D-ALBA Front-Line Sequential Treatment of Adult Philadelphia Chromosome Positive (Ph+) Acute Lymphoblastic Leukemia (ALL) Patients with Dasatinib and the Bispecific Monoclonal Antibody Blinatumomab

GIMEMA LAL2116

EudraCT number 2016-001083-11
Clinical Trial Number NTC02744768

Date 09.11.2016	*Final version #2.0*

This protocol has been written and will be conduct in respect of the Helsinki Declaration, of Good Clinical Practice and of applicable national regulations

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This protocol has been approved by Fondazione GIMEMA – Franco Mandelli ONLUS. The following signatures document this approval.

so delega:

Roma, 20.2.2017

Prof. Franco Mandelli
Scientific Coordinator

Date

21.2.2017

Prof. Sergio Amadori
AL Working Party Chairman

Date

16/2/2017

Prof. Roberto Foà
Study Coordinator

Date

Investigator statement

I have read the protocol, including all appendices, and I agree that it contains all necessary details for me and my staff to conduct this study as described. I will conduct this study as outlined herein and will make a reasonable effort to complete the study within the time designated.

I will provide all study personnel under my supervision copies of the protocol and access to all information provided by Fondazione GIMEMA – Franco Mandelli ONLUS. I will discuss this material with them to ensure that they are fully informed about the drugs and the study.

Principal Investigator, Name (Printed)

Signature

Date

Site Number

1 Synopsis

D-ALBA Front-Line Sequential Treatment of Adult Philadelphia Chromosome Positive (Ph+) Acute Lymphoblastic Leukemia (ALL) Patients with Dasatinib and the Bispecific Monoclonal Antibody Blinatumomab

Study Phase: II

Objectives: To explore the activity of a front-line chemo-free approach based on dasatinib plus steroids administration as induction treatment (day +85), followed by the infusion of Blinatumomab, in adult Ph+ ALL.

The primary objective of the trial is to evaluate the activity of a combination approach based on dasatinib and blinatumomab in obtaining a minimal residual disease MRD negativity (complete molecular response: CMR, e.g. BCR-ABL1/ABL1=0) in adult Ph+ ALL.

The secondary objectives are:

- The feasibility of a chemo-free approach, based on Dasatinib and Blinatumomab, in adult Ph+ ALL patients ≥18 years, with no upper age limit
- CMR achievement after induction with Dasatinib
- Capability of Blinatumomab to reduce the MRD levels (an extension of the primary objective)
- CMR duration
- Disease-free survival (DFS)
- Overall survival (OS)
- Cumulative incidence of relapse (CIR)
- Safety profile
- CMR achievement, duration of CMR, OS and DFS according to the clinical, biological and molecular characteristics: clinical and biological assessment at baseline, type of fusion protein (p190 *vs* p210) and presence of additional genomic lesions identified by SNP arrays.

Study design:
This study is an open-label, multicenter, phase II study based on the combination of Dasatinib - administered orally at a dose of 140 mg QD for a 12-week induction treatment - with Blinatumomab. Patients will initiate treatment with steroids 7 days prior to starting Dasatinib and will continue up to day 31. Thereafter, patients who obtain a complete hematological response (CHR) will receive a post-remissional treatment with Blinatumomab (28 µg/die) (minimum 2 cycles, up to a maximum of 5, all administered for 28 days continuous intravenous infusion (CIVI) with a 2 weeks wash-out interval). A minimum number of 2 cycles is mandatory; the administration of 3 additional cycles will depend on the level of response to therapy with Blinatumomab (for instance decrease in MRD, but not negativity) and medical decision (i.e. toxicity and compliance to treatment, possibility of undergoing transplant procedures depending on age, donor availability and presence of comorbidities that might contraindicate them).
During treatment with Blinatumomab, Dasatinib will not be discontinued. In case of toxicity deemed related to Dasatinib and/or the combination of the 2 compounds, Dasatinib might be reduced to 100 mg/day. Dasatinib will be continued for the whole study duration in these patients.
Patients not achieving a CHR after Dasatinib will also receive Blinatumomab (28 µg/die) (minimum 2 cycles, up to a maximum of 5, all administered for 28 days CIVI with a 2 weeks wash-out interval). The number of cycles will be established on the basis of response to therapy with Blinatumomab and medical decision.
Dasatinib will be interrupted during treatment with Blinatumomab only in these patients.

CNS prophylaxis will be carried out with medicated lumbar punctures (MTX 15 mg, Methylprednisolone 20 mg): 6 prophylactic injections will be administered during the induction phase (at days +14, +22, +43, +57 and +85). Subsequently, they will be administered at the end of each cycle with Blinatumomab (day +29).

Population:

Inclusion criteria:

- Newly diagnosed adult B-precursor Ph+ ALL patients.
- Age greater or equal to18 years.
- Signed written informed consent according to ICH/EU/GCP and national local laws.
- ECOG Performance Status 0 or 1 and/or WHO performance status less or equal to 2.
- Renal and hepatic function as defined below:
  - AST (GOT), ALT (GPT), and AP <2 x upper limit of normal (ULN)
  - Total bilirubin <1.5 x ULN
  - Creatinine clearance equal or greater than 50 mL/min
- Pancreatic function as defined below:
  - Serum amylase less or equal to 1.5 x ULN
  - Serum lipase less or equal to1.5 x ULN
- Normal cardiac function.
- Negative HIV test, negative HBV DNA and HCV RNA.
- Negative pregnancy test in women of childbearing potential.
- Bone marrow specimen from primary diagnosis available.

Exclusion criteria:

- Prior systemic chemotherapy for leukemia and/or CD19-directed therapy
- History of or current relevant CNS pathology (current ≥grade 2 epilepsy, seizure, paresis, aphasia, clinically relevant apoplexia, severe brain injuries, dementia, Parkinson's disease, organic brain syndrome, psychosis).
- Impaired cardiac function, including any one of the following:
  - LVEF (Left Ventricular Ejection Fraction) <45% as determined by MUGA (multigated acquisition) scan or echocardiogram.
  - Complete left bundle branch block.
  - Use of a cardiac pacemaker.
  - ST depression of >1mm in 2 or more leads and/or T wave inversions in 2 or more contiguous leads.
  - Congenital long QT syndrome.
  - History of or presence of significant ventricular or atrial arrhythmia.
  - Clinically significant resting bradycardia (<50 beats per minute).
  - QTc >450 msec on screening ECG (using the QTcF formula).
  - Right bundle branch block plus left anterior hemiblock, bifascicular block.
  - Myocardial infarction within 3 months prior to starting Dasatinib.
  - Angina pectoris.
- Other clinically significant heart disease (e.g., congestive heart failure, uncontrolled hypertension, history of labile hypertension, or history of poor compliance with an antihypertensive regimen).
- Impairment of gastrointestinal (GI) function or GI disease that may significantly alter the absorption of Dasatinib (e.g., ulcerative diseases, uncontrolled nausea, vomiting, diarrhea, malabsorption syndrome, or small bowel resection).
- History of or current autoimmune disease.
- Systemic cancer chemotherapy within 2 weeks prior to study.
- Known hypersensitivity to immunoglobulins or to any other component of the study drug formulation.
- Active malignancy other than ALL with the exception of basal cell or squamous cell carcinoma of the skin, or carcinoma "in situ" of the cervix.
- Active infection, any other concurrent disease or medical condition that are deemed to interfere with the conduct of the study as judged by the investigator.
- Nursing women or women of childbearing potential not willing to use an effective form of contraception during participation in the study and at least 3 months thereafter, or male patients not willing to ensure effective contraception during participation in the study and at least three months thereafter.

Treatment:

Adult Ph+ ALL (≥18 years old, with no upper age limit) patients will begin treatment with Dasatinib, 140 mg/day, from day 1 to day +84. Prednisone (PDN) will be administered from day -6 to day +0 (during which the presence of the BCR/ABL1 alteration will be established), at escalating doses up to 60 mg/m^2; PDN will be

continued up to day +24 and progressively tapered up to day +31.
HLA typing will be performed immediately after the diagnosis for eligible patients.
MRD will be evaluated by quantitative real-time PCR (Q-RT-PCR) at fixed time points (days +22, +45, +57) during the induction and at day +85, the latter for molecular response evaluation.

Upon induction:
- Patients in CHR will receive Blinatumomab at a dose of 28 µg/die as continuous intravenous infusion (CIVI) at a constant flow rate for four weeks, followed by a two-week infusion-free interval, defined as one treatment cycle. A variable number of cycles will be administered (minimum 2, up to a maximum of 5 cycles). Two cycles are mandatory; the administration of 3 additional cycles will depend on the level of response to therapy with Blinatumomab (for instance decrease in MRD, but not negativity) and medical decision (i.e. toxicity and compliance to treatment, possibility of undergoing transplant procedures depending on age, donor availability and presence of comorbidities that might contraindicate them). During treatment with Blinatumomab, Dasatinib will not be discontinued. In case of toxicity deemed related to Dasatinib and/or to the combination of the 2 compounds, Dasatinib may be reduced to 100 mg/day. In these patients, MRD will be evaluated at the end of each Blinatumomab cycle; Dasatinib will be continued for the whole study duration in these patients.

- Patients not achieving a CHR after induction with Dasatinib (day +85) will receive Blinatumomab (28 µg/die) (minimum 2 cycles, up to a maximum of 5); dasatinib will be interrupted during treatment with Blinatumomab only in these cases.

CNS prophylaxis will be performed with medicated rachicenteses (MTX 15 mg, Methylprednisolone20) at the following time points:
- during the induction phase (at diagnosis, at days +14, +22, +43, +57 and +85)
- during consolidation treatment with Blinatumomab: after completion of each cycles (day+29).

Sample Size: The number of patients required to demonstrate this hypothesis with a power of 90% and a Type I error probability of 5% and considering a 10% drop-out, is 60. In the proposal, to reject the null hypothesis that $p \leq 0.40$ vs. the alternative hypothesis that $p > 0.60$ with Type I error probability (α) equal to 0.05 and 90% power (1-β), 54 evaluable patients has to be accrued. Considering a 10% rate of non-evaluable patients due to ineligibility, toxicity, medical decision or refusal before treatment start, the estimated total number of patients to include in the study is 60. In the first stage of the study, 29 evaluable patients (32 considering a 10% of drop-out) will be enrolled and the trial will be terminated if 12 or fewer responses will be achieved; otherwise, 25 further evaluable patients (28 considering a 10% of drop-out) will be enrolled in the second stage. If the total number of responses will be less than or equal to 27, the combination therapy will not be recommended for further studies. If the total number of CMRs is at least 28, the association will be deemed worthy of further investigations. Calculations were implemented in PASS2008 using a Simon two stage (minimax) phase II study design.

Study duration: 41 months: 18 months of enrolment + 3 months of Dasatinib treatment + a maximum of 8 months of Blinatumomab treatment + 12 months of follow-up.

2 Background and introduction

2.1 ALL: treatment overview and rationale

Patients who have the Philadelphia (Ph) chromosome and/or the associated *BCR-ABL1* oncogene still have a poorer prognosis than other ALL subtypes (Ref. 1, Ref. 2). The Ph rearrangement gives rise to the BCR-ABL1 oncogene, whose gene product is a constitutively active tyrosine kinase that is associated with defects in cellular adhesion, apoptosis and DNA repair, as well as growth factor independence (Ref. 3). In addition, recent studies have demonstrated that deletions of *IKZF1* (encoding the transcription factor Ikaros) may be an important event in the development of Ph+ ALL (Ref. 4, Ref. 5); similarly, Src-family kinases (SFKs) may also contribute to the pathogenesis of Ph+ ALL. The frequency of the Ph chromosome increases with age, from approximately 2-5% in children, to about 25% in patients aged 21-50 years, with a progressive increase with age, and to over 40% in patients aged more than 50 years, making it the most common genetic abnormality in adult ALL (Ref. 6, Ref. 7). Prior to the introduction of tyrosine kinases inhibitors (TKI), the outcome of Ph+ ALL was extremely poor, both in terms of achievement of complete hematologic remission (CHR) and long-term survival (Ref. 2), and the only curative option was represented by allogeneic hematopoietic stem cell transplantation (allo-SCT) at the earliest opportunity. Even in the TKI era, this subgroup is still associated to a poor prognosis, because of the high relapse rates and the development of drug resistance. The long-term overall survival (OS) is in the range of 30-50% with current treatments (Ref. 8, Ref. 9, Ref. 10, Ref. 11, Ref. 12, Ref. 13, Ref. 14, Ref. 15, Ref. 16, Ref. 17, Ref. 18, Ref. 19), consisting of TKIs with or without chemotherapy, possibly followed by transplant procedures. Allo-SCT is still considered the only curative treatment for Ph+ ALL; despite treatment-related mortality rates of around 20-30% and significant post-transplant morbidity, long-term benefits are frequently achieved (Ref. 20, Ref. 21, Ref. 22). Currently, many groups administer an induction treatment based on the association of chemotherapy and TKI (first or second generations). Investigators at the M.D. Anderson Cancer Center (MDACC) have studied the efficacy of treatment with Imatinib in association with the hyper-CVAD regimen (Ref. 8), obtaining a CHR rate of 93%. The German Multicenter ALL (GMALL) study group tested two strategies in which Imatinib was incorporated either alternatively to or concurrently with chemotherapy, obtaining a CHR rate of 96% (Ref. 9). The Japan Adult Leukemia Study Group (JALSG) conducted a phase II study of Imatinib combined with chemotherapy and the CHR rate obtained was 95% (Ref. 11). The Northern Italy Leukemia Group (NILG) obtained a CHR of 92% in Ph+ ALL patients at diagnosis treated with chemotherapy plus Imatinib (Ref. 14). All these studies have shown that the combination of Imatinib with chemotherapy is effective in improving patients' chances to receive an allo-SCT during first CHR (Ref. 16, Ref. 17, Ref. 18, Ref. 20). Also in elderly patients, the use of Imatinib in combination with chemotherapy or alone obtained an increase of CHR (Ref. 23, Ref. 24). Dasatinib, the second generation TKI, utilized in the MDACC study (Ref. 19) in association with hyper-CVAD led a CHR rate of 94% even if the combination was accompanied by a high toxicity and side effects. It was later reported that achieving a major molecular response (MMR) at 3, 6, 9, and 12 months significantly impacted on survival (Ref. 25). In an another study by the European Working Group on Adult ALL (EWALL), the association between Dasatinib and chemotherapy in elderly patients induced a 95% of CHR (Ref. 26). All these trials showed that these approaches are feasible and capable of inducing high remission rates. However, induction deaths were consistently recorded combining a TKI with chemotherapy.

