants more than 10 years ago without hospitalization; occasional use of minor tranquilizers (e.g., BDZ; even if as hypnotherapy for insomnia); mild cognitive impairment (MMSE 25-28).

2 A history of major depression (according to DSM-IV criteria) within the last 10 years (treated or untreated); mild dementia (MMSE 20-25); previous admission to Psychiatric Department for any reason; history of substance abuse (more than ten years ago, including alcoholism).

3 Current major depression (according to DSM-IV criteria) or more than two previous major depression episodes in the past 10 years; moderate dementia (MMSE 15–20); current and usual usage of daily anti-anxiety meds (even as hypnotherapy for insomnia); current or within the past ten years substance abuse or dependence (according to DSM-IV criteria); requires daily antipsychotic medication; previous attempt at suicide.

4 Current mental illness requiring psychiatric hospitalization, institutionalization, or intensive outpatient management (psychiatric emergency, as attempt at suicide or severe depression with suicide purpose, acute psychosis or acute decompensation of chronic psychosis, severe substance abuse; severe agitation from dementia); severe dementia (MMSE < 15); delirium (acute confusion or altered mental status for medical (organic) reasons: in this case you have to codify also the medical cause in its own category with the appropriate level of severity).

A psychiatric consult could be requested for this category; dementia and depression, the most frequent diseases in the elderly, can be scored in details using the MMSE and GDS. The severity of any mental disorder (dementia, depression, anxiety, psychosis, substance abuse and all the others) has to be scored according to the level of functional impairment or disability they cause.

CHECKLIST

Medical history

- Timing of events and/or interventions (how long ago underwent surgery for…; how long ago had myocardial infarction or stroke, etc.) and evaluation of functional impairment
- Drugs list (fundamental), including laxatives and tranquilizers (even hypnoinducent)
- Symptoms of atherosclerotic disease (TIA, angina, claudication, amaurosis)
- Etiological diagnosis (reasonably reliable) of anemia
- Degree of vascular stenosis or aneurism dimension (by Doppler and/or ultrasound and/or TC data, when available)
- Information about smoking status (how many cigarettes per day for how many years, when stopped)
- Use of glasses? With this aid, the patient is able to read a newspaper? Requires an escort to venture out?
- Any hearing aid? (you should evaluate possibility to communicate with patient)
- "Peptic history" of the patient (including previous eradication therapy for H. Pylori)
- Urinary symptoms, incontinence, presence of bladder catheter (even from BADL)

Physical examination

- Height (m^2) and weight (kg) (measured, not reported, if possible) to calculate BMI
- Blood pressure, heart rate, cardiac murmurs, peripheral arterial pulses
- Joint pain or passive stiffness limitation (non–X-ray-based diagnosis of osteoarthritis)
- Residual neurological deficits (dysarthria/aphasia, hemiparesis/hemiplegia)

Baseline laboratory samples

- Blood count: hemoglobin, WBC and platelet count
- Creatinine, electrolytes
- AST, ALT, fractioned bilirubin
- Thyroid function and serum B12 (when indicated)
- Hemoglobin A_{1c} (for diabetic patients)

From: Miller MD, Paradi

Miller MD1, Paradis CF, et al Rating chronic medical illness burden in geropsychiatric practice and research: application of the Cumulative Illness Rating Scale. Psychiatry Res. 1992 Mar;41(3):237-48.

Appendix 6. Tumor Lysis prophylaxis

Risk categories for development of tumor lysis syndrome (TLS)

Based on review of all currently available data, three risk categories for developing TLS were defined including the size of the enlarged lymph nodes and the absolute lymphocyte count (ALC). In addition, the creatinine-clearance (CrCl) of the patient needs to be taken into account by the investigator (see below).

The following risk categories were defined:

	Low Risk	Medium Risk	High Risk	
Lymph Nodes	All measurable lymph nodes with the largest diameter <5 cm by radiographic assessment	Presence of any single measurable lymph node with the largest diameter ≥5 cm and <10 cm by radiologic assessment	A single measurable lymph node with the largest diameter ≥5 cm by radiologic assessment.	Any single measurable lymph node with the largest diameter ≥10 cm by radiologic assessment
	AND	**OR**	**AND**	
Absolute lymphocyte Count (ALC)	$< 25 \times 10^9$/L	$\geq 25 \times 10^9$/L	$\geq 25 \times 10^9$/L	

All patients enrolled in the trial will be assessed at screening and categorized based on their tumor burden, as described above. Patients can be restaged into a lower TLS risk group at any time according to their ALC.

