Study ITCC 021/I-BFM-SG study “bortezomib in relapsed ALL”

Final protocol: version 1.2, version date 08-12-2010
(including non-substantial amendment 2)

Bortezomib (Velcade®):
A feasibility and phase II study in childhood relapsed acute lymphoblastic leukemia

A study performed in the setting of

Innovative Therapies for Children with Cancer (ITCC)

&

International BFM Study Group

Sponsor:
Erasmus MC
Dr. Molewaterplein 60
3015 GD Rotterdam
the Netherlands

EudraCT number 2009-014037-25
**APPROVAL OF STUDY PROTOCOL**

Study Title: **Bortezomib (Velcade®): A feasibility and phase II study in childhood relapsed acute lymphoblastic leukaemia**

The following persons declare their consent with the study protocol:

**Principal Investigator:**

Prof. dr. G.J.L. Kaspers  
08-12-2010  
(Date, dd/mm/yr)  
(signature)

**Sponsor, on behalf of Erasmus MC:**

Assoc. Prof. dr. C.M. Zwaan  
08-12-2010  
(Date, dd/mm/yr)  
(signature)

Herewith I confirm that I read the study protocol carefully and declare my consent with it. I will treat and examine the patients in accordance with the study protocol, the national applicable laws, the international guidelines on good clinical practice (ICH-GCP) and the declaration of Helsinki.

**Local Investigator:**

_________________  ____________________  ________ ________

(name)  
(date, dd/mm/yr)  
(signature)

**Hospital site:**

_______________________________

(name)
Abbreviations

AE  adverse event
AIEOP  Associazione Italiana Ematologia ed Oncologia Pediatrica
ALAT/ALT  alanine transaminase
ALL  acute lymphoblastic leukemia
AML  acute myeloid leukemia
ASAT/AST  aspartate transaminase
AUC  area-under the-curve
BM  bone marrow
BMA  bone marrow aspiration
BM M1/2/3  bone marrow with <5%, 5-15% or ≥15% leukemic blasts, respectively
BTZ  bortezomib
CCG  Children's Cancer Group (USA)
CCR  continued complete remission
Cmax  maximum plasma concentration
CMV  cytomegalo virus
CNS  central nervous system
COG  Children’s Oncology Group (USA)
CR  complete remission
CRF  case report form
CRi  complete remission with incomplete peripheral blood regeneration
CRP  C-reactive protein
CSF  cerebrospinal fluid
CTC  common terminology criteria
CV  curriculum vitae
DCOG  Dutch Childhood Oncology Group
Dex  dexamethasone
DFS  disease-free survival
DLT  dose-limiting toxicity
DS  Down syndrome
EBV  Ebstein-bar virus
ECTC  Early Clinical Trial Consortium
EFS  event-free survival
Emax  maximum effect
EMEA  European Medicinal Agency
FAB  French American British Cooperative Group
FDA  Food and Drug Administration
FISH  fluorescent in situ hybridization
FRALLE  French ALL study group
GCP  good clinical practice
GGT  gamma glutamyl transferase
HSV  herpes simplex virus
I-BFM-SG  international Berlin-Frankfurt-Münster Study Group
ICH-GCP  international conference on harmonization good clinical practice
IEC  international ethics committee
IRB  international review board
ITCC  Innovative Therapies for Children with Cancer
Ith  intrathecal
### Synopsis/summary (references: see protocol)

<table>
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<th>MAIN STUDY OBJECTIVE</th>
<th>1. Determine the antileukemic activity of combination chemotherapy including bortezomib as reinduction therapy in childhood relapsed/refractory ALL</th>
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| ADDITIONAL STUDY OBJECTIVES | 2. Determine the feasibility and safety of combining bortezomib with conventional combination chemotherapy in children and adolescents with relapsed/refractory ALL  
3. Evaluate bortezomib levels and proteasome inhibition in cerebrospinal fluid, bone marrow and peripheral blood in patients with relapsed/refractory ALL, and assess the relationship to the efficacy and toxicity of bortezomib |
| STUDY RATIONALE | Childhood relapsed/refractory ALL remains one of the most significant problems in pediatric oncology, and innovative therapy is required. ALL is the most frequent type of childhood cancer, relapses in about 25% of newly diagnosed patients, and can still be cured in overall 40% of relapsing patients. Early first relapsed ALL, T-cell relapsed ALL, treatment-refractory first relapsed ALL and second relapsed ALL have a much worse prognosis with less than 10% probability of long-term survival. It is unlikely that conventional chemotherapy will bring major improvements, because relapsed ALL is characterized by increased cellular drug resistance in general, and by increased resistance to glucocorticoids in particular (Haarman 2003). Therefore, novel drugs with a new mechanism of action are needed. Bortezomib is such an innovative drug, being a proteasome inhibitor. Bortezomib (PS-341, Velcade®) is a dipeptidyl boronic acid, and has antitumor activity because of the inhibition of the 26S proteasome. It targets the chymotryptic-like site of the proteasome, and thereby interferes with the ubiquitin-proteasome pathway. This pathway affects both tumor cell proliferation and cell survival in several ways, among which is inhibition of NFκB activity. Preclinical studies showed antileukemic activity of bortezomib as single agent, and in their so-called pediatric preclinical testing program, it had highest activity towards ALL cell lines in preclinical and in vivo models (Houghton 2008). Bortezomib appeared to be synergistic in combination with several other drugs such as glucocorticoids and anthracyclines (Horton 2006, Mitsiades 2003). Clinically, bortezomib is FDA and EMEA approved for its use in multiple myeloma. In that setting, several studies suggest synergism between bortezomib and dexamethasone, which is highly relevant in the field of relapsed ALL (Hideshima 2001, Richardson 2003). It appeared feasible to combine bortezomib with several other drugs as well, such as anthracyclines, cytarabine and vincristine, in adults (Attar 2008, Jang 2007). In children, a single agent phase I study (ADVL0015) in refractory solid tumors has been reported by the Children's Oncology Group (COG; Blaney 2004), and so is the phase I study in children with recurrent or refractory leukemias (COG study ADVL0317) (Horton 2007). The ADVL0015 study showed no responses in 15 patients, but bortezomib was well-tolerated and the recommended phase II dose is 1.2 mg/m²/dose twice weekly for 2 weeks followed by 1 week rest in... |
solid tumors. In leukemias, a higher dose seems justified because of clinical experience in adults (dose-limiting toxicity at 1.5 mg/m²/dose twice weekly for 4 weeks) and improved inhibition of proteasome activity at higher doses. The COG ADVL0317 study showed no single agent activity in terms of CR or PR in 10 patients (both ALL and AML). Two patients experienced DLTs at the 1.7 mg/m² dose level, including altered mental status in one patient and in the other patient with febrile neutropenia a combination of Grade 4 hypotension, Grade 3 renal failure, and Grade 5 hypoxia. The MTD for bortezomib in pediatric leukemia patients was 1.3 mg/m², a dose at which NFkB activation was inhibited. The COG will further study bortezomib in both ALL and AML. There is a concern for bortezomib-induced peripheral neuropathy, which is relevant in ALL in view of the frequent use of vincristine. Since it is essentially unknown if bortezomib can be combined safely with vincristine in children, this study will provide important information on that issue. However, preliminary data from the so-called USA TACL consortium study on bortezomib combined with chemotherapy in relapsed ALL suggest that bortezomib can be combined with drugs such as dexamethasone, vincristine, asparaginase and doxorubicin, without excess toxicity (Messinger 2008). Bortezomib levels in CSF have not been studied previously and will be determined in this study in all patients.

In conclusion, both in vitro data and in vivo data in other disease entities strongly support the study of the combination of bortezomib with a glucocorticoid in ALL. In order to demonstrate antileukemic activity of that combination, its use as reinduction chemotheraphy in refractory or relapsed ALL will be most informative, which is the main purpose of this study. In reinduction therapy, vincristine is usually administered as well, and therefore will be included in this study. Thus, this study will also demonstrate if bortezomib and vincristine can be administered simultaneously to a steroid backbone without excess of neurotoxicity. Preliminary results in children (Messinger 2008) and several studies in adults demonstrate that bortezomib can be combined at its MTD of 1.3 mg/m² in combination chemotherapy.

**GENERAL STUDY DESIGN**

Multicenter, multinational, open label, comparative and randomised phase II study on the antileukemic activity of bortezomib with conventional combination chemotherapy in relapsed/refractory ALL in children and adolescents. The study will include one cohort of patients with relapsed/refractory ALL, with treatment guidelines for all patients for 3 weeks. Thereafter, bortezomib may be repeated in combination with the same conventional chemotherapy for patients with a good initial response to reinduction therapy, while treatment of all other patients will be left at the discretion of the patient, parents and the responsible clinician. Standard reinduction chemotherapy will consist of 2 weeks of dexamethasone plus vincristine given twice, plus intrathecal administration of methotrexate. In addition, all patients will be treated with one cycle of bortezomib, consisting of 4 doses in 2 weeks. However, they will be randomised 1:1 in 2 arms, group A getting “early” bortezomib, starting at day 1 of therapy, and group B getting...
“late” bortezomib, starting at day 8. Randomization will be stratified for the number of circulating leukemic blasts at inclusion in this study protocol. This design allows demonstrating an additional antileukemic effect of bortezomib when added to dexamethasone, after 1 week of therapy, measured by a reduction in ALL cells in peripheral blood and bone marrow. In addition, this design allows comparing the toxicity of limited (group A) and more extended (group B) overlap of administrations of bortezomib and vincristine. A total of 24 patients must be randomised and be fully evaluable in order to prove additional antileukemic effect of bortezomib when added to dexamethasone. Bortezomib will be available for further use in case of patients with a favourable early treatment response, i.e. bone marrow M1 or M2 (<15% blasts) 3 weeks after start of reinduction therapy, in combination with the same combination chemotherapy.

### STUDY POPULATION

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<td>- Age between 6 months and 19 years</td>
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<td>- Patients with a second or subsequent relapsed ALL</td>
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<td>- Patients with first relapsed ALL after prior allogeneic stem cell transplantation in first complete remission</td>
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<td>- Patients with refractory first relapse of ALL, as defined by the ALL relapse protocol these patientst were enrolled in before</td>
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<td>- Circulating leukemic blasts of at least 100/ul peripheral blood (i.e. at least 0.1x10^9/l)</td>
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<td>- Patients must take adequate contraceptives when of childbearing potential</td>
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<td>- Written informed consent</td>
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<th>Exclusion criteria:</th>
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<td>- Relapse not involving the bone marrow</td>
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<td>- Symptomatic CNS leukemia</td>
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<td>- Active uncontrolled infection</td>
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<td>- Performance status (Lansky or Karnofsky score) of 60% or less</td>
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<td>- Life expectancy of less than 6 weeks</td>
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<td>- Existing peripheral neuropathy NCI grade 2 or higher</td>
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<td>- Presence of acute diffuse infiltrative myocardial and/or pericardial disease</td>
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<td>- Clinical signs of cardiotoxicity</td>
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<td>- Previous allogeneic stem cell transplantation within 100 days</td>
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<td>- Pregnant or breastfeeding</td>
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<td>- Other contra-indications for chemotherapy, including no recovery from previous treatment</td>
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<td>- Previous exposure to bortezomib</td>
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<td>- Other experimental or conventional antileukemic treatment within 7 days from start of bortezomib</td>
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<td>- Allergy to boron and its metabolites</td>
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<td>- Concomitant anti-leukemic therapy other than according to this protocol</td>
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### DETAILED STUDY DESIGN

| The patients will be treated with bortezomib (1.3 mg/m²/dose twice weekly (days 1 and 4 of each week) for a total of 2 weeks. Group A will get bortezomib on days 1, 4, 8 and 11, while group B will get it on days |

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8, 11, 15 and 18. Bortezomib will be administered as i.v. push. In addition, patients will get standard reinduction chemotherapy consisting of dexamethasone and vincristine. Dexamethasone will be given from day 1 onwards for 2 weeks at 10 mg/m\(^2\)/day in 3 doses, orally. In addition, patients will receive vincristine on days 8 and 15, at 1.5 mg/m\(^2\)/dose (maximum 2.0 mg/dose), in a 60 minutes i.v. infusion (which is potentially less neurotoxic than i.v. push).

Finally, intrathecal methotrexate will be given on days 1 and 22 with age-adjusted dosing (figures 1 and 2; <2 yrs 8 mg, 2 yrs 10 mg, 3 yrs and above 12 mg).

Further therapy for good responders (bone marrow (BM) M1 or M2 on day 22) may consist of an additional cycle of bortezomib, but this decision is left to the discretion of the treating physician. A next cycle should start 11 days after the previous administration of bortezomib. Then, bortezomib must again be combined with 2 weeks of dexamethasone (same dose and schedule) and vincristine twice (same dose and schedule; figures 3 and 4). Such cycles may be repeated if justified by efficacy and (lack of) toxicity, which again is left to the discretion of the treating physician.

Patients with a BM M3 on day 22 and/or those with progressive disease will go off study.

**DRUGS**

Bortezomib is the investigational medicinal product (IMP) in this study and will be supplied free-of-charge by J&J. EMEA issued a marketing authorization valid throughout the European Union on 26 April 2004. The full summary of product characteristics is available at [http://www.emea.europa](http://www.emea.europa) under ‘human medicines/EPARs’. All other drugs will be derived from commercially available stock as part of regular treatment.

**STUDY DURATION AND TIME SCHEDULE**

A total of 24 patients will be randomised (12 patients in each arm) to provide the endpoints. Time of accrual will depend on the number of centers that will participate, which is specified in the procedure manual. However, we will aim to complete accrual within 1.5 years from the start of the study. The study is planned to open Q4 of 2009 in selected single centers, and to be open in 2010 in all centers.

**OBJECTIVES**

**Primary endpoint:**
1. Antileukemic activity of bortezomib when added to dexamethasone and vincristine and intrathecal methotrexate, as determined by the absolute peripheral blood (PB) blast count on day 8 of treatment. Morphology will be centrally reviewed.

**Secondary endpoints**
1. Antileukemic activity of bortezomib when added to demathasone and vincristine and intrathecal methotrexate, as determined by the absolute bone marrow (BM) blast percentage on day 8, and BM and PB analysis on day 22 of treatment, in absolute numbers and expressed as an M1, M2 or M3 marrow (<5%, 5-15%, 15% or above respectively). Morphology will be centrally reviewed.
<table>
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<th>Exploratory end points</th>
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<tr>
<td>1. Establish the pharmacokinetics of bortezomib in children. For this purpose, levels of bortezomib will be measured in bone marrow, peripheral blood and CSF samples obtained at several time-points as clarified in the protocol, limited to cycle one and the first and fourth administration of bortezomib.</td>
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<td>2. Explore the pharmacodynamics of bortezomib, in particular by proteasome inhibition as measured in BM and PB. This inhibition will be correlated with pharmacokinetic data and clinical response to bortezomib. Time-points of sampling will be detailed in the protocol.</td>
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**SAFETY ASSESSMENT AND GUIDELINES FOR DOSE REDUCTION OF BORTEZOMIB**

Toxicity monitoring will be done as usual, applying the most recent NCI CTC (currently CTCAEv3.0, December 12, 2003). All adverse events must be forwarded to the sponsor and principal investigator. For SAE’s an expedited procedure is in place.

**Neurotoxicity** will influence the administration of bortezomib as follows:
- Grade 1: no consequences
- Grade 2 and grade 3: withhold bortezomib until resolution to grade 1 or grade 0, and then resume bortezomib at 1.0 mg/m²
- Grade 4: stop bortezomib permanently

**Hematological toxicity** that is expected in these patients, including febrile neutropenia and infections, will not influence administration of bortezomib, unless the patient is in complete remission during follow-up cycles.

**Non-hematological toxicity**, other than neurotoxicity, will influence administration of bortezomib as follows:
- Grade 1 and 2: no consequences
- Grade 3: withhold bortezomib until resolution to grade 2 or lower, and then resume bortezomib at 1.0 mg/m².
- Grade 4: stop bortezomib permanently

Exceptions are nausea, vomiting and alopecia, which will not influence bortezomib administration, even in case of grade 4 toxicity.

