D'accord study
(Dasatinib Combination for CLL with Refractory Disease)

A phase II study in patients with fludarabine refractory CLL: Dasatinib treatment combination for Fludarabine-refractory Chronic Lymphocytic Leukemia (CLL)

PROTOCOL

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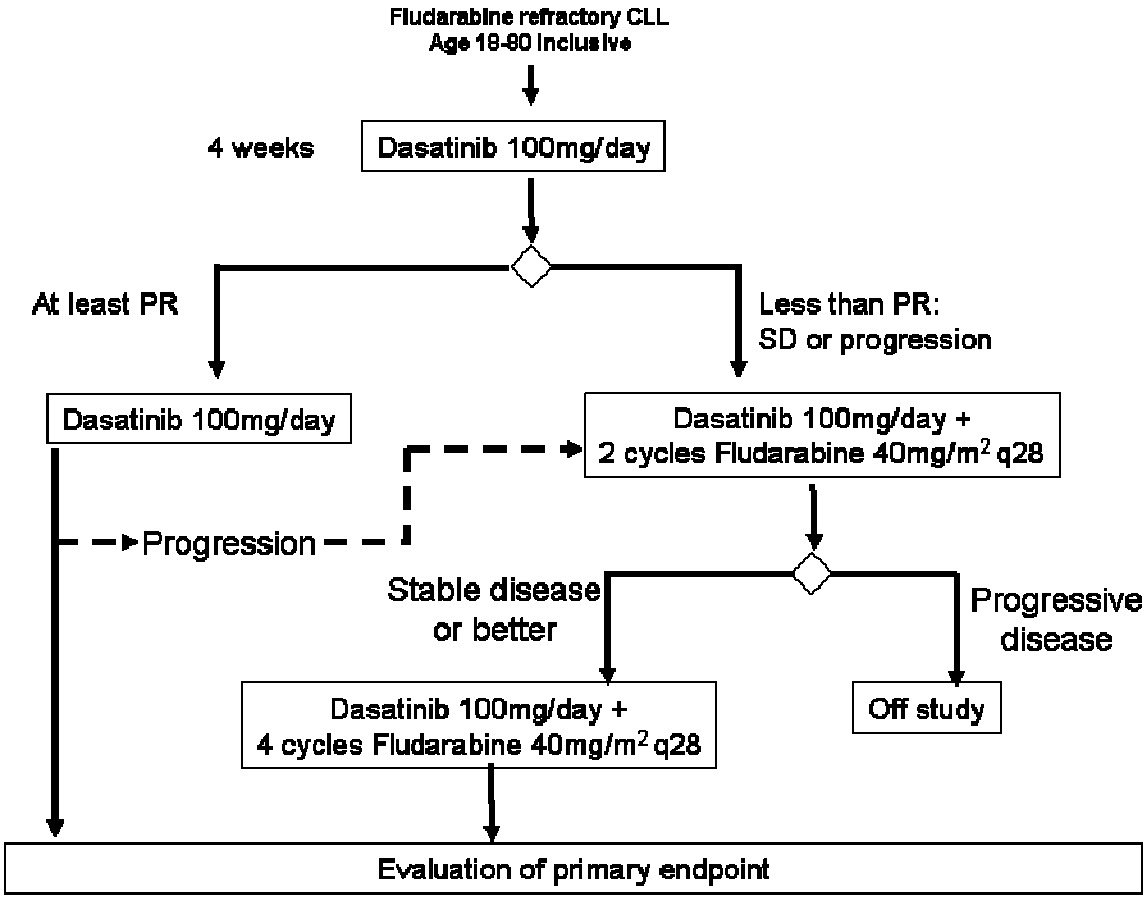
1 Scheme of the study



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3 Synopsis

Study type	Phase II
Study objectives	○ To determine the response rate and response quality of dasatinib monotherapy or dasatinib/fludarabine combination in fludarabine refractory CLL patients ○ To assess the overall safety profile of these treatment approaches ○ To assess event free survival, progression free survival and disease free survival following these treatment approaches ○ To assess influence of dasatinib on the expression profile of apoptosis regulating genes ○ To determine whether/how dasatinib acts synergistically with other drugs/agents as assessed by in vitro side studies
Patient population	Patients with CLL in symptomatic stage A or B or stage C, *AND* fludarabine refractory, age 18-80 years inclusive
Study design	Prospective, multicenter
Duration of treatment	Expected duration of treatment is 28 weeks.
Number of patients	35 patients registered
Adverse events	Adverse events will be documented if observed, mentioned during open questioning, or when spontaneously reported.
Planned start and end of Recruitment	Start of recruitment: 2008 End of recruitment: 2011

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5 Background

5.1 Biology of chemo-refractory CLL

Chronic lymphocytic leukemia (CLL) is a $CD5^+$ B-cell malignancy that is considered incurable[1]. Historically, the first-line treatment consisted of alkylating agents, which resulted in response rates in up to 70% of patients, but did not improve survival[2]. Treatment regimens with the nucleoside (purine) analogs, such as fludarabine monophosphate (fludarabine) or pentostatin, were found to yield higher response rates and to provide for longer progression-free survival[3], especially in combination with cyclophosphamide[4]. However, despite resulting in increased complete response (CR) rates, treatment with purine analogs alone does not appear to improve overall survival (OS)[3]. Newer treatment combinations have incorporated monoclonal antibodies in addition to chemotherapy[5;6]. Although treatment with these combinations suggest a survival benefit[7], such therapy is not considered curative. Most patients eventually develop drug resistance which is attributed to at least two independent mechanisms. The first mechanism is a shift in the balance between pro- and anti-apoptotic regulators[8]. Especially increased expression of both Mcl-1 and Bfl-1/A1[8;9] has been associated with resistance to chemotherapy. The second mechanism is based on acquired mutations resulting in a dysfunctional p53 response. Deletions of the short arm of chromosome 17 (17p-), which often is associated with loss of functional p53, or in the long arm of chromosome 11 (11q-) harboring the gene encoding the ataxia telangectasia mutated (ATM), which is a kinase required for p53 function, are uncommon in CLL at diagnosis, but increase in frequency as the disease progresses[10]. Since the cytolytic activity of most chemotherapy agents requires functional p53, loss of p53 is associated with drug resistance and poor prognosis[11].

The prognosis of CLL patients with chemotherapy refractory disease is very poor with an overall survival of approximately 10 months[12]. Although treatment with the monoclonal antibody alemtuzumab does induce responses in this subgroup of patients it makes patients vulnerable to life-threatening infections, especially following treatment with fludarabine and it is not considered curative[12]. An alternative and still experimental approach is treatment by reduced intensity allogeneic hematopoietic stem cell transplantation, which also has considerable toxicity and is only available for a minority of patients[13]. Therefore, novel strategies that improve the outcome in this subgroup of chemotherapy refractory patients are clearly needed for patients with drug-resistant CLL.

5.2 Influence of the micro-environment

CLL cells retain the capacity to respond to a variety of signals from the micro-environment, within lymph nodes, spleen and bone marrow (reviewed by Ghia and collegues[14]). Interactions between CLL cells and micro-environmental bystander cells can inhibit apoptosis of neoplastic B cells as shown in our recent study where we observed increased expression of anti-apoptotic proteins in CLL cells derived from lymph nodes as compared to CLL cells derived from peripheral blood[15;16]. Extended cell survival of tumor cells within this microenvironment may create not only an intracellular milieu permissive for genetic instability and for the accumulation of gene mutations that favor disease progression, but also these micro-environmental interactions may result in a safe haven from cytotoxic anticancer drugs, thus serving as a reservoir niche from which relapse occurs (reviewed by Pedersen and Reed[17]).