Please note: Patients with **CrCl < 80 ml/min** should be monitored very closely for signs of TLS, especially if they are in the intermediate or high risk TLS risk category (lymph node ≥ 5 and/or ALC ≥ 25×10^9/L).
Patients with **CrCl 30-50 ml/min** should be handled as high risk patients.

Initial dosing and monitoring

Low and medium risk patients

- Administration of an oral uric acid reducer (such as allopurinol 300 mg/day) beginning at least 24 hours prior to first dose and continued until at least 28 days after last dose escalation.
- Laboratory assessment of clinical chemistries (see under 'laboratory monitoring') and assessment of vital signs are required pre-dose, 6-8- and 24-hours post-dose at first dose of 20 mg and 50 mg. Pre-dose and 24 hours post-dose are required at subsequent ramp-up doses. The pre-dose laboratory samples are to be reviewed prior to dosing and should not demonstrate any clinically significant abnormalities prior to the first dose of venetoclax, or the patient should receive additional prophylactic treatment and hydration prior to the initiation of dosing.
- Post-dose Monitor clinical chemistries (see under 'laboratory assessments') for evidence of TLS at 6 – 8 hours after the first dose of 20 mg and 50 mg. The 6-8-hour post-dose laboratory values must be reviewed prior to a patient leaving the clinic and electrolyte abnormalities should be corrected promptly
- Serum chemistries must be re-assessed before administering the second dose of each ramp-up (i.e., at 24 hours).
- Any patient who, at any dose, develops clinically significant electrolyte abnormalities must have his or her subsequent venetoclax dose withheld until the electrolyte abnormalities resolve.
- Patients who develop electrolyte abnormalities should undergo aggressive management and further monitoring as outlined below (see under 'further actions')'.
- Oral hydration consisting of fluid intake of >2 L/day should be maintained during the whole ramp-up period of venetoclax.

High risk patients

- Administration of an oral uric acid reducer (such as allopurinol 300 mg/day) beginning at least 24 hours prior to first dose and continued until at least 28 days after last dose escalation.
- Laboratory assessment of clinical chemistries (see under 'laboratory monitoring') and assessment of vital signs are required pre-dose, 6-8 and 24 hours post-dose on the first day of each dose level, The pre-dose laboratory samples are to be reviewed prior to dosing and should not demonstrate any clinically significant abnormalities prior to the first dose of venetoclax, or the patient should receive additional prophylactic treatment and hydration prior to the initiation of dosing.
- Post-dose: Monitor clinical chemistries (see under 'laboratory assessments') for evidence of TLS at 6 – 8 hours at first dose of each dose level. The 6-8-hour post-dose laboratory values must be reviewed prior to a patient leaving the clinic and electrolyte abnormalities should be corrected promptly
- Serum chemistries must be re-assessed before administering the second dose of each ramp-up (i.e., at 24 hours).
- Any patient who, at any dose, develops clinically significant electrolyte abnormalities must have his or her subsequent venetoclax dose withheld until the electrolyte abnormalities resolve.
- Patients who develop electrolyte abnormalities should undergo aggressive management and further monitoring as outlined below (see under 'further actions')'.
- Oral hydration consisting of fluid intake of >2 L/day should be maintained during the whole ramp-up period of venetoclax.

The following table summarizes the safety precautions for mitigation of TLS depending on different risk categories:

Recommended TLS Prophylaxis Based on Tumor Burden from Clinical Trial.
Data (consider all patient co-morbidities before final determination of prophylaxis and monitoring schedule).

