Amendment to the protocol: ”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” (PreVent-ACaLL), version 2.0, dated 30 July 2019

Please find below clarifications for the Ethics Committee in Denmark concerning the protocol for the PreVent-ACaLL trial.

As the trial is an international investigator-initiated trial between the Nordic CLL study group and the Dutch HOVON group sponsored by Rigshospitalet and funded by the Novo Nordisk Foundation while investigational drugs and in part funding is provided by the multinational companies Abbvie and Acerta, the contracts are only available in English. No compensation is paid to the national coordinator or principal investigator; no compensation is paid to any of the trial participants. Publication is according to the Vancouver guidelines, the trial is registered at clinicaltrials.gov: NCT03868722.

Prescreening

Most patients diagnosed with CLL will not require treatment for CLL or acquire a serious infection within the first years of diagnosis. Thus, it is important to identify patients with high risk of infection and/or CLL treatment, who would be eligible for participation in the trial. To this end, the CLL-TIM algorithm has been developed as described in the main protocol. To avoid overload of information for patients not having high risk of infection and/or CLL treatment, a separate informed consent form for prescreening has been included for the trial. Patients may be troubled by reading and assessing extensive information when newly diagnosed with a potentially life-threatening disease. Thus, we have restricted the full patient information to patients eligible for the study, to save the approximately 80% of patients who will not be assessed as high risk from this. As patients may have an extra blood sample drawn for standard analyses during prescreening, informed consent for prescreening is necessary.

Biobank

A centralized research biobank is included for the trial, the research biobank is hosted at Rigshospitalet, Copenhagen University Hospital, some samples will be intermediately analyzed/prepared at Uppsala and Rotterdam University Hospital for logistic reasons prior to shipment to the centralized research biobank. The research biobank is centralized and managed as one research biobank. As detailed in the protocol, central laboratory assessment of several biological parameters is performed, based on biological samples sent to the reference laboratory in Copenhagen. As further detailed in the patient information, a research biobank is developed for translational studies. Thus, pseudonymized samples (identified only be the study-specific patient identification number) may be distributed for other laboratories in the EU and outside EU in the US, due to specialized functional analyses not being available within the EU at the moment.

Scientific examinations

The NGS-based assay covers approximately 500 megabases of DNA including 100-200 genes and regulatory elements with recurrent mutations described in CLL and the IGHV regions and regions with known cytogentic aberrations in CLL. The depth of sequencing will be up to approximately 100 000 X to allow for detection of subclonal aberrations. The analysis will be performed on MiSeq, HiSeq and or NovaSeq platforms. Bioinformatic pipeline for analyses will be custom-designed based on the GATK package. For some patientsamples, the targeted NGS assay described above will be supplemented with WGS up to 100 x coverage for tumor samples and 30 x coverage for germline

As germline samples will be used to subtract from tumor samples for detection of somatic tumor mutations, the risk of incidental detection of germline mutations is very low. However, a theoretical risk that a high penetrance germline mutation can be identified do exist. Thus, "Genomvejledningen" will be followed in the case of incidental identification of germline mutations. During discussion of informed consent with the patients, and in writing in the informed consent, the patients are informed about the risk of incidental detection of germline mutations with potential impact on health for the patient and/or relatives. The patient is given the option to indicate whether the patient wants to be informed about incidental detection of inheritable diseases. It is clarified that this information may also impact relatives and be given to relatives.

The objective of this scientific research is to improve the understanding of the disease CLL and to find better treatments for the disease as summarized in the protocol section 4.1.14, 4.1.15 and detailed in section 5.3.5.
Some of the samples which are taken during the trial, are not used for routine investigations, but will be sent to the central laboratory in Copenhagen (Denmark) for the analyses assessing immune function and the CLL disease as detailed in the protocol sections 4.1.14, 4.1.15 and 5.3.5. The identification of the samples will be made pseudonymous. This means that only the patient identification number will be used to identify the samples. The same will be done for the laboratory results and other data, which will be sent between the treating physicians and the central study office at Rigshospitalet.

Extensive genomic sequencing

To improve characterization of the CLL disease and to better understand how and why CLL and related diseases react differently from person to person, extensive genomic sequencing of biological samples will be included. These analyses may in rare instances reveal information about inheritable diseases. In such cases, a board representing independent members from department of Clinical Genetics, Rigshospitalet, will assess whether the individual patient should be informed. If decided to inform the patient about such findings, the patient will be offered genetic counseling.