If bortezomib is administered at 1.0 mg/m², and increased toxicity
again occurs, follow the guidelines above but then restart (if possible) at a further reduced dose of 0.7 mg/m²/dose. If that does not result in less toxicity, bortezomib administration should be stopped. Alternatively, in case of acceptable toxicity as defined above after bortezomib at 1.0 mg/m²/dose, subsequent doses of bortezomib may again be 1.3 mg/m²/dose.

STATISTICAL CONSIDERATIONS
This study design was chosen bearing in mind the apparent lack of effectiveness of single-agent bortezomib in heavily pretreated acute leukemias, but also the clear indications of synergistic interactions between bortezomib with other chemotherapeutic drug such as dexamethasone. Historical data on single agent dexamethasone for one week with one intrathecal administration of triple cytostatics on day 1 are available (data on file, kindly provided by P. Rohrlch, FRALLE). This shows in a cohort of 194 children with relapsed ALL, a decrease in BM blast percentage from median 80 (range, 5-100)% to 40 (0-100)% after 1 week. Similarly, PB blasts count decreased from median 538 (range, 0-300,000) blasts/µl to 22 (0-27,552), or mean 893 (SD 363) to 530 (SD) after 1 week. The percentage of “poor dexamethasone responders” was 14.4% in the total cohort of 160 patients that could be evaluated for this parameter, and 19.2% in the group of patients (n=120) with at least 1000 blasts/µl PB at start of treatment (ie, potentially poor responders).

Statistical comparison of response and toxicity data in both arms will be done using conventional tests, especially Pearson chi-square statistics for differences in the distribution of categorical variables (Fisher exact test in case of sparse data), and the Mann-Whitney-U test for differences in continuous variables.

The number of 12 patients in each group provides a power of 80% to detect a statistically significantly lower absolute number of circulating leukemic blasts on day 8 of treatment in patients exposed to bortezomib (“bortezomib early”) as compared to patients not yet exposed to bortezomib (“bortezomib late”) of at least 30% (2-sided test, alpha <0.05). In addition, based on calculations of 95%-confidence intervals, these numbers will allow predicting an incidence of <50% grade III/IV toxicity if <8 out of 24 patients suffer such toxicity. In case of early dropout before the day 8 time-point is reached, either due to early death, excess toxicity as described above, or as decided by patient/parents, patients will be replaced. However, the final study report will report on all patients that enrolled in the study and who received at least one dose of scheduled treatment.

FUTURE PERSPECTIVES
In case of positive results obtained in this feasibility study, bortezomib will be further investigated in the setting of the future international study on the treatment of first relapsed ALL. The following findings will be considered positive in this context. First, the feasibility to administer at least one cycle of bortezomib in at least 75% of patients. Second, an actual incidence of grade III/IV toxicity related to bortezomib in less than 8 out of 24 patients, excluding alopecia, nausea and vomiting, and haematological toxicity including febrile neutropenia and infections. Third, a significantly improved initial treatment response after 1 week of treatment with bortezomib, and/or a higher rate
of patients with a BM M1 result on day 22 with bortezomib. All three findings will be required to justify further study of bortezomib in relapsed ALL.
Treatment schedule: figure 1 (group A, “early” bortezomib)

**Study ITCC 021 bortezomib in Relapsed ALL - group A: bortezomib early -**

- Dex
- VCR
- BZM
- Ith mtx

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PB: x x x x x
BMA: x x x x x
LP: x x x x x

Treatment schedule: figure 2 (group B, “late” bortezomib)

**Study ITCC 021 bortezomib in Relapsed ALL - group B: bortezomib late -**

- Dex
- VCR
- BZM
- Ith mtx

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PB: x x x x x
BMA: x x x x x
LP: x x x x x
Full reinduction schedule for initially good responding patients: figure 3 ("early" bortezomib)

Extended reinduction schedule for “early bortezomib” and initially good responding patients

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Weeks: 1 2 3 4 5 6 7
Days: 1 8 15 22 29 36 43

PB: x x x x x
BMA: x x x x x
LP: x x x x x

Full reinduction schedule for initially good responding patients: figure 4 ("late" bortezomib)

Extended reinduction schedule for “late bortezomib” and initially good responding patients

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Weeks: 1 2 3 4 5 6 7
Days: 1 8 15 22 29 36 43

PB: x x x x x
BMA: x x x x x
LP: x x x x x
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11.1 Known Anticipated Risks of bortezomib (Velcade®)
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Bortezomib (Velcade®) and childhood relapsed ALL

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All participating centers will be mentioned in the separate procedure manual.
2. Objectives

Main study objective
Determine the antileukemic activity of combination chemotherapy including bortezomib as reinduction therapy in childhood relapsed/refractory ALL

Secondary study objectives
1. Determine the feasibility and safety of combining bortezomib with conventional combination chemotherapy in children and adolescents with relapsed/refractory ALL
2. Evaluate bortezomib levels and proteasome inhibition in cerebrospinal fluid, bone marrow and peripheral blood in patients with relapsed/refractory ALL, and assess the relationship to the efficacy and toxicity of bortezomib
3. Background/rationale

3.1 Relapsed/refractory ALL
Childhood relapsed ALL remains one of the most significant problems in pediatric oncology. ALL is the most frequent type of childhood cancer, relapses in about 25% of newly diagnosed patients, and can be cured in overall 40% of relapsing patients. Early first relapsed ALL, T-cell relapsed ALL and second relapsed ALL have a much worse prognosis with less than 10% probability of long-term survival. It is unlikely that conventional chemotherapy will bring major improvements, because relapsed ALL is characterized by increased cellular drug resistance in general, and by increased resistance to glucocorticoids in particular (Haarman 2003). Therefore, novel drugs with a new mechanism of action are needed. Bortezomib is such a drug, and has been shown to be synergistic in combination with several drugs, including dexamethasone.

3.2 Bortezomib
Introduction
Bortezomib is an innovative drug, being a proteasome inhibitor. Bortezomib (PS-341, Velcade®) is a dipeptidyl boronic acid, and has antitumor activity because of the inhibition of the 26S proteasome. It targets the chymotryptic-like site of the proteasome, and thereby interferes with the ubiquitin-proteasome pathway. This pathway affects both tumor cell proliferation and cell survival in several ways, among which is inhibition of NFκB activity (Vink 2006). Inhibition of NFκB activity was demonstrated to sensitize leukemic cells to glucocorticoids (Cloos 2004, Oerlemans 2007). Preclinical studies showed antileukemic activity of bortezomib as single agent, and in their so-called pediatric preclinical testing program, it had highest activity towards ALL cell lines in preclinical and in vivo models (Houghton 2008). Bortezomib appeared to be synergistic in combination with several other drugs such as glucocorticoids and anthracyclines (Horton 2006, Mitsiades 2003). Clinically, bortezomib is FDA and EMEA approved for its use in multiple myeloma. In that setting, several studies suggest synergism between bortezomib and dexamethasone, which is highly relevant in the field of relapsed ALL (Hideshima 2001, Richardson 2003). It appeared feasible to combine bortezomib with several other drugs as well, such as anthracyclines, cytarabine and vincristine, in adults (Attar 2008, Jang 2007).

Pre-clinical and nonclinical studies
Bortezomib has a novel pattern of cytotoxicity in National Cancer Institute (NCI) in vitro and in vivo assays (Adams 1999). In addition, bortezomib has shown cytotoxic activity in a variety of xenograft tumor models, both as a single agent and in combination with chemotherapy and radiation (Steiner 2001, Teicher 1999, Cusack 2001, LeBlanc 2002, Pink 2002). Notably, bortezomib induces apoptosis in cells that over express bcl-2, a genetic trait that confers unregulated growth and resistance to conventional chemotherapeutics (Williams 2003).

Pharmacokinetic/Pharmacodynamic Profile
Pharmacokinetic (PK) and pharmacodynamic (PD) studies were conducted in the rat and cynomolgus monkey. Upon intravenous (i.v.) bolus administration, bortezomib displays a rapid distribution phase ($t_{1/2a} < 10$ minutes) followed by a longer elimination phase ($t_{1/2b}$ 5 to 15 hours). Bortezomib has a large volume of distribution (range 5 to 50 L/kg). The plasma PK profile is well described by a 2-compartment model.
The PD action of bortezomib is well established and can be measured through an ex vivo assay (20S proteasome activity) (Lightcep 2000). This assay was used to determine the duration of drug effect in lieu of the PK data in the early preclinical toxicology studies as well as to set a guide for dose escalation in humans.

Following dosing with bortezomib in the rat and cynomolgus monkey, proteasome inhibition in peripheral blood had a half-life less than 24 hours, with proteasome activity returning to within 20% of pretreatment baseline within 48 hours in monkeys and within 48 to 72 hours in rats after a single dose of bortezomib. Further, intermittent but high inhibition (>70%) of proteasome activity was better tolerated than sustained inhibition.

### Toxicology

Single-dose i.v. toxicity studies were conducted with bortezomib in the mouse, rat, dog, and monkey to establish the single-dose maximum tolerated dose (MTD). The MTD were 0.25 mg/kg (1.5 mg/m^2) and 0.067 mg/kg (0.8 mg/m^2) in the 2 most sensitive species, rat and monkey, respectively.

Repeat-dose multicycle toxicity studies of 3 and 6 months in the rat and 9 months in the monkey, each with 8-week recovery periods, were conducted to characterize the chronic toxicity of bortezomib when administered by the clinical route and regimen of administration. The MTD in the 6-month rat study was 0.10 mg/kg (0.6 mg/m^2) and the key target organs were the gastrointestinal (GI) tract, hematopoietic, and lymphoid systems. The MTD in the 9-month monkey study was 0.05 mg/kg (0.6 mg/m^2) and the key target organs were the GI tract, hematopoietic and lymphoid systems, peripheral nervous system, and kidney. Full or partial reversibility was observed for each of the toxicities described to date.

In general, the nature of the toxicity of bortezomib is similar across species, and target organs of toxicity in animals have been largely predictive of human toxicity. The toxicity of bortezomib in animals is characterized by a steep dose-response with mortality seen at dosages above the MTD. The cause of death at acutely lethal dosages is considered to be related to cardiovascular effects of hypotension and vascular changes with secondary bradycardia and the cause of death in long-term studies has been attributed to GI or hematologic toxicity. The pharmacologic effects of bortezomib on the cardiovascular system have been extensively characterized. It has been demonstrated that indirect effects on cardiovascular function occur only at acutely lethal dosages and are abrogated by routine supportive care.

Additional detailed information regarding the nonclinical pharmacology and toxicology of bortezomib may be found in the Investigator Brochure (Velcade, Investigator Brochure).

### Phase I and phase II trial data in adults

#### Human Pharmacokinetics/Pharmacodynamics

The clinical pharmacology characterization of bortezomib has been determined from Phase 1 studies in subjects with solid tumors and hematologic malignancies, and confirmed in Phase 2 studies in subjects with multiple myeloma.

In subjects with solid tumors, the mean terminal elimination half-life of bortezomib was 9.06 hours. The mean maximum plasma concentration (C_{max}) and area under the curve (AUC_{last}) after the first dose (1.3 mg/m^2) of bortezomib were 173 ng/mL and 48.2 h*ng/mL, respectively. The average clearance of bortezomib following a single 1.3 mg/m^2 dose was 49.0 L/h. However, the AUC_{last} increased to 81.0 h*ng/mL after the third dose in the first cycle as a result of a reduction in systemic clearance to 28.2 L/h with a consequent increase in elimination half-life to 16.6 hours. Clinical experience has shown that the change in clearance does not result in overt toxicity from accumulation in this multidose regimen in humans.

In subjects with advanced malignancies, the maximum PD effect (inhibition of 20S activity) occurred within 1 hour post dose. At the therapeutic dose of 1.3 mg/m^2 in subjects with multiple myeloma, the mean proteasome inhibition at 1-hour post dose was approximately 61%.
The time course of proteasome inhibition in subjects is characterized by maximum inhibition observed within the first hour after administration, followed by partial recovery of proteasome activity over the next 6 to 24 hours to within 50% of the pretreatment activity. On the Day 1, 4, 8, and 11 schedule, variable (10% to 30%) levels of proteasome inhibition have been observed at next scheduled dosing. In theory, this advantage allows cells to recover proteasome activity for normal cellular housekeeping functions between doses.

The relationship between bortezomib plasma concentrations and proteasome inhibition can be described by a maximum effect (E_{max}) model. The E_{max} curve is initially very steep, with small changes in plasma bortezomib concentration over the range of 0.5 to 2.0 ng/mL relating to large increases in the percent inhibition (0% to 60%). After that, a plateau occurs where marginal increases of proteasome inhibition are observed in spite of large changes in plasma bortezomib concentrations.

**Efficacy/Safety Studies**

The clinical pharmacology characterization of bortezomib has been determined from Phase 1 studies in subjects with solid tumors and hematologic malignancies, and confirmed in Phase 2 and 3 studies in subjects with multiple myeloma.

In Phase 1 clinical studies, antitumor activity was reported in subjects with NHL, multiple myeloma, Waldenström’s macroglobulinemia, squamous cell carcinoma of the nasopharynx, bronchoalveolar carcinoma of the lung, renal cell carcinoma, and prostate cancer.

The safety and efficacy of bortezomib in subjects with multiple myeloma were investigated in two Phase 2 clinical studies, M34100-024 (subjects with first relapse) and M34100-025 (subjects with second or greater relapse and refractory to their last prior therapy). In the M34100-025 SUMMIT study, 202 heavily pre-treated subjects with refractory multiple myeloma after at least 2 previous treatments received bortezomib, 1.3 mg/m^2 on Days 1, 4, 8, and 11 of a 21-day treatment cycle. The European Group for Blood and Marrow Transplant response criteria, as described by Blade,^{12} were used to determine disease response. Complete responses (CR) were observed in 10% of subjects, including some cases in which monoclonal paraprotein (M-protein) was not detected by immunofixation. Partial response (PR) or CR was observed in 27% of subjects, and the overall response rate (CR, PR, and minor response combined) was 35%. Seventy percent of subjects experienced an M-protein reduction of 25% or more.

In a Phase 1 dose-escalation study of bortezomib in subjects with refractory hematologic malignancies, a subject with previously treated refractory follicular lymphoma who received bortezomib 1.38 mg/m^2/dose had a PR (50% shrinkage of mediastinal and intra-abdominal adenopathy) that was sustained for more than 12 months off therapy (Orlowski 2002, Data on file). Preliminary clinical data from two Phase 2 studies of bortezomib monotherapy for the treatment of indolent and aggressive NHL provide further indications of anti-tumor activity. In one study, 6 of 8 evaluable subjects with follicular lymphoma and 2 of 7 subjects with mantle cell lymphoma had a CR or PR (O’Connor 2005). In a separate study of heavily-pretreated NHL subjects, 1 of 8 evaluable subjects with diffuse large cell lymphoma had a PR, and 7 of 11 evaluable subjects with mantle cell lymphoma had a CR or PR (Goy 2005). In both Phase 2 studies, bortezomib has been well tolerated and the side effects observed have been similar to those seen in other bortezomib studies.

Studies using bortezomib as monotherapy and in combination with other chemotherapy agents are continuing. Further details on clinical studies of bortezomib may be found in the Investigator Brochure (VELCADE, Investigator Brochure) and in preliminary and mature clinical data presentations (Cusack 2001, Papandreou 2004, Aghajanian 2002, Jagannath 2004, Richardson 2003, Williams 2003a).
Potential Risks of bortezomib

The most common side effects of bortezomib (i.e., incidence ≥30%) observed in patients are weakness, fatigue, and general discomfort; gastrointestinal (GI) effects such as constipation, diarrhea, nausea, vomiting, and loss of appetite, which may result in dehydration and/or weight loss; fever; peripheral neuropathy (including painful sensations or numbness and tingling in hands and feet that may not get better after discontinuation of bortezomib); thrombocytopenia that may increase the risk of bleeding; and anemia.