The latter is strongly supported by in vitro experiments using CD40 activation. We recently showed that prolonged CD40 activation induces expression of anti-apoptotic proteins independent of functional p53, which largely mimics the anti-apoptotic expression profile of LN derived CLL cells[18]. On the functional level, prolonged CD40 activation results in resistance of CLL cells to commonly used chemotherapeutic drugs, notably purine analogues[18;19].
C-Abl kinase is over-expressed in CLL cells and levels of c-Abl protein expression correlate positively with tumor burden and disease stage[20]. In chronic myeloid leukemia it has been shown that treatment with tyrosine kinase inhibitors (TKIs) that target BCR-Abl, induces apoptosis through alterations in apoptotic regulators (downmodulation of the anti-apoptotic molecules Bcl-xL and Mcl-1, induction of the pro-apoptotic molecule Bim)[21;22]. Interestingly, the pattern of expression of these molecules in CML is quite similar to the expression profile found in LN derived CLL cells[15].

5.3 Abl-kinase inhibitors in CLL

We recently studied the impact of tyrosine kinase inhibition of CLL cells with both the TKI imatinib and with dasatinib. The latter Dasatinib [SPRYCEL®] is a potent, broad spectrum ATP-competitive inhibitor of 5 critical oncogenic tyrosine kinase/kinase families: BCR-ABL, SRC, c-KIT, PDGF receptor β (PDGFRβ), and ephrin (EPH) receptor kinases. Although both drugs have only a moderate effect on apoptosis induction when used as single agent, treatment with imatinib and especially dasatinib reverted the anti-apoptotic profile of CD40-stimulated ('lymph node type') CLL cells in a p53 independent fashion. This resulted in a dose-dependent sensitization of these cells to various cytotoxic drugs, notably fludarabine. Dasatinib proved to be effective in the nanomolar range. These effects were observed both when cells were treated with dasatinib *prior and during* CD40 stimulation but also when cells were treated with dasatinib *after* CD40 stimulation. From these results we can conclude that in vitro (a) dasatinib prevents CD40 dependent acquired chemoresistance, and (b) that dasatinib treatment has the ability to reverse the CD40 mediated chemo-resistance of CLL cells. Our data indicate that c-Abl inhibitors, notably dasatinib, overcome the protective profile within the micro-environment resulting in susceptibility to cytotoxic drugs, notably fludarabine[23]. A recent phase 2 evaluation of dasatinib as single agent in relapsed and refractory CLL showed limited effects, but in good correlation with our data a reduction of lymph node size was observed in a major fraction of patients. In this study patients were treated with 140mg of dasatinib daily. This dose resulted in major hematological toxicity in seven of the 14 patients[24].

5.4 Hypothesis

We hypothesize that: 1. Dasatinib treatment is clinically active in chemo-refractory CLL, and 2. Dasatinib will restore responsiveness to fludarabine in chemo-refractory CLL.
Phase I and II clinical data show that dasatinib is well tolerated and highly effective for the treatment of imatinib-resistant/imatinib-intolerant chronic myelogenous leukemia (CML) and Philadelphia chromosome-positive acute lymphoblastic leukemia[25]. We plan to determine clinical responses, toxicities and progression free survival of this drug in chemotherapy refractory CLL patients. Also we will study whether dasatinib treatment sensitizes CLL cells from fludarabine-refractory patients to cytotoxic drugs, notably fludarabine.

5.5 Dasatinib

5.5.1 Clinical pharmacokinetics

The pharmacokinetics of dasatinib have been evaluated in 229 healthy subjects and in 137 patients with leukemia (CML or Ph+ALL) from a Phase I clinical study (CA180002).

5.5.1.1 Absorption

Maximum plasma concentrations (Cmax) of dasatinib are observed between 0.5 and 6 hours (Tmax) following oral administration dasatinib exhibits dose proportional increases in AUC and linear elimination characteristics over the dose range of 15 mg to 240 mg/day. The overall mean terminal half-life of dasatinib is 3–5 hours[26].
Data from a study of 54 healthy subjects administered a single, 100-mg dose of dasatinib 30 minutes following consumption of a high-fat meal resulted in a 14% increase in the mean AUC of dasatinib. The observed food effects were not clinically relevant.

5.5.1.2 Distribution

In patients, dasatinib has an apparent volume of distribution of 2505 L, suggesting that the drug is extensively distributed in the extravascular space. Binding of dasatinib and its active metabolite to human plasma proteins in vitro was approximately 96% and 93%, respectively, with no concentration dependence over the range of 100–500 ng/mL[26].

5.5.1.3 Metabolism

Dasatinib is extensively metabolized in humans, primarily by the cytochrome P450 enzyme 3A4. CYP3A4 was the primary enzyme responsible for the formation of the active metabolite. Flavin-containing monooxygenase 3 (FMO-3) and uridine diphosphate-glucuronosyltransferase (UGT) enzymes are also involved in the formation of dasatinib metabolites. In human liver microsomes, dasatinib was a weak time-dependent inhibitor of CYP3A4.
The exposure of the active metabolite, which is equipotent to dasatinib, represents approximately 5% of the dasatinib AUC. This indicates that the active metabolite of dasatinib is unlikely to play a major role in the observed pharmacology of the drug. dasatinib also had several other inactive oxidative metabolites.
Dasatinib is a time-dependent inhibitor of CYP3A3. At clinically relevant concentrations, dasatinib does not inhibit CYP1A2, 2A6, 2B6, 2C9, 2C19, 2D6, or 2E1. Dasatinib is not an inducer of human CYP enzymes.1

5.5.1.4 Elimination

Elimination is primarily via the feces. Following a single oral dose of [14C]-labeled dasatinib, approximately 4% and 85% of the administered radioactivity was recovered in the urine and feces, respectively, within 10 days. Unchanged dasatinib accounted for 0.1% and 19% of the administered dose in urine and feces, respectively, with the remainder of the dose being metabolites[26].

5.5.2 Clinical experience in CML and Ph+ ALL: Randomized studies

A Phase 2, randomized, open-label study was conducted in patients with chronic phase CML whose disease was resistant to prior imatinib therapy at doses of 400 or 600 mg. The primary endpoint was molecular cytogenetic remission at 12 weeks.

One hundred fifty patients were randomized in a 2:1 ratio to either dasatinib 70 mg twice daily or imatinib 800 mg daily (400 mg twice daily). Crossover to the alternate therapy was permitted in the event of disease progression or intolerable toxicity. Median follow-up was 15 months. Median duration of treatment prior to crossover was 14 months for dasatinib and 3 months for imatinib.
Prior to crossover, 93% of the dasatinib-treated patients and 82% of the imatinib-treated patients achieved a complete hematologic remission. At 12 weeks, molecular cytogenetic remission was achieved in 36% of the dasatinib-treated patients and 29% of the imatinib-treated patients. With longer treatment and follow-up, molecular cytogenetic remission was achieved in 52% of the dasatinib-treated patients and 33% of the imatinib-treated patients prior to crossover. Since the median follow-up was 15 months, there were too few progressions to reliably estimate the duration of molecular cytogenetic remission.
A Phase 3, randomized, open-label, dose-optimization study was conducted in patients with chronic phase CML, whose disease was resistant to or who were intolerant to imatinib, to evaluate the efficacy of dasatinib administered once daily compared with dasatinib administered twice daily. Patients with significant cardiac diseases including myocardial infarction within 6 months, congestive heart failure within 3 months, significant arrhythmias, or QTc prolongation were excluded from the study. The primary endpoint was molecular cytogenetic remission in patients with imatinib-resistant chronic phase CML. The main secondary endpoint was molecular cytogenetic remission by total daily dose level in the same population. A total of 670 patients, of whom 498 had imatinib resistant disease, were randomized to the dasatinib 100 mg once daily, 140 mg once daily, 50 mg twice daily, or 70 mg twice daily group. Minimum follow-up was 6 months and median duration of treatment was approximately 8 months.
Efficacy was achieved across all dasatinib treatment groups with the once daily schedule demonstrating comparable efficacy (non-inferiority) to the twice daily schedule on the primary efficacy endpoint (difference in molecular cytogenetic remission 2.8%; 95% confidence interval [-6.0%–11.6%]). The main secondary endpoint of the study also showed comparable efficacy (non-inferiority) between the 100 mg total daily dose and the 140 mg total daily dose (difference in molecular cytogenetic remission -0.8%; 95% confidence interval [-9.6%–8.0%]). Since the minimum follow-up was only 6 months, there were too few progressions to estimate the duration of molecular cytogenetic remission.