The GIMEMA experience with the first generation TKI Imatinib in Ph+ ALL is represented by the LAL 0201 protocol. This study included Imatinib for the treatment of Ph+ positive ALL for adult (A study) or elderly (B study) patients. The first report showed a strong activity of an induction treatment based only on a TKI plus steroids and CNS prophylaxis, with a 100% rate of CHR obtained in the elderly patients (>60 years) (Ref. 24). These results represented the prerequisite starting point for the design of the GIMEMA LAL1205 protocol, based on the use of the second generation TKI Dasatinib alone for 12 weeks as first-line induction treatment for all Ph+ ALL ≥18 years of age (with no upper age limit); the protocol took advantage of the central handling laboratory network operating in Italy over the last 20 years, coordinated by the Hematology Center at the "Sapienza" University in Rome, and aimed at a broad, integrated and uniform characterization of all adult ALL cases entering in the GIMEMA protocols. This allows to identify in all ALL cases the presence of the BCR/ABL1 gene fusion within the 7-day steroid pre-phase, to set up a bank of material and to centrally perform a monitoring of MRD at pre-defined time points during the course of the disease. In this protocol, 53/53 of the evaluable patients (100%) obtained a CHR with an overall good compliance and no deaths (or relapses) during the induction phase. According to the study design, post-induction treatment was left to the investigator's choice. The results of this study have been presented orally twice at ASH, as well as at EHA, and published in Blood (Ref. 27). This study showed that remission induction

with Dasatinib (12 weeks) is highly effective and safe. Nevertheless, most patients at the end of the induction with Dasatinib were still MRD positive, by means of immunophenotype and/or molecular biology. DFS was significantly better in patients with low MRD levels (cut-off $=10^{-3}$) by day +22. These observations led to the development of the subsequent protocol, the GIMEMA LAL 1509, in which patients not achieving a complete molecular remission (CMR) after Dasatinib induction were further stratified, according to allo-SCT eligibility, to proceed directly to transplant or otherwise to receive one or two consolidation cycles with clofarabine and cyclophosphamide. Also in this setting, a CHR was achieved in all 60 eligible patients by day +57, although the CHR was lost in two cases (both carrying the p210 fusion protein). A CMR with Dasatinib alone was obtained in 19% of cases; OS and DFS at 24 months are of 71.1% and 50.3%. Although not significant, also in this study, a CMR achievement correlated with a better DFS. The first results of the 1509 protocol have been presented orally twice at the ASH meetings (Ref. 28, Ref. 29**Ref. 28**). Thus, these latter studies indicate that the administration of dasatinib in induction is capable of inducing sustained molecular responses in a proportion of patients, while most patients remain MRD+, and that a CMR should be regarded as a primary endpoint of induction/consolidation treatment for Ph+ ALL patients, that should be reached as soon as possible, e.g. 3 months, as previously reported (Ref. 25), and maintained overtime.
Blinatumomab is bispecific T-cell engager antibody. Blinatumomab was initially used in refractory non-Hodgkin lymphomas (Ref. 30), but neurologic side effects were observed. This compound has so far found its largest clinical use in ALL, in both the MRD+ setting (Ref. 31, Ref. 32, Ref. 33), as well as in adults with relapsed/refractory ALL (Ref 34, Ref. 35) . Thus, the rationale for the current trial relies on the fact that we previously documented that by administering Imatinib or Dasatinib and steroids as induction treatment, virtually all adult patients - including the elderly - can achieve a CHR, without experiencing relevant toxicities and without deaths in induction. However, the majority of patients remain MRD+. If not treated further, MRD+ patients will inevitably relapse. We have shown that the degree of MRD debulking associates with prognosis. Thus, MRD negativity should be the primary endpoint in the management of Ph+ ALL, that can be achieved at early time points (end of induction, 3 months) by Dasatinib administration only in a small fraction of patients; we hypothesize that following induction with Dasatinib the subsequent administration of Blinatumomab, which has proven particularly effective in a context of MRD positivity, should significantly increase the percentage of cases reaching, and maintaining, a MRD negative status (i.e. BCR/ABL copy number=0) and reduce the incidence of relapses, ultimately leading to a significant improvement in the outcome of adult Ph+ ALL patients, in terms of OS and DFS. Following previous experiences with Blinatumomab, at least 2 cycles of the compound will be administered by CIVI for 28 days followed by a 14-days period wash-out. In order to increase MRD clearance, in patients achieving a CHR Dasatinib will not be discontinued during Blinatumomab administration, as well as following it. Allo-SCT will be offered to eligible patients, according to donor availability and at investigator's discretion. While this approach is valid for all adult patients, it acquires further relevance for the more elderly patients for whom intensive systemic chemotherapy (particularly if associated with a TKI) and/or an allogeneic transplant are often coupled to unacceptable toxicity. It should be further underlined that in patients over the age of 50 the prevalence of the BCR/ABL1 fusion transcript is in the order of 50% of cases.

2.2 Dasatinib (Sprycel®)

2.2.1 Introduction

Dasatinib (Sprycel®, Bristol-Myers Squibb Pharmaceutical Research Institute) is a novel, oral, multi-targeted kinase inhibitor of BCR-ABL and SRC family kinases that bind both the active and inactive conformations of the ABL kinase domain. Additional background information on preclinical pharmacology, toxicology and pharmacokinetics may be found in the Investigator Brochure.

2.2.2 Clinical studies

Dasatinib was first evaluated in a phase 1, open-label, dose-escalation study in patients with CML or Ph+ ALL who could not tolerate or were resistant to Imatinib. A CHR was achieved in 37 of 40 patients with chronic phase CML, and major hematologic responses were seen in 31 of 44 patients with accelerated phase CML, CML with blast crisis, or Ph+ ALL. In these two phases, the rates of major cytogenetic response were 45% and 25%, respectively. Responses were maintained in 95% of patients with chronic phase disease and in 82% of patients with accelerated phase disease, with a median follow-up greater than 12 months and 5 months, respectively. Nearly all patients with lymphoid blast crisis and Ph+ ALL had a relapse within 6 months. Responses occurred among all BCR-ABL1 genotypes, with the exception of the T315I

mutation, which confers resistance to both Dasatinib and Imatinib in vitro (Ref. 37). Following promising phase I results, results of phase II clinical trials of Dasatinib in patients with Imatinib-resistant or intolerant blast-crisis CML (74 myeloid and 42 lymphoid blast crisis) have been reported. With a minimum follow-up of 8 months, treatment with Dasatinib resulted in substantial hematologic and cytogenetic response rates. Major hematologic responses were achieved in 42% (15/36) of patients, 67% of whom remained progression-free. Complete cytogenetic responses were attained by 58% (21/36) of patients. The presence of BCR-ABL1 mutations conferring Imatinib resistance did not preclude a response to Dasatinib. Dasatinib was also tolerable, with 6% (2/36) of patients discontinuing therapy as a result of study drug toxicity. Most AEs were grade 1 or 2; febrile neutropenia was the most frequent severe AE, but this and other cytopenias were manageable with dose reduction (Ref. 38. Results from the START-L, an open label, multi-center, global phase II study clearly established the durable efficacy and safety of Dasatinib 70 mg twice-daily (BID) in patients with Ph+ ALL resistant or intolerant to Imatinib (Ref. 38). The phase III CA180-035 study assessed Dasatinib 140 mg once daily and 70 mg BID in patients following resistance or intolerance to Imatinib. A first analysis from this study (follow-up = 6.5 months) indicatted that once-daily dosing was associated with equivalent efficacy and less frequent key side effects compared to the BID dosing (Ref. 39). Data at 24 months confirmed that Dasatinib 140 mg once daily has similar efficacy to 70 mg BID in patients with Ph+ ALL following Imatinib resistance or intolerance; with both schedules, responses were observed allowing to perform an allo-SCT in some patients in this high-risk population. The rate of pleural effusion (grade 3/4) with 140 mg once daily was 3% vs 14% for 70 mg BID (Ref. 40). Imatinib failed to inhibit intracranial tumor growth, while stabilization and regression of CNS disease were achieved with continued Dasatinib administration. The drug also demonstrated substantial activity in 11 adult and pediatric patients with CNS Ph+ leukemia. Eleven evaluable patients had clinically significant, long-lasting responses, which were complete in 7 patients (Ref. 41). The GIMEMA experience with the LAL 1205 and LAL 1509 protocols has shown that induction treatment of adults with newly diagnosed Ph+ ALL with Dasatinib alone (plus steroids) without systemic chemotherapy is associated with a CHR in virtually all patients, irrespective of age, good compliance and a very rapid debulking of the neoplastic clone (Ref. 27, Ref. 28, Ref. 29).

2.2.3 Toxicity profile

Serious Adverse Events (SAEs)

Roughly, 30% of subjects treated in the LAL 1205 protocol reported SAEs (any grade) that were considered by the investigator possibly related to the study drug. The non-hematologic drug-related SAEs (any grade) included, but were not limited to, pleural effusion, pyrexia, gastrointestinal hemorrhage, pneumonia and congestive cardiac failure. In the induction phase of the LAL 1509, no SAE were recorded, as well as no pleural effusions. Among non-hematologic toxicities, an increase in the ALT levels (grade ≥3) was observed in 5 patients.

2.2.4 Blinatumomab (Blincyto®)

2.2.5 Introduction

Blinatumomab (Blyncyto®, Amgen Inc.) is a murine recombinant single-chain antibody construct combining both the binding specificity for the pan B-cell antigen CD19 and the epsilon chain of the T-cell receptor/CD3 complex on one polypeptide chain. It is monomeric, not glycosylated and weighs approximately 55 kilo Daltons (kDa). It belongs to a new class of bispecific antibody constructs called bispecific T-cell engagers (BITE).

It belongs to a new class of bispecific antibody constructs called bispecific T-cell engagers.

Additional background information on preclinical pharmacology, toxicology and pharmacokinetics may be found in the Investigator Brochure.

2.2.6 Clinical studies

Blinatumomab was initially used in refractory non-Hodgkin lymphomas (NHL) (Ref. 42 Goebeler, M., Viardot, A., Noppeney, R. et al. Blinatumomab (CD3/CD19 Bite (R) Antibody) results in a high response rate in patients with relapsed non-Hodgkin lymphoma (NHL) including mantle cell lymphoma (MCL) and diffuse large B cell lymphoma (DLBCL). Ann. Oncol. 2011; 22:190

Ref. 43 Goebeler ME, Knop S, Viardot A., et al. A Bispecific T-Cell Engager (BiTE) Antibody Construct Blinatumomab for the Treatment of Patients With Relapsed/Refractory Non-Hodgkin Lymphoma: Final Results From a Phase I Study. J Clin Oncol. 2016 Apr 1;34(10):1104-11.

Ref. 44 Viardot A, Goebeler ME, Hess G, et al. Phase 2 study of the bispecific T-cell engager (BiTE) antibody blinatumomab in relapsed/refractory diffuse large B-cell lymphoma., Blood. 2016 Mar 17;127(11):1410-6. d

), but neurologic side effects were observed: currently, a Phase I trial (Ref. 43) showed that the MTD is 60 µg/m²/day; furthermore, a Phase II study (Ref. 44), based on a stepwise dose escalation of 9-28-112 µg/day, has led to an overall response rate of 43% in diffuse large B-cell lymphomas. Subsequently, this compound was extensively used in ALL. The MT103-202 trial was conducted in collaboration with the German Multicenter Study group for ALL (GMALL). Twenty-one patients were enrolled and received a dose of 15 µg/m² for 4 weeks per cycle: this study showed that a MRD negative status was achieved in 80% molecularly relapsed/refractory cases. An updated follow-up (33 months) reports a relapse-free survival (RFS) of 61% for the whole cohort, with no significant differences among allografted patients vs non allografted individuals (Ref. 31, Ref. 32). Importantly, 5 patients with Ph+ ALL were included in this trial and 3 of them achieved a molecular response: 4 of them did not receive an allo-SCT and 2, both receiving TKIs as consolidation treatment, are still in CHR, whereas the other 2 patients, who did not receive further consolidation, relapsed after 4.2 and 5.1 months. Interestingly, of the 4 patients who did relapse, 2 relapses were CD19- and two occurred in potentially immunoprivileged sites (CNS and testis), suggesting a possible immune escape. Following this trial, a large phase II study, MT103-203, has been designed for adult patients with MRD positivity (Ref. 33). Study MT103-206, enrolling 36 patients, was a single-arm phase II study for relapsed/refractory(r/r) patients which included a dose-finding component followed by an extension cohort in adults with r/r ALL. The final established dose was 9 µg for the first 7 days and then escalated at 28 µg. A CR/CRh (CRh = CR with partial recovery of peripheral blood counts) was achieved in 69% of patients. Among patients with a prior allo-SCT, the CR/CRh rate was 53% (8 out of 15). Importantly, 88% (22 out of 25) of all responders achieved a molecular remission and most achieved this following the first cycle (Ref. 34). The median RFS was 7.6 months and median OS 9.8 months. The results of a large pivotal phase II trial MT103-211 have been recently published in Lancet Oncology (Ref. 35). A total of 189 adults with r/r Ph- B-ALL were treated with the 9–28 µg schedule of Blinatumomab for up to 5 cycles: 43% had either a CR or a CRh within the first 2 treatment cycles, with 79% of cases achieving Ca R/CRh within the first cycle. Notably, a MRD negative status was achieved in 82% of responding and evaluable patients. A allo-HSCT was feasible in 32 patients (40%). The median RFS was 5.9 months for the 82 CR/CRh patients, 6.9 months for patients in CR and 5 months for those in CRh, with a median follow-up of 8.9 months. The median OS was 6.1 months for all 189 patients, with a median follow-up of 9·8 months. Finally, the median RFS and OS for MRD responders vs MRD non-responders were 6.9 vs 2.1 months and 11.5 months vs 6.7 months, respectively. A randomized phase III trial comparing Blinatumomab to standard of care chemotherapy (311 TOWER study) and a phase II trial evaluating Blinatumomab in Ph+ ALL (216 Alcantara study) have recently closed patients' accrual; the results of the Alcantara study showed that 36% of cases achieved a CHR, and a MRD negativity was also documented in the majority of these cases (86%). (Ref. 36).