Very common side effects of bortezomib (i.e., incidence 10–29%) observed in patients are neutropenia; abdominal pain; symptoms of flu and other respiratory tract infections (chills, sore throat, runny nose); aches and pains in muscles and joints; skin rash with itching and redness; hypotension; fluid retention; dizziness; cough; dyspnea; lung infections including pneumonia; headache; blurred vision; changed sense of taste; herpes zoster that occasionally results in persistent local pain; heartburn; insomnia; and anxiety.

Common side effects of bortezomib (i.e., incidence 1–9%) observed in patients are changes in heart rate and heart rhythm possibly with the feeling of confusion, light-headedness, dizziness, fainting, shortness of breath, and/or chest discomfort (Rarely, potentially life threatening heart rhythm abnormalities have been observed.); new or worsening heart failure or decreased heart function (Patients with heart failure or other diseases that put them at risk of developing heart failure should be closely monitored.); vertigo, dizziness and/or tinnitus; conjunctivitis; retinal hemorrhage; painful sores of the mouth and/or throat that may make swallowing difficult; severe bleeding, including bleeding in the stomach and intestines associated with low platelet counts, and blood clotting changes that may result in bloody diarrhea and/or bloody vomit (preventative platelet transfusions to reduce the risk of bleeding may be necessary); deterioration in kidney function; infections of the bladder, sinuses, throat, stomach and intestines; yeast infections in the mouth, throat, fingernails and vagina; sepsis; hypoglycemia and hyperglycemia (Patients on oral antidiabetic agents may require close monitoring of their blood sugar levels.); convulsions; hematuria; hemoptysis; epistaxis; orthostatic hypotension; confusion; and depression.

Uncommon side effects of bortezomib (i.e., incidence <1%) observed in patients are injection site reaction (pain, redness, and swelling); hearing impairment; small bowel obstruction that may resolve on its own and not require surgery; skin infection; deterioration in liver function including abnormal liver function tests, hepatitis, and liver failure (reported in patients receiving multiple concomitant medications and other serious medical conditions.); severe heart failure; cerebral hemorrhage; tumor lysis syndrome; and allergic reactions that can be severe or life threatening. Complications arising from these bortezomib toxicities may result in death.

The effect of bortezomib on reproduction and its safety in pregnancy are unknown. Laboratory tests show that bortezomib may damage DNA therefore it is possible that bortezomib may cause infertility in men and women.

Further details on the potential risks of bortezomib may be found in the Investigator Brochure.

Phase I trial data: children

Pharmacokinetics/pharmacodynamics

There is actually very limited information on these important topics in children. Horton et al. (2007) reported a phase 1 study in 12 children, and concluded that drug disposition was similar to that observed in adults. In four out of five patients, the three-compartment model provided the best model fit according to Akaike’s criteria (Yamakawa 1978). At doses of 1.3 mg/m2, clearance was 11 ml/hr/m2, central volume of distribution 6.7 l/m2, and terminal half-life 12.6 hours. This pharmacokinetic analysis was actually based on data from 5 patients only. Pharmacodynamics was studied in 2 pediatric studies. Horton et al. (2007) reported a transient increase in NFkB activity followed by a 4 to 6-fold decrease by 24 hours following bortezomib in two patients.
Accordingly, IκB protein expression showed an initial decrease after administration of bortezomib, followed by a normalisation of its expression. Blaney et al. (2994) reported a dose-dependent inhibition of 20S proteasome activity, with an average inhibition 1 hour after administration of 67% at 1.2 mg/m² and of 76% at 1.6 mg/m², rapidly decreasing to 53% 4 hours after administration and 18% after 24 hours at 1.2 mg/m², and to 64% 4 hours after administration and 32% after 24 hours at 1.6 mg/m².

**Efficacy/Safety**

The single agent phase I study (ADVL0015) in children with refractory solid tumors has been reported by the Children’s Oncology Group (COG; Blaney 2004), while the phase I study in children with recurrent or refractory leukemias (COG study ADVL0317) has more recently been completed and published (Horton 2007). The ADVL0015 study showed no responses in 15 patients, but bortezomib was well-tolerated and the recommended phase II dose will be 1.2 mg/m²/dose twice weekly for 2 weeks followed by 1 week rest in solid tumors. In leukemias, a higher dose seems justified because of clinical experience in adults (dose-limiting toxicity at 1.5 mg/m²/dose twice weekly for 4 weeks) and improved inhibition of proteasome activity at higher doses. The COG ADVL0317 study showed no single agent activity in terms of CR or PR in 10 patients (both ALL and AML). Two patients experienced DLTs at the 1.7mg/m² dose level, including altered mental status in one patient and in the other patient with febrile neutropenia a combination of Grade 4 hypotension, Grade 3 renal failure, and Grade 5 hypoxia. The MTD for bortezomib in pediatric leukemia patients was 1.3 mg/m², a dose at which NFkB activation was inhibited. COG will study bortezomib in both ALL and AML. For ALL, the protocol has not opened yet because of another competing ongoing study. There is a concern for bortezomib-induced peripheral neuropathy, which is relevant in ALL in view of the frequent use of vincristine. Since it is essentially unknown if bortezomib can be combined safely with vincristine in children, this study will address this issue. However, preliminary data from the so-called USA TACL consortium study on bortezomib combined with chemotherapy in relapsed ALL suggest that bortezomib can be combined with drugs such as dexamethasone, vincristine, asparaginase and doxorubicin, without excess toxicity (Messinger 2008).

**Sanctuary sites**

Bortezomib levels in CSF has not been studied previously and will be determined in this study in all patients.

### 3.3 Rationale for the proposed study

In conclusion, in vitro data and in vivo data in other disease entities strongly support the study of potential synergy between bortezomib and dexamethasone in ALL, a disease with constitutive NFkB activation. In order to demonstrate antileukemic synergy, its use as reinduction chemotherapy in refractory or relapsed ALL will be most informative.

In reinduction therapy in relapsed/refractory ALL, vincristine is usually administered as it is an active drug in relapsed/refractory ALL. Therefore, vincristine will be included in this study. Vincristine and bortezomib may have overlapping neurotoxicity, and hence this study will clarify if bortezomib and vincristine can be administered simultaneously without excess of neurotoxicity. Methotrexate will be given intrathecally as routine CNS-prophylaxis.
In support of this study, preliminary results in children (Messinger 2008) and several studies in adults demonstrate that bortezomib can be combined at its MTD of 1.3 mg/m\(^2\) in combination chemotherapy, including drugs such as glucocorticoids, anthracyclines, vincristine, and rituximab (Hideshima 2001, Richardson 2003, Orlowski 2005, Leonard 2005, Jang 2007, Attar 2008). Also, the pharmacokinetic profile of bortezomib in combination with carboplatin was shown to be similar to that observed in single agent studies (Aghajanian 2005). Thus, major interactions at the pharmacokinetic level are not expected.
4. **Eligibility criteria**

**Inclusion criteria:**
- Age between 6 months and 19 years
- Patients with a second or subsequent relapsed ALL
- Patients with first relapsed ALL after prior allogeneic stem cell transplantation in first complete remission
- Patients with refractory first relapse of ALL refractory, as defined by the ALL relapse protocol these patients were enrolled in
- Circulating leukemic blasts of at least 100/ul peripheral blood (i.e. at least $0.1 \times 10^9/l$)
- Patients must take adequate contraceptives when of childbearing potential
- Written informed consent

**Exclusion criteria:**
- Relapse not involving bone marrow
- Symptomatic CNS leukemia
- Active uncontrolled infection
- Performance status (Lansky or Karnofsky score) of 60% or less
- Life expectancy of less than 6 weeks
- Existing peripheral neuropathy NCI grade 2 or higher
- Presence of acute diffuse infiltrative myocardial and/or pericardial disease
- Clinical signs of cardiotoxicity
- Previous allogeneic stem cell transplantation within 100 days
- Pregnant (requiring a pregnancy test in post-pubertal females) or breastfeeding
- Other contra-indications for chemotherapy, including no recovery from previous treatment
- Previous exposure to bortezomib
- Other experimental or conventional antileukemic treatment within 7 days from start of bortezomib
- Allergy to boron and its metabolites (present in vials of bortezomib)
- Concomitant anti-leukemic therapy other than according to this protocol
5. Trial design

Multicenter, multinational, open label, comparative and randomised phase II study on the antileukemic activity of bortezomib with conventional combination chemotherapy in relapsed/refractory ALL in children and adolescents. The study will include one cohort of patients, with treatment guidelines for all patients for 3 weeks. Thereafter, bortezomib may be repeated in combination with the same conventional chemotherapy for patients who benefit and have no major toxicities, but this and treatment of all other patients will be left at the discretion of the investigator.

Standard reinduction chemotherapy will consist of 2 weeks of dexamethasone plus vincristine given twice, plus intrathecal administration of methotrexate.

In addition, all patients will be treated with one cycle of bortezomib, consisting of 4 doses in 2 weeks. However, they will be randomised 1:1 in 2 arms, group A getting “early” bortezomib, starting at day 1 of therapy, and group B getting “late” bortezomib, starting at day 8. This design allows demonstrating an additional antileukemic effect of bortezomib when added to dexamethasone, after 1 week of therapy. In addition, this design allows comparing the toxicity of limited (group A) and more extended (group B) overlap of administrations of bortezomib and vincristine. A total of 24 patients must be randomised and be fully evaluable in order to prove additional antileukemic effect of bortezomib when added to dexamethasone.

Bortezomib will be available for further use in case of patients with a favourable early treatment response, i.e. bone marrow M1 or M2 (see definitions section 8.2) 3 weeks after start of reinduction therapy according to this protocol, in combination with the same combination chemotherapy.

Duration of Study

All study endpoints will be evaluable at day 22. However, patients are allowed to receive subsequent cycles of combination chemotherapy including bortezomib as described in this protocol, as long as the BM examination shows BM M1 or M2 (see definitions section 8.2), there is no progressive disease otherwise, and toxicity is manageable and does not exceed the limits as described in this protocol to give bortezomib. Each next cycle should start 11 days after the previous administration of bortezomib.
6. Therapeutic regimen, PK/PD sampling and dose modifications

6.1 Antileukemic regimen, drug dosing schedule and PK/PD sampling

The patients will be treated with bortezomib 1.3 mg/m\(^2\)/dose twice weekly. Group A will get bortezomib on days 1, 4, 8 and 11, while group B will get it on days 8, 11, 15 and 18. Bortezomib will be administered as i.v. push, over 3-5 seconds. Please refer to appendix B for precise instructions. In addition, patients will get standard reinduction chemotherapy consisting of dexamethasone and vincristine. Dexamethasone will be given from day 1 onwards for 2 weeks at 10 mg/m\(^2\)/day in 3 doses, orally, to be started 1 hour before intravenous drug administration. In addition, patients will receive vincristine on days 8 and 15, at 1.5 mg/m\(^2\)/dose (maximum 2.0 mg/dose), as 60 minutes i.v. infusion (which potentially is less neurotoxic).

Group A (bortezomib early). Intravenous/intrathecal drug administration and PK/PD sampling

<table>
<thead>
<tr>
<th>Drug</th>
<th>Day 1, time</th>
<th>Day 4, time</th>
<th>Day 8, time</th>
<th>Day 11, time</th>
<th>Day 15, time</th>
<th>Day 18, time</th>
<th>Day 22, time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dexamethasone 10 mg/m(^2)/day in 3 doses orally for 2 weeks</td>
<td>T=-1h T=11h</td>
<td>T=-1h T=11h</td>
<td>T=-1h T=11h</td>
<td>T=-1h T=11h</td>
<td></td>
<td></td>
<td>T=-1h T=11h</td>
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<tr>
<td>Anti-emet</td>
<td>T=-1h T=11h</td>
<td>T=-1h T=11h</td>
<td>T=-1h T=11h</td>
<td>T=-1h T=11h</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vincristine 1.5 mg/m(^2)/dose max 2.0 mg/dose 1 hour infusion</td>
<td>T=0</td>
<td>T=0</td>
<td>T=0h</td>
<td>T=0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bortezomib 1.3 mg/m(^2)/dose i.v. push</td>
<td>T=0</td>
<td>T=0</td>
<td>T=0h</td>
<td>T=0</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Intrathecal</td>
<td>T=15'</td>
<td>T=15'</td>
<td>T=15'</td>
<td>T=15'</td>
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<td>PK-CSF</td>
<td>T=15'</td>
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<td>T=15'</td>
<td>T=15'</td>
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<td></td>
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<tr>
<td>PK-PB</td>
<td>T=0-15'-3h-8h-24h-48h-72h (i.e., t=72h day 1)</td>
<td>T=0</td>
<td>T=30'</td>
<td>T=0-15'-3h-8h-24h-48h-72h (i.e., t=72h day 1)</td>
<td>T=30'</td>
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<td></td>
</tr>
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<td>PK-BM</td>
<td>T=30'</td>
<td>T=30'</td>
<td>T=30'</td>
<td>T=30'</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PD-BM</td>
<td>T=30'</td>
<td>T=30'</td>
<td>T=30'</td>
<td>T=30'</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PD-PB</td>
<td>T=0-15'-3h-8h-24h-48h-72h (i.e., t=72h day 1)</td>
<td>T=0</td>
<td>T=30'</td>
<td>T=0-15'-3h-8h-24h-48h-72h (i.e., t=72h day 1)</td>
<td>T=30'</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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Group B (bortezomib late). Intravenous/intrathecal drug administration and PK/PD sampling.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Day 1, time</th>
<th>Day 4, time</th>
<th>Day 8, time</th>
<th>Day 11, time</th>
<th>Day 15, time</th>
<th>Day 18, time</th>
<th>Day 22, time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dexamethasone 10 mg/m²/day in 3 doses orally for 2 weeks</td>
<td>T=-1h T=11h</td>
<td>T=-1h T=11h</td>
<td>T=-1h T=11h</td>
<td>T=-1h T=11h</td>
<td>T=-1h T=11h</td>
<td>T=-1h T=11h</td>
<td></td>
</tr>
<tr>
<td>Anti-emetic</td>
<td>T=-1h T=11h</td>
<td>T=-1h T=11h</td>
<td>T=-1h T=11h</td>
<td>T=-1h T=11h</td>
<td>T=-1h T=11h</td>
<td>T=-1h T=11h</td>
<td></td>
</tr>
<tr>
<td>Vincristine 1.5 mg/m²/dose max 2.0 mg/dose 1 hour infusion</td>
<td>T=-1h</td>
<td>T=-1h</td>
<td>T=-1h</td>
<td>T=-1h</td>
<td>T=-1h</td>
<td>T=-1h</td>
<td></td>
</tr>
<tr>
<td>Bortezomib 1.3 mg/m²/dose i.v. push intrathecal</td>
<td>T=0</td>
<td>T=0</td>
<td>T=0</td>
<td>T=0</td>
<td>T=0</td>
<td>T=0</td>
<td></td>
</tr>
<tr>
<td>PK-CSF</td>
<td>T=15’</td>
<td>t=15’</td>
<td>T=15’</td>
<td>T=15’</td>
<td>T=15’</td>
<td>T=15’</td>
<td></td>
</tr>
<tr>
<td>PK-PB</td>
<td>T=0</td>
<td>T=0-15’-3h-8h-24h-48h-72h</td>
<td>T=0 (i.e., t=72 h from day 8)</td>
<td>T=30’</td>
<td>T=0-15’-3h-8h-24h-48h-72h</td>
<td>T=30’</td>
<td></td>
</tr>
<tr>
<td>PK-BM</td>
<td>T=30’</td>
<td>T=30’</td>
<td>T=30’</td>
<td>T=30’</td>
<td>T=30’</td>
<td>T=30’</td>
<td></td>
</tr>
<tr>
<td>PD-BM</td>
<td>T=30’</td>
<td>T=30’</td>
<td>T=30’</td>
<td>T=30’</td>
<td>T=30’</td>
<td>T=30’</td>
<td></td>
</tr>
<tr>
<td>PD-PB</td>
<td>T=0h</td>
<td>T=0-15’-3h-8h-24h-48h-72h</td>
<td>T=0 (i.e., t=72 h from day 1)</td>
<td>T=30’</td>
<td>T=0-15’-3h-8h-24h-48h-72h</td>
<td>T=30’</td>
<td></td>
</tr>
</tbody>
</table>

PK and PD sampling will be further described in the procedure manual. It requires 3 ml of blood/bone marrow/CSF, of which 2 ml is meant for PK and 1 ml for PD. It is crucial to flush the canula or central line with at least 10 ml of saline before each blood sampling that is done after bortezomib administration through the same access. Whenever possible, use another access point for bortezomib administration than for blood sampling.