5.5.3 Safety and toxicity in clinical studies in CML and Ph+ ALL

The data discussed below reflect exposure to dasatinib in 2182 patients with leukemia in clinical studies (starting dosage 100 mg once daily, 140 mg once daily, 50 mg twice daily, or 70 mg twice daily). The median duration of therapy was 11 months (range 0.03–26 months).
The majority of dasatinib-treated patients experienced adverse reactions at some time. Drug was discontinued for adverse reactions in 9% of patients in chronic phase CML, 10% in accelerated phase CML, 15% in myeloid blast phase CML, and 8% in lymphoid blast phase CML or Ph+ ALL. In a Phase 3 dose-optimization study in patients with chronic phase CML, the rate of discontinuation for adverse reaction was lower in patients treated with 100 mg once daily than in patients treated with 70 mg twice daily (4% and 12%, respectively).

The most frequently reported adverse reactions (reported in ≥20% of patients) included fluid retention events, diarrhea, headache, skin rash, nausea, hemorrhage, fatigue, and dyspnea.
The most frequently reported serious adverse reactions included pleural effusion (9%), pyrexia (3%), pneumonia (3%), infection (2%), febrile neutropenia (4%), gastrointestinal bleeding (4%), dyspnea (3%), sepsis (1%), diarrhea (2%), congestive heart failure (2%), and pericardial effusion (1%).

5.5.3.1 Laboratory Abnormalities
Myelosuppression was commonly reported in all patient populations. The frequency of Grade 3 or 4 neutropenia, thrombocytopenia, and anemia was higher in patients with advanced CML or Ph+ ALL than in chronic phase CML. Myelosuppression was reported in patients with normal baseline laboratory values as well as in patients with pre-existing laboratory abnormalities (Table 3).
In patients who experienced severe myelosuppression, recovery generally occurred following dose interruption and/or reduction; permanent discontinuation of treatment occurred in 1% of patients.
Grade 3 or 4 elevations of transaminases or bilirubin and Grade 3 or 4 hypocalcemia and hypophosphatemia were reported in patients with all phases of CML but were reported with an increased frequency in patients with myeloid or lymphoid blast CML and Ph+ ALL. Elevations in transaminases or bilirubin were usually managed with dose reduction or interruption. Patients developing Grade 3 or 4 hypocalcemia during the course of dasatinib therapy often had recovery with oral calcium supplementation.

5.5.4 Anticipated Adverse Events
Myelosuppression
Treatment with dasatinib in CML is associated with severe (NCI CTC Grade 3 or 4) thrombocytopenia, neutropenia, and anemia. Since dasatinib directly targets the BCR-Abl positive stem cell this phenomenon is at least partly linked to the underlying disease as illustrated by the fact that their occurrence is more frequent in patients with advanced CML or Ph+ ALL than in chronic phase CML. Complete blood counts should be performed regularly. Myelosuppression was generally reversible and usually managed by withholding dasatinib temporarily or dose reduction. In a Phase 3 dose-optimization study in patients with chronic phase CML, Grade 3 or 4 myelosuppression was reported less frequently in patients treated with 100 mg once daily than in patients treated with 70 mg twice daily.1
Bleeding Related Events
In addition to causing thrombocytopenia in human subjects, dasatinib caused platelet dysfunction in vitro. In all clinical studies, severe central nervous system (CNS) hemorrhages, including fatalities, occurred in <1% of patients receiving dasatinib. Severe gastrointestinal hemorrhage occurred in 4% of patients and generally required treatment interruptions and transfusions. Other cases of severe hemorrhage occurred in 2% of patients. Most bleeding events were associated with severe thrombocytopenia.
Patients were excluded from participation in dasatinib clinical studies if they took medications that inhibit platelet function or anticoagulants. In some trials, the use of anticoagulants, aspirin, and non-steroidal anti-inflammatory drugs (NSAIDs) was allowed concurrently with dasatinib if the platelet count was >50,000. Caution should be exercised if patients are required to take medications that inhibit platelet function or anticoagulants.

Fluid Retention
Dasatinib is associated with fluid retention. In all clinical studies, severe fluid retention was reported in 8% of patients, including pleural and pericardial effusion reported in 5% and 1% of patients, respectively. Severe ascites and generalized edema were each reported in <1% of patients. Severe pulmonary edema was reported in 1% of patients. Patients who develop symptoms suggestive of pleural effusion such as dyspnea or dry cough should be evaluated by chest X-ray. Severe pleural effusion may require thoracentesis and oxygen therapy. Fluid retention events were typically managed by supportive care measures that include diuretics or short courses of steroids.
In the Phase 3 dose-optimization study in patients with chronic phase CML, fluid retention events were reported less frequently in patients treated with 100 mg once daily than in patients treated with 70 mg twice daily..

QT Prolongation
In vitro data suggest that dasatinib has the potential to prolong cardiac ventricular repolarization (QT interval). In single-arm clinical studies in patients with leukemia treated with dasatinib, the mean QTc interval changes from baseline using Fridericia's method (QTcF) were 3–6 msec; the upper 95% confidence intervals for all mean changes from baseline were <8 msec. Nine patients had QTc prolongation reported as an adverse event. Three patients (<1%) experienced a QTcF >500 msec.
Dasatinib should be administered with caution to patients who have or may develop prolongation of QTc. These include patients with hypokalemia or hypomagnesemia, patients with congenital long QT syndrome, patients taking anti-arrhythmic medicines or other medicinal products that lead to QT prolongation, and cumulative high-dose anthracycline therapy. Hypokalemia or hypomagnesemia should be corrected prior to dasatinib administration

5.6 Study set up
In this phase II clinical study we will examine whether chemo-refractory CLL patients will respond to dasatinib treatment and whether dasatinib can act synergistically with the purine analogue fludarabine. According to our in vitro data in CLL, dasatinib should have some activity as single agent, but importantly has the ability to render chemotherapy refractory CLL cells sensitive for fludarabine by reversion of the anti-apoptotic profile of these cells. Indeed, as earlier mentioned, the study of Dr. Amrein, et al. showed that the activity of dasatinib in CLL when used as monotherapy is rather low[24]. In this study, treatment was interrupted in large number of the patient due to hematological toxicity. Therefore, in this protocol a lower dose will be given. Our intention is to study efficacy and safety of dasatinib in combination with fludarabine. This phase II feasibility study will provide the data on the kinetics of responses of dasatinib in patients with relapsed/ refractory CLL after a purine analogue containing treatment. All patients will start with dasatinib monotherapy. All patients with less than a PR will receive fludarabine in addition to dasatinib.