2.2.7 Toxicity profile

Serious Adverse Events (SAEs)

The most common AEs are represented by pyrexia, rigor and fatigue, ranging from mild to moderate, and are likely to be related mechanistically to polyclonal T-cell activation and/or tumor lysis (Ref. 30). The production of pro-inflammatory cytokines is thought to play a role, their release appears to be transient and more sustained with the onset of treatment, while it is negligible during subsequently cycles (Ref. 45). More importantly, neurologic events such as aphasia, ataxia, disorientation and seizure have been observed: these events were fully reversible but led to drug discontinuation in 12 patients. The underlying mechanism is not completely understood, though one hypothesis is that T cells become activated and adhere to the endothelium, potentially transmigrating across the blood-brain barrier (BBB) and entering into the CNS space (Virchow-Robin space). Activated T cells, upon encountering CD19-expressing B cells in the CNS, potentially cause a local inflammatory phenomenon that results in local cytokine release at the BBB/parenchyma and disturbance of the BBB, possibly leading to neurologic events in some patients. Prophylaxis with Levitiracetam efficiently prevents neurologic side effects.

3 Objectives of the trial

3.1 Primary objective

The primary objective of the trial is to evaluate the activity of a combination approach based on Dasatinib and Blinatumomab in obtaining a MRD negativity (complete molecular response: CMR, e.g. BCR-ABL1/ABL1=0) in adult Ph+ ALL.

3.2 Secondary objectives

To explore:

- The feasibility of a chemo-free approach, based on Dasatinib and Blinatumomab, in adult Ph+ ALL patients ≥18 years, with no upper age limit
- CMR achievement after induction with Dasatinib
- Capability of Blinatumomab to reduce the MRD levels (an extension of the primary objective)
- CMR duration
- Disease-free survival (DFS)
- Overall survival (OS)
- Cumulative incidence of relapse (CIR)
- Safety profile
- CMR achievement, duration of CMR, OS and DFS according to the clinical, biological and molecular characteristics: clinical and biological assessment at baseline, type of fusion protein (p190 *vs* p210) and presence of additional genomic lesions identified by SNP arrays.

4 Patient selection criteria

4.1 Eligibility Criteria

- Newly diagnosed adult B-precursor Ph+ ALL patients.
- Age greater or equal to 18 years.
- Signed written informed consent according to ICH/EU/GCP and national local laws.
- ECOG Performance Status 0 or 1 and/or WHO performance status less or equal to 2.
- Renal and hepatic function as defined below:
  - AST (GOT), ALT (GPT), and AP <2 x upper limit of normal (ULN).
  - Total bilirubin <1.5 x ULN.
  - Creatinine clearance equal or greater than 50 mL/min.
- Pancreatic function as defined below:
  - Serum amylase less or equal to 1.5 x ULN
  - Serum lipase less or equal to1.5 x ULN.
- Normal cardiac function.
- Negative HIV test, negative HBV DNA and HCV RNA.
- Negative pregnancy test in women of childbearing potential.
- Bone marrow specimen from primary diagnosis available.

4.2 Non-eligibility criteria

- Prior systemic chemotherapy for leukemia and/or CD19-directed therapy.
- History of or current relevant CNS pathology (current ≥grade 2 epilepsy, seizure, paresis, aphasia, clinically relevant apoplexia, severe brain injuries, dementia, Parkinson's disease, organic brain syndrome, psychosis).
- Impaired cardiac function, including any one of the following:

- LVEF (Left Ventricular Ejection Fraction) <45% as determined by MUGA (multigated acquisition) scan or echocardiogram.
- Complete left bundle branch block.
- Use of a cardiac pacemaker.
- ST depression of >1mm in 2 or more leads and/or T wave inversions in 2 or more contiguous leads.
- Congenital long QT syndrome.
- History of or presence of significant ventricular or atrial arrhythmia.
- Clinically significant resting bradycardia (<50 beats per minute).
- QTc >450 msec on screening ECG (using the QTcF formula).
- Right bundle branch block plus left anterior hemiblock, bifascicular block.
- Myocardial infarction within 3 months prior to starting Dasatinib.
- Angina pectoris.

– Other clinically significant heart disease (e.g., congestive heart failure, uncontrolled hypertension, history of labile hypertension, or history of poor compliance with an antihypertensive regimen).
– Impairment of GI function or GI disease that may significantly alter the absorption of Dasatinib (e.g., ulcerative diseases, uncontrolled nausea, vomiting, diarrhea, malabsorption syndrome, or small bowel resection).
– History of or current autoimmune disease.
– Systemic cancer chemotherapy within 2 weeks prior to study.
– Known hypersensitivity to immunoglobulins or to any other component of the study drug formulation.
– Active malignancy other than ALL with the exception of basal cell or squamous cell carcinoma of the skin, or carcinoma "in situ" of the cervix.
– Active infection, any other concurrent disease or medical conditions that are deemed to interfere with the conduct of the study as judged by the investigator.
– Nursing women or women of childbearing potential not willing to use an effective form of contraception during participation in the study and at least 3 months thereafter or male patients not willing to ensure effective contraception during participation in the study and at least three months thereafter.

5 Trial Design

5.1 General Design

This study is an open-label, multicenter, phase II study based on the combination of Dasatinib - administered orally at a dose of 140 mg QD for a 12-week induction treatment - with Blinatumomab. Patients will initiate treatment with steroids 7 days prior to starting Dasatinib and will continue up to day 31. Thereafter:
- Patients who obtain a CHR will receive a post-remissional treatment with Blinatumomab (28 µg/die) (minimum 2 cycles, up to a maximum of 5, all administered for 28 days CIVI with a 2 weeks wash-out interval). A minimum number of 2 cycles is mandatory; the administration of 3 additional cycles will depend on the basis of response to therapy with Blinatumomab (for instance decrease in MRD, but not negativity) and medical decision (i.e. toxicity and compliance to treatment, possibility of undergoing transplant procedures depending on age, donor availability and presence of comorbidities that might contraindicate them). During treatment with Blinatumomab, Dasatinib will not be discontinued. In case of toxicity deemed related to Dasatinib, and/or the combination of the 2 compounds, Dasatinib might be reduced to 100 mg/day. Dasatinib will be continued for the whole study duration in these patients.
- Patients not achieving a CHR will receive Blinatumomab (28 µg/die) (minimum 2 cycles, up to a maximum of 5, all administered for 28 days CIVI with a 2 weeks wash-out interval). The number of cycles will be established on the basis of response to therapy with Blinatumomab and medical decision. In these cases, Dasatinib will be interrupted during treatment with Blinatumomab.

CNS prophylaxis will be carried out with medicated lumbar punctures (MTX 15 mg, Methylprednisolone 20 mg): 6 prophylactic injections will be administered during the induction phase (at days +14, +22, +43, +57 and +85) and, subsequently, they will be administered at the end of each cycle of Blinatumomab (day +29).

5.2 Treatment scheme: overall scheme of the protocol

Adult Ph+ ALL (≥18 years old, with no upper age limit) patients will begin treatment with Dasatinib, 140 mg/day, from day 1 to day +84. Prednisone (PDN) will be administered from day -6 to day +0 (during which the presence of the BCR/ABL1 alteration will be established), at escalating doses up to 60 mg/m²; PDN will be continued up to day +24 and progressively tapered up to day +31.
HLA typing will be performed immediately after the diagnosis for eligible patients.
MRD will be evaluated by (Q-RT-PCR) at fixed time points (days +22, +45, +57) during the induction and at day +85, the latter for molecular response evaluation.

Upon induction, patients will receive Blinatumomab as described in the trial design (section 5.1):

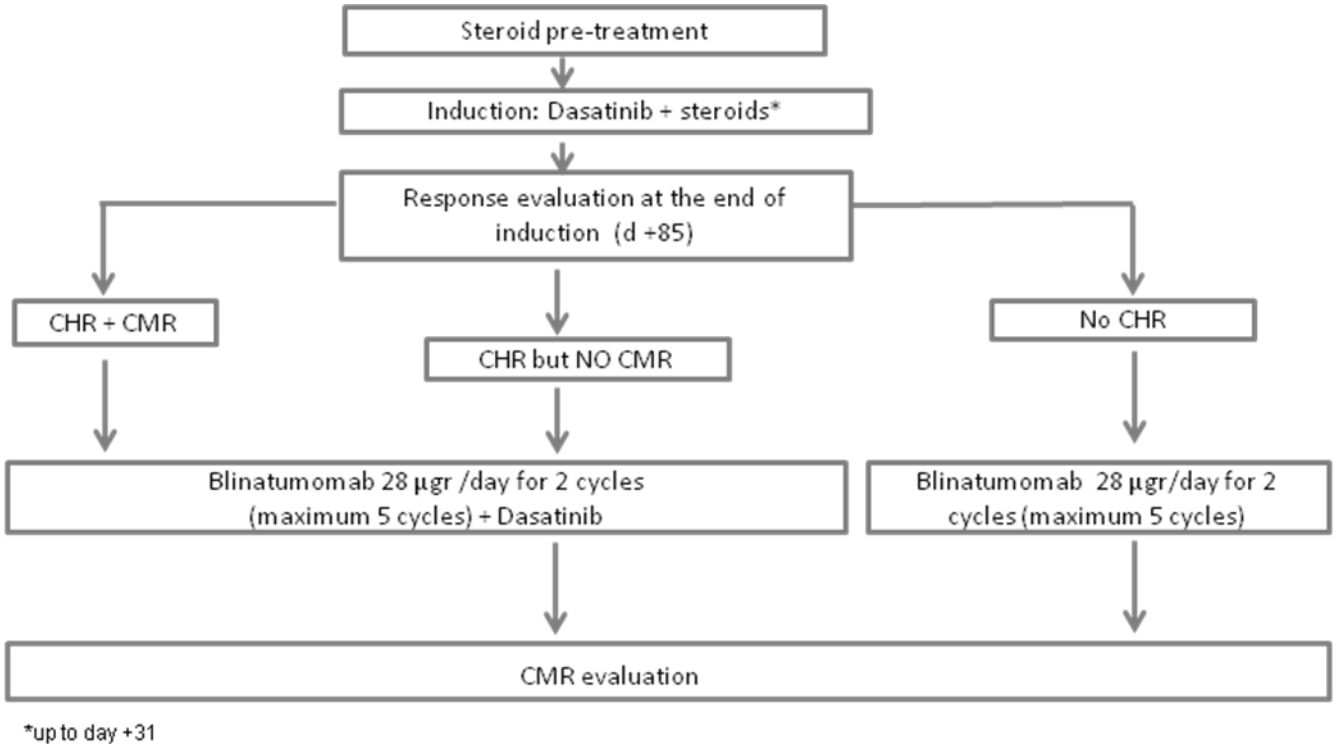

Figure 1: Treatment scheme

5.3 Endpoints

5.3.1 Primary

The primary endpoint is the rate of patients who achieve MRD negativity (CMR) upon treatment, in particular: the rate of patients in CMR after 2 cycles of Blinatumomab.

5.3.2 Secondary

- Feasibility, calculated on the rate of patients completing the 2 cycles of Blinatumomab treatment and alive in first CHR from day +85 at 12 months
- Rate of patients in CMR at day +22, +45, +57 and +85
- CMR duration
- DFS
- OS

- CIR
- Safety profile in terms of incidence of grade >3 CTC-NCI side effects and toxicities.
- Role of clinical and biological assessment at baseline, type of fusion protein (p190 vs p210) and presence of additional genomic lesions identified by SNP arrays on CMR achievement, duration of CMR, OS and DFS.

5.4 Risks and benefits assessment

Overall, the balance between risks and benefits associated to the present study protocol are considered to be favorable. The likelihood of AE and risks of the protocol are outweighed by the benefits that are hypothesized to be related to therapy. In particular, the protocol has been developed on the basis of the last 15 years' GIMEMA experience in the treatment of adult Ph+ ALL, during which the possibility of obtaining a CHR without systemic chemotherapy has been documented for the first time and then confirmed (Ref. 22 Ref. 26, Ref. 27, Ref. 28). This protocol will offer an induction treatment without systemic chemotherapy and will be based only on the oral administration of targeted therapy (a 2nd generation TKI). After the completion of the induction period, an immunotherapeutic approach with Blinatumomab aimed at increasing the probability of MRD negativity achievement – that with the 2nd generation TKI alone is in the range of 19%.

Risks to participating in the study

Toxicity

Concerning the first part of the treatment - the induction period -, toxicity with the TKI alone is reduced in comparison with the standard chemotherapy protocols usually administered to these patients. In the post-remission phase, a consolidation therapy with an immunotherapeutic approach, whose side effects are now largely manageable, will be offered. As for treatment with Blinatumomab, the most common AEs are represented by pyrexia, rigor and fatigue, that are usually mild to moderate. More serious side effects might be represented by neurologic events such as aphasia, ataxia, disorientation and seizure that are usually fully reversible and may only rarely lead to treatment discontinuation; to prevent these side effects, prophylaxis with Levitiracetam will be mandatory.

Psychosocial distress

Patients with acute leukemia experience a physical and psychological distress mainly due to the malignant disease and the treatment will be administered with the aim of obtaining a remission of the disease and of prolonging survival. Thus, no distress secondary to the treatment "per se" must be considered, but rather all the psychological problems related to the diagnosis of an acute leukemia. In particular, this protocol permits an induction treatment that may partly be conducted on an out-patient basis, is oral and does not include systemic chemotherapy, while the approaches normally utilized for the treatment of this disease require a long period – up to two months – of admission to the hospital. As for the consolidation phase with Blinatumomab, since the drug must be administered as CIVI for 28 days, patients will receive it through a portable infusion pump, which must not be removed. Therefore, psychosocial distress might derive from the fact that the infusion device must be kept in all circumstances (for example, showering, driving and physical exercise) for the 28-days infusion period. Hospitalization period will be mandatory for the first 3 days of cycle 1 and for at least 2 days of the subsequent cycles: if no AEs occur, treatment will be continued on an out-patient basis.

Benefits to participating patients

The previous GIMEMA experience has shown a near-100% potential likelihood of obtaining a CHR of the disease with a TKI and no systemic chemotherapy (Ref. 22, Ref. 26, Ref. 27, Ref. 28). The post-remission treatment has been scheduled with the aim of increasing as much as possible the probability of achieving a MRD negative status (CMR) and, as a consequence, a longer remission and survival. In addition, if patients will undergo an allo-SCT, toxicity-related procedures should be alleviated, since patients will be fitter, without chemotherapy-related complications and with less tumor burden. If the hypothesis of the study will be confirmed, this will represent the proof of principle that a chemo-free strategy based on Dasatinib and Blinatumomab could be considered as the new standard option for the management of Ph+ ALL of all ages (including the elderly), being capable of eradicating MRD, and might be adopted also by other groups and/or countries. Upon study completion, Dasatinib will be further provided free of charge by BMS until deemed of benefit for the patient.