Storage of biological samples

Some samples are analysed in the clinic's own laboratories at local sites. The samples are managed according to the laboratory's own procedure, which means that they are destroyed immediately after the analysis (few days). For every visit blood test will be taken (20 ml). This is similar to what patients would get if they were not in the study. For the treatment group additional 10 blood samples will be taken for tumor lysis syndrome (TLS) risk.

Other samples from biopsies, blood samples and microbiome samples are stored in a research biobank, which is set up for research purposes as detailed below, due to logistics, these samples may be stored for prolonged periods of time at the local laboratories prior to shipment to the central laboratory. During the research blood (100 ml) will be taken for the research biobank at a maximum of 10 different times over a period of 3 years. The samples and the results are stored in the research biobank adjoined to the study at Rigshospitalet, Copenhagen.
A bone marrow biopsy will be performed minimal two times (10 ml per biopsy) and is analyzed to measure the number of CLL cells and their activity. If considered necessary on the discretion of the treating physician or due to signs of progression of the disease, a lymph node biopsy may be done as part of the standard care.
All samples are managed without name or personal identification number. A code links the participant with the samples via a list of names. This means that the information is made pseudonymous. The list, which links to the name with the study-specific patient identification number, is stored only at the hospital, and only authorized trial personnel has access to the list.

At any time during the trial, the participant may request that their samples are destructed. The information, which has already been collected, will not be destroyed, but new analyses will not be performed.

The samples and personal information will be treated in agreement with the Act on Processing of Personal Data and the Danish Health Act.

The centralized research biobank included for the study is developed for the following specified purposes, as stated in the consent for collection of samples to the research biobank:

The samples in the research biobank will be used by researchers:

- to better understand, why some people has a higher probability to develop resistance towards treatment
- to better understand, why some people has a higher probability to develop immune dysfunction and infections
- to better understand how and why CLL and related diseases react differently from person to person
- to develop better ways for prevention or earlier treatment of diseases
- to develop better ways for prevention or reduction of side effects to CLL treatment
- to develop or improve tests, which help with diagnosing or understanding of CLL and related diseases and find the right medication to the right patient

The samples will be used for functional characterization of cells (immunophenotyping, assessment of intracellular pathway activity including phosphoflowcytometry, effects of different drugs on cellular function including BH3 profiling), assessment of cellular function in mixed cell populations of blood, bone marrow and lymph nodes (coagulation, immune function and microenvironmental interactions) and genetic characterization (sequencing including next generation sequencing to obtain information about clonal and subclonal changes in the cells (see section on extensive genomic sequencing above) and minimal residual disease (MRD) assessment) as detailed in the protocol sections 4.1.14, 4.1.15 and 5.3.5.

The biological material will be handled according to the legislation of Denmark and EU, for samples analyzed outside EU, i.e. for this trial US (up to 30 mL of blood, bone marrow aspirate and lymph node samples), an agreement on handling samples accordingly will be assured. The biological samples are planned to be kept in the research biobank until all analysis in the study are completed. All material will be destroyed no later than 2035. The research biobank will be covered in terms of data protection by the application by the sponsor to the Danish Data Protection Agency under the RegionH umbrella approval according to EU and Danish legislation in accordance with GDPR regulations.
Possible risks due to procedures that are not considered due to participation in the study but due to the assessment of the disease itself, have not been included for the main protocol text. However, these potential risks are addressed here for blood sampling: The blood sampling is part of the standard of care for patients with CLL. However, additional amounts of blood may be drawn as part of the protocol. During blood sampling, there is a risk of light pain at the site of puncture, a small risk of a minor bruise, very rarely infections or damage to nearby nerves may occur. Some patients may experience dizziness or fainting due to blood sampling. As stated in the patient information, possible risks due to the assessment of disease as follows:

Possible risks and discomfort related to biopsies

The risks related to the biopsies include pain, redness, swelling, excessive bleeding, bruises or leak at the area, where the needle is inserted, abnormal wound healing, fever, infection and allergic reaction towards the drug, which is used to anaesthetize the skin around the area where the biopsy is taken. The risks related to blood sampling includes pain, redness, bleeding and rarely infections.