5.7 Rationale of the study
CLL is the most common leukemia in the western world predominantly occurring in the elderly. Because of demographic changes in our society, CLL is expected to become an increasing clinical problem within the near future. At this moment chemo-naïve patients have the choice between chlorambucil and fludarabine monotherapy

or a fludarabine containing combination treatment, all of which are proven to be effective and relatively safe. Also for first relapse effective approaches like fludarabine (for fludarabine naïve patients) or immuno-chemotherapeutic combinations are available. However, there is currently no well-defined alternative for fludarabine refractory patients except (potentially highly toxic) allogeneic stem cell transplantation which is available to a small minority of patients and the outcome of fludarabine refractory patients is very poor[12]. Our in vitro data indicated that dasatinib sensitizes chemo-refractory CLL cells to fludarabine treatment. If we will be able to show that dasatinib is active in fludarabine-refractory CLL and that in can sensitize patients to fludarabine it will pave the way for randomized trials not only in chemo-refractory patients but also as additional drug in second-line treatment strategies.

5.8 side study

In at least 3 patients enrolled in Academic Medical Center with palpable enlarged lymph nodes who have given informed consent for the core biopsy side study the following will be performed: Prior to start of treatment, and after one month of dasatinib monotherapy ultrasound-guided core biopsies of an involved lymph node. More details can be found in the study lab manual.
Studies will include:

1. Profiling (RNA and Kinase activity) of LN derived CLL cells before and after Dasatinib treatment
   - Confirmation of findings of the profiling studies by qPCR, western blot, FACS

6 Study objectives

Primary Objective

To determine the response rate and response quality of dasatinib monotherapy or dasatinib/fludarabine combination in fludarabine refractory CLL patients.

Secondary Objective(s)

- To assess the overall safety profile of these treatment approaches
- To assess event free survival (i.e. time from registration to induction failure, progression, relapse or death whichever occurs first), progression free survival (i.e. time from registration to disease progression, relapse or death due to CLL whichever occurs first) and disease free survival (i.e. time from CR to relapse)
- To assess influence of dasatinib on the expression profile of apoptosis regulatoring genes.
- To determine by in vitro analysis whether dasatinib acts synergistically with other immuno-chemotherapeutic agents by co-culture experiments.

7 Study design

Patients with fludarabine refractory CLL meeting all eligibility criteria (see 8.1) will be treated with dasatinib monotherapy 100mg daily**.** Four weeks after initiation of dasatinib patients will be re-evaluated. Patients with less than a partial response will receive fludarabine (orally 40mg/daily for 3 days q28) in addition to dasatinib (first cycle at the beginning of week 5). Patients with at least a partial response will continue dasatinib monotherapy. After two cycles of fludarabine, responses will be

evaluated (end of week 12). In case of progressive disease following 2 cycles of fludarabine in combination with dasatinib, patients will go off study. All other patients will be treated with four more cycles of fludarabine in combination with daily dasatinib treatment (see flowchart). Patients that receive monotherapy after the initial 28 days and that develop progressive disease will 'cross-over' to the combination treatment. In case of CR, quality of the response will be analyzed by minimal residual disease measurement using flow cytometry.

8 Study population

8.1 Eligibility for registration

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

8.1.1 Inclusion criteria

- CLL confirmed according to the IWCLL Working Group criteria[27]
- Binet[28] stages A or B with indication for treatment according to IWCLL guidelines (appendix A and B)[27], Binet C AND
  Fludarabine refractory, defined as relapse (any sign of disease recurrence or progression with or without indication for treatment ≤ 6 months following fludarabine containing chemo(immuno)therapy[27]. A patient that has received other drugs following fludarabine but who has been proven fludarabine refractory in the past according to above definition can be included;
- Age 18-80 years inclusive;
- WHO performance status ≤ 2 (appendix E);
- No possibility for rapid reduced intensity allogeneic hematopoietic stem cell transplantation;
- At least 4 weeks without any treatment before study entry;
- Negative pregnancy test;
- Written informed consent;

8.1.2 Exclusion criteria

- Richter's transformation;
- Suspected or documented CNS involvement by CLL;
- Grade 3 cytopenia not due to bone marrow infiltration
- Concurrent medical condition which may increase the risk of toxicity, including:
  - The presence of pleural or pericardial effusion of any grade
- Cardiac Symptoms, including:
  - Uncontrolled angina, congestive heart failure or MI within (6 months)
  - Diagnosed congenital long QT syndrome
  - Any history of clinically significant ventricular arrhythmias (such as ventricular tachycardia, ventricular fibrillation, or Torsades de pointes)
  - Prolonged QTc interval on pre-entry electrocardiogram (> 450 msec)
  - Subjects with hypokalemia or hypomagnesemia if it cannot be corrected prior to dasatinib adminstration
- Severe pulmonary dysfunction (CTCAE grade III-IV);
- Active hepatitis B infection;
- History of significant bleeding disorder unrelated to the CLL, including:
  - Diagnosed congenital bleeding disorders (e.g., von Willebrand's disease)
  - Diagnosed acquired bleeding disorder within one year (e.g., acquired anti-factor VIII antibodies)
  - Ongoing or recent (≤ 3 months) significant gastrointestinal bleeding
- Known HIV positivity
- Clinically significant auto-immune hemolytic anemia (AIHA)

- Severe neurological or psychiatric disease;
- Significant hepatic dysfunction (Total bilirubin < 2.0 times ULN; Hepatic enzymes (AST, ALT) ≤ 2.5 times ULN) except when caused by leukemic infiltration;
- Significant renal dysfunction (serum creatinine ≥ 150 uM/L after rehydration);
- History of active malignancy during the past 5 years with the exception of basal carcinoma of the skin or stage 0 cervical carcinoma;
- Concurrent use of CYP3A4 inducers or inhibitors, or QT_c-prolonging agents*;
- Active, uncontrolled infections;
- Any psychological, familial, sociological and geographical condition potentially hampering compliance with the study protocol and follow-up schedule;
- Female patients of reproductive potential who are not using effective contraception;

* The following medications should be considered for exclusion:

a) Category I drugs that are generally accepted to have a risk of causing torsades de pointes including: (patients must discontinue drug 7 days prior to starting dasatinib)
quinidine, procainamide, disopyramide
amiodarone, sotalol, ibutilide, dofetilide
erythromycin, clarithromycin
chlorpromazine, haloperidol, mesoridazine, thioridazine, pimozide, zyprasidone, cisapride, bepridil, droperidol, methadone, arsenic, chloroquine, domperidone, halofantrine, levomethadyl, pentamidine, sparfloxacin, lidoflazine.

b) The concomitant use of H2 blockers or proton pump inhibitors with dasatinib is not recommended. The use of antacids should be considered in place of H2 blockers or proton pump inhibitors in patients receiving dasatinib therapy. If antacid therapy is needed, the antacid dose should be administered at least 2 hours prior to or 2 hours after the dose of dasatinib. Patient may not be receiving any prohibited CYP3A4 inhibitors.

9 Treatments

9.1 Dasatinib monotherapy

Agent	Dose	Route	Time
Dasatinib	100 mg	Oral	daily for28 days

All patients will be treated with dasatinib 100mg once a day for 28 days.
- In case a PR or CR is achieved at day 28 the patient will continue with dasatinib monotherapy for a total of 28 weeks. Patients should be evaluated for efficacy every month during this time. In case of progression before week 28 the patient will be treated with the combination of dasatinib and fludarabine (see section 9.2).
- All patients with less than a PR at day 28 will be treated with the combination of dasatinib and fludarabine (see section 9.2).

9.1.1 Dose adjustments during dasatinib

9.1.1.1 Hematological toxicity
Hematological toxicity as defined by the IWCLL guielines[27] (appendix D) will be handled as follows:

Grade 1 and 2:
No dose adjustments for grade 1 and 2 hematological toxicity will be made.