Intrathecal methotrexate will be given on days 1 and 22, with age-adjusted dosing (figures 1 and 2; and Table 1 below for age-adjusted doses).

Table 1. Age-adjusted dosing of methotrexate for CNS prophylaxis.

<table>
<thead>
<tr>
<th>Methotrexate intrathecally</th>
<th>Age &lt;2 years</th>
<th>Age 2 years</th>
<th>Age 3 years and above</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose</td>
<td>8 mg</td>
<td>10 mg</td>
<td>12 mg</td>
</tr>
</tbody>
</table>

Please refer to page 12 and 13 for figures outlining the drug schedules.
6.2  Further therapy and dose modifications

6.2.1  Criteria for continuation of treatment
Further therapy for good responders (BM M1 or M2 on day 22) may consist of an additional cycle of bortezomib, but this decision is left to the discretion of the treating physician. A next cycle should start not earlier than 11 days after the last administration of bortezomib. Then, bortezomib must again be combined with 2 weeks of dexamethasone (same dose and schedule) and vincristine twice (same dose and schedule; figures 3 and 4). Such cycles may be repeated if justified by efficacy and (lack of) toxicity, which again is left to the discretion of the treating physician. According to local policy, intrathecal chemotherapy can be given at the end of each cycle.

6.2.2  Dose modification and dose-limiting toxicity in general
Toxicity monitoring will be done as usual, applying the most recent NCI CTC (currently CTCAEv3.0, December 12, 2003).

6.2.3  Dose modifications and dose-limiting toxicity for neurological toxicity
Neurotoxicity will influence administration of bortezomib as follows:
- Grade 1: no consequences
- Grade 2 and grade 3: withhold bortezomib until resolution to grade 1 or grade 0, and then resume bortezomib at 1.0 mg/m²
- grade 4: stop bortezomib permanently

6.2.4  Dose modifications and dose-limiting toxicity for hematological toxicity
Hematological toxicity that is expected in these patients, including febrile neutropenia and infections, will not influence administration of bortezomib.

6.2.5  Dose modifications and dose-limiting toxicity for non-hematological toxicity
Non-hematological toxicity, other than neurotoxicity, will influence administration of bortezomib as follows:
- grade 1 and 2: no consequences
- grade 3: withhold bortezomib until resolution to grade 2 or lower, and then resume bortezomib at 1.0 mg/m².
- grade 4: stop bortezomib permanently
An exception is nausea, vomiting and alopecia, which will not influence bortezomib administration, even in case of grade 4 toxicity.

6.2.6  Dose modifications of bortezomib once dose-reduction was necessary
If bortezomib is administered at 1.0 mg/m² and increased toxicity again occurs, follow the guidelines above and restart once possible at a further reduced dose of 0.7 mg/m²/dose. If that does not result in less toxicity, bortezomib administration should be stopped. Alternatively, in case of acceptable toxicity as defined above after bortezomib at 1.0 mg/m²/dose, subsequent doses of bortezomib may again be 1.3 mg/m²/dose.

6.2.7  Discontinuation of therapy
Patients with a BM M3 on day 22 and/or those with progressive disease will go off study, as well as patients that are going to be treated with other drugs than those described in this protocol. In addition, patients that experience grade 4 neurotoxicity or grade 4 non-hematological toxicity (except nausea, vomiting and alopecia) that is assumed to be related to bortezomib, should discontinue bortezomib permanently. Finally, patients that experience excess toxicity (grade 2 and 3 neurotoxicity, and grade 3 non-hematological toxicity other than nausea, vomiting and/or alopecia) with bortezomib already reduced to 0.7 mg/m² should also discontinue bortezomib.
6.3 Concomitant antileukemic treatment

All other anti-cancer therapy should be discontinued at least one week before treatment with bortezomib, and should not be prescribed during the treatment.

6.4 Supportive care

Supportive care often is given according to local institution guidelines, i.e. with regards to blood-product support and treatment of infections. Guidelines for supportive care are provided below, and should be applied as much as possible.

Because of toxicity concerns, azoles such as fluconazole, itraconazole and voriconazole should not be used as prophylactic drugs within the week before, during, nor the week after bortezomib administrations. However, its use is allowed in case of an infection, either proven or assumed. Such patients will remain to be included in the study.

Be aware that in case of hepatotoxicity, the metabolism of other drugs may be influenced (for instance antibiotics, anti-convulsive treatment), which may necessitate dose modifications for such drugs. Drugs other than vincristine that may be neurotoxic, such as amiodarone, antivirals, isoniazid, nitrofurantoin, or statins, should be avoided. Similarly, drugs that are nephrotoxic, such as aminoglycosides, should be avoided if possible.

Tumor-lysis syndrome

To prevent tumor-lysis syndrome, the following is advised:

1. Vigorously hydrate the patient (2.5-3.0 liter/m^2/day) with an IV solution starting at ideally at least 24 hours before administration of the chemotherapy, and lasting until the risk of tumor lysis syndrome has disappeared.
2. Hydrate the patient with a solution containing NaHCO_3 (for instance NaHCO_3 4.2% at 4 ml/m^2/hour; to alkalinize the urine; which needs to be adjusted according to urine pH; aiming at a urine output with a pH between 7.0-8.0) and without potassium. This is not necessary in case of the use of rasburicase.
3. Begin allopurinol 200 mg/m^2/day, divided into 2 doses, ideally 24 hours before therapy begins. Rasburicase can replace allopurinol (as well as the need to alkalinize the urine).

In case of high WBC (>50x10^9/l), use rasburicase. The recommended dose is 0.20 mg per kilogram body weight given as a daily infusion for up to seven days. The duration of treatment is adjusted depending on the patient’s uricaemia (blood levels of uric acid). The infusion should last 30 minutes.

Patients should have indwelling silastic catheters, preferably prior to the initiation of treatment. Therapy may be started with a temporary deep line until a permanent one can be placed. In exceptional cases, a peripheral catheter may be sufficient.

Nausea and vomiting

Bortezomib can induce nausea and vomiting and anti-emetic drugs should be prescribed prophylactically, such as 5HT3 antagonist.
Blood Products
Use of leukocyte-reduced blood products is encouraged, according to each institution’s guidelines. Also, CMV- and ParvoB19 negative patients should receive CMV/ParvoB19 negative blood products, according to each institution’s guidelines.
Persistent bleeding may be attributable to thrombocytopenia and patients should receive platelet transfusions as quickly as possible. Platelet transfusions should also occur if the platelet count is <10,000/ul and falling (relative indication). Splenomegaly, DIC, active bleeding, fever/sepsis, amphotericin B and platelet antibodies are reasons for poor recovery or survival of transfused platelets.

Infection Prophylaxis
The use of prophylactic antibacterial, antifungal, and antiviral agents is recommended according to each institution’s guidelines. Given the potential for liver toxicity by bortezomib it is recommended to at least interrupt prophylactic azoles the week before, during, and until the week after bortezomib administrations, whenever possible. Do give prophylaxis for pneumocystic carinii pneumonia.

Treatment of Fever and Neutropenia
Patients with ANC of ≤ 500/ul (or < 1,000 and falling) and an oral temperature > 38°C twice in 12 hours, or > 38.5°C once, should have empiric systemic antibiotics started immediately. Broad-spectrum antibiotics should be initiated according to institutional guidelines, and should cover major gram-negative pathogens, as well as alpha hemolytic streptococcus and staphylococci.
The persistence of fever during broad spectrum antibiotic coverage or the emergence of a new fever in neutropenic patients with negative blood cultures warrants the initiation of IV antifungal treatment, unless other causes are apparent.

Colony Stimulating Factors
G-CSF or any other growth factors are not allowed during the administration of chemotherapy, and should be stopped at least 3 days prior to administration of chemotherapy.
Only in case of serious infections in neutropenia G-CSF therapy can be initiated.

Suppression of menstruation
All menstruating females should receive oral contraceptives during the entire course of this protocol. Suppression of menses should be continued until the platelet count is 50,000/ul without transfusion support.

Nutrition
Active measures should be used to prevent weight loss of greater than 10% of pre-illness body weight. If possible, enteral feedings are preferred to parenteral. However, enteral feedings should be withheld at any time during therapy if gastrointestinal problems such as severe nausea, vomiting, or diarrhea, or signs and symptoms of illness (e.g. typhlitis) occur.

Hyperleukocytosis and Metabolic Derangement
Patients with high peripheral blast counts (> 100,000/ul, i.e. >100x10^9/l) and significant organomegaly have increased problems related to metabolic abnormalities, bleeding, and hyperviscosity. Patients with high WBCs and/or evidence of disseminated intravascular coagulation (DIC) may be prone to more complications of general anesthesia. In these patients,
platelets should be transfused in patients with platelet counts < 20,000. Hemoglobin levels should not be raised above 10 mg/dl ~ 6 mmol/l. The use of leukapheresis, exchange transfusion, or hemodialysis may be necessary with some patients.
7. **Clinical evaluation, laboratory tests and follow up**

7.1 **Before start of treatment**

Within 7 days from the start of treatment the following parameters need to be obtained, partly as routine work-up in case of relapsed ALL:

- Medical history and full physical examination, including full neurological examination with signs of peripheral neuropathy
- Height, weight, body surface area
- Performance status (depending on age, either the Karnofsky or Lansky Performance scale; see appendix A)
- Complete hematological count (hemoglobin, platelets, white blood cell count and differentiation, to calculate absolute blast count). This should be determined within 1 day prior to the start of dexamethasone and (if applicable) bortezomib.
- Serum chemistry: CRP, creatinine, blood urea nitrogen, albumin, sodium, potassium, calcium, magnesium, phosphate, ASAT, ALAT, bilirubin, uric acid, LDH, bicarbonate and glucose. This should be determined within 2 days prior to the start of treatment.
- Bone marrow aspiration (examination of marrow cellularity and percentage of blasts, by morphology and flowcytometry). In case of dry tap perform a trephine biopsy. This should be performed within 3 days prior to the start of treatment.
- Six unstained bone marrow slides and 3 peripheral blood slides should be sent to Amsterdam, for central review of the diagnosis. More information is provided in the separate procedure manual
- Documentation of (suspected) extramedullary leukemia by appropriate imaging (normally ultrasound) and/or biopsies.
- 1-3 ml of bone marrow and 10-20 ml of peripheral blood should be sent to the Research Laboratory of Pediatric Oncology in Amsterdam, for translational research, see appendix C
- Lumbar puncture (quantification of cells and protein, pathological examination), immediately followed by intrathecal administration of methotrexate.
- Chest X-ray
- Ultrasound abdomen
- Echocardiography
- Pregnancy test (in female patients with childbearing potential)
- PK/PD sampling, please refer to page 26 for the schedule of sampling, and to the separate procedure manual for storage and shipment instructions. Bortezomib should be administered 15 minutes before sampling of CSF and PB and 30 minutes before sampling BM. From a practical point of view, CSF should thus be sampled first, followed by peripheral blood and then BM. Please also refer to the separate procedure manual for instructions on storage and shipment of the material.
7.2 During treatment

* Day 4 (group A only)
- PK and PD sampling should be done according to the guidelines on page 26, with storage and shipments details provided in the separate procedure manual.

* day 8
- complete hematological count and serum chemistry as described above, consider chemistry to be determined more often
- physical including neurological examination
- Peripheral blood sample and a bone marrow aspiration sample (or trephine biopsy in case of dry tap) to determine response. Again, 6 unstained bone-marrow slides should be sent to Amsterdam and then will be reviewed by the reference laboratory, together with 3 slides of a peripheral blood sample taken at the same time. Please refer to the separate procedure manual.
- Lumbar puncture for CSF examination.
- PK/PD sampling, please refer to page 26 for the schedule of sampling, and to the separate procedure manual for storage and shipment instructions. Bortezomib should be administered 15 minutes before sampling of CSF and PB and 30 minutes before sampling BM. From a practical point of view, CSF should thus be sampled first, followed by peripheral blood and then BM. Please also refer to the separate procedure manual for instructions on storage and shipment of the material.
- Document adverse events according to the NCI CTCAE, version 3.0

* day 11
- PK and PD sampling should be done according to the guidelines on page 26, with storage and shipments details provided in the separate procedure manual.

* day 15
- complete hematological count and serum chemistry as described above, consider chemistry to be determined more often
- physical including neurological examination
- PK and PD sampling should be done according to the guidelines on page 26, with storage and shipments details provided in the separate procedure manual (group B only).

* day 18 (group B only)
- PK and PD sampling should be done according to the guidelines on page 26, with storage and shipments details provided in the separate procedure manual (group B only).

* day 22
- complete hematological count and serum chemistry as described above, consider chemistry to be determined more often
- physical including neurological examination
- Peripheral blood and bone marrow aspiration and send 6 unstained bone marrow and 3 peripheral blood slides to Amsterdam for central review (which will be done by independent investigators of the DCOG laboratory). Please refer to the separate procedure manual. In case of bone marrow hypoplasia or aplasia, repeat the bone marrow aspirate at first sign of
hematopoietic regeneration, but no later than day 35 after the start of treatment. In case of dry tap: perform a trephine biopsy.
- Lumbar puncture together with the bone marrow aspiration (cells/protein/pathology), and apply intrathecal chemotherapy according to local policy.
- With the third lumbar puncture on day 22, again CSF and blood levels of bortezomib and peak proteasome inhibition in (remaining blasts in) bone marrow, blood and CSF will be measured in these patients.
- Echocardiography
- PK and PD sampling should be done according to the guidelines on page 26, with storage and shipments details provided in the separate procedure manual. CSF should be samples 15’ before obtaining PB and BM. Please also refer to the separate procedure manual for instructions on storage and shipment of the material.

### 7.3 After treatment (after day 22)

- time to allogeneic transplant, if any, and main events (relapse/death) after completing this protocol treatment should be recorded, independent of further treatment.
- AE’s are recorded until 30 days after completing study treatment, and SAE’s until resolution, stabilisation or death.
- In case of multiple cycles of chemotherapy including bortezomib, cumulative toxicity will be recorded weekly and at the end of each cycle
**Table 2.** Investigations before, during and after treatment with bortezomib. Please refer to the separate procedure manual for practical details, and to the table on page 26 for PK/PD sampling.

<table>
<thead>
<tr>
<th>Investigation</th>
<th>Before start of treatment (=week 1, day 1)</th>
<th>Weekly</th>
<th>Day 22 (=week 4, before start of next cycle)</th>
<th>Weekly thereafter (only in case of 2nd cycle)</th>
<th>6 weeks after start of treatment (=week 7, day 43); only in case of 2nd cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medical history</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical examination</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>Height, weight, BSA</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Performance status</td>
<td>*</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hematology</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Chemistry etc.</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>X-thorax</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ultrasound abdomen</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Echocardiography</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bone marrow aspiration</td>
<td>*</td>
<td></td>
<td>Day 8 (=week 2)</td>
<td></td>
<td>*</td>
</tr>
<tr>
<td>Send BM and PB slides to research laboratory in Amsterdam</td>
<td>*</td>
<td></td>
<td>Day 8 (=week 2)</td>
<td></td>
<td>*</td>
</tr>
<tr>
<td>Send BM+PB+CSF to Amsterdam for translational research</td>
<td>*</td>
<td></td>
<td>Day 8 (=week 2)</td>
<td>Sampled 1 hour after administration of bortezomib!</td>
<td>*</td>
</tr>
<tr>
<td>Lumbar puncture</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adverse events</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
</tbody>
</table>

1 See text;
2 In case of dry tap: perform trephine biopsy;
3 In case of peripheral blood aplasia, the bone marrow aspirate can be postponed until hematological recovery is present, but not later than 35 days after the start of the 2nd week of each cycle of bortezomib. Material should be sent to Amsterdam, which center will organize central review of the slides.
4 Only in case of asymptomatic CNS-involvement (see paragraph 7.1).
8. Response and toxicity evaluation

8.1 Study endpoints

Primary endpoint:
1. Antileukemic activity of bortezomib when added to dexamethasone and vincristine and intrathecal methotrexate, as determined by the absolute peripheral blood (PB) blast count on day 8 of treatment. Morphology will be centrally reviewed.