Tumor Burden		**Prophylaxis**		**Blood Chemistry Monitoring[c,d]**
		Hydration[a]	**Anti-hyperuricemics**	**Setting and Frequency of Assessments**
Low	All LN <5 cm AND ALC <25 x10^9/L	Oral (1.5-2 L)	Allopurinol[b]	Outpatient 1. Pre-dose, 6 to 8 hours, 24 hours at first dose of 20 mg and 50 mg 2. Pre-dose and 24 hours at at subsequent ramp-up doses
Medium	Any LN 5 cm to <10 cm OR ALC ≥25 x10^9/L	Oral (1.5-2 L) and consider additional intravenous	Allopurinol	Outpatient 3. Pre-dose, 6 to 8 hours, 24 hours at first dose of 20 mg and 50 mg 4. Pre-dose and 24 hours at subsequent ramp-up doses 5. Consider hospitalization for subjects with CrCl <80mL/min at first dose of 20 mg and 50 mg; see below for monitoring in hospital
High	Any LN ≥10 cm OR ALC ≥25 x10^9/L AND any LN ≥5 cm	Oral (1.5-2 L) and intravenous (150-200 mL/hr as tolerated)	Allopurinol; rasburicase is recommended if baseline uric acid is elevated **(ALLOPURINOL SHOULD NOT BE GIVEN AT SAME DAY AS RASBURICASE)**	In hospital (recommended) at first dose of 20 mg and 50 mg 6. Pre-dose, 6 to 8, and 24 hours Outpatient at subsequent ramp-up doses 7. Pre-dose, 6 to 8 hours, 24 hours

ALC = absolute lymphocyte count; LN = lymph node.

a Administer intravenous hydration for any patient who cannot tolerate oral hydration.

b Start allopurinol or xanthine oxidase inhibitor at least 1 day prior to initiation of venetoclax.

c Evaluate blood chemistries (potassium, uric acid, phosphorus, calcium, and creatinine); review in real time.

d For subjects at low/medium risk of TLS, monitor blood chemistries pre-dose and 24 hours at each subsequent ramp-up dose. For subjects at high risk of TLS, monitor blood chemistries pre-dose, at 6-8 hours and at 24 hours at each dose level.

Hydration: Ensure adequate hydration prior to initiating therapy with venetoclax and throughout the ramp-up phase, especially the first day of each ramp-up dose. Administer intravenous (IV) fluids as indicated based on overall risk of TLS or for those who cannot maintain adequate oral hydration.

Anti-hyperuricemic agents: Administer uric acid reducing agents (e.g., allopurinol). Start at least 1 day prior to initiation of venetoclax; consider continuing through the ramp-up phase.

Laboratory Assessments:

Pre-dose (within 24 hours prior to ramp-up dose):

- Sodium, potassium, calcium, phosphate, chloride
- uric acid, urea, total bilirubin, serum creatinine
- LDH
- complete blood count (CBC), including hemoglobin, platelets and white blood cell count with differentials
- liver enzymes (AST, ALT, alkaline phosphatase)
-

Assess blood chemistries mentioned above prior to initiating venetoclax to evaluate kidney function and correct pre-existing hyperuricemia, hyperkalemia, hyperphosphatemia, or hypocalcemia to normal levels. Reassess blood chemistries before starting each subsequent ramp-up dose of venetoclax.

Post-dose: For subjects at low or medium risk of TLS, monitor blood chemistries at 6-8 hours and at 24 hours after initiating venetoclax at the 20 mg and 50 mg doses. For subjects at high risk of TLS, monitor blood chemistries in the hospital at 4, 8, 12 and 24 hours after initiating venetoclax at the 20 mg and 50 mg doses; subsequent ramp-up doses can be administered in the outpatient setting with monitoring of blood chemistries at 6-8 hours and at 24 hours after initiating venetoclax. Electrolyte abnormalities should be corrected promptly. The next dose of venetoclax should not be administered until the 24-hour blood chemistry results have been evaluated.

Hospitalization:
Based on physician assessment, some patients, especially those at greater risk of TLS, may require hospitalization on the day of the first dose of venetoclax for more intensive prophylaxis and monitoring during the first 24 hours. Hospitalization should be considered for subsequent dose increases based on reassessment of risk.

Scoring of tumor lysis syndrome and further actions
The below listed criteria defined by Cairo and Bishop should be used to diagnose a laboratory or clinical TLS.

Laboratory Tumor Lysis Syndrome (LTLS):

Laboratory Parameter	Laboratory Result
Uric Acid	≥ 476 µmol/L (≥ 8.0 mg/dL) or 25% increase from baseline
Potassium	≥ 6.0 mmol/L (≥ 6.0 mEq/L) or 25% increase from baseline
Phosphorous	≥1.45 mmol/L (≥ 4.5 mg/dL) or 25 % increase from baseline
Calcium	≤ 1.75 mmol/L (≤ 7.0 mg/dL) or 25% decrease from baseline

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, phosphate, 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 hypouricaemic agent(s) (Cairo, 2004).

Clinical Tumor Lysis Syndrome (CTLS):

Clinical tumor lysis syndrome (CTLS) assumes the laboratory evidence of metabolic changes and significant clinical toxicity that requires clinical intervention.