Grade 3 and 4:
If a patient experiences a grade ≥ 3 neutropenia and/or thrombocytopenia (i.e., ANC < 1.0x109/l, or decrease in platelet count > 50%) not due to bone marrow infiltration, dasatinib must be withheld and patients will be monitored weekly until the toxicity has resolved to grade ≤ 2 within 4 weeks.
If the hematological toxicity resolves to grade ≤ 2, dasatinib must be resumed at a dose of 70mg once daily. If the grade ≥ 3 hematological toxicity recurs, dasatinib must be withheld and patients will be monitored weekly until the toxicity has resolved to grade ≤ 2. If the hematological toxicity resolves to grade ≤ 2, dasatinib must be resumed at a dose of 50mg once daily. If the grade ≥ 3 hematological toxicity recurs again the patient should go off protocol treatment.

No dose reductions will be performed for grade ≥ 3 anemia or lymphopenia. Patients developing anemia may be transfused.

9.1.1.2 Non-hematological toxicity
Non-hematological toxicity of dasatinib can occur at any time during dasatinib therapy and are scored according to the NCI Common Terminology Criteria for Adverse Events, version 3.0 (appendix D).

Grade 1 and 2:
No dose adjustments for grade 1 non-hematological toxicity will be made.

Grade 3 and 4:
If a patient experiences a grade 2 non-hematological toxicity that does not resolve despite therapeutic intervention (see section 9.1.2), dasatinib must be withheld until

the toxicity has resolved to grade ≤ 1. Dasatinib may then be resumed at a dose of 100mg once a day. If the grade 2 toxicity recurs, dasatinib must again be withheld until the toxicity has resolved to grade ≤ 1, and the dose of dasatinib must be reduced to 70mg daily. If the grade 2 toxicity recurs at 70mg, dasatinib must be withheld until the toxicity has resolved to grade ≤ 1, and resumed at 70mg daily. If the grade 2 toxicity recurs at 70mg, dasatinib must again be withheld until the toxicity has resolved to grade ≤ 1, and dasatinib must be resumed at 50mg daily. If at any time the non-hematological toxicity does not resolve to grade ≤ 2 in 28 days, consult with the study coordinator. A documented grade ≥ 3 non-hematological toxicity that recurs despite dose reduction to 50mg daily is considered intolerance of treatment and the patient should discontinue study treatment.

9.1.2 Special management orders and concomitant medication in conjunction with dasatinib

- All patients should receive allopurinol 300 mg daily day 0 until day 7 after the start of dasatinib. In case of allopurinol allergy, alternative drugs (probenecid) may be used. During the first week of dasatinib monotherapy all patients will be instructed to secure relevant fluid intake, and will be monitored for tumor lysis syndrome with blood tests day 2 and day 7.
- In most cases rash is mild, self-limiting and manageable with antihistamines or topical steroids. A short course of oral steroids may be initiated for the management of more severe cases. Prednisone 25 mg is recommended for one week or until rash has resolved.
- Patients should be monitored closely for peripheral edema and rapid weight gain. The use of diuretics may be initiated for the management of edema. In severe cases dasatinib should be withheld according to section 9.1.1 and the edema may be controlled with diuretics. Dasatinib can be resumed, while maintaining or increasing diuretic therapy.
- Patients can develop pleural effusion at any time during treatment. Both the use of diuretics and prednisone 25mg can be useful to alleviate symptoms and may be initiated. In severe cases dasatinib should be withheld according to section 9.1.1 and the edema may be controlled with diuretics. Dasatinib can be resumed, while maintaining or increasing diuretic therapy.
- Routine liver function tests should be performed throughout the study. Dose reduction may be warranted and the decision to continue dasatinib needs to be made in light of the clinical situation.

9.2 Dasatinib and fludarabine combination

Agent	Dose	Route	Time
Dasatinib	50mg q 12 hrs	Oral	daily
Fludarabine	40mg/m2	Oral	Day 1,2,3 q28

Patients eligible for the combination treatment (less than a PR at day 28 or progression on dasatinib monotherapy after day 28) with ANC > 1.0x109/l, and a platelet count < 50% decrease as compared to baseline unless related to bone marrow infiltration) will receive fludarabine p.o. (total 120 mg/m2 per cycle) combined with dasatinib p.o. 100mg/day.

In case the patient has progressive disease after 2 cycles of fludarabine the patient should go off study treatment. In all other cases a total of 6 cycles will be given.

9.2.1 Dose modification during combination treatment

9.2.1.1 Hematological toxicity

Dose modifications will not be made during the first cycle. During the next cycles modifications of the treatment schedule will only be made as follows:

- If at day 1 of any cycle, there is a grade ≥3 cytopenia (excluding anemia), not related to bone marrow infiltration, treatment should be delayed for up to two weeks and given with a 25% dose reduction of fludarabine in the following cycles. If after two weeks, the grade ≥3 cytopenia, not related to bone marrow infiltration, still prevails, the dose of dasatinib must be adjusted as described in section 9.1.1.
- If there is further grade ≥3 cytopenia in subsequent cycles despite the first 25% dose reduction treatment should again be delayed for up to two weeks and the dose of fludarabine is further reduced to 50% of the full dose.
- If there is further grade ≥3 cytopenia in subsequent cycles despite 50% dose reduction the patient should go off protocol treatment.
- If there is any grade ≥3 neutropenia with infection during any cycle, G-CSF should be administered and G-CSF should be given in all subsequent cycles.

9.2.1.2 Dose modification for impaired renal function

Fludarabine is partly (40-60%) excreted by the kidneys. If the creatinine clearance is reduced to 30-60 ml/min, the fludarabine dose should be reduced to 50%. Patients with a creatinine clearance below 30 ml/min are excluded from the study. Patients who develop renal dysfunction (serum creatinine >150 µmol/l or creatinine clearance < 30 ml/min) during treatment should go off protocol treatment.

9.2.1.3 Dose modification for other non-hematological toxicity

- If other grade ≥3 non-hematological, non-renal toxicity not likely due to dasatinib occurs during any cycle, treatment should be delayed until recovery to grade ≤2, for up to two weeks. All subsequent cycles should be given with a 25% dose reduction of fludarabine. If after two weeks, the grade ≥3 non-hematological, non-renal toxicity still prevails, the patient should go off protocol treatment.
- If there is further grade ≥3 non-hematological, non-renal toxicity in subsequent cycles despite the first 25% dose reduction treatment should again be delayed for up to two weeks and the dose of fludarabine is further reduced to 50% of the full dose.
- If there is further grade ≥3 non-hematological, non-renal toxicity in subsequent cycles despite 50% dose reduction the patient should go off protocol treatment.

9.2.3 Special management orders and concomitant medication during/after combination treatment

- All patients should receive pneumocystis carinii pneumonia (PCP) prophylaxis: sulphametoxazol with trimetoprim 400/80 mg daily and viral prophylaxis with valaciclovir 500mg twice daily from the start of fludarabine treatment throughout the study period until at least 3 months after the last treatment day. In case of intolerance to the PCP prophylaxis pentamidine inhalation 300 mg every month, dapsone 100 mg three times a week or any other documented PCP prevention is recommended.
- All blood products should be irradiated for one year after treatment to prevent transfusion-related GVHD.