Possible risks and discomfort related to CT-scan

A CT-scan is a special X-ray test, which is used for examinations of inner organs and bones in your body. It is necessary to measure the effect of your treatment. CT-scans involve exposure to radiation. In Denmark the background radiation is approximately 4 mSv (milliSievert) per year. The exposure to radiation after a CT-scan is approximately 10 milliSievert (mSv).

In connection with the CT-scan a contrast medium may be taken orally and/or injected in the vein to make certain organs and disease places visible. An oral contrast medium might cause side-effects such as nausea, constipation, diarrhoea and bloated stomach. Pain, bruises, redness, swelling or infection may occur at the place, where the needle is inserted to administer the contrast medium in your vein. It is normal to experience warmth and flushing, when the contrast medium is given. The participant may get allergic reactions towards the contrast medium, which causes rash, hives, difficulty in breathing, wheezing breathing, itching and very rarely cardiac arrest. The use of contrast medium during these examinations will be a normal part of the measurement of treatment effect, even if the patients do not participate in this research trial.

The study has been submitted to the data protection agency through the umbrella approval for RegionH by the sponsor, according to the attached document. Based on the EU regulations, this submission also covers the protection of patients' integrity and privacy in accordance with GDPR regulation in other EU countries.
The disclosure of patient-related information i.e. results from blood sample analyses, clinical response data, information about adverse events and medication for the sponsor within the trial is covered in the main protocol. These data are disclosed to the sponsor through the eCRF system REDCap. All

data that are disclosed are pseudonymized according to EU legislation as detailed in the protocol and patient information. The types of patient-related information that will be disclosed for the sponsor are further detailed in the main protocol.

Economy

The trial is an international investigator-initiated trial between the Nordic CLL study group and the Dutch HOVON group sponsored by Rigshospitalet The financial sponsors are the Novo Nordisk Foundation, which has given a grant of DKK 10 500 000 DKK. for the study and the pharmaceutical company Acerta Pharma, which has given € 1.19 million to the first part of the study. Acerta Pharma and Abbvie, the companies that are the marketing authorization holders of the drugs involved in the study, provide trial medication free of charge. Financial support is paid to the participating sites. The Danish study sites is paid 7,000 euros as start-up fee and then they are paid on average approx. € 10000 per participant that also covers visits, data collection, tests and procedures. The investigators in the study receive no financial compensation for their participation in the study. The national coordinator and the other principal investigators in Denmark do not have any economic connection to the companies that support the trial, the trial economy is managed through a research account at Rigshospitalet.
Expected number of participating patients in Denmark: 30 for the Phase 2-part and additional 70 for phase 3.
Participating sites in Denmark: 3

The participants of the study do not get any reimbursement for participation, as also stated in the patient information.

Optional extension from phase 2 to phase 3 part

As detailed in the analysis plan, based on the safety outcome of the phase 2 part of the study, it may be extended to the optional phase 3 part. The treatment schedule and study assessments will be similar for the two parts of the study, although the immune phenotyping and translational side studies will be more extensive for the phase 2 part. As the primary outcome for the phase 2 part is different from and prior to the primary outcome for the optional phase 3 part, the patients participating in the phase 2 part will automatically be part of the phase 3 cohort, if it is decided to extent the study to phase 3. This will be further detailed in an amendment to the protocol, if extended to phase 3 – the following sections are detailing this optional amendment.

Optional phase 3 part

The primary outcome is the grade ≥3-infection-free, CLL-treatment-free survival two years after inclusion in the trial. This will be assessed by a chi-squared test of proportions at 2 years. An absolute improvement of 20% in the treatment arm compared to the observation arm in grade ≥3-infection-free, CLL-treatment-free survival (from 35% to 55%, i.e. 57% increase) will be considered a success. At an alpha level of 0.05 and a power (1-β) of 0.80, 106 patients will be needed in each group to detect a 20% absolute increase in the primary outcome (with continuity correction).(37) Allowing for 10% of patients to be lost to follow-up the required sample size is 117 patients in each group.
Based on a yearly incidence of 450 newly diagnosed patients with CLL in Denmark with a population of 6,000,000 people, a yearly incidence of approximately 3,000 newly diagnosed CLL patients in the Netherlands – Belgium and Nordic region with more than 40,000,000 inhabitants will make approximately 600 patients eligible for the trial each year. Based on prior trial experience, a 12 months

enrollment period for the phase 2 part and a three-year enrollment period for the full trial (including both the phase 2 part and the optional extension to a phase 3 part and the pause of enrollment waiting for the outcome of the phase 2 part) is thus expected with 1/6 of the eligible subpopulation actually being enrolled on the trial.
For the optional extension to the phase 3 part, a total of 106 patients in each arm is planned, thus 212 patients in total, including the 50 patients from the phase 2 part. As the primary outcome for the phase 2 part is different from and prior to the primary outcome for the phase 3 part, the patients participating in the phase 2 part can count for the phase 3 part primary outcome, if it is decided to extent the study to phase 3.