6 Therapeutic regimens, expected toxicity, dose modifications

6.1 Steroid-pretreatment

Day -6 to 0 (total 7 days):

- Start steroid pre-treatment: p.o. Prednisone (or the equivalent dose of i.v. Methylprednisolone) at escalating doses from 20 up to 60 mg/m^2.
- Steroid pre-treatment will start at day -6 at 20 mg/m^2 of Prednisone, escalating the dose of 10 mg/ m^2/day up to day -4, and then, reaching the full dosage of 60 mg/m^2 at day -3. This therapy must be continued until day +24 and, thereafter, it must be tapered from day +25 to suspension within one week, reducing the dose of 20 mg/m^2 every two days (days 25-26: 40 mg/m^2; days 27-28: 20 mg/m^2; days 29-30: 10 mg/m^2; day +31: 5 mg/m^2; day +32: tapering up to stop), as depicted in Figure 2.
- Diagnostic and therapeutic (1st) rachicentesis (Methotrexate 15 mg with Methylprednisolone 20 mg) should be performed as soon as possible.

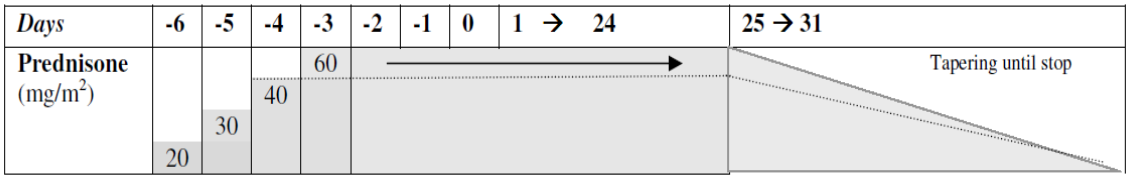

Figure 2: Steroid pretreatment

Steroid response evaluation:
Steroid response evaluation will be carried out using the classic method (blast count in the peripheral blood expressed as cells x 10^9/L at the end of steroid pre-treatment) and with the method based on the blast reduction rate (Ref. 46).

6.2 Induction therapy

Patients will begin treatment with 140 mg QD of Dasatinib from day 1 to day 84. PDN will be continued up to day 31. In case of disease progression during the induction treatment, Dasatinib will be discontinued and patients will go off study.

6.2.1 Dasatinib (Sprycel®) General information

Dasatinib will be supplied by Bristol-Myers Squibb Pharmaceutical Research Institute. Patients should be instructed to take the first daily dose of Dasatinib in the morning after a light breakfast. No fasting is requested at the time of Dasatinib administration. If vomiting occurs during the course of treatment, no re-dosing of the patient is allowed before the next scheduled dose. If the dose is delayed (ie, missed by a few hours), patients should take the dose and wait a minimum of 8 hours before the next dose; if that is not possible, the patient should omit the missed dose and take the next dose as scheduled. If, for any reason, a light meal cannot be consumed, the patient should still take the scheduled dose. Patients should be instructed to take their daily doses at approximately the same time each day. Patients should quickly swallow the tablets with a glass of water and not chew them. Grapefruit juice should not be consumed during study drug therapy, as CYP450 enzyme inhibition may increase drug exposure.

6.2.2 Dosage and administration during induction therapy

Dose escalation of Dasatinib is not allowed in any case:

Day +1: Start Dasatinib at 140 mg once a day (total planned treatment is 12 weeks, i.e. 84 days).
Continue p.o. Prednisone (or the equivalent dose of i.v. methyl-prednisolone) at 60 mg/m^2/day.

Day +14: Prophylactic IT injection (2nd): i.t. Methotrexate 15 mg with Methylprednisolone 20 mg.

Day +22: Prophylactic IT injection (3rd): i.t. Methotrexate 15 mg with Methylprednisolone 20 mg.

Day +25: Start tapering steroids.

Day +45: Prophylactic IT injection (4th): i.t. Methotrexate 15 mg with Methylprednisolone 20 mg.

Day +57: Prophylactic IT injection (5th): i.t. Methotrexate 15mg with Methylprednisolone 20 mg.

Day +85: Prophylactic IT injection (6th): i.t. Methotrexate 15 mg with Methylprednisolone 20 mg.

6.2.3 Toxicity of Dasatinib

Dasatinib Summary of adverse reactions (source Summary of Product Characteristics 15/01/2015)

Infections and infestations	
Very common	Infection (including bacterial, viral, fungal, non-specified)
Common	Pneumonia (including bacterial, viral, and fungal), upper respiratory tract infection/inflammation, herpes virus infection, enterocolitis infection, sepsis (including uncommon cases with fatal outcomes)
Blood and lymphatic system disorders	
Very Common	Myelosuppression (including anemia, neutropenia, thrombocytopenia)
Common	Febrile neutropenia
Uncommon	Lymphadenopathy, lymphopenia
Rare	Pure red cell aplasia
Immune system disorders	
Uncommon	Hypersensitivity (including erythema nodosum)
Endocrine Disorders	
Uncommon	Hypothyroidism
Rare	Hyperthyroidism, thyroiditis
Metabolism and nutrition disorders	
Common	Appetite disturbances[a], hyperuricaemia
Uncommon	Tumor lysis syndrome, dehydration, hypoalbuminemia, hypercholesterolemia
Rare	Diabetes mellitus
Psychiatric disorders	
Common	Depression, insomnia
Uncommon	Anxiety, confusional state, affect lability, libido decreased
Nervous system disorders	
Very common	Headache
Common	Neuropathy (including peripheral neuropathy), dizziness, dysgeusia, somnolence
Uncommon	CNS bleeding[b], syncope, tremor, amnesia, balance disorder
Rare	Cerebrovascular accident, transient ischaemic attack, convulsion, optic neuritis, VIIth nerve paralysis, dementia, ataxia
Eye disorders	
Common	Visual disorder (including visual disturbance, vision blurred, and visual acuity reduced), dry eye
Uncommon	Visual impairment, conjunctivitis, photophobia, lacrimation increased
Ear and labyrinth disorders	
Common	Tinnitus
Uncommon	Hearing loss, vertigo
Cardiac disorders	
Common	Congestive heart failure/cardiac dysfunction[c], pericardial effusion, arrhythmia (including tachycardia), palpitations
Uncommon	Myocardial infarction (including fatal outcome), electrocardiogram QT prolonged, pericarditis, ventricular arrhythmia (including ventricular

	tachycardia), angina pectoris, cardiomegaly, electrocardiogram T wave abnormal, troponin increased
Rare	Cor pulmonale, myocarditis, acute coronary syndrome, cardiac arrest, electrocardiogram PR prolongation, coronary artery disease, pleuropericarditis
Not known	Atrial fibrillation/atrial flutter
Vascular disorders	
Very common	Hemorrhage[d]
Common	Hypertension, flushing
Uncommon	Hypotension, thrombophlebitis
Rare	Deep vein thrombosis, embolism, livedo reticularis
Respiratory, thoracic and mediastinal disorders	
Very common	Pleural effusion, dyspnoea
Common	Pulmonary oedema, pulmonary hypertension, lung infiltration, pneumonitis, cough
Uncommon	Pulmonary arterial hypertension, bronchospasm, asthma
Rare	Pulmonary embolism, acute respiratory distress syndrome
Not known	Interstitial lung disease
Gastrointestinal disorders	
Very common	Diarrhoea, vomiting, nausea, abdominal pain
Common	Gastrointestinal bleeding, colitis (including neutropenic colitis), gastritis, mucosal inflammation (including mucositis/stomatitis), dyspepsia, abdominal distension, constipation, oral soft tissue disorder
Uncommon	Pancreatitis (including acute pancreatitis), upper gastrointestinal ulcer, oesophagitis, ascites, anal fissure, dysphagia, gastroesophageal reflux disease
Rare	Protein-losing gastroenteropathy, ileus, anal fistula
Not known	fatal gastrointestinal haemorrhage
Hepatobiliary disorders	
Uncommon	Hepatitis, cholecystitis, cholestasis
Skin and subcutaneous tissue disorders	
Very common	Skin rash[e]
Common	Alopecia, dermatitis (including eczema), pruritus, acne, dry skin, urticaria, hyperhidrosis
Uncommon	Neutrophilic dermatosis, photosensitivity, pigmentation disorder, panniculitis, skin ulcer, bullous conditions, nail disorder, palmar-plantar erythrodysesthesia syndrome, hair disorder
Rare	Leukocytoclastic vasculitis, skin fibrosis
Musculoskeletal and connective tissue disorders	
Very common	Musculoskeletal pain
Common	Arthralgia, myalgia, muscular weakness, musculoskeletal stiffness, muscle spasm
Uncommon	Rhabdomyolysis, osteonecrosis, muscle inflammation, tendonitis, arthritis
Renal and urinary disorders	
Uncommon	Renal impairment (including renal failure), urinary frequency, proteinuria
Pregnancy, puerperium and perinatal conditions	
Rare	Abortion
Reproductive system and breast disorders	
Uncommon	Gynecomastia, menstrual disorder
General disorders and administration site conditions	
Very common	Peripheral oedema[f], fatigue, pyrexia, face oedema[g]
Common	Asthenia, pain, chest pain, generalised oedema[h], chills
Uncommon	Malaise, other superficial oedema[i]
Rare	Gait disturbance
Investigations	
Common	Weight decreased, weight increased
Uncommon	Blood creatine phosphokinase increased, gamma-glutamyltransferase

	increased
Injury, poisoning, and procedural complications	
Common	Contusion

a Includes decreased appetite, early satiety, increased appetite.

b Includes central nervous system haemorrhage, cerebral haematoma, cerebral haemorrhage, extradural haematoma, haemorrhage intracranial, haemorrhagic stroke, subarachnoid haemorrhage, subdural haematoma, and subdural haemorrhage.

c Includes brain natriuretic peptide increased, ventricular dysfunction, left ventricular dysfunction, right ventricular dysfunction, cardiac failure,cardiac failure acute, cardiac failure chronic, cardiac failure congestive, cardiomyopathy, congestive cardiomyopathy, diastolic dysfunction, ejection fraction decreased and ventricular failure, left ventricular failure, right ventricular failure, and ventricular hypokinesia.

d Excludes gastrointestinal bleeding and CNS bleeding; these adverse reactions are reported under the gastrointestinal disorders system organ class and the nervous system disorders system organ class, respectively.

e Includes drug eruption, erythema, erythema multiforme, erythrosis, exfoliative rash, generalised erythema, genital rash, heat rash, milia, miliaria, pustular psoriaisis, rash, rash erythematous, rash follicular, rash generalised, rash macular, rash maculo-papular, rash papular, rash pruritic, rash pustular, rash vesicular, skin exfoliation, skin irritation, toxic skin eruption, urticaria vesiculosa, and vasculitic rash.

f Gravitational oedema, localised oedema, oedema peripheral.

g Conjunctival oedema, eye oedema, eye swelling, eyelid oedema, face oedema, lip oedema, macular oedema, oedema mouth, orbital oedema, periorbitalo edema, swelling face.

h Fluid overload, fluid retention, gastrointestinal oedema, generalised oedema, oedema, oedema due to cardiac disease, perinephric effusion, post procedural oedema, visceral oedema.

i Genital swelling, incision site oedema, oedema genital, penile oedema, penile swelling, scrotal oedema, skin swelling, testicular swelling, vulvovaginal swelling.

Frequencies are defined as: very common (≥1/10); common (≥1/100 to <1/10); uncommon (≥1/1,000 to <1/100); rare (≥1/10,000 to <1/1,000); not known (cannot be estimated from available post-marketing data).

6.2.4 Dasatinib dose and schedule modifications

No dose reduction is allowed for hematological toxicities during induction treatment.

During treatment with Blinatumomab, Dasatinib will not be discontinued for cases achieving a CHR, while it will be interrupted in cases not entering a CHR upon induction (day +85). In case of toxicity deemed related to Dasatinib, and/or the combination of the 2 compounds, Dasatinib might be reduced to 100 mg/day.

Dose reduction for non-hematological toxicities [Common Toxicity Criteria – Adverse Event (**CTCAE**) version 4.0 Criteria (**"Appendix C"**)]:

- Grade 1: no dose reduction;
- Grade 2/3 1st and 2nd time: the investigator will evaluate the clinical conditions of the patient and the hematological status (in particular, whether the CHR has been already obtained or not) and will decide if interruption of Dasatinib is indicated, taking into account that the drug is administered for obtaining the first hematological response in a patient with acute leukemia. If interruption of Dasatinib is decided, check daily and resume 140 mg/day when grade <2.
- Grade 2/3 3rd time: see above. If interruption of Dasatinib is decided, check daily, resume Dasatinib at 100 mg/day when grade <2 and at 140 mg/day after 3 days.
- Grade 2/3 4th time: see above. If interruption of Dasatinib is decided, check daily, resume

Dasatinib at 100 mg/day when grade <2, and contact the Principal Investigator.
- Grade 4: stop Dasatinib, check daily and contact the Principal Investigator.

Dose reduction for hematological and non-hematological toxicities during follow-up is planned according to **CTCAE** version 4.0 Criteria (**"Appendix C"**). In case of pleural effusion, refer to Appendix E.

Treatment in case of severe adverse events (SAE)
Stop Dasatinib immediately and contact the Principal Investigator.

6.2.5 Supportive care

• The use of double strength trimethoprim-sulfamethoxazole tablets twice daily 2 days per week, is mandatory.
• Coadministration of dasatinib with CYP3A4 inhibitors (macrolides, fluoroquinolones, and triazoles) should be carefully evaluated, in view of the potential drug-drug interaction.
• Transfusional products are allowed, if needed.

6.3 Blinatumomab

6.3.1 Blinatumomab (Blincyto®) General information

Blinatumomab will be administered in all patients as a CIVI at the dosage of 28 µg/die. A single cycle of Blinatumomab is defined as 6 weeks in duration, which includes 4 weeks of CIVI of Blinatumomab followed by a 2-week treatment-free interval (Figure 3).