10 End of protocol treatment

Reasons for going off protocol treatment are:

- Progressive disease after 2 cycles of fludarabine and dasatinib
- Progressive disease at subsequent cycles of fludarabine and dasatinib
- Excessive toxicity (including toxic death) requiring permanent discontinuation of protocol treatment
- No compliance of the patient (especially refusal to continue treatment)
- Intercurrent death
- Major protocol violation
- Completion of protocol treatment
- Withdrawal by the investigator for clinical reasons not related to protocol treatment

11 Required clinical evaluations

11.1 Time of clinical evaluations

- At entry: at time of randomization, within 14 days prior to start dasatinib
- At day 2, 7, 14 and 28 from the start of dasatinib
- Dasatinib monotherapy: monthly until week 28
- Combination treatment: prior to each cycle: within 7 days prior to each cycle
- End of protocol: day 28 after end of cycle 6
- Follow up: every 3 Months until 1,5 years after last treatment

11.2 Required investigations at entry, during treatment and during follow up

	At entry	Day 2, 7, 14 dasatinib mono-therapy	Day 28 dasatinib mono-therapy	Monthly dasatinib mono therapy	Prior to each cycle combination treatment	After cycle 2 combination treatment	End of Protocol	Follow up
Medical history	X							
Physical examination	X	X	X	X	X	X	X	X
Adverse events	X	X	X	X	X	X	X	X
Blood tests:								
Hematology	X	X	X	X	X	X	X	X
Blood Chemistry	X	X	X	X	X	X	X	X
Bone marrow Aspirate/Biopsy	A and B						A and B	
Molecular evaluations:								
PB Flow cytometry	X		X			X	X	
BM Flow cytometry	X[1]		X			X	X	
PB FISH	X[2]							
Mutational status	X[3]							
PB MRD studies			X[4]			X[4]	X[4]	
BM MRD studies							X[4]	
PB storage future	X	X	X	X	X	X	X	
BM storage future studies	X		X			X	X	
Specific investigations:								
ECG	X							
CT scan	X		X	X[5]		X	X	
Additional investigations	o.i.	o.i.	o.i.	o.i.	o.i.	o.i.	o.i.	o.i.

[1] Only if not done on PB
[2] Only when not performed after last treatment
[3] Only if not done at any time prior to study
[4] In case of CR
[5] In case of clinical suspected progression
o.i. on indication

11.2.1 Medical history
Standard medical history, including B symptoms and concomitant medications.

11.2.2 Physical examination
Standard physical examination, with special attention to:
- vital signs
- WHO performance status (see appendix E)
- Documentation of lymphadenopathy, liver and spleen size

11.2.3 Hematology
- Hb
- WBC and differential count
- Platelet count
- DAT (direct antiglobulin test/Coombs test) (only at entry and on indication)

11.2.4 Blood chemistry

- Sodium
- Potassium
- Creatinine
- Uric acid
- ASAT
- ALAT
- Alkaline phosphatase
- Bilirubin
- LDH
- Haptoglobin

11.2.5 Additional blood chemistry at entry

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

* IgG, IgM and IgA should be repeated at the end of protocol

11.2.6 Bone marrow aspirate and biopsy

At entry and at the end of protocol treatment a bone marrow aspirate and/or biopsy is performed as indicated. For pathology study of infiltration pattern and immunocytochemistry (required markers: CD5, CD19, CD20, CD23,CD79b, kappa, lambda, cyclin D1) will be performed.

11.2.7 Molecular evaluations

Flow cytometry

At entry, after the first 28 days, after the seconds cycle of fludarabine and at the end of protocol treatment.

- Diagnosis of classical CLL immunophenotype (required markers: CD5/CD19/CD23 triple positive with light chain restriction)

FISH

- At entry on PB if not done after last given treatment before registration (required markers: 17p13 deletion, 11q22-23 deletion, trisomy 12 and 13q14 deletion).

Mutational status and MRD studies

At entry mutational status on PB if not performed at any time prior to registration. After the first 28 days, after cycle 2 and at the end of the treatment MRD studies on PB and BM will be performed by flow cytometry only as confirmation of CR (required markers: CD43, CD19, CD20, CD5).

In addition to these investigations, all patients are asked for informed consent to store biological material for future studies.

11.2.8 CT scan

At entry, after the first 28 days, after the seconds cycle of fludarabine and at the end of protocol treatment and in case of clinical suspected progression at dasatinib monotherapy.

Imaging including CT of neck, thorax, abdomen and pelvis.

11.3 Evaluation of response

- Response will be evaluated:
- After the first 28 days,
- In case of continuous monotherapy:
    - After third cycle;
    - At the end of protocol treatment (following 7 cycles monotherapy);
    - In case of clinical suspected progression
- In case of combination treatment:
    - After the second cycle of fludarabine;
    - At the end of protocol treatment (following 6 cycles combination therapy);. Assessment of response is described in appendix C according to the IWCLL Working Group criteria[27].

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11.4 Toxicities

All agents used in the protocol can cause pancytopenia and can induce septic or hemorrhagic complications.
In addition:

- Dasatinib can cause nausea, muscle spasms, arthralgia, headache, peripheral edema including peri-orbital edema and pleural effusion (transudate or exudates), and liver function abnormalities.
- Fludarabine can cause immune suppression and auto-immune hemolytic anemia

Hematological toxicities will be scored according to IWCLL guidelines (appendix D). Non-hematological toxicities will be scored according to the NCI Common Terminology Criteria for Adverse Events, version 3.0 (appendix D)

12 Serious adverse events

12.1 Documentation of serious adverse events

Adverse event (AE)
An adverse event (AE) is any untoward medical occurrence in a patient or clinical study subject during protocol treatment. An AE does not necessarily have a causal relationship with the treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

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

Serious adverse event (SAE)

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

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

Unexpected SAE
Unexpected Serious Adverse Events are those SAE's of which the nature or severity is not consistent with information in the relevant source documents.

Suspected unexpected serious adverse reaction (SUSAR)
All suspected AR's which occur in the trial and that are both unexpected and serious.

Protocol treatment period
The protocol treatment period is defined as the period from the first dose of dasatinib until 30 days following the last dose of protocol treatment or until the start of another systemic anti-cancer treatment off protocol, if earlier.

12.2 Reporting of serious adverse events
During protocol treatment all deaths, all SAE's that are life threatening and any unexpected SAE must be reported to the study coordinators by fax **within 24 hours of the initial observation of the event**. All details should be documented on the **Serious Adverse Event and Death Report**. In circumstances where it is not possible to submit a complete report an initial report may be made giving only the mandatory information. Initial reports must be followed-up by a complete report within a further 14 calendar days. All SAE Reports must be dated and signed by the responsible clinical investigator or one of his/her authorized staff members. At any time after the protocol treatment period, unexpected Serious Adverse Events that are considered to be at least suspected to be related to protocol treatment must also be reported to the study coordinators using the same procedure**, within 48 hours after the SAE or death was known to the investigator**.
The investigator will decide whether the serious adverse event is related to the treatment (i.e. unrelated, unlikely, possible, probable, definitely and not assessable) and the decision will be recorded on the Serious Adverse Event and Death Form.
The assessment of causality is made by the investigator using the following relationship description:
UNRELATED: There is no evidence of any causal relationship
UNLIKELY: There is little evidence to suggest there is a causal relationship (e.g. the event did not occur within a reasonable time after administration of the trial

medication). There is another reasonable explanation for the event (e.g. the patient's clinical condition, other concomitant treatments).
POSSIBLE: There is some evidence to suggest a causal relationship (e.g. because the event occurs within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (e.g. the patient's clinical condition, other concomitant treatments).
PROBABLE: There is evidence to suggest a causal relationship and the influence of other factors is unlikely.
DEFINITELY: There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.
NOT ASSESSABLE: There is insufficient or incomplete evidence to make a clinical judgement of the causal relationship.
The study coordinators will forward all reports within one working day of receipt to the central datamanager. The report of an SAE will be the signal for the datamanager to ask the investigator to complete and send as soon as possible all relevant information for the involved patient with details of treatment and outcome. It is of utmost importance that all SAE's (including all deaths due to any cause) are reported in a timely fashion.
Patients without a report of an SAE are implicitly considered alive without SAE. This information will be used in monitoring the incidence of SAE's, the estimation of overall survival and safety monitoring.
The manufacturer will notify the study coordinators of any new information, which becomes available during the course of the study, which may affect the overall safety profile of dasatinib. Any SUSAR's, from any source, which are considered by the study coordinator or manufacturer to be reportable to investigators, Health Authorities and Ethics Committees will be sent to clinical investigators within 12 calendar days of the study coordinator becoming aware of such events, or 5 calendar days for fatal or life-threatening reports. The study coordinator has responsibility for reporting such events to the Ethics Committee, which approved the study within the required timelines. The study coordinator will report to all applicable Health Authorities within required timelines. All events will also be reported to BMS in parallel to reporting to Ethics Committees and Health Authorities, according to agreement.