Efficacy Endpoints for optional Phase 3 part

- Grade ≥3-infection free, CLL-treatment-free survival 2 years after enrollment. This is a superiority analysis.
    - 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
    - Response rate and duration according to IWCLL criteria
    - Immune function as assessed by immune phenotyping, functional TruCulture assays and measurements of cytokine levels
    - MRD levels in bone marrow and peripheral blood
- Quality of life during and for 2 years after treatment, QLQC30 and CLL17

Primary Endpoint, optional phase 3 part

Grade ≥3-infection free, CLL-treatment-free survival 2 years after enrollment. This will be assessed using a chi-squared test of proportions between the treatment arm and the observation arm two years after enrollment.
If a grade ≥3 infection or an infection considered an SAE or new CLL treatment or death occurs, this is considered an event for the Grade ≥3-infection free, CLL-treatment-free survival

Secondary Efficacy Endpoint

For the optional phase 3 study, a secondary endpoint will consider the time to a combined endpoint of death, infection or initiation of treatment for CLL. Kaplan Meier survival curves will be used to compare the time to event in the treatment and observation arms, censored at two years of follow-up. Conducting a log-rank test comparing two survival rates with a sample size of 212 (106 in each arm), we will have 80% power to detect a hazard ratio of 0.67.

Interim Analysis for the optional phase 3 part

One interim analysis will be performed when half the patients have been enrolled in each arm and have been follow-up for 1 year. The proportion of patients with Grade 3-infection free, treatment-free survival at 1 year will be assessed. An alpha of 0,0001 will be used as our significance criterion for stopping due to superiority of the treatment arm.

Publication of the trial results

Publication of the trial results are given in the main protocol. The results will be published whether positive, negative or inconclusive. The results will be published in scientific paper if possible and at conferences.

Recruitment and Informed Consent

Patients with chronic lymphocytic leukemia are diagnosed at the Departments of Hematology. The patients are admitted from their GP or from another hospital department.

There are two Informed Consents in the trial, one for the pre-screening and one for the trial.
At the pre-screening visit the patients will receive both oral and written information of the pre-screening part of the protocol, and the first Informed Consent (pre-screening) will be used, to get the patients permission to pre-screen (as also detailed above), where it will be assessed whether the patient is at high risk for infections and/or early treatment. Pre-screening will be done with data already collected as part of the standard of care and for some patients an extra blood sample for standard analyses.
If it is confirmed that the patient is at high risk a new visit where both oral and written information of the trial will be given. At this visit – the second Informed Consent will be used to ask if patients at will participate in the trial.
The screening data part of the standard of care, that are taken prior to the signing of the second Informed consent, will then be disclosed to the study investigator.
The patients who after the pre-screening are not at high risk will not be offered participation in the trial and will not receive the second Informed consent.

A medical report will be done by a hematologist and if the patient is eligible for the study, the patient will be informed about the existence of the protocol. The hematologist will encourage the patients to bring a relative or friend to the following information consultation of the protocol. The hematologist in charge of the protocol or another hematologist appointed the task will inform the patient in a consultation room. Written information about the study will be provided for the patient and the patient is encouraged to discuss the clinical protocol with friends or family. The patient will be given the time needed to decide on whether to participate in the protocol, however for some patients early treatment initiation may be needed, thus a decision within 24 hours may be needed for a subset of patients. If the patient wants to participate in the study, a written informed consent will be collected and examination and evaluation will be done to clarify if the inclusion criteria to participate in the protocol are fulfilled.

The procedure for the oral and written information will be followed for both the pre-screening and the actual trial. Patients who are eligible for the actual trial will have two information consultations and two Informed Consents and the patients that are not at high risk of infections after pre-screening will only have one information consultation and one Informed Consent.

Ethical considerations

Please see the attached document in Danish. “Etiske overvejelser” dated 01 April 2019.