The presence of LTLS and one or more of the following criteria:
Creatinine: ≥ 1.5 ULN *
Cardiac arrhythmia / sudden death*
Seizure*

(*Not directly or probably attributable to a therapeutic agent (e.g. rise in creatinine after amphotericin administration)). (Cairo, 2004)

Cairo-Bishop Tumor Lysis Syndrome Grading

[#]	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
LTLS	-[*]	+	+	+	+	+
Creatinine[#]	≤ 1.5 x ULN	1.5 x ULN	> 1.5-3.0 x ULN	> 3.0-6.0 x ULN	> 6.0 x ULN	Death[$]
Cardiac arrythmia[#]	None	Intervention not indicated	Nonurgent medical intervention indicated	Symptomatic and incompletely controlled medically or controlled with device (e.g., defibrillator)	Life-threatening (e.g., arrhythmia associated with CHF, hypotension, syncope, shock	Death[$]
Seizure[#]	None	-	One brief, generalized seizure; seizures(s) well controlled by anticonsulvants; or infrequent focal motor seizures not interfering with ADL	Seizures in which consciousness is altered; poorly controlled seizure disorder, with breakthrough generalized seizure despite medical intervention	Seizures of any kind which are prolonged, repetitive, or difficult to control (e.g., status epilepticus, intractable epilepsy)	Death[$]

CTLS requires one or more clinical manifestations along with criteria for LTLS
Maximal CTLS manifestation (renal, cardiac, neuro) defines the grade
* No LTLS
[#] Not directly or probably related to a therapeutic agent
[$] Attributive probably or definitely to CTLS

<u>Further actions</u> need to be taken in case of:

- potassium increase by ≥0.5mmol/l from baseline and/or any potassium value >5.0 mmol/l
- phosphorus increase of >0.5 mg/dl 0.16 mmol/l <u>and</u> >4.5 mg/dl 1,45 mmol/l
- any other significant laboratory change, especially electrolyte imbalances

These actions should be according to the institutional practice and include but are not limited to:

- discontinuation of administration of venetoclax, no further dosage should be taken until resolution
- hospitalization for more aggressive monitoring (especially regular laboratory assessments, telemetry/ECG monitoring, observation for signs/symptoms of TLS (e.g. fever, chills, tachycardia, nausea/vomiting, diarrhea, sweating, hypotension, muscle aches, weakness, paresthesias, mental status changes, confusion, and seizures)
- administration of intravenous fluids at a rate of ≥1 ml/kg/h (≥50 ml/hr., target 150 to 200 ml/hr.; as clinically appropriate)
- in case the diagnosis of TLS is established, further measures, such as urine alkalization with intravenous sodium bicarbonate or administration of rasburicase as per institutional practice should be considered
- consultation of nephrology (or acute dialysis service) to ensure emergency dialysis is available

Please note: A rapidly rising serum potassium level is a medical emergency.

Reassessment of risk category

Patients classified as high risk for developing TLS who presented at screening with BOTH an ALC≥ 25 × 10^9/L AND a measurable lymph node with the largest diameter ≥ 5 cm by radiologic assessment may have their TLS risk category reassessed.

Prior to dose increases above 50 mg of venetoclax, patients may have a reassessment of their disease status based on their most recent ALC. Based on those results, one of the following two options may be implemented:

– If the patient's ALC decreases to < 25 × 10^9/L, patients may be re-categorized as medium risk and follow the management guidelines for the medium-risk category for the subsequent increases in dose during the dose ramp-up period.
– If the patient's ALC remains ≥ 25 × 10^9/L, they will remain in the high-risk category and continue to follow management guidelines for high-risk patients for subsequent dose increases of venetoclax during the dose ramp-up period.

Reassessment of the patient's risk category can occur prior to each subsequent dose increase.