13 Endpoints

Primary:

- Clinical response rate and quality (CR, PR) at 28 weeks according to the IWCLL Working Group criteria[27]
- In case of complete responses: minimal residual disease status as assessed by flow cytometry

Secondary :

- Overall safety profile as determined by the incidence of clinically significant adverse events according to the National Cancer Institute Common Toxicity Criteria version 3.0.
- Event free survival (i.e. time from registration to induction failure, progression, relapse or death whichever occurs first), progression free survival (i.e. time from registration to disease progression, relapse or death due to CLL whichever occurs first) and disease free survival (i.e. time from CR to relapse)
- Extensive (functional) In vitro studies of dasatinib treated cells will be performed:

- mRNA Expression profile of apoptosis regulatory genes including the complete Bcl-2 family, IAP family and most important miscellaneous genes (A1/Bfl-1, AIF, APAF, APAF-XL, APOLLON, BAD, Bak, Bax1, Bax2, Bcl-2, Bcl-G, Bcl-Rmb, Bcl-W, Bcl-xL, Bid, BIK, Bim, BMF, DIABLO, Flip, FLT, HRK, IAP-1, IAP-2, LIVIN, MAP-1, Mcl-1L, Mcl-1S, NIAP, NIP-3, NIX, Noxa, PI-9, Puma, SURVIVIN, XIAP) will be measured by Multiple Ligation-dependent Probe Amplification (MLPA)[16],
- Western blot analyses to study expression of pro- and anti-apoptotic regulatory proteins (e.g. Bcl-xL, Bcl-2, Mcl-1, Bfl-1, Bim, Bid, Bax), and to study the potential target for dasatinib (Abl, Lyn, BK, potential correlation to za-70 expression etc
- In vitro synergy tests with different chemotherapeutic and immunotherapeutic drugs (fludarabine, chloorambucil, cyclophosphamide and rituximab, alemtuzumab, bendamustine).

14 Data collection

Data will be collected in the FDA proven database Oracle®. Data collected in this database are derived from the protocol and will include at least:

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

15 Monitoring

Data monitoring will be performed by a certified clinical research associate of our institute. The monitor will compare the data entered into the database with the hospital or clinic records (source documents). The nature and location of all source documents will be identified to ensure that all sources of original data required to complete the database are known to the investigational staff and are accessible for verification. At a minimum, source documentation must be available to substantiate: subject identification, eligibility and participation; proper informed consent procedures; dates of visits; adherence to protocol procedures; records of safety and efficacy parameters; adequate reporting and follow-up of adverse events; administration of concomitant medication; drug receipt/dispensing/return records; study drug administration information; date of subject completion, discontinuation from treatment, or withdrawal from the study, and the reason if appropriate.
Direct access to source documentation (medical records) must be allowed for the purpose of verifying that the data recorded in the database are consistent with the original source data.

16 Statistical considerations

16.1 Sample size justification

An overall response rate of at least 20% is considered to be clinically relevant in this poor prognosis patient group. According to the 95% confidence intervals table and normogram (original source: Pearson ES, Hartley HO [editors]: Biometrika tables for statisticians. 3rd ed. Vol 1. Cambridge Univ Press, 1966) if no partial or complete responses are observed at week 12 within the first 14 patients, there is a less than 5% chance that the real overall response will be at least 20% and the study will be terminated. Otherwise we will include 35 patients in total which would give an exact 95% CI of 8.4%-36.9% (width=28.5%) for a RR of 20%; the width of the CI increasing for RRs closer to 50% and decreasing for RRs further from 50% than 20%.[$]Clopper CJ and Pearson ES. The use of confidence or fiducial limits illustrated in the case of the binomial. Biometrika 1934; 26: 404-413.

16.2 Statistical analysis

All analyses will be according to the intention-to-treat principle.
The primary endpoint is the Clinical Response Rate. This is defined as the proportion of all treated subjects with best response of CR or PR by 28 weeks (CR or PR measured at any of the official 3 evaluation timepoints (28 days, after 2 cycles combination, or at end of protocol treatment) even if PD is established at the subsequent evaluation, will be regarded as a Response). The Clinical Response Rate will be presented with 95% confidence interval.

16.3 Interim analysis/stopping rules

At the interim analysis**,** we will formally assess toxicity and efficacy results as soon as the first 14 patients **have been followed for 12 weeks after** first day of drug administration. At this assessment it will be decided whether the study can be continued as planned:
Toxicity: In case the toxicity (>=grade 3) of dasatinib monotherapy exceeds 25% of cases **(i.e: >=4 cases)** the initial dose of dasatinib will be modified. In case the toxicity (>=grade 3) of dasatinib and fludarabine combination exceeds 25% of cases the initial dose of fludarabine will be modified. In case excess of five or more SAEs are reported with at least probable causal relationship with the treatment, the study will be closed.
Efficacy: In case no on-study responses of PR or CR during the 12 weeks following the first day of drug administration are observed amongst any of the first 14 patients treated (including any going off-study early), the study will be closed.

17 Ethics

17.1 Independent ethics committee or Institutional review board

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

17.2 Ethical conduct of the study

The study will be conducted in accordance with the ethical principles of the Declaration of Helsinki and the ICH-GCP Guidelines.

17.3 Patient information and consent

Written Informed consent of patients is required before randomization.

18 Trial insurance

In accordance with Dutch law and the W.M.O., an insurance policy, covering all participating patients, has been effected as mentioned in the patient information form.

19 Publication policy

The final publication of the trial results will be written by the Study Coordinator(s). The manuscript will be sent to a peer reviewed scientific journal.
Authors of the manuscript will include the study coordinator(s),clinicians who referred a significant number evaluable patients, and others who have made significant scientific contributions.

20 Glossary of abbreviations

(in alphabetical order)
ALAT Alanine Amino Transferase
ANC Absolute Neutrophil Count
AR Adverse Reaction
ASAT Aspartate Animo Transferase
BM Bone Marrow
BMS Bristol-Myers Squibb
CLL Chronic Lymphocytic Leukemia
CML Chronic myeloid leukemia
CR Complete Remission/response
CT Computerized Tomography
CTCAE Common Terminology Criteria for Adverse Events
DAT Direct Antiglobulin Test
F Fludarabine
Gamma-GT Gamma Glutamyl Transferase
GCP Good Clinical Practice
G-CSF Granulocyte Colony Stimulating Factor
GVHD Graft Versus Host Disease
Hb Hemoglobin
HIV Human Immunodeficiency Virus
IWCLL International Workshop on CLL
LDH Lactate Dehydrogenase
LVEF Left Ventricular Ejection Fraction
MRD Minimal Residual Disease
OI On Indication
OS Overall Survival
PB Peripheral Blood
PD Progressive Disease
Ph+ ALL Philadelphia chromosome positive Acute Lymphoblastic Leukemia
PR Partial Response
RBC Red Blood Cells
RR Response rate
SAE Serious Adverse Event
SD Stable disease
SUSAR Suspected Unexpected Serious Adverse Reaction
WBC White Blood Count
WHO World Health Organization

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Appendices

Appendix A

A IWCLL criteria for active CLL

1. Evidence of progressive marrow failure as manifested by the development of, or worsening of, anemia and/or thrombocytopenia

2. Massive (i.e., > 6 cm below the left costal margin) or progressive or symptomatic splenomegaly

3. Massive nodes (i.e., >10 cm in longest diameter) or progressive or symptomatic lymphadenopathy

4. Progressive lymphocytosis with an increase of >50% over a 2-month period, or lymphocyte doubling time (LDT) of less than 6 months. LDT can be obtained by linear regression extrapolation of absolute lymphocyte counts (ALC) obtained at intervals of two weeks over an observation period of 2-3 months; patients with initial blood lymphocyte counts of less than 30.000/µl may require a longer observation period to determine the LDT. Also, factors contributing to lymphocytosis or lymphadenopathy other than CLL (e.g., infections) should
   be excluded.