Secondary endpoints
1. Antileukemic activity of bortezomib when added to dexamethasone and vincristine and intrathecal methotrexate, as determined by the absolute bone marrow (BM) blast percentage on day 8, and BM and PB analysis on day 22 of treatment, in absolute numbers and expressed as an M1, M2 or M3 marrow (<5%, 5-15%, 15% or above respectively). Morphology will be centrally reviewed.
2. Feasibility of combining bortezomib with dexamethasone and vincristine and intrathecal methotrexate, as determined by the percentage of patients in whom bortezomib had to be dose-reduced or withdrawn because of toxicity.
3. Toxicity of bortezomib when combined with dexamethasone and vincristine and intrathecal methotrexate, as determined by the percentage of patients suffering from grade III/IV toxicity in any field
4. Determine the feasibility of combining a second cycle of bortezomib with combination chemotherapy, its toxicity and antileukemic activity, as measured after 6 weeks of therapy by bone marrow, peripheral blood and cerebrospinal fluid

Exploratory end points
1. Establish pharmacokinetics of bortezomib in children. For this purpose, levels of bortezomib will be measured in peripheral blood and CSF samples obtained at several time-points as clarified in the protocol, limited to cycle one and the first and second administration of bortezomib.
2. Explore pharmacodynamics of bortezomib, in particular by proteasome inhibition as measured in BM, PB and CSF. This inhibition will be correlated with pharmacokinetic data and clinical response to bortezomib. Time-points of sampling will be detailed in the protocol.

8.2 Definitions
In the analyses and reporting of the data of this study we will use several definitions, which are explained and defined below.

8.2.1 Response Rate
The proportion of patients with complete remission (CR), CR but without complete regeneration (CRi), partial remission (PR) and treatment failure will be determined after the first (week 4) and, if applicable, second (week 7) cycle of study treatment. In addition, events will be defined as early death, relapse and death in remission. The patient-group will also be characterised by calculating
probabilities of event-free and overall survival, and duration of remission (if any). Definitions are explained below. This is additional to the primary endpoints as explained above.

8.2.2 Response definitions

Bone marrow day 8 and day 22 response
In these bone marrow aspirations, the percentage of leukemic cells will be determined morphologically, and in addition to the absolute percentage of leukemic blasts, results will be expressed as an M1, M2 or M3 bone marrow. The latter indicates <5%, 5-15%, and >15% leukemic blasts, respectively.

The definitions below will be used after day 22.

Morphologic complete remission with incomplete blood count recovery (CRi): After chemotherapy, some patients fulfill all of the criteria for CR except for residual neutropenia (<1,000/µL) or thrombocytopenia (<100,000/µL). Although this category of response indicates activity, it should not be included with CR. CRi fills the gap, but requires platelet transfusion independence.

Morphologic complete remission (CR) requires that the patient achieve the morphologic leukemia-free state, and have an absolute neutrophil count of ≥1,000/µL and platelets of ≥100,000/µL. Hemoglobin concentration or hematocrit has no bearing on remission status, although the patient must be independent of transfusions. There should be no residual evidence of extramedullary leukemia.

Partial remission (PR). This designation requires all of the hematologic values for a CR but with a decrease of at least 50% in the percentage of blasts to 5-25% in the bone marrow aspirate. Thus, if the pretreatment bone marrow blast percentage was 50% to 100%, the percentage of blasts must decrease to a value between 5% and 25%; if the pretreatment blast percentage was 20% to less than 50%, they must decrease by at least half to a value of more than 5%.

Treatment failure. Treatment failure includes those patients for whom treatment has failed to achieve less than a PR.

8.2.3 Time to and Duration of Responses

Time to response is defined as the time from first dose of study treatment until the first day the response criteria are met, computed only for subjects whose best response is CR, CRi, or PR. Duration of response will be computed from the first day all criteria are met for response until the date that progressive disease is reported or death.

Overall Survival
Overall survival (OS) is defined as time from first dose of study treatment to date of death. All subjects will be followed until death or (if study drug is stopped) as long as possible in case of no events. Subjects lost to follow-up will be censored on the last date the subject was known to be alive. Probabilities of survival will be estimated by the method of Kaplan and Meier and will be compared using the log-rank test.

Event-free survival
Event-free survival (EFS) will be defined as the time between diagnosis and first event (relapse, death of any cause, failure to achieve remission or second malignancy) or date of last follow-up. Patients who were classified as treatment failures will be considered as failures at time zero. Probabilities of survival will be estimated by the method of Kaplan and Meier and will be compared using the log-rank test.
8.2.4 Other Definitions

- CNS disease: \( \geq 5 \) white blood cells/mm\(^3\) (ie, \( \geq 5/\mu l \), or \( \geq 15/3 \mu l \)) and unequivocal evidence of blasts on cytospin examination, and/or clinical (seizures, cranial nerve palsy and symptoms of increased cranial pressure or other signs/symptoms not readily explained by another disease) and/or radiological evidence of leukemic infiltration in the central nervous system. Patients with symptomatic CNS disease are excluded from this study.

- Early Death: Death during the first 3 weeks of treatment (ie, before the time that day 22 BM results could have been documented). The cause of death (disease, therapy or both) should be recorded as well as the last known percentage of blasts in the BM.

- Relapse: After a documented CR/CRi, this designation is defined as a reappearance of leukemic blasts in the peripheral blood, or \( \geq 5\% \) blasts in the bone marrow not attributable to any other cause (eg, bone marrow regeneration after consolidation therapy) (Cheson 2003). In case of leukemic cells demonstrable in the peripheral blood, and confirmed with flowcytometry, a bone marrow aspirate is not needed to diagnose relapse. In case of CSF with <5 cells/mm\(^3\), but with blasts on the cytospin examination, a second lumbar puncture should be performed at least 7 days later to confirm relapse. In the setting of recent treatment, if there are no circulating blasts and the bone marrow contains 5\% to 20\% blasts, a repeat bone marrow performed at least a week later is necessary to distinguish relapse from bone marrow regeneration, unless there is confirmation of the leukemic origin with flowcytometry or other techniques. In such instances the date of recurrence is retrospectively defined as the first date that more than 5\% blasts were observed in the marrow. The reappearance or development of cytologically proven extramedullary disease also indicates relapse.

8.3 Toxicity and safety

Toxicity monitoring will be done as usual for all patients, applying the most recent NCI CTC manual (currently CTCAEv3.0, December 12, 2003). All adverse events must be recorded on the CRF’s. Safety and toxicity measurements must be performed as soon as the patient is enrolled in the study, and continuously during treatment until week 4 (in case of 1 cycle only) or week 7 (in case of 2 cycles).

In addition, safety and toxicity measurements must be done upon recovery from aplasia, if that occurs later than at the indicated weeks.
8.4 Adverse events (AEs), serious adverse events (SAE) and suspected unexpected serious adverse reactions (SUSARs)

Adverse events (AEs) are defined as any untoward medical occurrence in a patient or a clinical investigation subject administered a pharmaceutical product, and which does not necessarily have a causal relationship with the treatment. An AE can therefore be any unfavorable and unintended sign, symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related. Pre-existing conditions which worsen during this study are to be reported as AEs. AEs will be assessed continuously and graded according to NCI Common Terminology Criteria for Adverse Events (CTCAE) version 3.0 (a copy can be downloaded from the CTEP website [http://ctep.cancer.gov/reporting/ctc.html]). Frequency and severity, and outcomes of AEs will be determined.

The causality relationship of bortezomib to the AEs will be assessed by the investigator as:

1. Certain: There is a reasonable causal relationship between bortezomib treatment and the AE. The event responds to withdrawal of study treatment (dechallenge), and recurs with rechallenge when clinically feasible.
2. Probable: There is a reasonable causal relationship between bortezomib treatment and the AE. The event responds to dechallenge. Rechallenge is not required.
3. Possible: There is reasonable causal relationship between bortezomib treatment and the AE. Dechallenge information is lacking or unclear.
4. Not likely: There is a temporal relationship to bortezomib treatment, but there is not a reasonable causal relationship between bortezomib and the AE.
5. Not related: There is not a temporal relationship to bortezomib (too early, or late, or study drug not taken), or there is a reasonable causal relationship between another drug, concurrent disease, or circumstance and the AE.

AEs should be followed to resolution or stabilization, and reported as SAEs as they become serious. AEs occurring in the first cycle will be tabulated separately from the 2nd or subsequent cycles. AEs will be collected until 30 days after the administration of the last dose of bortezomib.

Serious adverse events
A serious adverse event (SAE) is any adverse drug experience that occurs at any dose and results in any of the following outcomes:

1. Death.
2. Life-threatening adverse drug experience.
3. Requires unexpected inpatient hospitalization or prolongation of existing hospitalization.
4. Persistent or significant disability/incapacity.
5. A congenital anomaly/birth defect.
6. Requires medical or surgical intervention to prevent one of the outcomes listed above.

NOTE 1:
- Pregnancy: Incidence of pregnancy is not considered a SAE; pregnancy must, however, be reported immediately by E-mail to the sponsor of this study (A completed Pregnancy Notification Form is necessary);
- Overdose: All cases of overdose must be reported immediately by E-mail to sponsor of this study.
The sponsor will notify the P.I. in such situations.

**NOTE 2:**
Criteria for hospitalizations not reported as SAEs because they are not unexpected include admissions for:
- Planned as per protocol medical/surgical procedure
- Routine health assessment requiring admission for baseline/trending of health status documentation
- Medical/surgical admission for purpose other than remedying ill health state (planned prior to entry into study trial; appropriate documentation required)
- Admission encountered for other life circumstance that carries no bearing on health status and requires no medical/surgical intervention (e.g. lack of housing, economic inadequacy, care-giver respite, family circumstances, administrative)
- Admissions for protocol-scheduled procedures or blood product transfusions.
- Uncomplicated admissions for neutropenic fever

An SAE report should be completed for any event where doubt exists regarding its status of seriousness.

The P.I. will have the responsibility to monitor all SAE’s and to decide whether they should be labelled as SUSARs. A suspected unexpected serious adverse reaction (SUSAR) indicates a serious adverse reaction related to the study drug bortezomib (hence, a side effect of bortezomib), which was not anticipated, as was not mentioned in the summary of known risks of bortezomib (according to section 11 and the SPC-text). In case of a SUSAR, the sponsor will make a SUSAR report and will inform the parties involved in this study.

AE’s, SAE’s and SUSAR’s will be monitored and reported as described in section 10.

**8.5 Follow up**
In addition to the prescribed monitoring, all patients will be followed for time to allogeneic transplant, if any, and for time to progression and death, or as long as possible if no event occurs, with yearly follow-up.
9. REGULATORY AND REPORTING REQUIREMENTS

9.1 Adverse Event Monitoring and Reporting
The local investigator is responsible for reporting the AEs of patients who enroll in the study. The sponsor is responsible for reporting SAEs/SUSARs to the IRB. The P.I. is responsible for monitoring the AEs. All AEs will be followed until resolution. The descriptions and grading scales found in the revised NCI CTCAE version 3.0 will be used for adverse event reporting. A copy of the CTCAE version 3.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov/reporting/ctc.html).

9.2 Reporting Serious Adverse Events
All SAEs occurring during the study or within 30 days of the last administration of the combination chemotherapy treatment, or until a new treatment is started, whichever occurs first, must be reported to the DCOG – ECTC Safety Desk, which will notify the P.I. and J&J, all within 24 hours of occurrence.

Adverse events classified as "serious" must be recorded on the SERIOUS AE (SAE) page of the CRF and require expeditious handling and reporting (within 24 hours after occurrence) to the DCOG–ECTC Safety Desk to comply with regulatory requirements.

SAE reporting by FAX (telephone and email):
DCOG-Early Clinical Trial Consortium Safety Desk
Fax: +31 (0) 8778-41-723
Tel: +31 (0) 10 703 6568 / 6325 / 6691
E-mail: research-kocr@erasmusmc.nl and to gjl.kaspers@vumc.nl and c.m.zwaan@erasmusmc.nl

Collection of complete information concerning SAEs is extremely important. If only limited information is initially available, follow-up reports are required. Thus, follow-up information which becomes available as the SAE evolves, as well as supporting documentation (e.g., hospital discharge summaries and autopsy reports), should be collected subsequently, if not available at the time of the initial report, and immediately sent using the same procedure as the initial SAE report. For ongoing SAEs a follow-up report should be sent at least once-monthly. The investigator is responsible for submitting these follow-up reports for all SAEs, until the SAE has resolved or until the patient’s condition stabilizes (in the case of persistent impairment), or the patient dies.

The Sponsor, together with the national co-sponsors, is responsible for reporting SAEs/SUSARS to the IRB or other applicable regulatory authority.

In accordance with local regulations, the DCOG-ECTC Safety Desk will notify Local Investigators of all AEs that are serious, unexpected, and certainly, probably, or possibly related to the investigational product bortezomib. This notification will be in the form of a SUSAR report.

Upon receiving such notices, the Local Investigator must review and retain the SUSAR reports with the Investigator Brochure.
Where required by local regulations or when there is a central Institutional Review Board (IRB)/Independent Ethics Committee (IEC) for the study, the Sponsor will submit the SUSAR report to the appropriate IRB/IEC. The sponsor, together with the principal investigator, will determine if the informed consent requires revision.

9.3 Compliance with the protocol

The study shall be conducted as described in this approved protocol. All revisions to the protocol must be discussed with, and be prepared by the P.I. The Investigator should not implement any deviation or change to the protocol without prior review and documented approval/favorable opinion from the IRB/IEC of an Amendment, except where necessary to eliminate an immediate hazard(s) to study subjects.

If a deviation or change to a protocol is implemented to eliminate an immediate hazard(s) prior to obtaining IRB/IEC approval/favorable opinion, as soon as possible the deviation or change will be submitted to:

- IRB/IEC for review and approval/favorable opinion;
- the sponsor;
- Regulatory Authority(ies), if required by local regulations.

Documentation of approval signed by the chairperson or designee of the IRB(s)/IEC(s) must be sent to the sponsor.

If the revision is a non-substantial amendment, investigators must inform their IRB(s)/IEC(s).

If an Amendment substantially alters the study design or increases the potential risk to the subject:

1. the consent form must be revised and submitted to the IRB(s)/IEC(s) for review and approval/favorable opinion;
2. the revised form must be used to obtain consent from subjects currently enrolled in the study if they are affected by the Amendment; and
3. the new form must be used to obtain consent from new subjects prior to enrollment.

9.4 CRFs and Monitoring

The investigator will receive case report forms (CRF) for documentation. A CRF completion guideline will be provided by the DCOG-ECTC Trial Office. Web-based data management training will be organized. All relevant data collected during the study for all of the patients enrolled into the study have to be entered into these CRF’s by the responsible investigator or someone authorized by him in a timely manner so that they are clear and legible. The local investigator will review all the CRF’s of each patient and confirm the completeness, medical correctness and plausibility of the documented data by his signature on all CRF pages. The entries should be made with black ball-point pen.

Additions and corrections in the CRF will be dated and signed by the responsible physician or an authorised person. Reasons must be given for corrections that are not self-explanatory. Copies of the originals will be retained at the centre. If corrections and/or additions are needed after collection of the original CRF, a corresponding query has to be filled out on a Query Form and forwarded to the physician for his response. The Query Form is part of the CRF.
Monitors will ensure that the clinical trial is conducted, recorded, and reported in accordance with the protocol, ICH-GCP, and the applicable regulatory requirement(s). The sponsors may delegate monitoring activities to national co-sponsors or to a free-lance monitor in each of the participating countries, provided they are appropriately qualified and approved by the sponsor. The site of the sponsor will be monitored by one of the co-sponsors or by an independent free-lance monitor.