Appendix 7. Charlson Comorbidity Index

Clinical Condition	Weight
Myocardial infarction	1
Congestive heart failure	1
Peripheral vascular disease	1
Dementia	1
Cerebrovascular disease	1
Chronic pulmonary disease	1
Connective tissue disease	1
Ulcer disease	1
Mild liver disease	1
Slight diabetes without complications	1
Hemiplegia	2
Moderate or severe renal disease	2
Diabetes with end organ damage	2
Tumors	2
Leukemia	2
Lymphoma	2
Moderate or severe liver disease	3
Metastatis solid tumor	6
Acquired immunodeficiency syndrome	6

M. Charlson, T.P. Szatrowski, J. Peterson, et al. Validation of a combined comorbidity index
J Clin Epidemiol, 47 (1994), pp. 1245–1251

Appendix 8. Response and Outcome assessment

According to IWCLL guidelines 2018, Hallek et al., Blood, 2018

Table 4. Response definition after treatment of CLL patients

Group	Parameter	CR	PR	PD	SD
A	Lymph nodes	None ≥1.5 cm	Decrease ≥50% (from baseline)*	Increase ≥50% from baseline or from response	Change of −49% to +49%
	Liver and/or spleen size†	Spleen size <13 cm; liver size normal	Decrease ≥50% (from baseline)	Increase ≥50% from baseline or from response	Change of −49% to +49%
	Constitutional symptoms	None	Any	Any	Any
	Circulating lymphocyte count	Normal	Decrease ≥50% from baseline	Increase ≥50% over baseline	Change of −49% to +49%
B	Platelet count	≥100 × 10^9/L	≥100 × 10^9/L or increase ≥50% over baseline	Decrease of ≥50% from baseline secondary to CLL	Change of −49 to +49%
	Hemoglobin	≥11.0 g/dL (untransfused and without erythropoietin)	≥11 g/dL or increase ≥50% over baseline	Decrease of ≥2 g/dL from baseline secondary to CLL	Increase <11.0 g/dL or <50% over baseline, or decrease <2 g/dL
	Marrow	Normocellular, no CLL cells, no B-lymphoid nodules	Presence of CLL cells, or of B-lymphoid nodules, or not done	Increase of CLL cells by ≥50% on successive biopsies	No change in marrow infiltrate

For a detailed description of the response parameters, see section 5.

*Sum of the products of 6 or fewer lymph nodes (as evaluated by CT scans and physical examination in clinical trials or by physical examination in general practice).

†Spleen size is considered normal if <13 cm. There is not firmly established international consensus of the size of a normal liver; therefore, liver size should be evaluated by imaging and manual palpation in clinical trials and be recorded according to the definition used in a study protocol.

CR, complete remission (all of the criteria have to be met); PD, progressive disease (at least 1 of the criteria of group A or group B has to be met); PR, partial remission (for a PR, at least 2 of the parameters of group A *and* 1 parameter of group B need to improve if previously abnormal; if only 1 parameter of both groups A and B is abnormal before therapy, only 1 needs to improve); SD, stable disease (all of the criteria have to be met; constitutional symptoms alone do not define PD).

Complete remission

CR requires all of the following criteria (see Table 4 above).

5.1.1. Peripheral blood lymphocytes (evaluated by blood and differential count) ,≤4.3 10^9/L.
5.1.2. Absence of significant lymphadenopathy by physical examination. In clinical trials, a CT scan of the neck, abdomen, pelvis, and thorax is desirable if previously abnormal. Lymph nodes should be ≤1.5 cm in longest diameter. Once this is determined, further imaging should not be required until disease progression is apparent by clinical examination or on blood testing.
5.1.3. No splenomegaly or hepatomegaly by physical examination. In clinical trials, a CT scan of the abdomen should be performed at response assessment and should show no evidence for lymphadenopathy and splenomegaly. We propose to use a recent consensus response cutoff for splenomegaly of 13 cm in craniocaudal length. However, the persistence of splenomegaly may not correlate with outcome. The quantitative determination of hepatomegaly seems more difficult; changes such as focal or disseminated hepatic nodules support liver involvement.
5.1.4. Absence of disease-related constitutional symptoms.
5.1.5. Blood counts need to show the following values:
5.1.5.1. Neutrophils ≥1.5 10^9/L.