Inpatient administration
Patients will require hospitalization for the first 3 days of the first cycle and for 2 days for all subsequent cycles. However, it must be underlined that the hospitalization time will depend on the investigator's judgment, as well as safety and tolerability of Blinatumomab. Close monitoring of the patient during the first 48 hours of the first two cycles is deemed essential, because of the potential AEs associated with T-cell redistribution and potential cytokine release effects triggered by Blinatumomab administration. The infusion bags will be changed every 96 hours (maximum) by site nursing personnel.
Prior to each cycle, subjects will be premedicated with dexamethasone 20 mg i.v. within 1 hour prior to start of treatment in each treatment (**Errore. L'origine riferimento non è stata trovata.**).
Premedication with dexamethasone is intended to prevent CRS events associated with Blinatumomab treatment.

Outpatient administration
In the outpatient setting, either the subject will return to the study site to change the infusion bag or subjects will be visited by a well-trained ambulant/home care service provider at specific intervals to change the infusion bag. The subject and the ambulant/home care provider will be trained and will receive written instructions for storage of the IV bags. For the ambulant/home care provider, study-specific requirements and recording of source documentation must be completed before any study-related tasks are started.

Post-CHR: at least 2 cycles of Blinatumomab
A single cycle of Blinatumomab is defined as 6 weeks in duration, which includes 4 weeks of CIVI Blintumomab, followed by a 2-week treatment-free interval

Table 1: Blinatumomab treatment

6.3.2 Toxicity of Blinatumomab

Source: Blinatumomab Core Informed Consent Form Risks and Discomforts Section V. 4 17.2.2015

As of 10 October 2014, a total of 615 adults and 93 pediatric subjects have been treated with Blinatumomab by intravenous infusion in research studies.

Side effects that other people have had in research studies that are thought to have been caused by Blinatumomab are as follows.

Very common side effects (which may affect more than 1 person in 10)
Decreased blood levels of white blood cells, red blood cells, and platelets (for clotting)
Abdominal pain
Diarrhea
Constipation
Fatigue
Chest pain
Bone and joint pain
Back pain
Pain in the arms, legs, and hands
Cough
Rash
Difficulty falling and/or staying asleep
Weight gain
Swelling of hands, legs, ankles, feet, face, or trunk
Increased level of blood sugar, and decrease in magnesium and/or decrease in potassium level in the blood

The patient may experience cytokine release syndrome during the Blinatumomab infusion. These signs and symptoms of cytokine release syndrome generally are mild to moderate but occasionally can be serious or life threatening, or may even lead to death. Very rarely, a condition called hemophagocytic histiocytosis has been reported. The Investigator may give patient medications such as steroids and/or other medications to prevent or treat cytokine release syndrome.

The patient may experience nervous system problems such as shaking, dizziness, seizures, changes in alertness and brain malfunction, abnormal skin sensation such as burning, prickling, or tingling, difficulty speaking or slurred speech, difficulty understanding words, difficulty walking, loss of consciousness, memory loss, confusion and/or disorientation, or loss of balance. These nervous system problems can be serious or life threatening, or may even lead to death. The investigator will be closely monitoring the patient and may give him medications such as steroids and/or other medications to treat nervous system problems or stop the patient treatment with blinatumomab.

Serious infections can occur during and after treatment and can lead to death. These infections may be bacterial, fungal, or viral. Serious infections such as sepsis, and pneumonia have been reported in patients treated with Blinatumomab. The investigator may give patient antibiotics to treat the infection or stop patient treatment with Blinatumomab.

Common side effects (which may affect between 1 and 10 people in every 100)
Increased heart rate
Increased level of bilirubin in the blood, and decreased levels of blood immunoglobulins
Decreased levels of albumin in the blood
Increased levels of white blood cells
Tumor lysis syndrome. Tumor lysis syndrome may cause kidney failure, abnormal heart rhythm, and can even lead to death

The investigator may give patient medicines before patient treatment to help prevent tumor lysis syndrome.

Uncommon side effects (which may affect between 1 and 10 people in every 1000)
Capillary leak syndrome
Disseminated intravascular coagulation
Leukoencephalopathy. Symptoms can include difficulty thinking, loss of balance, changes in speech or walking, weakness on one side of your body, or blurred or lost vision

If patients present any of these side effects, they may be treated with a steroid (dexamethasone or similar medication) or other medication or procedure to reduce any discomfort. The patient may also be referred for further treatment if necessary. If the patient experiences any of these symptoms, he/she should contact the study doctor or his/her study staff immediately
Infusion reactions to blinatumomab have been reported. The patient may experience symptoms of infusion reaction including headache, rash, itching, flushing, swelling, shortness of breath, nausea and sometimes vomiting. Severe infusion reactions can cause dizziness, severe skin reactions, difficulty in breathing or swallowing, a decrease in blood pressure, that could be life threatening. Signs and symptoms of infusion reaction can be very similar to cytokine release syndrome discussed above.
Allergic reactions to blinatumomab, including hypersensitivity, have been reported. Signs and symptoms of allergic reactions can be very similar to infusion reaction. If the patient has symptoms of an allergic reaction, he/she should contact the study doctor or his/her study staff immediately.
After the patient starts taking blinatumomab, it is possible that he/she may produce antibodies that can cause blinatumomab not to work.
No studies of the effects of blinatumomab on the ability to drive and use machineries have been performed. However, due to the potential for nervous system problems, the patient should not drive or engage in hazardous occupations or activities such as operating heavy or potentially dangerous machinery while blinatumomab is being infused.
At last, further to rare cases of pancreatitis, if the patient experiences one of the following symptoms – stiffening and upper abdominal pain (worsening while eating), nausea and vomit - he should contact the hospital in order to get a medical advice.

The severity of AEs will be assessed according to the NCI Common Terminology Criteria for Adverse Events version 4.0 (NCI- CTCAE).

6.3.3 Dose and schedule modifications and overdose

The drug administration should not be interrupted. In case of infusion interruption, due to any technical or logistic reason, the interruption should be as short as possible and the infusion continued at the earliest time possible. Every interruption longer than 1 hour should be documented. If the interruption is longer than 4 hours, re-start of the infusion should be performed in the hospital, under the supervision of the investigator. The subject should be observed over night for possible side effects after the re-start, either in the hospital or in the outpatient setting as applicable. In such cases, administration of the dexamethasone premedication at the dosage of 16 mg IV within 1 hour prior to re-start of treatment should be performed. A dose of up to 10% higher than the intended dose may not require specific intervention. In case of overdose or medication error, the infusion should be immediately stopped. Routine supportive and symptomatic care according to standard medical practice is recommended. Once the subject is stable and no clinically relevant safety findings due to Blinatumomab are observed, resumption of Blinatumomab at a correct dose can be considered after consultation with the Investigator. If the overdose results in an adverse event, the subject should be followed carefully until all signs of toxicity are resolved and the adverse event/s is to be recorded/reported.

Dose Modifications (Interruptions, Withholdings, and Criteria for Restarting Treatment)
(Source: Blinatumomab Summary of Product Characteristics 07/12/2015)

Consideration to discontinue Blinatumomab temporarily or permanently as appropriate should be made in the case of the following severe (grade 3) or life-threatening (grade 4) toxicities: cytokine release syndrome, tumor lysis syndrome, neurological toxicity, elevated liver enzymes and any other clinically relevant toxicities. If the interruption of treatment after an adverse event is no longer than 7 days, continue the same cycle to a total of 28 days of infusion inclusive of days before and after the interruption in that cycle. If an interruption due to an adverse event is longer than 7 days, start a new cycle. If the toxicity takes more than 14 days to resolve, discontinue Blinatumomab permanently, except if described differently in the table below

Toxicity	Grade*	Action
Cytokine release syndrome, tumor lysis syndrome	Grade 3	Interrupt Blinatumomab until resolution, then restart Blinatumomab at 9 mcg/day. Escalate to 28 mcg/day after 7 days if the toxicity does not recur.
	Grade 4	Discontinue Blinatumomab permanently.

Neurological toxicity	Convulsion	Discontinue Blinatumomab permanently if more than one convulsion occurs.
	Grade 3	Interrupt Blinatumomab until resolved to grade ≤1 (mild) for at least 3 days, then restart Blinatumomab at 9 mcg/day. Escalate to 28 mcg/day after 7 days if the toxicity does not recur. For reinitiation, premedicate with a 24 mg dose of dexamethasone. Then reduce dexamethasone stepwise over 4 days. If the toxicity occurred at 9 mcg/day, or if the toxicity takes more than 7 days to resolve, discontinue Blinatumomab permanently.
	Grade 4	Discontinue Blinatumomab permanently.
Elevated liver enzymes	Grade 3	If clinically relevant, interrupt Blinatumomab until resolved to grade ≤1 (mild), then restart Blinatumomab at 9 mcg/day. Escalate to 28 mcg/day after 7 days if the toxicity does not recur.
	Grade 4	Consider discontinuing Blinatumomab permanently.
Pancreatitis	Grade 3	Interrupt Blinatumomab until resolved to grade ≤1 (mild),then restart Blinatumomab at 9 mcg/day. Escalate to 28 mcg/day after 7 days if the toxicity does not recur
	Grade 4	Consider discontinuing Blinatumomab permanently
Other clinically relevant (as determined by treating physician) adverse reactions	Grade 3	Interrupt Blinatumomab until resolved to grade ≤1 (mild), then restart Blinatumomab at 9 mcg/day. Escalate to 28 mcg/day after 7 days if the toxicity does not recur.
	Grade 4	Consider discontinuing Blinatumomab permanently.

*Based on the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. Grade 3 is severe, and grade 4 is life-threatening.

Infusion Interruption/Dose Modification Due to CNS Events

To prevent CNS event, prophylaxis with Levitiracetam (500 mg/BID) will be mandatory throughout the whole period of Blinatumomab administration, starting one day prior to infusion.

In case of CNS-related adverse events, dexamethasone should be administered at a dose of at least 24 mg/day. The dexamethasone dose will then be reduced step-wise over 4 days (**Errore. L'origine riferimento non è stata trovata.Errore. L'origine riferimento non è stata trovata.**).

In case of CTCAE grade 3 or higher CNS-related AEs, blinatumomab will be stopped immediately and a physical exam, vital signs and safety laboratories will be performed.

Additional measures can be taken upon discretion of the investigator, depending on the nature of the AE. Diagnostic measures to exclude potential infectious causes should be conducted after CTCAE grade ≥3 CNS events; an assessment of cerebrospinal fluid should be performed for cytology, cell count, B- and T-cell measurement (flow cytometry at local lab), and viral studies (HSV 1/2, HSV6, JC virus and adenovirus). Additional investigations of the CSF should be performed as clinically appropriate.

For subjects who experience a CTCAE grade 3 CNS AE or serious AEs leading to treatment interruption, if the event has decreased to at least CTCAE grade 1 within 1 week, treatment may be restarted within 2 weeks, but not earlier than 72 hours (3 days) after the infusion was stopped.

After treatment interruption, a new treatment cycle may be started after consultation with the Investigator. A contrast-enhanced magnetic resonance imaging (MRI) of the head will be performed for subjects who had to interrupt treatment because of a CNS event grade 3 before treatment is resumed. Infusion should be restarted in the hospital, under supervision of the investigator and the subject should remain hospitalized for at least 2 days.

Following dexamethasone premedication as described in **Errore. L'origine riferimento non è stata trovata.**, a new treatment cycle will start with a dose of 9 µg/die. After restarting Blinatumomab, vital sign measurements, a neurological examination and writing tests should be performed for the next 3 days.

In case of CNS-related events CTCAE grade 4 or in case of occurrence of more than one seizure, the infusion of Blinatumomab will be stopped immediately and treatment will be permanently discontinued. The investigations associated with and previously described for a CTCAE grade 3 or higher CNS-related AEs, must also be performed.

A MRI is recommended for subjects who permanently discontinue treatment because of a CNS event grade ≥3.

Criteria for discontinuation of Blinatumomab

Treatment with Blinatumomab should be discontinued in the event of any of the following:
- Hematological or extramedullary relapse subsequent to CR/CRh/CRi on protocol treatment.
- Occurrence of a CTCAE grade 4 AE event possibly related to Blinatumomab. For CTCAE grade 4 AEs that are numerically defined laboratory parameters, independent investigator assessment should be used to determine the risk: benefit for each individual patient to continue or discontinue Blinatumomab treatment.
- Occurrence of an AE which makes discontinuation from treatment necessary due to protocol specified safety criteria or desirable in the investigator's and/or the subject´s opinion.
- An infusion interruption of ≧2 weeks due to an AE related to Blinatumomab (exception: in case of logistical difficulties, restart of treatment can be postponed for up to 7 additional days without resulting in permanent treatment discontinuation).
- Occurrence of CNS-related AEs meeting one or more of the following criteria:
    - More than one seizure
    - A CNS-related CTCAE grade 4 AE
    - A CNS-related AE leading to treatment interruption requiring more than 1 week to resolve to a CTCAE grade ≤1.

For subjects who discontinue treatment because of a CNS event ≥grade 3 a MRI is recommended.
- Investigator's decision that a change of therapy (including immediate allo-SCT) is in the subject´s best interest.
- Administration of relevant non-permitted concomitant medications.
- Investigator's decision that a subject no longer benefits from the treatment (eg, non-response or evidence of progressive disease).
- Intercurrent medical condition, which in the opinion of the investigator or the subject precludes further treatment.
- Withdrawal of subject's consent to study treatment.
- All reasons for treatment discontinuation will be documented in the CRFs. If a subject fails to keep the appointments for study visits, the investigator will document the reason and circumstances as completely and accurately as possible.

6.3.4 Supportive care

- Hematopoietic growth factor (G-CSF) is permitted at the discretion of the investigator for patients with neutropenia (ANC <0.5 x 10^9/L) during induction therapy with Dasatinib.
- The use of trimethoprim-sulfamethoxazole and ciprofloxacin is mandatory during treatment.
- Fluconazole and acyclovir will be given to prevent fungal infections and herpes simplex virus, respectively.
- Anticonvulsant prophylaxis with Levitiracetam (500 mg/BID) is mandatory throughout the whole period of Blinatumomab administration.
- Transfusional products are allowed, if needed.

Dexamethasone administration is mandatory as premedication before the start of Blinatumomab and in case of dose interruption as outlined in the table below (Table 2).

Treatment Phase	Target Patient	Dexamethasone Dose
Pre-dose Dexamethasone prior to each Blinatumomab treatment	All patients (before each cycle)	Dexamethasone 20 mg IV: within 1 hour prior to start of treatment.
Infusion Interruption/Dose modification due to AE	Patients who interrupt treatment >4 hours	Dexamethasone 20 mg IV: within 1 hour prior to re-start of treatment.
In case of signs of cytokine release (CRS)	Patients with signs of CRS	Dexamethasone orally or IV at a dose maximum 3 x 8 mg/day for up to 3 days. The dose should then be reduced stepwise over 4 days.
Infusion interruption/Dose modification due to CNS events	Patients with CNS-related AE	Dexamethasone should be administered at a dose of at least 24mg/day. Dexamethasone will then be reduced stepwise over 4 days.