5. Autoimmune anemia and/or thrombocytopenia poorly responsive to corticosteroids or other standard therapy.

6. Presence of a minimum of any one of the following disease-related symptoms:
   - Unintentional weight loss ≥ 10% within the previous 6 months.
   - Significant fatigue (i.e., ECOG PS 2 or worse; cannot work or unable to perform usual activities).
   - Fevers of greater than 100.5° F or 38.0° C for 2 or more weeks without other
   - evidence of infection.
   - Night sweats for more than 1 month without evidence of infection.

Appendix B

B. Binet classification system

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

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

Stage C: Lymphocytosis and Hb < 6,2 mmol/l (< 10 g/dl) or platelet count < 100 x 109/l

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

Appendix C

C. Response criteria for CLL (IWCLL criteria with adjustments)

Definition of Response

While subjects will be entering the study in remission, changes in status to disease progression or even from PR to CR will be determined according to the definitions of response in the IWCLL updated NCI-WG guidelines (Hallek, 2008). For a tabular summary of all criteria of response definition in CLL patients see table.

6.2.1.1. Complete Remission (CR)

CR requires all of the following criteria as assessed at least 2 months after therapy:

1. Peripheral blood lymphocytes (evaluated by blood and differential count) below 4x109 (4000/µL)
2. Absence of significant lymphadenopathy (e.g. lymph nodes > 1.5 cm diameter).
3. No hepatomegaly or splenomegaly.
4. Absence of constitutional symptoms
5. Blood counts above the following values:
    a. Neutrophils > 1.5 x 10^9/L*
    b. Platelets > 100 x 10^9/L*
    c. Hemoglobin > 11.0 g/dL (6.8mmol/L)**

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

6. Bone marrow aspirate and biopsy should be performed at least 2 months after the final treatment and if clinical and laboratory results listed above in Section 6.2.1.1 (items 1 to 5) demonstrated that a CR has been achieved. The marrow sample should be analyzed by flow cytometry to demonstrate that the marrow is free of clonal CLL cells. Cases with residual CLL cells by conventional flow cytometry are defined as PR.

   To define a CR, the marrow sample must be at least normocellular for age, with less than 30% of nucleated cells being lymphocytes. Lymphoid nodules should be absent. If lymphoid nodules can be found, immunohistochemistry analysis should be performed to assess if nodules are comprised primarily of T-cells or lymphocytes other than CLL cells or of CLL cells. If marrow is hypocellular, repeat after 4-6 weeks, provided the blood counts have . Marrow biopsies should be compared with any pretreatment marrow. In some cases, it is necessary to postpone the marrow biopsy until after all other criteria to define a CR have been satisfied, however, this time interval should not exceed 6 months after treatment.

Subjects who fulfill all the preceding criteria for CR but have persistent anemia, thrombocytopenia or neutropenia, apparently unrelated to CLL but related to drug toxicity, should be considered as CR with incomplete bone marrow recovery.

6.2.1.2. Partial Remission (PR)

PR is defined by the criteria described in at least two of the items 1, 2, and /or 3 (if abnormal prior to therapy), as well as one or more of the features listed in item 4. To define a PR at least one of these parameters needs to be documented for a minimal duration of 2 months. Constitutional symptoms persisting for more than 1 month should also be documented.

1. A decrease in the number of peripheral blood lymphocytes by 50% or more from the value prior to therapy

2. Reduction in lymphadenopathy as defined by:

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

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

4. The blood count should show at least one of the following results:

- Neutrophils more than 1.5 x 10^9/L (1500/µL) *
- Platelet counts greater than 100 x 10^9/L (100,000/µL) or 50% improvement over baseline*
- Hemoglobin greater than 110g/L (11.0g/dL, 6.8mmol/L) or 50% improvement over baseline **

**without need for exogenous growth factors*

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

6.2.1.3. Progressive Disease (PD)

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

1. Lymphadenopathy. Progression of lymphadenopathy, if one of the following is observed:

- Appearance of new lesion such as enlarged lymph nodes (>1.5cm), splenomegaly, hepatomegaly or other organ infiltrates
- An increase by 50% or more in greatest determined diameter of any previous site.

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

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

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

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

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

- After treatment: The progression of any cytopenia (unrelated to autoimmune cytopenia), as documented by a decrease of Hb levels by more than 20g/L (2 g/dL) or to less than 100g/L (10g/dL), or by a decrease of platelet counts by more than 50% or to less than 100 x 10^9/L (100.000/µL), which occurs at least 3 months after treatment, defines disease progression, if the marrow biopsy demonstrates an infiltrate of clonal CLL cells.

6.2.1.4. Stable Disease (SD)

Subjects who have changed from a CR or a PR, but who have not exhibited PD, may be considered to have SD.

Appendix D

D. Toxicity criteria

The grading of hematological toxicity will be done using the IWCLL guidelines as follows:

GRADE#	DECREASE IN PLATELETS* OR HB° (NADIR) FROM PRETREATMENT VALUE (%)	ABSOLUTE NEUTROPHIL COUNT/ML§ (NADIR)
0	No chance to 10%	≥ 2,000
1	11%-24%	≥ 1,500 and < 2,000
2	25%-49%	≥ 1,000 and < 1,500
3	50%-74%	≥500 and < 1,000
4	≥75%	< 500

* Platelet counts must be below normal levels for grades 1-4. If, at any level of decrease the platelet count is <20.000/µL, this will be considered grade 4 toxicity, unless a severe or life threatening decrease in the initial platelet count (e.g., 20.000/µL) was present pretreatment, in which case the patient is not evaluable for toxicity referable to platelet counts.
° Hb levels must be below normal levels for grades 1-4. Baseline and subsequent Hb determinations must be performed before any given transfusions. The use of erythropoietin is irrelevant for the grading of toxicity, but should be documented.
Grades: 1, mild; 2, moderate; 3, severe; 4, life-threatening; 5, fatal. Death occurring as a result of toxicity at any level of decrease from pretreatment will be recorded as grade 5.
§ If the absolute neutrophil count (ANC) reaches less than 1,000/µL, it should be judged to be grade 3 toxicity. Other decreases in the white blood cell count, or in circulating granulocytes, are not to be considered, since a decrease in the white blood cell count is a desired therapeutic end point. A gradual decrease in granulocytes is not a reliable index in CLL for stepwise grading of toxicity. If the ANC was less than 1,000/µL prior to therapy, the patient is not evaluable for toxicity referable to the ANC. The use of G-CSF is irrelevant for the grading of toxicity, but should be documented.

The grading of toxicity and adverse events will be done using the NCI Common Terminology Criteria for Adverse Events, CTCAE version 3.0, published December 12, 2003. A complete document (72 pages) may be downloaded from the following site:
http://ctep.info.nih.gov/reporting/ctc.html

Appendix E

E. ZUBROD-ECOG-WHO Performance Status Scale

0 Normal activity
1 Symptoms, but nearly ambulatory
2 Some bed time, but to be in bed less than 50% of normal daytime
3 Needs to be in bed more than 50% of normal daytime
4 Unable to get out of bed