5.1.5.2. Platelets ≥100 10^9/L.
5.1.5.3. Hemoglobin ≥11.0 g/dL (without red blood cell transfusions).
5.1.6. MRD assessment.
In clinical trials aimed at maximizing the depth of remission, the presence of MRD after therapy should be assessed (see section 5.9). The sensitivity of the method used to evaluate for MRD should be reported, as should the tissue studied (blood or marrow). The proportion of patients achieving undetectable MRD should be reported with the total number of patients treated with the specific therapy as the denominator (not as a proportion of responders or those in CR).
5.1.7. For patients in clinical trials, a bone marrow aspirate and biopsy should be performed if clinical and laboratory results listed in sections 5.1.1 to 5.1.5 demonstrate that a CR may have been achieved. To define a CR, the cytological or pathological evaluation of the bone marrow smear or biopsy must be at least normocellular for age, without evidence for typical CLL lymphocytes by morphological criteria. This evaluation is not based on a flow cytometry–based MRD assessment.
In a clinical trial, the time point of marrow biopsy should be defined by the protocol. For example, in patients receiving chemo(immuno)therapy, the time point of marrow biopsy is typically 2 months posttherapy. When performing marrow biopsies in clinical trials, lymphoid nodules can be found that may reflect residual disease. These nodules may be recorded as “nodular partial remission.” Immunohistochemistry may be performed to define whether the nodules comprise primarily T cells, B cells other than CLL cells, or CLL cells. If nodules are not composed of CLL cells, a CR can be documented provided all other criteria are met. If the marrow is hypocellular, a repeat determination should be performed 4 weeks or later, when peripheral blood counts have recovered; however, this interval should not exceed 6 months after the last treatment. In cases in which a marrow biopsy was obtained at baseline, a comparison of pre- vs posttherapy biopsies should be performed. In general practice, the use of a marrow biopsy for evaluating a CR is at the discretion of the physician. In clinical trials aimed at maximizing the response rate, the quality of the response should be assessed in the marrow for MRD by highly sensitive molecular-based assays or immunophenotyping (see section 5.9).
5.1.8. Some patients fulfill all the criteria for a CR (including the marrow examinations described in section 5.1.7), but have a persistent anemia, thrombocytopenia, or neutropenia apparently unrelated to CLL, but related to drug toxicity. These patients should be considered as a different category of remission, CR with incomplete marrow recovery (CRi). For the definition of this category, the marrow evaluation (see section 5.1.7) should be performed with scrutiny and not show any clonal disease infiltrate. In clinical trials, patients having CR with incomplete marrow recovery should be monitored prospectively to determine whether their outcome differs from that of patients with detectable residual disease or with noncytopenic CR.

Partial remission
To define a partial remission, at least 2 parameters of group A and 1 parameter of group B need to improve, if previously abnormal (Table 4; sections 5.2.1 to 5.2.5). If only 1 parameter of both groups A and B was abnormal before therapy, only 1 needs to improve. Constitutional symptoms persisting for ≥1 month should be recorded.
5.2.1. A decrease in the number of blood lymphocytes to 50% or less from the value before therapy.
5.2.2. Reduction in lymphadenopathy compared with baseline (by cross-sectional imaging scans in clinical trials or by palpation in general practice) as defined by:
5.2.2.1. A decrease in lymph node size by 50% or more in n the sum of the products of the same enlarged lymph nodes selected at baseline as assessed by imaging (an established number in clinical trials of lymph nodes has been up to 6). n and the sum of longest diameters of the same enlarged lymph nodes selected at baseline as assessed by physical examination (an established number in clinical trials of lymph nodes has been a maximum of 6).

5.2.2.2. No increase in any lymph node and no new enlarged lymph node (diameter ≥1.5 cm). For small lymph nodes (longest diameter ≤1.5 cm), an increase ≤25% is not considered significant.
5.2.3. A regression ≥50% of the extent of enlargement of the spleen below the costal margin defined by palpation, or normalization in size. When assessed by CT, scan spleen size must have regressed by ≥50% in length beyond normal.96 A persistence of splenomegaly posttherapy may have limited influence on outcome in CLL.
5.2.4. A regression of ≥50% of the extent of enlargement of the liver below the costalmargin defined by palpation, or normalization in size. Given the impact of numerous medical conditions, liver size by physical examination or CT scan is not a reliable measure of hepatic involvement by CLL and should only be counted if hepatomegaly is clearly attributable to lymphoid involvement.
5.2.5. The blood count should show 1 of the following results:
5.2.5.1. Platelet counts ≥100 10^9/L or 50% improvement over baseline.
5.2.5.2. Hb ≥11.0 g/dL or 50% improvement over baseline without red blood cell transfusions or erythropoietin support.

Infectious event
Grading of all adverse events are according to Common Terminology Criteria for Adverse Events (CTCAE), Version 5. For the primary outcome of the optional phase 3 part of the study, any infectious events leading to an SAE will be considered at least grade 3, thus counting as an event for the primary outcome.