Representatives of the sponsor and/or co-sponsor must be allowed to visit all study site locations periodically to assess the data, quality and study integrity. On site they will review study records and directly compare them with source documents, and discuss the conduct of the study with the Investigator, and verify that the facilities remain acceptable. Source documents are defined as: patient files, letters, laboratory / histology records. The type and scope of monitoring will be defined in the Monitoring Manual and documented on study specific Source Data Verification Forms.

With his participation in the study the investigator is obligated to support the activities of the monitors, provide them with direct access to the files and give them the opportunity to inspect the laboratory facilities, storage of the investigational product, etc.

9.5 Quality assurance
Apart from the monitoring process described above, the sponsor may implement procedures to assure the quality of every aspect of the study. During the course of the study, external auditors contracted by the Sponsor may conduct an onsite audit visit.

In addition, the study may be evaluated by government inspectors who must be allowed access to CRFs, source documents and other study files. THE INVESTIGATOR MUST NOTIFY THE SPONSOR PROMPTLY OF ANY INSPECTIONS SCHEDULED BY REGULATORY AUTHORITIES, AND PROMPTLY FORWARD COPIES OF INSPECTION REPORTS TO THE SPONSOR.

9.6 Delegation of authority
The Investigator will maintain a Delegation of Authority Form in the Investigator File to document signatures and initials of all persons to provide a legal delegation of study specific principal responsibilities. Each member of study staff should sign to agree the acceptance of these, and each delegation should be signed by the Investigator.

9.7 Records retention
The confidentiality of records that could identify subjects must be protected, respecting the privacy and confidentiality rules in accordance with the applicable regulatory requirement(s).

The Investigator must retain investigational product disposition records, copies of CRFs and source documents for the maximum period required by applicable regulations and guidelines, or Institution procedures, or for the period specified by the Sponsor, whichever is longer. The Investigator must contact the sponsor prior to destroying any records associated with the study.
9.8 Institutional Review Board/Independent Ethics Committee (IRB/IEC)

Before study initiation, the Investigator must have written and dated approval/favorable opinion from the local IRB/IEC for the protocol, consent form, subject recruitment materials/process (e.g., advertisements), and any other written information to be provided to subjects. The Investigator or Sponsor should also provide the IRB/IEC with a copy of the Investigator Brochure or product labeling, and information to be provided to subjects.

The Investigator or Sponsor should provide the IRB/IEC with reports, updates and other information (e.g., Amendments, Administrative Letters) according to regulatory requirements or Institution procedures.

Freely given written informed consent must be obtained from every subject or their legally acceptable representative prior to clinical trial participation, including informed consent for any screening procedures conducted to establish subject eligibility for the trial. For minors, according to local legislation, one or both parents or legally acceptable representative must be informed of the study procedures and must sign the informed consent form approved for the study prior to clinical trial participation. The explicit wish of a minor who is capable of forming an opinion and assessing this information to refuse participation in, or to be withdrawn from, the clinical trial at any time should be considered by the Investigator.

9.9 Informed Consent Procedures

Preparation of the consent form is the responsibility of the Investigator and must include all elements required by ICH, GCP and applicable regulatory requirements, and must adhere to GCP and to the ethical principles that have their origin in the Declaration of Helsinki. The consent form must also include a statement that the sponsor and regulatory authorities have direct access to subject records.

Prior to the beginning of the study, the Investigator must have the IRB/IEC’s written approval/favorable opinion of the written informed consent form and any other information to be provided to the subjects.

The Investigator must provide the subject or legally acceptable representative with a copy of the consent form and written information about the study in the language in which the subject is most proficient. The language must be non-technical and easily understood. The Investigator should allow time necessary for subject or subject's legally acceptable representative to inquire about the details of the study, then informed consent must be signed and personally dated by the subject or the subject's legally acceptable representative, and by the person who conducted the informed consent discussion. The parents/legal guardians and/or the child should sign an date before the investigator signs and dates the informed consent. The subject or legally acceptable representative should receive a copy of the signed informed consent and any other written information provided to study subjects prior to subject's participation in the trial.

The parents information form, patient information form and informed consent form are provided separately from this protocol.

9.10 Update of Informed Consent

The informed consent and any other information provided to subjects or the subject's legally acceptable representative, should be revised whenever important new information becomes
available that is relevant to the subject's consent, and should receive IRB/IEC approval/favorable opinion prior to use. The Investigator, or a person designated by the Investigator should fully inform the subject or the subject's legally acceptable representative of all pertinent aspects of the study and of any new information relevant to the subject's willingness to continue participation in the study. This communication should be documented. During a subject's participation in the trial, any updates to the consent form and any updates to the written information will be provided to the subject.
10. Study completion/study withdrawal and end of complete study

A subject will be considered as having completed the study if he/she has completed all assessments at week 3 (day 22) of the reinduction chemotherapy with or without bortezomib. Subjects who discontinue study treatment because of lack of efficacy are also considered to have completed the study, unless bortezomib was not given according to protocol.

The treatment will always be discontinued in case of:
- disease progression (see chapter 9 for definitions)
- unacceptable toxicity occurs per investigator’s discretion
- general or specific changes in the patient’s condition which render the patient unacceptable for further treatment per the investigator’s judgment
- the patient chooses to withdraw from the study
- the patient becomes pregnant or fails to use adequate birth control if able to conceive
- the patient is not able to comply with the protocol requirements
- the sponsor decides to terminate the study for significant risk-benefit concerns
- death or lost to follow-up

If the treatment is discontinued in the absence of disease progression patients should not receive other therapy until progression develops, unless this is not in the best interest of the patient.

The sponsor will notify the accredited METC and the competent authority of the end of the study within a period of 90 days. The end of the study is defined as the last visit of the patient that enrolled as last in the study.

In case the study is ended prematurely, the sponsor will notify the accredited METC and the competent authority within 15 days, including the reasons for the premature termination.

Within six months after the end of the study, the principal investigator in collaboration with sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC and the Competent Authority and the company providing study drug.
11. Study drug information including known risks of bortezomib

General information
Bortezomib (Velcade®) for injection is an antineoplastic agent available for i.v. use only. Each single dose vial contains 3.5 mg of bortezomib as a sterile lyophilized (white to off-white) cake or powder. Inactive ingredient: 35 mg mannitol, USP. EMEA issued a marketing authorization valid throughout the European Union on 26 April 2004. The full summary of product characteristics is available at www.emea.europa.eu under human medicines/EPARs.

Drug supply
Bortezomib will be supplied in boxes of bulk vials. Bortezomib will be supplied free-of-charge by J&J, all other drugs will be provided by the individual study centers as part of regular treatment.

Packaging, dispensing and storage
Unopened vials of bortezomib may be stored at controlled room temperature 25°C (77°F); excursions permitted from 15°C to 30°C (59°F to 86°F). It should be retained in the original package to protect from light. Bortezomib contains no antimicrobial preservative. When reconstituted as directed, bortezomib may be stored (at these same temperatures) in the original vial or syringe. The product may be stored for up to 3 hours in a syringe, however total storage time for the reconstituted material must not exceed 8 hours.

Bortezomib injections should be prepared and handled according to the instructions provided in Appendix B, Instructions for the Preparation and Handling of bortezomib Injections.

The other drugs are not investigational and as common and conventional agents, drug information on dexamethasone, vincristine and (intrathecal) methotrexate is not provided here.

11.1 Known Anticipated Risks of bortezomib (Velcade®)

The known anticipated risks of bortezomib are presented in table 3 ‘Known Anticipated Risks of bortezomib by MedDRA System Organ Class, Observed Incidence, and Preferred Term’, and are also available in the SPC text.

Adverse events are grouped according to the combined frequency observed in a pooled analysis of studies as of 27 March 2006 evaluating single agent bortezomib at a dose of 1.3 mg/m², administered on days 1, 4, 8, and 11 of a 21-day schedule. The analysis included the phase 3 study (M34101-039) (Currie 2004) and the phase 2 studies (M34100-024, M34100-025, M34101-040, and M34103-053). (Richardson 2003, Jagannath 2004, Currie 2005). Additional events reported since the pooled analysis was completed are reviewed as part of the ongoing safety review of bortezomib. These additional events have been incorporated into table 3 as of 04 December 2007.
### Table 3 Known Anticipated Risks of bortezomib by MedDRA System Organ Class, Observed Incidence, and Preferred Term

<table>
<thead>
<tr>
<th>System Organ Class</th>
<th>Preferred Term</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Blood and Lymphatic System Disorders</strong></td>
<td></td>
</tr>
<tr>
<td>Most common</td>
<td>Thrombocytopenia that may increase the risk of bleeding*, anemia*</td>
</tr>
<tr>
<td>Very common</td>
<td>Neutropenia*</td>
</tr>
<tr>
<td>Common</td>
<td>Lymphopenia, pancytopenia*, leukopenia*</td>
</tr>
<tr>
<td>Uncommon</td>
<td>Febrile neutropenia</td>
</tr>
<tr>
<td><strong>Cardiac Disorders</strong></td>
<td></td>
</tr>
<tr>
<td>Common</td>
<td>Arrhythmias including tachycardia, atrial fibrillation, and palpitations; acute development or exacerbation of cardiac failure, including congestive heart failure*; pulmonary edema*</td>
</tr>
<tr>
<td>Uncommon</td>
<td>Cardiogenic shock*, new onset of decreased left ventricular fraction*, atrial flutter, cardiac tamponade*, bradycardia, atrioventricular block (complete), cardiac arrest, cardiopulmonary failure</td>
</tr>
<tr>
<td><strong>Ear and Labyrinth Disorders</strong></td>
<td></td>
</tr>
<tr>
<td>Uncommon</td>
<td>Deafness, hearing impairment</td>
</tr>
<tr>
<td><strong>Eye Disorders</strong></td>
<td></td>
</tr>
<tr>
<td>Common</td>
<td>Blurred vision, conjunctival infection and irritation</td>
</tr>
<tr>
<td>Uncommon</td>
<td>Conjunctival hemorrhage</td>
</tr>
<tr>
<td><strong>Gastrointestinal Disorders</strong></td>
<td></td>
</tr>
<tr>
<td>Most common</td>
<td>Constipation, diarrhea*, nausea, vomiting*</td>
</tr>
<tr>
<td>Very common</td>
<td>Gastrointestinal and abdominal pain, excluding oral and throat</td>
</tr>
<tr>
<td>Common</td>
<td>Dyspepsia, pharyngolaryngeal pain, gastroesophageal reflux, abdominal distension, stomatitis and mouth ulceration, dysphagia, gastrointestinal hemorrhage (upper and lower gastrointestinal tract)*, rectal hemorrhage (includes hemorrhagic diarrhea)</td>
</tr>
<tr>
<td>Uncommon</td>
<td>Eructation, tongue ulceration, retching, upper gastrointestinal hemorrhage*, hematemesis*, oral mucosal petechiae, ileus paralytic*, odynophagia, enteritis, colitis, oesphagitis, fungal oesphagitis, enterocolitis, acute pancreatitis*, gastritis</td>
</tr>
<tr>
<td><strong>General Disorders and Administration Site Conditions</strong></td>
<td></td>
</tr>
<tr>
<td>Most common</td>
<td>Asthenic conditions, including weakness, fatigue, lethargy, and malaise; pyrexia</td>
</tr>
<tr>
<td>Very common</td>
<td>Rigors, edema of the lower limbs</td>
</tr>
<tr>
<td>Common</td>
<td>Neuralgia, chest pain, mucosal inflammation*</td>
</tr>
<tr>
<td>Uncommon</td>
<td>Injection site pain and irritation, injection site phlebitis, general physical health deterioration*, injection site cellulitis, catheter site cellulitis, injection site infection</td>
</tr>
<tr>
<td>System Organ Class</td>
<td>Preferred Term</td>
</tr>
<tr>
<td>--------------------------------------------</td>
<td>---------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Hepatobiliary Disorders</td>
<td></td>
</tr>
<tr>
<td>Common</td>
<td>Abnormal liver function tests</td>
</tr>
<tr>
<td>Uncommon</td>
<td>Hyperbilirubinemia, hepatitis*</td>
</tr>
<tr>
<td>Immune System Disorders</td>
<td></td>
</tr>
<tr>
<td>Uncommon</td>
<td>Drug hypersensitivity, angioedema</td>
</tr>
<tr>
<td>Infections and Infestations</td>
<td></td>
</tr>
<tr>
<td>Very common</td>
<td>Upper respiratory tract infection, nasopharyngitis, lower respiratory tract and</td>
</tr>
<tr>
<td></td>
<td>lung infections*, pneumonia*, Herpes zoster*</td>
</tr>
<tr>
<td>Common</td>
<td>Herpes zoster disseminated*, postherpetic neuralgia, sinusitis, pharyngitis,</td>
</tr>
<tr>
<td></td>
<td>oral candidiasis, urinary tract infection*, catheter-related infection*,</td>
</tr>
<tr>
<td></td>
<td>sepsis and bacteremia*, cellulitis and other skin infections*, Herpes simplex</td>
</tr>
<tr>
<td>Uncommon</td>
<td>Bronchitis, gastroenteritis*, septic shock*, urosepsis*, aspergillosis*, tinea</td>
</tr>
<tr>
<td></td>
<td>infections, Herpes zoster ophthalmic, Herpes simplex ophthalmic, meningoenec</td>
</tr>
<tr>
<td></td>
<td>phalitis herpetic, varicella, empyema</td>
</tr>
<tr>
<td>Injury, Poisoning, and Procedural</td>
<td></td>
</tr>
<tr>
<td>Complications</td>
<td></td>
</tr>
<tr>
<td>Common</td>
<td>Catheter-related complication</td>
</tr>
<tr>
<td>Uncommon</td>
<td>Subdural haematoma</td>
</tr>
<tr>
<td>Investigations</td>
<td></td>
</tr>
<tr>
<td>Common</td>
<td>Increased ALT, increased AST, increased alkaline phosphatase</td>
</tr>
<tr>
<td>Uncommon</td>
<td>Increased GGT, oxygen saturation decreased*, blood albumin decreased</td>
</tr>
<tr>
<td>Metabolism and Nutritional Disorders</td>
<td></td>
</tr>
<tr>
<td>Most common</td>
<td>Decreased appetite and anorexia, which may result in dehydration and/or weight</td>
</tr>
<tr>
<td>Very common</td>
<td>Dehydration*</td>
</tr>
<tr>
<td>Common</td>
<td>Hyperglycemia, hypoglycemia, hyponatremia, hypokalemia, hypercalcemia*</td>
</tr>
<tr>
<td>Musculoskeletal and Connective Tissue</td>
<td></td>
</tr>
<tr>
<td>Disorders</td>
<td></td>
</tr>
<tr>
<td>Very common</td>
<td>Bone pain, pain in limb, myalgia, arthralgia</td>
</tr>
<tr>
<td>Nervous System Disorders</td>
<td></td>
</tr>
<tr>
<td>Most common</td>
<td>Peripheral neuropathy (including all preferred terms under the MedDRA high-</td>
</tr>
<tr>
<td></td>
<td>level term peripheral neuropathy NEC)</td>
</tr>
<tr>
<td>Very common</td>
<td>Paresthesia and dysesthesia; dizziness, excluding vertigo; headache</td>
</tr>
<tr>
<td>Common</td>
<td>Polyneuropathy, syncope, dysgeusia</td>
</tr>
<tr>
<td>Uncommon</td>
<td>Convulsions, loss of consciousness, ageusia, encephalopathy, paralysis*,</td>
</tr>
<tr>
<td></td>
<td>reversible posterior leukoencephalopathy syndrome, autonomic neuropathy</td>
</tr>
</tbody>
</table>
Table 3  Known Anticipated Risks of bortezomib by MedDRA System Organ Class, Observed Incidence, and Preferred Term