Table 2: Dexamethasone premedication

6.3.5 Other concomitant therapies

All concomitant medication and therapies should be recorded in the case report from (CRF).

6.4 Study duration

41 months: 18 months of enrolment + 3 months of Dasatinib treatment + a maximum of 8 months of Blinatumomab treatment + 12 months of follow-up.

7 Drug supply

7.1 Dasatinib

7.1.1 Accountability

The investigator and the trial site are responsible for investigational product accountability. To this end, it is assumed that all clinical trial supplies will be delivered to and by the responsibility of a suitably qualified and authorized person such as a hospital pharmacist, who will document drug disposition and accountability for the duration of the trial.

Dasatinib will be supplied by Bristol-Myers Squibb.
The contact for Bristol-Myers Squibb will be:

Cosimo Paga
Disease Area Specialist Onco-Hematology - Italy
Bristol-Myers Squibb
Via Virgilio Maroso 50
00142 Roma
Phone: 06 50396564
mail: cosimo.paga@bms.com

7.1.2 Packaging, dispensing and storage

Packaging and labelling will be in accordance with Good Manufacturing Practice (GMP) for clinical trials.
Dasatinib will be supplied as film-coated tablets. Dasatinib does not require any special storage conditions

Each bottle will bear a single label, which will state the following GIMEMA protocol code, EudraCT number, patient code, date of treatment start, site, Dasatinib dose, pharmaceutical form, for clinical trial use only, storage conditions, batch number, expiry date, sponsor, sponsor's contacts.

Investigators and pharmacists should note that the clinical trial supplies may only be used for the clinical trial for which they are indicated. They must not be employed for any other trial or for any other clinical use.

7.1.3 Drug reconciliation procedures

The drug formulation, dose, number of bottles received, dispensed and returned for each patient must be recorded. Research personnel must not destroy any drug labels, or any partly used or unused bottle. If any bottle is lost or damaged, its disposition should be documented in the source documents. At the end of the study, and, as appropriate during the course of the study, the investigator will destroy all used and unused bottles and provide GIMEMA with the certificate of destruction.

7.2 Blinatumomab

7.2.1 Accountability

The investigator and the trial site are responsible for investigational product accountability. To this end, it is assumed that all clinical trial supplies will be delivered to and by the responsibility of a suitably qualified

and authorized person such as a hospital pharmacist, who will document drug disposition and accountability for the duration of the trial.

Blinatumomab will be supplied by Amgen.
The contact for Amgen will be:
Alessandra Balduzzi,
Senior Medical Advisor Immunooncology,
Amgen, s.r.l., Via E. Tazzoli 6, 20154, Milano
Phone: 02624112204, mobile: 3351097242
mail: balduzzi@amgen.com

7.2.2 Packaging, dispensing and storage

Packaging and labeling will be in accordance with GMP for clinical trials. Each vial will bear a single label, which will state the following GIMEMA protocol code, EudraCT number, patient code, date of treatment start, site, Blinatumomab dose, pharmaceutical form, for clinical trial use only, storage conditions, batch number, expiry date, sponsor, sponsor's contacts.
Blinatumomab will be supplied as 4 mL single-use glass injection vials as a sterile, preservative-free, white to off-white lyophilized powder for reconstitution and administration by IV infusion.
To prepare Blinatumomab for IV administration, the lyophilized powder is reconstituted with sterile water for injection (sWFI). The reconstituted solution is added to an infusion bag containing 0.9% NaCl and a product-specific stabilizer (IV Solution Stabilizer). The IV Solution Stabilizer functions to prevent adsorption of Blinatumomab to surfaces of the infusion components. The IV Solution Stabilizer is supplied in 10 mL single-use glass injection vials as a sterile, preservative-free, clear, colorless-to-slightly-yellow liquid concentrate. It consists of 25 mM citric acid monohydrate, 1.25 M L-lysine hydrochloride, and 0.1% (weight/volume [w/v]) polysorbate 80, pH 7. Following dilution in 0.9% NaCl, the ingredient concentrations are 25 mM L-lysine hydrochloride, 0.002% (w/v) polysorbate 80, and 0.5 mM citric acid monohydrate.
Blinatumomab solution for infusion may be administered using IV bags and infusion lines made of polyolefin/polyethylene, ethylene vinyl acetate (EVA), or PVC non-DEHP with an in-line 0.2 µm filter.
Blinatumomab must be administered using portable infusion pumps approved for use by the appropriate regulatory authority for the country in which the patient is undergoing treatment, in both the inpatient and outpatient settings. Infusion pumps should be programmable, lockable, non-elastomeric and have an alarm.
Both the Blinatumomab lyophilized drug product and IV Solution Stabilizer vials must be stored at 2°C to 8°C in their original outer package to maintain product integrity and prevent exposure to light. Do not freeze.
Reconstituted vials should not be stored for longer than 4 hours between 23°C and 27°C or longer than 24 hours between 2°C and 8°C.
Blinatumomab solution for infusion must be administered at ambient temperature and must not be kept at ambient temperature more than 96 hours.
For storage prior to administration, the prepared infusion solution must be kept at 2°C to 8°C. The total storage and administration time must not exceed 10 days.
Investigators and pharmacists should note that the clinical trial supplies may only be used for the clinical trial for which they are indicated. They must not be employed for any other trial or for any other clinical use.

7.2.3 Drug reconciliation procedures

The drug formulation, dose, number of vials received, dispensed and returned for each patient must be recorded. Research personnel must not destroy any drug labels, or any partly used or unused vial. If any vial is lost or damaged, its disposition should be documented in the source documents. At the end of the study, and, as appropriate during the course of the study, the investigator will destroy all used and unused vials and provide GIMEMA with the certificate of destruction.

7.3 Blinatumomab infusion pump

7.3.1 Dispensing

Blinatumomab infusion pumps will be supplied by GIMEMA as sponsor of the study. An infusion pump will be sent from GIMEMA to the participating center upon patient's enrolment in the protocol; furthermore, each center will receive an additional "back-up" an infusion pump, required for training and in case of malfunctioning of the other infusion pump.

7.3.2 Reconciliation procedures

At study completion, infusion pumps will be returned to GIMEMA.

8 Clinical evaluation, laboratory tests and follow-up

8.1 Before treatment starts

Assessment at enrolment/baseline (BL)

- Informed consent
- Demography
- General medical history and present medical condition
- Prior and concomitant medications
- Present disease signs and symptoms
- Physical examination and vital signs (height, weight, adenomegaly, spleen and liver size in cm below costal margin, other relevant findings)
- Performance status (WHO)
- ECG & Echocardiogram
- Chest X-RAY
- Abdominal ultrasound
- BM cytomorphology status performed by local investigator
- Blood count and differential, hemoglobin, platelet, total WBC, PMN, blasts including the absolute blasts count (at day -6)
- Biochemistry: BUN, urea, creatinine, uric acid. Albumin, total protein, total bilirubin, alkaline phosphatase, AST (SGOT), ALT (SGPT) and LDH, potassium, calcium
- Coagulation: PT, PTT/INR, fibrinogen; ATIII
- HIV, HBV DNA and HCV RNA test
- Rachicentesis (diagnostic and therapeutic) as soon as possible
- Biological sample centralization procedures (see "**Appendix F**"): BM and PB for cytomorphology, immunophenotype, cytogenetics, molecular studies.

Assessment at the end of pre-treatment phase

- Physical examination and vital signs (height, weight, adenomegaly, spleen and liver size, in cm below costal margin, other relevant findings)
- Performance status (WHO)
- Blood count and differential, hemoglobin, total WBC, PMN, blasts and platelet count
- Response evaluation to steroid pre-treatment (absolute blast count at day 0)
- Biochemistry: BUN, urea, creatinine, uric acid. Albumin, total protein, total bilirubin, alkaline phosphatase, AST (SGOT), ALT (SGPT) and LDH

8.2 During induction treatment

- Physical examination/vital signs, Performance status (WHO), hemoglobin, total WBC, PMN, blasts and platelet count:
  - 1st week: daily
  - from 2nd to 5th week: twice a week
  - from the 6th week: weekly
- Biochemistry: BUN, urea, creatinine, uric acid. Albumin, total protein, total bilirubin, alkaline phosphatase, AST (SGOT), ALT (SGPT), LDH, electrolytes:
  - 1st week: every other day
  - from 2nd to 4th week: twice a week
  - from the 5th week: weekly
- Coagulation: PT, PTT/INR, fibrinogen; ATIII before rachicenteses
- Rachicentesis (diagnostic and therapeutic): days +14, +22, +45, +57, +85
- BM and PB for cytomorphology evaluation of the hematological status performed by the local investigator at least on the following days: +22, +45, +57

- Biological sample (BM and PB) centralization procedures at days +22, +45 and +57 (see "**Appendix F**") only for patients either in CHR or without evidence of disease in BM and in PB (i.e., no "biological" studies will be performed if disease is still evident by cytomorphology).

8.3 End of induction treatment

- Physical examination/vital signs
- Performance status (WHO)
- ECG & Echocardiogram
- Chest X-RAY
- Abdominal ultrasound
- Hemoglobin, total WBC, PMN, blasts and platelet count
- Biochemistry: BUN, urea, creatinine, uric acid. Albumin, total protein, total bilirubin, alkaline phosphatase, AST (SGOT), ALT (SGPT) and LDH
- Rachicentesis (diagnostic and therapeutic) at day +85
- BM and PB for cytomorphology evaluation of the hematological response performed by the local investigator ad day +85.
- Biological sample (BM and PB) centralization procedures at day +85 (see **"Appendix F"**). Patients who have a CMR (i.e. BCR/ABL1 to ABL1 ratio=0) must repeat a centralized confirmatory BM 2 weeks later.
  Subjects found to have grade 1 asymptomatic pleural effusion on day 85 should be closely monitored for the development of symptoms requiring Dasatinib dose interruption and supportive care.

8.4 During post-induction treatment (prior to Blinatumomab administration - Cycle 1)

- Physical examination/vital signs
- Performance status (WHO)
- ECG & Echocardiogram
- Chest X-RAY
- Abdominal ultrasound
- Hemoglobin, total WBC, PMN, blasts and platelet count
- Biochemistry: BUN, urea, creatinine, uric acid. Albumin, total protein, total bilirubin, alkaline phosphatase, AST (SGOT), ALT (SGPT) and LDH
- Creatinine Clearance (calculated)
- Diagnostic and medicated rachicentesis, in case of delayed start of Blinatumomab (more than 2 weeks)
- Neurological examination and electroencephalogram (EEG), together with subject's writing test
- BM for cytomorphology to evaluate the persistence of hematologic response (undertaken by the local investigator) and molecular biology in case of delayed start of Blinatumomab (more than 2 weeks)
- Pregnancy test
- IgG
- HIV, HBV DNA and HCV RNA test
- Biological sample (BM and PB) centralization in case of delayed start of Blinatumomab (more than 2 weeks): see time points at the section of translational research and centralization procedures in **"Appendix F"**
- Disease status

8.5 During Blinatumomab cycles

- Physical examination, vital signals, neurological examination and performance status (WHO): days +1, +2, +3, +8, +15, +29
- Lumbar puncture (medicated): day +29
- BM aspirate/biopsy for cytomorphology and molecular biology: day +29
- Hemoglobin, total WBC, PMN, blasts and platelet count: days +1, +2, +3, +8, +15, +29

- Biochemistry: BUN, urea, creatinine, uric acid. Albumin, total protein, total bilirubin, alkaline phosphatase, AST (SGOT), ALT (SGPT) and LDH: days +1, +2, +3, +8, +15, +29
- IgG: day +29

Chemistry	Coagulation	Urinalysis	Hematology
Sodium	PT	Blood	Hemoglobin
Potassium	PTT	Protein	Hematocrit
Chloride	Fibrinogen	Glucose	Reticulocytes
Total protein	ATIII		Platelets
Albumin			WBC
Calcium			Differential: Neutrophils Eosinophils Basophils Lymphocytes Monocytes Atypical lymphocytes
Magnesium			
Phosphorus			
Glucose			
BUN or Urea			
Creatinine			
Uric acid			
Alk phos			
LDH			
AST (SGOT)			
ALT (SGPT)			
C-reactive Protein			
Amylase			
Lipase Bilirubin			
γGT			

Table 3: Chemistry, coagulation, urinalysis, hematology

8.6 During follow-up after Blinatumomab cycles

All subjects, including subjects who withdraw early, should complete a safety follow-up visit 30 days (±3 days) after the last dose of Blinatumomab, or prior to HSCT/chemotherapy, if applicable. The following procedures will be completed at the visit:

• Physical examination including weight
• Vital signs (eg, systolic/diastolic blood pressure, pulse rate, respirations, and temperature)
• WHO performance status assessment
• Complete neurological examination
• BM aspirate/biopsy for morphological and molecular analysis will be performed every month for the first 6 months and then every 2 months for the first year.
• Local laboratory assessments:
− Hematology with differential
− Chemistry
− Coagulation (includes INR and PTT)
− Immunoglobulins
− Urinalysis via dipstick

- Urine or serum pregnancy test (if indicated)
- Central laboratory assessments including
- Lymphocyte subsets
- Subject writing test
- AEs/SAEs reporting
- Documentation of concomitant medications

8.6 Summary table

8.6.1 Induction

Time (weeks from starting Dasatinib)	**Baseline**	**End of pretreatment**	1st	2nd	3rd	4th **Day +22**	5th	6th	7th **Day +45**	8th	9th **Day +57**	10th	11th	12th	**Response evaluation**
Time (days from starting Dasatinib)	-6	0	1 to 7	8 to 14	15-21	22-28	29-35	36-42	43-49	50-56	57-63	64-70	71-77	78-84	**Day +85**
Examination															
Informed consent	*														
Eligibility criteria (Inclusion/Exclusion)	*														
Demographics	*														
General medical history/present medical conditions	*														
Present disease leukemia history	*														
Present disease signs & symptoms	*														
Physical examinations/Vital signs	*	*	Daily	Every other day	Every other day	Twice a week	Twice a week	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly	*
Performance status (WHO)	*	*	Daily	Every other day	Every other day	Twice a week	Twice a week	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly	*
Hematology (Hemoglobin; total WBC, PMN, blast and Platelet count expressed as cells x10^9/L)	*	*	Daily	Every other day	Every other day	Twice a week	Twice a week	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly	*
HIV, HBV DNA and HCV RNA test	*														
Serum biochemistry [BUN, urea, creatinine, uric acid, albumin, total protein, total bilirubin, alkaline phosphatase, AST (SGOT), ALT (SGPT), LDH, electrolytes].	*	*	Every other day	Every other day	Twice a week	Twice a week	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly	*
ECG & visit	*														*
Echocardiogram	*														*
Chest X-Ray	*														*
Abdominal ultrasound	*														*
Rachicentesis	*			Day +14		Day +22			Day +45		Day +57				Day +85
BM cytomorphology status performed by local investigator	*					Day +22			Day +45		Day +57				Day+85
BM cytomorphology centralized	*														*
BM immunophenotype centralized	*														*a
BM cytogenetics centralized	*														
BM molecular studies centralized	*					*			*		*				*
Peripheral blood sample	*					*			*		*				*
Pregnancy test	*														
Concomitant treatment			Continuously throughout the study												
AE and SAE	Continuously throughout the study														

a: In case of CMR, e.g.BCR-ABL1/ABL1=0, a second confirmatory and centralized bone marrow aspirate must be repeated 2 weeks later.