Appendix 9. Questionnaires

ENGLISH

EORTC QLQ-C30 (version 3)

We are interested in some things about you and your health. Please answer all of the questions yourself by circling the number that best applies to you. There are no "right" or "wrong" answers. The information that you provide will remain strictly confidential.

Please fill in your initials: ⌊ ⌋⌊ ⌋⌊ ⌋⌊ ⌋

Your birthdate (Day, Month, Year): ⌊ ⌋⌊ ⌋⌊ ⌋⌊ ⌋⌊ ⌋⌊ ⌋⌊ ⌋⌊ ⌋

Today's date (Day, Month, Year): 31 ⌊ ⌋⌊ ⌋⌊ ⌋⌊ ⌋⌊ ⌋⌊ ⌋⌊ ⌋⌊ ⌋

	Not at All	A Little	Quite a Bit	Very Much
1. Do you have any trouble doing strenuous activities, like carrying a heavy shopping bag or a suitcase?	1	2	3	4
2. Do you have any trouble taking a long walk?	1	2	3	4
3. Do you have any trouble taking a short walk outside of the house?	1	2	3	4
4. Do you need to stay in bed or a chair during the day?	1	2	3	4
5. Do you need help with eating, dressing, washing yourself or using the toilet?	1	2	3	4

During the past week:	Not at All	A Little	Quite a Bit	Very Much
6. Were you limited in doing either your work or other daily activities?	1	2	3	4
7. Were you limited in pursuing your hobbies or other leisure time activities?	1	2	3	4
8. Were you short of breath?	1	2	3	4
9. Have you had pain?	1	2	3	4
10. Did you need to rest?	1	2	3	4
11. Have you had trouble sleeping?	1	2	3	4
12. Have you felt weak?	1	2	3	4
13. Have you lacked appetite?	1	2	3	4
14. Have you felt nauseated?	1	2	3	4
15. Have you vomited?	1	2	3	4
16. Have you been constipated?	1	2	3	4

Please go on to the next page

ENGLISH

During the past week:	Not at All	A Little	Quite a Bit	Very Much
17. Have you had diarrhea?	1	2	3	4
18. Were you tired?	1	2	3	4
19. Did pain interfere with your daily activities?	1	2	3	4
20. Have you had difficulty in concentrating on things, like reading a newspaper or watching television?	1	2	3	4
21. Did you feel tense?	1	2	3	4
22. Did you worry?	1	2	3	4
23. Did you feel irritable?	1	2	3	4
24. Did you feel depressed?	1	2	3	4
25. Have you had difficulty remembering things?	1	2	3	4
26. Has your physical condition or medical treatment interfered with your family life?	1	2	3	4
27. Has your physical condition or medical treatment interfered with your social activities?	1	2	3	4
28. Has your physical condition or medical treatment caused you financial difficulties?	1	2	3	4

For the following questions please circle the number between 1 and 7 that best applies to you

29. How would you rate your overall health during the past week?

1	2	3	4	5	6	7
Very poor						Excellent

30. How would you rate your overall quality of life during the past week?

1	2	3	4	5	6	7
Very poor						Excellent

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ENGLISH

EORTC QLQ – CLL16

Patients sometimes report that they have the following symptoms or problems. Please indicate the extent to which you have experienced these symptoms or problems during the past week. Please answer by circling the number that best applies to you.

During the past week:	Not at All	A Little	Quite a Bit	Very Much
31. Have you lost weight?	1	2	3	4
32. Have you had a dry mouth?	1	2	3	4
33. Did you bruise?	1	2	3	4
34. Did you have abdominal discomfort?	1	2	3	4
35. Has your temperature been going up and down?	1	2	3	4
36. Did you have night sweats?	1	2	3	4
37. Have you had skin problems (e.g. itchy, dry)?	1	2	3	4
38. Did you feel ill or unwell?	1	2	3	4
39. Did you feel lethargic?	1	2	3	4
40. Have you felt "slowed down"?	1	2	3	4
41. Were you limited in planning activities, for example meeting friends, in advance?	1	2	3	4
42. Were you worried about your health in the future?	1	2	3	4

During the past four weeks:	Not at All	A Little	Quite a Bit	Very Much
43. Have you had trouble with chest infections?	1	2	3	4
44. Have you had trouble with other infections?	1	2	3	4
45. Have you needed repeated courses of antibiotics?	1	2	3	4
46. Have you worried about picking up an infection?	1	2	3	4

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