<table>
<thead>
<tr>
<th>System Organ Class</th>
<th>Preferred Term</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neoplasms, Benign, Malignant, and Unspecified (including cysts and polyps)</td>
<td></td>
</tr>
<tr>
<td>Uncommon</td>
<td>Tumor lysis syndrome*</td>
</tr>
<tr>
<td>Psychiatric Disorders</td>
<td></td>
</tr>
<tr>
<td>Very common</td>
<td>Anxiety</td>
</tr>
<tr>
<td>Common</td>
<td>Confusion, insomnia</td>
</tr>
<tr>
<td>Uncommon</td>
<td>Delirium</td>
</tr>
<tr>
<td>Renal and Urinary Disorders</td>
<td></td>
</tr>
<tr>
<td>Common</td>
<td>Renal impairment, including renal failure and increased serum creatinine*; hematuria</td>
</tr>
<tr>
<td>Uncommon</td>
<td>Difficulty in micturition</td>
</tr>
<tr>
<td>Respiratory, Thoracic, and Mediastinal Disorders</td>
<td></td>
</tr>
<tr>
<td>Very common</td>
<td>Cough, dyspnea</td>
</tr>
<tr>
<td>Common</td>
<td>Epistaxis, exertional dyspnea, pleural effusion*, rhinorrhea, hypoxia*</td>
</tr>
<tr>
<td>Uncommon</td>
<td>Hemoptysis*, acute respiratory distress*, respiratory failure*, pneumonitis*, lung infiltrates, pulmonary alveolar hemorrhage*, interstitial lung disease*, pulmonary hypertension*, pleurisy, pleuritic pain</td>
</tr>
<tr>
<td>Skin and Subcutaneous Tissue Disorders</td>
<td></td>
</tr>
<tr>
<td>Very common</td>
<td>Skin rash, which can be pruritic, erythematous, and can include evidence of leukocytoclastic vasculitis</td>
</tr>
<tr>
<td>Common</td>
<td>Urticaria</td>
</tr>
<tr>
<td>Uncommon</td>
<td>Leukocytoclastic vasculitis</td>
</tr>
<tr>
<td>Vascular Disorders</td>
<td></td>
</tr>
<tr>
<td>Very common</td>
<td>Hypotension*</td>
</tr>
<tr>
<td>Common</td>
<td>Orthostatic/postural hypotension, petechiae</td>
</tr>
<tr>
<td>Uncommon</td>
<td>Cerebral hemorrhage*</td>
</tr>
</tbody>
</table>

Most common = ≥ 30%, Very common = 10% to 29%, Common=1% to 9%, Uncommon= < 1%

* Fatal outcomes have been reported

Abbreviations: ALT = alanine transaminase; AST = aspartate transaminase; GGT = gamma glutamyl transferase
Medical Events from Postmarketing Experience

Adverse drug reactions, as listed in the postmarketing section of the bortezomib (VELCADE®) PI (Velcade package insert or SPC (Velcade 2007)) are listed in table 4.

<table>
<thead>
<tr>
<th>System Organ Class</th>
<th>Preferred Term</th>
<th>Observed Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood and lymphatic system disorders</td>
<td>Disseminated intravascular coagulation</td>
<td>Rare</td>
</tr>
<tr>
<td>Cardiac Disorders</td>
<td>Atrioventricular block complete</td>
<td>Rare</td>
</tr>
<tr>
<td></td>
<td>Cardiac tamponade</td>
<td>Rare</td>
</tr>
<tr>
<td>Ear and labyrinth disorders</td>
<td>Deafness bilateral</td>
<td>Rare</td>
</tr>
<tr>
<td>Eye Disorders</td>
<td>Ophthalmic herpes</td>
<td>Rare</td>
</tr>
<tr>
<td>Gastrointestinal Disorders</td>
<td>Acute pancreatitis</td>
<td>Rare</td>
</tr>
<tr>
<td></td>
<td>Ischemic colitis</td>
<td>Rare</td>
</tr>
<tr>
<td>Hepatobiliary disorders</td>
<td>Hepatitis</td>
<td>Uncommon</td>
</tr>
<tr>
<td></td>
<td>Liver failure</td>
<td>Unknown</td>
</tr>
<tr>
<td>Infections and infestations</td>
<td>Herpes meningoencephalitis</td>
<td>Rare</td>
</tr>
<tr>
<td>Immune System Disorders</td>
<td>Angioedema</td>
<td>Rare</td>
</tr>
<tr>
<td>Nervous System Disorders</td>
<td>Autonomic neuropathy</td>
<td>Rare</td>
</tr>
<tr>
<td></td>
<td>Dysautonomia</td>
<td>Unknown</td>
</tr>
<tr>
<td></td>
<td>Encephalopathy</td>
<td>Rare</td>
</tr>
<tr>
<td>Respiratory, thoracic and mediastinal disorders:</td>
<td>Acute diffuse infiltrative pulmonary disease</td>
<td>Rare</td>
</tr>
<tr>
<td></td>
<td>Acute respiratory distress syndrome (ARDS)</td>
<td>Unknown</td>
</tr>
<tr>
<td></td>
<td>Interstitial pneumonia</td>
<td>Unknown</td>
</tr>
<tr>
<td></td>
<td>Pneumonitis</td>
<td>Unknown</td>
</tr>
<tr>
<td>Skin and subcutaneous system disorders</td>
<td>Toxic epidermal necrolysis</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

a Incidence is assigned using the following convention: very common (>1/10); common (>1/100 and <1/10); uncommon (>1/1000 and <1/100); rare (>1/10,000 and <1/1000); very rare (<1/10,000, including isolated reports).
12. Statistical design

This study design was chosen bearing in mind the apparent lack of effectiveness of single-agent bortezomib in heavily pretreated acute leukemias, but also the clear indications of synergistic interactions between bortezomib with other chemotherapeutic drug such as dexamethasone. Historical data on single agent dexamethasone for one week (5 mg/m^2/day) with one intrathecal administration of triple cytostatics on day 1 are available (data on file, kindly provided by P. Rohrlich, FRALLE). This shows in a cohort of 194 children with relapsed ALL, a decrease in BM blast percentage from median 80 (range, 5-100)% to 40 (0-100)% after 1 week. Similarly, PB blasts count decreased from median 538 (range, 0-300,000) blasts/µl to 22 (0-27,552), or mean 893 (SD 363) to 530 (SD) after 1 week. The percentage of “poor dexamethasone responders” was 14.4% in the total cohort of 160 patients that could be evaluated for this parameter, and 19.2% in the group of patients (n=120) with at least 1000 blasts/µl PB at start of treatment (ie, potentially poor responders).

Statistical comparison of response and toxicity data in both arms will be done using conventional tests, especially Pearson chi-square statistics for differences in the distribution of categorical variables (Fisher exact test in case of sparse data), and the Mann-Whitney-U test for differences in continuous variables.

The number of 12 patients in each group provides a power of 80% to detect a statistically significant at least 30% lower absolute number of circulating leukemic blasts (2-sided test, alpha <0.05) on day 8 in patients exposed to bortezomib (“early bortezomib”) as compared to patients not yet exposed to bortezomib (“late bortezomib”). The randomization will be stratified for the number of circulating leukemic blasts at inclusion in this study: 0.1-10 x 10^9/l versus ≥ 10 x 10^9/l.

In addition, based on calculations of 95%-confidence intervals, these numbers will allow predicting an incidence of <50% grade III/IV toxicity if <8 out of 24 patients suffer such toxicity. In view of early dropout, approx. 30 patients should be enrolled.
13. Ethical issues

This study will be conducted in accordance with the ethical principles that have their origin in the current Declaration of Helsinki, and will be consistent with International Conference on Harmonization Good Clinical Practice (ICH GCP) and applicable regulatory requirements.

The study will be conducted in compliance with the protocol. The protocol and any Amendments and the subject informed consent will receive Institutional Review Board (IRB)/Independent Ethics Committee (IEC) approval/favorable opinion prior to initiation of the study.

The rights, safety and well-being of the trial subjects are the most important considerations and should prevail over interests of science and society.

Study personnel involved in conducting this trial will be qualified by education, training, and experience to perform their respective task(s), and will be authorized to perform study related procedures as laid down in the delegation of authority log.

Systems with procedures that assure the quality of every aspect of the study will be implemented.
14. Publication policy

The main results of the clinical trial will be published after complete data collection and evaluation. Publications and/or presentations are to be initiated and/or authorized by the principal investigator. As a guideline, abstracts and manuscripts will be prepared by the principal investigator, who will be first author, and will include the sponsor's representative as last author.

The following persons will be included as co-authors:

- investigators who have recruited at least 1 patient into the trial. In case the journal to which the manuscript is submitted does not allow a large number of co-authors this may have to be restricted to investigators enrolling at least 2 patients.
- the co-investigators representing their country/national group as co-sponsor.
- The order of authorship will be determined by the enrolled number of patients, followed by alphabet of the last (family) name

The final manuscript should be prepared within six months after the last visit of the last patient who was enrolled in the study. The co-authors must notify the main author in writing concerning their approval or proposed changes to the manuscript within four weeks after receiving the publication draft. If such comments are not received within these four weeks, approval will be assumed. If the principal investigator fails to submit a manuscript on the multicenter study results within twelve (12) months after conclusion, abandonment or termination of the Study at all sites, the investigators may publish the results from each site individually. In the meantime, any publication in the form of a lecture, poster or publication of data must be approved by the principal investigator. Such publications can not occur before the joint main publication of the study results as described above. Enquiries from the press and/or general public concerning study results may only be answered by the principal investigator of the clinical trial after consultation with the sponsor.

Manuscripts will acknowledge the support by ITCC and I-BFM-SG.
15. Responsibilities of Sponsor, Co-sponsors and Principal Investigator

Erasmus MC is the coordinating sponsor of the study. There is a co-sponsor for each country involved in the study. Certain tasks will be delegated by the sponsor to the co-sponsors.

The co-sponsor in each country will provide insurance or indemnity in accordance with the applicable regulatory requirements for all patients within that country.

Any investigator or co-investigator who signed this protocol agrees to carry out this research in accordance with the protocol approved by the ethic committee, GCP and regulatory requirements.

Study personnel involved in conducting this trial will be qualified by education, training, and experience to perform their respective task(s).

Each local investigator (including co-investigators and pharmacists) should provide the Sponsor with his/her recent signed and dated CV.

The Sponsor and the PI have the right to prematurely discontinue the study for significant efficacy or safety problems and will notify the co-investigators in writing, as well as the ethics committees and the competent authorities according to local law and regulations.
16. Patient registration and randomization procedure

Patients will be recruited from a population of children and adolescents treated at or referred to one of the participating study sites.

A study site can only start enrolling patients once the local ethical committee and other relevant authorities have agreed with the study, if the contract with the sponsor has been signed, and if the study drug (bortezomib) is available at the study site. It is the responsibility of the local investigator to ensure that the patient is eligible for the study before enrolling him/her.

The study site will be able to enroll patients into the study by procedure of registration and randomization using the ALEA randomization program available via the website http://www.skion.nl/d cog-ectc

Randomization will be done with the method of minimisation. This method of dynamic randomization will be done utilizing a web-based randomization service called TenALEA. This web-based randomization program will provide continuous service during 24 hours 7 days per week. Notification of a randomization will be send automatically to all involved parties. Each study site will request a login for the local person responsible for the randomization of the patients. Detailed instructions will be available in the Procedure Manual.

Alternatively, patient registration and randomization may be done by faxing the signed Randomization Form to:
DCOG – ECTC Trial Office
Fax: +31 (0) 10 703 6681 (in case of dysfunction, fax +31 (0) 10 703 1134)
The signed Randomization Form may also be sent by e-mail to: research-kocr@erasmusmc.nl

The following information will be required to register and randomize a patient:
1. Date of birth
2. Number of circulating leucemic blasts at inclusion (stratification factor) leukemia type and phase; date of initial diagnosis and data of first relapse (if relevant)
3. All eligibility criteria

In case any enrollment issues need to be discussed prior to randomization, please send an e-mail to the P.I. (Gertjan Kaspers) for discussion: gil.kaspers@vumc.nl. Please send a cc to the trial office (research-kocr@erasmusmc.nl) and to the sponsor’s representative (Michel Zwaan): c.m.zwaan@erasmusmc.nl.

After enrolling a patient, treatment according to this protocol should start within 3 days.
17. References


18. Appendices

A  performance scales
B  preparation and handling of bortezomib injections
C  translational research
## Appendix A  Performance scales

<table>
<thead>
<tr>
<th>Lansky score: 0-16 years</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fully active</td>
<td>100</td>
</tr>
<tr>
<td>Minor restriction in normal physical activity</td>
<td>90</td>
</tr>
<tr>
<td>Active, but tires more quickly</td>
<td>80</td>
</tr>
<tr>
<td>Both greater restriction and less time spent in active play</td>
<td>70</td>
</tr>
<tr>
<td>Minimal active play, busy with quieter activities</td>
<td>60</td>
</tr>
<tr>
<td>Gets dressed, but no active play, able to participate in all quiet play and activities</td>
<td>50</td>
</tr>
<tr>
<td>Mostly in bed, participates in quiet activities</td>
<td>40</td>
</tr>
<tr>
<td>In bed, needs assistance even for quiet play</td>
<td>30</td>
</tr>
<tr>
<td>Often sleeping, play limited to passive activity</td>
<td>20</td>
</tr>
<tr>
<td>No play, does not get out of bed</td>
<td>10</td>
</tr>
<tr>
<td>Unresponsive</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Karnofsky score: 16 years and older</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal, no complaints, no evidence of disease</td>
<td>100</td>
</tr>
<tr>
<td>Able to carry on normal activities</td>
<td>90</td>
</tr>
<tr>
<td>Normal activity with effort</td>
<td>80</td>
</tr>
<tr>
<td>Cares for self, unable to carry on normal activity or to do active work</td>
<td>70</td>
</tr>
<tr>
<td>Requires occasional assistance, is able to care for most of own needs</td>
<td>60</td>
</tr>
<tr>
<td>Requires considerable assistance, frequent medical care</td>
<td>50</td>
</tr>
<tr>
<td>Disabled, requires special care/assistance</td>
<td>40</td>
</tr>
<tr>
<td>Severely disabled, hospitalization</td>
<td>30</td>
</tr>
<tr>
<td>Hospitalization, very sick, active treatment</td>
<td>20</td>
</tr>
<tr>
<td>Moribund, fatal processes in progression</td>
<td>10</td>
</tr>
<tr>
<td>Dead</td>
<td>0</td>
</tr>
</tbody>
</table>
Appendix B Instructions for the Preparation and Handling of bortezomib (VELCADE®) Injections

A licensed pharmacist or a properly trained designee will prepare all doses of bortezomib. It is very important that the exact dose is accurately dispensed and administered. If possible, the same person should prepare all doses (on all dosing days). Bortezomib will be administered only to eligible subjects under the supervision of the investigator or identified subinvestigator(s).

Calculation of Dose
The amount (in mg) of bortezomib to be administered will be determined based on body surface area (BSA). BSA will be calculated based on body weight and height using a standard calculation (Attachment 8, Suggested Body Surface Area Calculation). The bortezomib dose will not be corrected for obese subjects. The dose should be calculated on Day 1 of each cycle and remain consistent during an individual cycle. The dose administered should remain consistent across cycles unless a notable change in weight (e.g., loss or gain of ≥10%) is documented during the Day 1 weight assessment of the cycle, in which case the subject’s dose should be recalculated at that time. In the event of bortezomib-associated toxicity during the study, the dose may be decreased according to the dose reduction schedules provided in the protocol.

Preparation and Handling of Solution
Bortezomib is cytotoxic. As with all cytotoxic drugs, caution should be exercised when handling and preparing bortezomib solution. Caution should be taken to avoid accidental ingestion, inhalation, and dermal or ocular contact when handling the test article. Cytotoxic drugs should only be handled by staff who are specially trained in the safe handling of such preparations. The pharmacist will prepare the bortezomib dose under aseptic conditions. The use of gloves and other appropriate protective clothing is recommended.

Study drug will be supplied in sterile, single-use vials containing 3.5 mg of bortezomib. Each vial of bortezomib for injection should be reconstituted with 3.5 mL of normal (0.9%) saline, Sodium Chloride Injection USP, so that the reconstituted solution contains bortezomib at a concentration of 1 mg/mL. Reconstitution should take place under a vertical laminar flow biological cabinet (hood) within 8 hours before dosing. Dissolution is completed in approximately 10 seconds. The reconstituted solution should be clear and colorless, with a final pH of 5 to 6. Reconstituted bortezomib should be administered promptly and in no case more than 8 hours after reconstitution.