Table 4: Induction treatment exams.

8.6.2 Post-induction treatment (prior and during treatment with Blinatumomab)

Examination	Prior to blinatumomab start	During treatment					
		D1	D2	D3	D8	D15	D29 ± 1 day
Physical examinations/Vital signs	*	*	*	*	*	*	*
Performance status (WHO)	*	*	*	*	*	*	*
Neurological examination and electroencephalogram (EEG)	*						
Hematology (hemoglobin; WBC, PMN, blast and platelet counts expressed as cells x10^9/L)	*	*	*		*	*	*
Serum biochemistry (BUN, urea, creatinine, uric acid, albumin, total protein, total bilirubin, alkaline phosphatase, AST (SGOT), ALT (SGPT), LDH, electrolytes.	*	*	*		*	*	
Coagulation (PT; PTT; fibrinogen, ATIII)	*	*	*		*	*	*
Pregnancy test	*						
IgG	*						
HIV, HBV DNA and HCV RNA test	*						
ECG & visit	*						
Echocardiogram	*						
Chest X-Ray	*						
Abdominal ultrasound	*						
Rachicentesis [a]	*[a]						*
BM cytomorphology (performed by the local investigator)	*						*
BM molecular studies	*						*
Biological samples centralization (see Appendix F)	*						*
Disease status	*						*
Pregnancy test	*						
Subject's writing test[b]	Continuously throughout the study						
Concomitant treatment	Continuously throughout the study						
AE and SAE	Continuously throughout the study						

a: Only in case of delayed start of Blinatumomab (more than 2 weeks); b: Subject writing test completed in the morning and evening on day 1 and day 2, then once daily through day 29 for each cycle.

Table 5: Post-induction treatment exams

8.7.3.1 During follow-up

Examination	1st month	2nd month	3rd month	4th month	5th Month	6th month	8th month	10th month	12th month
Physical examinations/Vital signs	Every two weeks	*	*	*	*	*	*	*	*
Performance status (WHO)	Every two weeks	*	*	*	*	*	*	*	*
Hematology (hemoglobin; WBC, PMN, blast and platelet counts expressed as cells x10^9/L)	Every two weeks	*	*	*	*	*	*	*	*
Serum biochemistry (BUN, urea, creatinine, uric acid, albumin, total protein, total bilirubin, alkaline phosphatase, AST (SGOT), ALT (SGPT), LDH, electrolytes.	Every two weeks	*	*	*	*	*	*	*	*

ECG & visit			*		*	*			*
Echocardiogram			*		*	*			*
Chest X-R[a]			*		*	*			*
Abdominal ultrasound[a]									
Rachicentesis	*	*	*	*	*	*	*	*	*
BM cytomorphology (performed by the local investigator)	*	*	*	*	*	*	*	*	*
Biological samples centralization (see Appendix F)[b] for BM molecular studies	*	*	*	*	*	*	*	*	*
Disease status	*	*	*	*	*	*	*	*	*
Patient status	*	*	*	*	*	*	*	*	*
AE and SAE	Continuously throughout the study								

[a]: According to medical opinion; [b]: According to time points in the section of the translational research

Table 6: Follow-up examinations

8.7 Biological samples conservation: timelines and procedures

Samples will be stored (cryopreserved in DMSO, dry pellet or pellet in GTC) centrally - Laboratorio Centralizzazione, Ematologia, Azienda Policlinico "Umberto I", Dipartimento Biotecnologie Cellulari ed Ematologia, Università "Sapienza", Via Benevento 6 - 00161 Roma - for up to 10 years after database closure.

9 Criteria of evaluation

9.1 Evaluation of response

Complete hematologic remission (CHR):
BM cellularity of at least 20% containing less than 5% blast cells.
PB smears without blasts.
No evidence of extramedullary involvement from leukemia.

CHRi:
Less than or equal to 5% blasts in the BM with incomplete recovery of peripheral blood counts (llatelets >100,000/µl or PMN >1000).

Failure:
Defined as absence of CHR or CHRi.
It can be classified in 3 different types:
- Partial remission (PR): 5-20% blast present in the BM.
- Resistance (RES): >20% blast in the BM.
- Disease progression (DP): increasing of blast rate in BM and/or PB after diagnosis and during treatment; appearance or extension of extramedullary localizations after diagnosis and during treatment.

Hematologic relapse:
Re-appearance of blasts in the PB or the finding of >5% blasts in the BM, not attributable to another cause (e.g. marrow regeneration). Relapse can be suspected in case of unexplained cytopenia. In any case, relapse must be documented by blood and marrow smears.

Extra-medullary relapse:
A diagnosis of extra-medullary relapse should be based on tissue histology or liquor cytology.

MRD assessment:
MRD will be evaluated at diagnosis and during follow-up using a Quantitative QRT- PCR system:

Complete molecular remission (CMR):
CMR, defined as BCR-ABL1/ABL1 ratio of zero. In patients in CMR at the end of induction, a centralized confirmatory BM must be repeated 2 weeks later for confirmatory purposes.

MRD reduction:
- Major molecular remission, defined as BCR-ABL1/ABL1 reduction of 2 logs compared to the value detected at diagnosis.
- Optimal molecular remission, defined as BCR-ABL1/ABL1 reduction of 3 logs compared to the value detected at diagnosis.

Molecular relapse
An increase of at least 2 logs detected and confirmed in two consecutive controls during the patient follow-up.

9.2 Evaluation of toxicity

All toxicities encountered during therapy will be evaluated according to the Common Toxicity Criteria – Adverse Event (CTCAE) version 4.0 Criteria (Appendix C).

9.3 Time to event analysis

Disease-free survival (DFS): DFS is defined as the time interval between the evaluation of CHR (day +85) and hematologic relapse of the disease or death in first CHR; patients still alive, in first CHR, will be censored at the time of the last follow-up.

Overall survival (OS): OS is defined as the time interval between treatment start and death for any cause; patients still alive will be censored at the time of the last follow-up.

Cumulative incidence of relapse (CIR): the CIR will be calculated from the date of evaluation of CHR until the date of first hematologic relapse of the disease, using the cumulative incidence method considering death in CHR as a competing risk. Patients still alive, without a date of relapse, will be censored at the time of the last follow-up.

10 Statistical considerations

10.1 Sample size

This study is designed to evaluate the activity of Dasatinib plus Blinatumumab in eradicating MRD in adult Ph+ ALL, in terms of percentage of patients who achieve a CMR after the induction phase based on Dasatinib and steroids only, followed by 2 (up to a maximum of 5) cycles of Blinatumomab to improve the CMR rate from 40% to 60%; this percentage was estimated on the basis of a preliminary analysis of the LAL 1509. The number of patients required to demonstrate this hypothesis with a power of 90% and a Type I error probability of 5%, and considering a 10% drop-out, is 60.
In the proposal, to reject the null hypothesis that $p \leq 0.40$ vs. the alternative hypothesis that $p > 0.60$ with Type I error probability (α) equal to 0.05 and 90% power ($1-\beta$), 54 evaluable patients has to be accrued. Considering a 10% rate of non-evaluable patients due to ineligibility, toxicity, medical decision or refusal before treatment starts the estimated total number of patients to include in the study is 60. In the first stage of the study, 29 evaluable patients (32 considering 10% of drop out) will be enrolled and the trial will be terminated if 12 or fewer responses after the completion of the 2 cycles of Blinatumomab will be achieved; otherwise, 25 further evaluable patients (28 considering 10% of drop out) will be enrolled in the second stage. If the total number of responses will be less than or equal to 27, the combination therapy will not be recommended for further studies. If the total number of CMRs is at least 28, the association will be deemed

worthy of further investigations. Calculations were implemented in PASS2008 using a Simon two stage (minimax) phase II study design.

10.2 Analysis

Response achievement will be evaluated in terms of percentage of successful responses over all eligible and evaluable patients enrolled in the study (following an Intention-To-Treat principle). All AEs will be tabulated. All reported toxicities will be correlated with clinical outcome. Patients' characteristics will be summarized by means of cross-tabulations for categorical variables or by means of quantiles for continuous variables. In univariate analysis non-parametric tests will be performed for comparisons between groups (Chi-Squared and Fisher Exact test in case of categorical variables or response rate, Mann-Whitney and Kruskal-Wallis test in case of continuous variables) and logistic regression will be performed in multivariate analysis to assess the effect of clinical and biologic factors on CMR rate. OS will be defined as the time from treatment start to death from any cause. DFS will be defined as the time from the achievement of CHR to relapse, death, or date of last follow-up for patients alive in first CHR. CMR duration will be defined as the time form the achievement of CMR to molecular relapse, death or date of last follow-up for patients alive in first CMR. The OS, DFS and duration of CMR probabilities will be estimated using the Kaplan-Meier method. CIR will be calculated from the achievement of CHR to relapse or date of last follow-up for patients alive in first CHR, using the cumulative incidence method and considering death in CHR as competing risk. Subgroups comparisons will be performed for descriptive purposes. Differences in terms of OS, DFS duration of CMR will be evaluated by means of Log-Rank test in univariate analysis and by means of the Cox regression model in multivariate analysis, after assessment of proportionality of hazards. CIR will be estimated by cumulative incidence curves using the proper non-parametric method. The Gray test will be applied for significance tests on cumulative incidence curves. Median follow-up time will be estimated by reversing the codes for the censoring indicator in a Kaplan-Meier analysis. Confidence intervals will be calculated at 95% level and forest plots wil be used to summarize differences among subgroups. All analysis will be performed using the SAS software (release 9.4 or later).

10.3 Safety analyses

Analysis of safety data will be conducted on the safety population, which includes all subjects who receive at least 1 dose of study medication. This population will be used for all safety analyses and all analyses of treatment compliance and exposure. All data will be analyzed according to the treatment subjects actually received. The safety variables to be analyzed include AEs, clinical laboratory tests (hematology and chemistry), physical examination results, ECGs, and deaths. Safety variables will be tabulated by descriptive statistics (n, mean, median, standard deviation, minimum, and maximum; or n and percent). No formal statistical testing is planned.

10.4 Interim analysis

Interim analysis will be performed after the first stage according to the Simon design (see chapter 10.1).

11 Independent data monitoring committee

No independent data monitoring committee has been appointed for this study. No formal interim statistical analysis will be performed.

12 Quality of life assessment

Quality of life will not be assessed in this study.

13 Economic evaluation

No economic evaluation will be performed in this study.

14 Pharmacokinetic evaluation

No pharmacokinetic evaluation will be performed in this study.

15 Translational research

15.1 Rationale and objectives

This trial is based on an innovative approach, based on a chemo-free, targeted therapy, aimed not only at obtaining a rapid CHR, but also - as a novelty - at eradicating MRD disease.
A molecular assay of MRD disease is required to monitor the molecular response and in case of any MRD increase to shift therapy to best treatment available.
Moreover, a detailed knowledge of the genetic and molecular mechanisms of sensitivity and resistance will lead to a refined understanding of the disease itself, and ultimately will pave the way for any further treatment improvement.
For these reasons, it is necessary to collect, analyze and store biologic material at presentation and at different time points during treatment.
BM cells and PB cells for the studies that are listed herewith:

- Extended immunophenotypic characterization at presentation, at day +85 and at relapse.
- Cell karyotype by conventional cytogenetics at presentation and at relapse.
- Determination of the type of fusion transcript (p190, p210, p190/p210). Quantitative determination of the BCR-ABL1 transcript to assess MRD at the days +22, +45 +57 and +85 during the induction therapy, every month for the first 6 months after induction, every 2 months for the first year and then every 3 months.
- Screening of *IKZF1* and additional copy number aberrations (CNA) by single nucleotide polimorphisms (SNP) arrays (HD Cytoscan arrays, Affymetrix, CA) at diagnosis and in case of relapse.
- Evaluation of ABL1 mutations on MRD+ positive cells, in case of MRD increase and/or persistence.
- Next-generation sequencing (NGS), either by whole exome sequencing or RNA sequencing, of leukemic cases to evaluate the presence of additional lesions (mutations, other than those affecting *ABL1,* or rearrangements) at diagnosis and at relapse.
- Gene expression profiling of enrolled cases on diagnostic material, if feasible.
- Finally, if feasible, germline material collection at CHR for comparative purposes with leukemic cells for SNP array and NGS approaches.

15.2 Samples collection and schedule

Samples will be collected to promote, facilitate and improve the individualized health care, by better understanding the study efficacy, the safety mode of action and progression of the disease.
PB and BM samples will be taken at baseline and during induction therapy at defined timepoints (days +22, +45, +57 and +85); during post-induction treatment (see above); thereafter, at any time in case of failure or disease progression.
Samples are collected at all investigational sites and sent to the central reference laboratory as described in the "**Appendix F**".
The sampling is subject to patients' signature on the informed consent.
The central reference laboratory will take care of supplying to competent GIMEMA network laboratories the samples for the assigned analyses, according to the laboratory area of expertise.

15.3 Methods to be used

15.3.1 Morphology

Smears of bone marrow and peripheral blood will be analyzed with the standard May-Grünwald and Giemsa stain.