Preparation of Syringe for Injection
The appropriate amount of bortezomib will be drawn from the injection vial according to the dosage calculations. Designated study site personnel will administer bortezomib as an i.v. push over 3- to 5-seconds followed by a standard saline flush (e.g., 0.9% Sodium Chloride). Indwelling cannulas may be used for injections. Vials are for single-use administration only. Bortezomib should be administered promptly after drawing into syringe and must be administered within 3 hours of drawing into syringe and 8 hours of reconstitution (maintained at controlled room temperature 25°C (77°F). Conditions for storage of bortezomib are provided in Section 12. There must be at least 72 hours between bortezomib doses. Procedures to follow in case of bortezomib contact with the skin are provided below.
Discarding of Vial and Syringe
After administration all materials that have been used for preparation should be disposed of according to standard practices. All unused vials must be saved for reconciliation by the study monitor. A log must be kept of all disposed materials.

Procedures to follow in case of skin contact:
Wash the affected area immediately and thoroughly with soap and water and diluted hydrogen peroxide. Remove contaminated clothing and dispose of according to standard procedures. In case of contact with mucous membranes, flush thoroughly with water. Always contact the investigator after any form of body contact.
Appendix C Translational Research

1. Title and Summary

Title: Application of bortezomib in pediatric acute lymphoblast leukemia (ALL)

Projectleaders: J. Cloos and G.J.L. Kaspers.

Full address: Dept. of Pediatric Oncology / Hematology
VU University Medical Center,
De Boelelaan 1117, NL-1081 HV, Amsterdam, The Netherlands.
Phone: +31 20 444 2420, Fax: +31 20 444 2422,
E-mail: gjl.kaspers@vumc.nl

Background: Childhood relapsed ALL remains one of the most significant problems in pediatric oncology. Therefore, novel drugs with a new mechanism of action are needed to improve prognosis. The proteasome inhibitor bortezomib (PS341, Velcade®, BTZ) represents a novel class of therapeutics drugs that revealed promising clinical activity in Multiple Myeloma (MM) and Lymphoma patients. Recently, it has been shown that BTZ also exerts activity against Acute Lymphoblastic Leukemia (ALL) both in vitro and in vivo in mice. Moreover, BTZ administration has shown to be feasible in a phase I trial in adult and children as well as combined with conventional chemotherapy in pediatric relapsed ALL patients. However, determinants for BTZ response are still not fully understood. Therefore, we developed three different BTZ resistant cell line models, to obtain more insight this matter. In addition, we initiated a multicenter, multinational, open label, comparative and randomised phase II study on the antileukemic activity of BTZ with conventional combination chemotherapy in 24 (12 in each arm) pediatric relapsed ALL patients. In this trial, the feasibility and efficacy of BTZ addition to the standard re-induction treatment will be evaluated. That clinical study enables important translational research on the antileukemic activity of bortezomib.

Aim of the study: In this study, we aim to elicit cell biological and proteasome characteristics that can explain and/or predict sensitivity to the proteasome inhibitor BTZ in a preclinical ALL cell line model and in clinical pediatric ALL samples before and after BTZ treatment.

Methods: Preclinical in vitro study: In order to elucidate the mechanism of action and resistance of BTZ, we generated subclones of the ALL cell line CCRF-CEM with various levels of BTZ resistance (10 fold to 170 fold). In order to further clarify the mechanistic basis for this resistant phenotype, expression microarray experiments will be conducted. These data will be compared to expression data, recently obtained at our laboratory, of the two other BTZ resistant cell line models; the AML THP-1 cell line and the Multiple Myeloma RPMI-8226 cell line. A selected set of alternatively expressed genes will be validated by RT-PCR and Western blot to serve as a potential predictive marker of BTZ response.

Add-on study on the clinical trial: In order to monitor the efficacy of the proteasome inhibition, direct and indirect markers for proteasome activity will be utilized. Moreover, possible molecular mechanisms of resistance to BTZ will be investigated. This includes measurement of activity and composition of the catalytic subunits of the proteasome, NF-kB activity, TNF-alpha production, MHC-I expression and stabilization of several pro apoptotic proteins in peripheral blood and bone marrow samples. In addition, in a selected number of patients, BTZ levels and, if possible, proteasome inhibition will be measured in the cerebrospinal fluid (CSF). Furthermore, relevant findings form the preclinical study will be confirmed in these patient samples. The time schedule for each type of experiment will be dependent on recruitment rate of the clinical study which is expected to be completed within 2 years.

Relevance: Clinical patient samples derived from this clinical trial will give a unique opportunity to investigate the in vivo effect of BTZ in a pediatric cohort. Data derived from this study may give more insight in the main mechanism of action of BTZ and thereby contribute to the improvement of the treatment of pediatric ALL and relapsed ALL in particular.
2. **Introduction/background:**
Childhood relapsed Acute Lymphoblastic Leukemia (ALL) remains one of the most significant problems in pediatric oncology. ALL is the most frequent type of childhood cancer, relapses in about 25% of newly diagnosed patients, and can be cured in only 40% of relapsing patients. Early first relapsed ALL, T-cell relapsed ALL, treatment-refractory first relapsed ALL and second relapsed ALL have a much worse prognosis with less than 10% probability of long-term survival (Nguyen 2008). Therefore, novel drugs with a new mechanism of action are needed to improve prognosis and ultimately avoid relapses.

The proteasome inhibitor bortezomib (PS341, Velcade®, BTZ) represents a novel class of therapeutic drugs that revealed promising clinical activity in Multiple Myeloma (MM) and Lymphoma patients (Richardson 2003). The ubiquitin-proteasome pathway (UBP), which is inhibited by BTZ, degrades the majority of misfolded, damaged, short-, or long-lived regulatory proteins in the cell. Proteins targeted for degradation are tagged by the ligation of multiple ubiquitins by a family of ubiquitin ligases. Subsequently, the ubiquinated proteins are trafficked towards the proteasome where they are unfolded and degraded by the 26S proteasome complex. This complex consists of two 19S caps that deubiquinate and unfold the tagged protein, and a 20S core unit, which actually cleaves the protein in small peptides. The 20S unit is constructed of two structural alpha rings and to catalytic beta rings, each harboring 7 subunits. The beta rings harbor the catalytic beta 1, beta 2 and beta 5 subunits. BTZ mainly inhibits the beta 5 activity, at higher concentrations beta 1 activity is inhibited, while it stimulates beta 2 activity (Reviewed by Ravid et al. 2008).

Although it is known that by blocking the 26S proteasome, BTZ inhibits several cell cycle and pro-survival pathways, including NF-kB (Franke 2008a), the exact mechanism of action is still not completely elucidated. Concerning BTZ sensitivity determinants some recent preliminary data became available. Kraus et al. (2007) showed in several hematological malignancies ex vivo that the ratio of the activity of the catalytic subunits (beta 2 / (beta 1 + beta 5)) correlates with ex vivo sensitivity to BTZ. Furthermore, it has been shown that proteasome subunit expression also correlates with sensitivity to BTZ in several cell line models (Xu 2008; Fuchs 2008; Brusse 2008). In order to get more insight in this topic, we generated 3 different BTZ resistant cell lines. We have shown that in vitro BTZ resistance coincides with upregulation of the catalytic beta subunits expression and mutation of the beta 5 subunit in an AML(Oerlemans & Franke 2008), ALL (Franke 2008b) and MM model (Franke 2008c). These data are concurrent with the results of Lu et al. (2008) in an ALL model.

Previously we have shown that AML cells can be sensitized towards glucocorticoids by the NF-kB inhibitor sulfasalazine (Oerlemans & Vink 2007). Because BTZ also exerts NF-kB inhibiting properties, we also tested in primary patient samples the combination of BTZ with dexamethasone ex vivo. Figure 1 shows that BTZ alone already exerts promising activity against primary pediatric AML and in particular against ALL patients’ samples. Moreover, the ex vivo combination of BTZ with dexamethasone conveys additive or even synergistic activity (Franke 2007). Recently, in a pediatric preclinical testing program, BTZ was confirmed to be very effective against ALL cell lines and primary patient samples, in vitro as well.
as in vivo in a NOD/Scid mouse model (Houghton 2008). In addition, it has been shown in a phase I trial that administration of BTZ to adult relapsed ALL patients is feasible (Cortes 2004). Furthermore, the feasibility of BTZ administration as a single drug has also been shown in a pediatric patients suffering from leukemia, although single-agent antileukemic activity in that setting was not observed (Horton 2007). Finally, preliminary unpublished data from a study in the USA, show promising activity of BTZ co-administration with dexamethasone (Messinger 2008).

Due to these encouraging results, we have initiated a multicenter, multinational, open label, comparative and randomised phase II study on the antileukemic activity of BTZ with conventional combination chemotherapy in 24 (12 in each arm) relapsed ALL patients (children and adolescents). The clinical trial is a collaboration between Innovative Therapies for Children with Cancer Consortium (ITCC), represented by Dr. P. Kearns, and International BFM Study Group (I-BFM-SG), represented by Dr. C.M. Zwaan. The DCOG, UK CCLG, French FRALLE group, German BFM group and AIEOP will join the clinical study. In this trial, the feasibility and efficacy of BTZ addition to the standard re-induction treatment will be evaluated. Furthermore, the “early” BTZ administration (arm A) will be compared to BTZ administration in week 2 (“late”, arm B).

Time period of accrual will depend on the number of participating centers, currently scheduled to be 15-20 centers. Accrual is aimed to be completed within 2 years from the start of the study. The study is planned to open Q2 of 2009.

Three clinical parameters will be analyzed in this phase II trial:

1. Antileukemic activity of BTZ when added to dexamethasone and vincristine and intrathecal methotrexate as determined by the absolute peripheral blood (PB) blast count and the absolute bone marrow (BM) blast percentage on day 8 of treatment, and BM and PB analysis on day 22 of treatment, in absolute numbers and expressed as an M1, M2 or M3 marrow (<5%, 5-15%, 15% or above respectively).

2. Feasibility of combining BTZ with dexamethasone and vincristine and intrathecal methotrexate, as determined by the percentage of patients in whom BTZ had to be dose-reduced or withdrawn because of toxicity.

3. Toxicity of BTZ when combined with dexamethasone and vincristine and intrathecal methotrexate, as determined by the percentage of patients suffering from grade III/IV toxicity in any field.

Clinical patient samples derived from this clinical trial will give a unique opportunity to investigate which parameters are relevant for the in vivo effect of BTZ in a pediatric cohort. Data derived from this study may provide more insight in the exact of the mechanism of action of BTZ and thereby contribute to the improvement of the treatment of pediatric ALL and relapsed ALL in particular.
3. **Aims of the study: (max ½ A4)**

In this study, we aim to elicit cell biological and proteasome characteristics that can explain and/or predict sensitivity to the proteasome inhibitor bortezomib (BTZ, PS-341, Velcade®) in a preclinical Acute Lymphoblastic Leukemia (ALL) cell line model and in clinical pediatric ALL samples.

In the preclinical *in vitro* study we will:

1. Characterize subclones of the CCRF-CEM ALL cell line with various levels of BTZ resistance.
   a. Elucidate mechanism of action
   b. Elucidate mechanism of resistance
   c. Identify determinants for BTZ response
2. Identify possible universal resistance mechanisms by comparing the resistant subclones with those of BTZ resistant THP-1 (AML) and RPMI-8226 (MM).
3. Correlate selected determinants, identified in BTZ resistant cell lines, with *ex vivo* sensitivity of primary pediatric ALL to BTZ.

In the add-on study on the clinical trial we will:

1. Evaluate BTZ levels and proteasome inhibition in peripheral blood of all patients and, whenever feasible, in cerebrospinal fluid (CSF) of a limited number of patients.
2. Compare selected read-out systems, both direct and indirect, as potential markers for efficacy of proteasome inhibition.
3. Correlate the different read-outs, before and during treatment, with clinical response.
4. Determine expression, activity and mutation status of the catalytic subunits of the proteasome in blood and bone marrow cells of BTZ treated pediatric ALL patients.
5. Confirm the clinical value of the newly discovered *in vitro* derived determinants for BTZ response as determined by the absolute peripheral blood (PB) blast count and the absolute bone marrow (BM) blast percentage on day 8 of treatment, and BM and PB analysis on day 22 of treatment, in absolute numbers and expressed as an M1, M2 or M3 marrow (<5%, 5-15%, 15% or above respectively).

4. **Materials and methods: (max 1 A4)**

*Preclinical in vitro study*

In order to elucidate the mechanism of action and resistance of BTZ, we generated BTZ resistant subclones of the ALL cell line CCRF-CEM. These subclones show upregulation of the catalytic subunits of the proteasome together with mutations in the beta 5 subunit. However, these data do not fully explain the resistant phenotype. In order to further clarify this resistant phenotype, data of expression microarrays will be compared with that of the two other BTZ-resistant cell line models; the AML THP-1 cell line and the Multiple Myeloma RPMI-8226 cell line at various levels of BTZ resistance. Finally, a selected set of differentially expressed genes will be validated by RT-PCR and Western blot. Relevant determinants for BTZ response will be confirmed *ex vivo* in primary patient samples.

*Add-on study on the clinical trial*

Samples of the clinical trial of all participating centers will be collected in the VU University Medical Center in Amsterdam. Samples will be redrawn at several time points, including before and 24 hours after BTZ administration. In order to monitor the efficacy of the proteasome inhibition, direct and indirect markers for proteasome activity will be utilized. Moreover, possible molecular mechanisms of resistance to BTZ will be investigated. Relevant determinants for BTZ response that are found in the preclinical *in vitro* study will also be included. The priority given to the different measurements is depicted between brackets.
• **Direct proteasome activity.** Analysis of the beta 1, 2 and 5 associated proteasome catalytic activity will be performed at several time point using an intact cell based bioluminescent assay (Promega) which is currently being validated on primary patient samples (1).

• **Indirect proteasome activity**
  - Part of the mode of action of BTZ is attributed to its ability to inhibit the transcription factor NF-kB by stabilizing the natural inhibitor of NF-kB (IkB). To this end, inhibition of NF-kB activity will be determined using a lentiviral reporter construct (Wurdinger 2008) (2).
  - TNF-alpha production is dependent on the NF-kB activity. Given the notion that in a recent study in lymphoma patients a correlation was observed between a decrease in TNF-alpha production and response to BTZ (Strauss 2006), we will measure this parameter in the current study for pediatric lymphoblastic leukemia (4).
  - As indirect marker for BTZ efficacy, a decrease in MHC-I expression (shown in Multiple Myeloma (Shi 2008)) will be monitored using flow cytometry (3) as well as the stabilization of several pro apoptotic proteins (6).

• **BTZ resistance.** To determine a possible correlation between proteasome properties and antileukemic activity, catalytic subunit expression of the proteasome will be determined by Western blot analysis (Oerlemans & Franke 2008) (5). Finally, the DNA of the genes of the catalytic proteasomal subunits of non-responding patients will be sequenced to identify possible mutations resulting in amino acid changes in the BTZ-binding pocket of the proteasome (7).

• **Ex vivo BTZ sensitivity.** The ex vivo cytotoxicity of BTZ will be determined using a MTT assay. This test will be performed on t = 0 and t = day 8 samples, if the leukemic cell percentage can be enriched above 70% at least (8). Efficacy of BTZ will be determined by comparing the treatment response in the first week in arm A with arm B, and by absolute treatment response as defined in the clinical trial. For this study, patient samples will be collected, cryopreserved and analyzed simultaneously for every type of experiment. The time schedule for each type of experiment will be dependent on recruitment rate of the clinical study which is expected to be completed within 2 years.

**References translational research**


in Human RPMI-8226 Multiple Myeloma Cells: Molecular Characterization, Cross-Resistant with Other Proteasome Inhibitors but Marked Sensitization to Glucocorticoids. Blood (ASH Annual Meeting Abstracts), 112: 2640.