15.3.2 Immunophenotype

The evaluation of immunophenotype will be done through cell suspension of BM samples or through PB samples (only if the blastosis is >50% of circulating cells) and will mean the analysis by cytofluorimetry of a minimum number of markers: cyCD79a e/o cyCD22, CD19, CD10, CD20, cyμ, cyCD3, CD7, CD5, CD2, CD1a sCD3, CD4, CD8, CD45, TdT, HLA-DR, CD34, cyMPO, CD13, CD33. Usually, surface markers are considered as positive if the percentage of antigen expressed by blasts is ≥20%, while with intracytoplasmatic markers the percentage is ≥10%. The immunophenotype will be performed centrally in the immunophenotypic laboratory of Ematologia, Azienda Policlinico "Umberto I", Dipartimento Biotecnologie Cellulari ed Ematologia, Università "Sapienza" di Roma. Immunophenotyping will be repeated at the end of induction and at the end of each Blinatumomab cycle to evaluate T-cell subpopulations.

15.3.3 Cytogenetics

Cytogenetic analysis will be performed in the cytogenetic laboratory of Ematologia, Azienda Policlinico "Umberto I", Dipartimento Biotecnologie Cellulari ed Ematologia, Università "Sapienza" di Roma.
Conventional cytogenetics: analysis is performed on BM cells after short term culture (24 and/or 48 hours). Cells are treated with colchicine and with hypotonic solution. Pellet is fixed and washed in methanol-acetic acid (3:1). Cells are resuspended in fixative and dropped on slides. Karyotypes are examined after G-banding or Q-banding and are described according to the ISCN (Ref. 47). Karyotypes with a number of metaphases ≥10 will be considered evaluable. In cases with conventional cytogenetics not evaluable (due to lack of cells in mitosis) or normal, other analysis could be done.

15.3.4 Molecular biology

Briefly, according to the timing of blood and marrow sampling, RNA will be extracted from mononuclear cells collected from patients enrolled in the trial following the Chomczynski method (Ref. 48). Molecular analysis will be performed in the molecular biology laboratory of Ematologia, Azienda Policlinico "Umberto I", Dipartimento Biotecnologie Cellulari ed Ematologia, Università "Sapienza" di Roma. Mutational analysis
At diagnosis, during follow-up and at the relapse, blood and marrow samples will be collected in all subjects for descriptive analysis of the spectrum of mutations. During the follow-up, a cDNA sample must be stored in order to eventually perform the analysis at the discretion of the investigator.
Mutational analysis of ABL1 will be performed at MRD increase and at relapse, either by Sanger sequencing, or NGS techniques. T315I mutations will be also detected by a specific PCR assay (Ref. 52, Ref. 53).
Alternatively, NGS will be applied by using the Genome Sequencer Junior 454 (Roche Applied Science®) and specific primers and MIDs. Raw images and internal control analysis of each run will be performed using settings of the GS Run Browser Software version 2.3 (Roche Applied Science®). A cut-off of 80 reads in forward and in reverse (combined n=160 reads/amplicon/sample) will be used as pass filter for subsequent variants analysis. The Sequence Pilot software version 3.4.0 (JSI Medical System, Kippenheim, Germany) will be used for sequence analyses and variants detection.

15.3.5 Gene expression profiling

Gene expression analysis will be performed on the Affymetrix platform using the Human genome U133 Plus 2.0 arrays (that contains over 54,0000 genes – corresponding approximately to the whole genome). Data analysis will be performed with unsupervised and supervised approaches (dChip software). Functional classification of identified pathways will follow (based on Gene Ontology categories, using the DAVID functional software and Gene Set Enrichment Analysis -GSEA). Real time RT-PCR and Western-blot analyses will then be performed to validate results.

15.3.6 Analysis of genomic copy-number alterations by SNP array and analysis of IKZF1 (Ikaros) deletions and CNA

The identification of genomic abnormalities is important in diagnosis and risk stratification. In this study we will perform a molecular karyotype in order to identify cryptic genomic abnormalities and common clusters of alterations. The experiments will be performed starting from genomic DNA extracted from ALL patients (500 ng) prior to treatment using Affymetrix® Cytoscan HD Array.
Genomic DNA (gDNA) will be extracted using the Wizard® Genomic DNA Purification Kit (Promega) from mononuclear cells isolated from bone marrow or peripheral blood samples by Ficoll gradient centrifugation. DNA will be quantified using the Nanodrop Spectrophotometer and quality assessed using the Nanodrop and by agarose gel electrophoresis. Genome-wide CNA analysis will be performed by using CytoScan® HD arrays (Affymetrix, Santa Clara, CA) and standard protocols. The identification of CNAs, as well as the recognition of minimal common regions of aberration, will be performed as previously reported. *(Pasqualucci L, Trifonov V, Fabbri G, et al. Analysis of the coding genome of diffuse large B-cell lymphoma. Nat Genet. 2011;43(9):830-837.25. Mullighan CG, Goorha S, Radtke I, et al. Genome-wide analysis of genetic alterations in acute lymphoblastic leukaemia. Nature. 2007; 446(7137):758-764.)* CEL files will be created using the GeneChip® System 3000 7G according to the manufacturer's instructions (Affymetrix) and analyzed using Chromosome Analysis Suite v2.0 (ChAS) software. Positive (Genomic DNA Control supplied by Affymetrix) and negative controls (Low EDTA TE Buffer) will be used to validate chip, reagents, and instruments and every CytoScan® HD array has internal quality control metrics used to determine pass/fail for individual samples. Identification of segments of abnormal copy numbers will be carried out using the dChipSNP software, and a karyotype-guided normalization procedure according to a published workflow (Pasqualucci et al., 2011b). When available, to exclude inherited copy number variants, a comparison with paired constitutional DNA or with reference samples was performed. Normal genomic variants (present in the Genomic Variants Database [http://projects.tcag.ca/variation]) and/or found in constitutional DNA of patients will be excluded from the analysis.
As for *IKZF1* deletion detection, two different approaches will be used to detect *IKZF1* deletions from DNA samples (they are not mutually exclusive):
1. Single Nucleotide Polymorphism (SNP) arrays by using Cytoscan arrays (Affymetrix, Santa Clara, CA) and according to the manufacturer's instructions.
2. MLPA (Multiplex Ligation-dependent Probe Amplification).

15.3.8 Whole exome sequencing (WES) and RNA-sequecing (RNA-seq)

If feasible, WES and RNA-sequecing will be carried out to evaluate the presence of potentially novel and noxious mutations. Both analyses will be carried out at diagnosis, and if feasible, at MRD increase or hematologic relapse.

15.4 Shipping instructions

See appendix F.

15.5 Data transfer to the Data Center

The results will be transferred to the data center and to the study sponsor at the end of the study, within two months from the shipment of all the samples.

16 Investigator authorization procedure

Investigators will be authorized to register or randomize patients in this trial only when they have returned to the Data Center:

- The (updated) list of the normal ranges, in their own institution, of all laboratory data required by the protocol, preferably signed and dated by the head of the laboratory.
- A signed conflict of interest disclosure form.
- A copy of the favorable opinion of their local ethics committee mentioning the documents that have been reviewed (incl. version number and date of documents) and indicating the list of the ethics committee members.

- A copy of the translated, if applicable, and adapted, if changed by the Ethics Committee, Informed Consent, clearly mentioning the version number and the date.
- The coordinator of the pharmacist who will be responsible for the trial medication (for any trial where the drug will be provided)
- List of co-investigators who are authorized to work for this study

The center specific applicable list of required documents will be included in the protocol activation package, with proper instructions as required.

17 Patient registration procedure

Patient registration will only be accepted from authorized investigators (see "Authorization procedure"). A patient can be registered after verification of eligibility criteria.
A patient who has not been registered before the first treatment administration will not be accepted in the study at a later date. An exhaustive list of questions to be answered during the registration procedure is included in the registration check-list, which is part of the case report forms. This check-list should be completed by the responsible investigator before the patient is registered.

18 Forms and procedures for collecting data

18.1 Case report forms and schedule for completion

A web system data entry will be used for this trial. Still, SAE/SUSAR report forms must be sent by e-mail to the Data Center.

18.2 Data flow

In all cases, it remains the responsibility of the investigator to check that e-CRFs are sent to the Data Center as soon as possible and that they are completely and correctly filled out. GIMEMA Data Center will perform extensive consistency checks and issue electronic Query Forms in case of inconsistent data. The investigator (or an authorized staff member) will electronically answer these queries and sign the query forms.
The GIMEMA data manager will subsequently verify the modifications. If an investigator (or an authorized staff member) needs to modify a CRF after the e-form has been sent to the GIMEMA Data Center, he/she should notify the Data Center in creating a query.

19 Reporting adverse events

19.1 Adverse Event Definitions and Classifications

Adverse event (AE)
An AE is any untoward medical occurrence in a clinical study subject administered a medicinal (investigational or non-investigational) product. An AE does not necessarily have a causal relationship with the treatment. An AE may therefore be any unfavorable and unintended sign (including an abnormal finding), symptom, or disease temporally associated with the use of a medicinal (investigational or non-investigational) product, whether or not related to that medicinal (investigational or non-investigational) product. (Definition per International Conference on Harmonisation [ICH]). This includes any occurrence that is new in onset or aggravated in severity or frequency from the baseline condition, or abnormal results of diagnostic procedures, including laboratory test abnormalities (GVP Annex 1 – European directive 2001/20/EC).

Adverse reaction
An adverse reaction is defined as a response to a medicinal product which is noxious and unintended. Response in this context means that a causal relationship between a medicinal product and an AE is at least a reasonable possibility (see Annex IV, ICH-E2A Guideline). Note: The sponsor collects AEs starting with the signing of the ICF (refer to Section Adverse Events, for time of last AE recording).

Serious adverse event (SAE)
A SAE based on ICH and EU guidelines on pharmacovigilance for Medicinal Products for Human Use is any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening (the subject was at risk of death at the time of the event. It does not refer to an event that hypothetically might have caused death if it were more severe)
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- Is a suspected transmission of any infectious agent via a medicinal product
- Is medically important*

*Medical and scientific judgment should be exercised in deciding whether expedited reporting is also appropriate in other situations, such as important medical events that may not be immediately life threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above. These should usually be considered serious.

Unlisted (unexpected) AE/reference safety information
An AE is considered unlisted if the nature or severity is not consistent with the applicable product reference safety information. The expectedness of an AE will be determined by whether or not it is listed in the Investigator's Brochure.

Special reporting situations
Safety events of interest on a sponsor study drug that may require expedited reporting and/or safety evaluation include, but are not limited to:

- Overdose of a sponsor study drug
- Suspected abuse/misuse of a sponsor study drug
- Inadvertent or accidental exposure to a sponsor study drug
- Medication error involving a sponsor product (with or without subject/patient exposure to the sponsor study drug, eg, name confusion)

Special reporting situations should be recorded in the CRF. Any special reporting situation that meets the criteria of a SAE should be recorded on the SAE form.

AE associated with the use of the drug
An AE is considered associated with the use of the drug if the attribution is possible, probable, or very likely by the definitions listed below.

19.2 Attribution definitions

Not related
An AE that is not related to the use of the drug.

Doubtful
An AE for which an alternative explanation is more likely, eg, concomitant drug(s), concomitant disease(s), or the relationship in time suggests that a causal relationship is unlikely.

Possible
An AE that might be due to the use of the drug. An alternative explanation, eg, concomitant drug(s), concomitant disease(s), is inconclusive. The relationship in time is reasonable; therefore, the causal relationship cannot be excluded.

Probable
An AE that might be due to the use of the drug. The relationship in time is suggestive (eg, confirmed by dechallenge). An alternative explanation is less likely, eg, concomitant drug(s), concomitant disease(s).

Very likely
An AE that is listed as a possible adverse reaction and cannot be reasonably explained by an alternative explanation, eg, concomitant drug(s), concomitant disease(s). The relationship in time is very suggestive (eg, it is confirmed by dechallenge and rechallenge).

19.3 Severity criteria

An assessment of severity grade will be made using the NCI-CTCAE (version 4.03). The investigator should use clinical judgment in assessing the severity of events not directly experienced by the subject (eg, laboratory abnormalities).

19.4 Reporting procedures

19.4.1 All adverse events

All AEs and special reporting situations, whether serious or non-serious, will be reported from the time a signed and dated ICF is obtained until completion of the subject's last study-related procedure (which may include contact for follow-up of safety). Serious AEs, including those spontaneously reported to the investigator within 30 days after the last dose of study drug, must be reported. All events that meet the definition of a SAE will also be reported as SAE, regardless of whether they are protocol-specific assessments. All AEs, regardless of seriousness, severity, or presumed relationship to study drug, must be recorded using medical terminology in the source document and the CRF. Whenever possible, diagnoses should be given when signs and symptoms are due to a common etiology (eg, cough, runny nose, sneezing, sore throat, and head congestion should be reported as "upper respiratory infection"). Investigators must record in the CRF their opinion concerning the relationship of the AEs to study therapy. All measures required for AE management must be recorded in the source document. The sponsor assumes responsibility for appropriate reporting of AEs to the regulatory authorities. The sponsor must report these events to the appropriate Independent Ethics Committee/Institutional Review Board (IEC/IRB) that approved the protocol unless otherwise required and documented by the IEC/IRB

19.4.2 Serious adverse events

All SAEs occurring during the study must be reported to GIMEMA safety-desk within 24 hours of their knowledge of the event. All SAEs that have not resolved by the end of the study, or that have not resolved upon discontinuation of the subject's participation in the study, must be followed until any of the following occurs:

- The event resolves.
- The event stabilizes.
- The event returns to baseline, if a baseline value/status is available.
- The event can be attributed to agents other than the study drug or to factors unrelated to study conduct.
- It becomes unlikely that any additional information can be obtained (subject or health care practitioner refusal to provide additional information, lost to follow-up after demonstration of due diligence with follow-up efforts).

Suspected transmission of an infectious agent by a medicinal product will be reported as a SAE. Any event requiring hospitalization (or prolongation of hospitalization) that occurs during the course of a subject's participation in a study must be reported as a SAE, except hospitalizations for the following:

- Hospitalizations not intended to treat an acute illness or AE (eg, social reasons such as pending placement in long-term care facility).
- Surgery or procedure planned before entry into the study (must be documented in the CRF). Note: Hospitalizations that were planned before the signing of the ICF, and where the underlying condition for which the hospitalization was planned has not worsened, will not be considered SAEs. Any AE that results in a prolongation of the originally planned hospitalization is to be reported as a new SAE.

- Disease progression should not be recorded as an AE or SAE; instead, signs and symptoms of clinical sequelae resulting from disease progression/lack of efficacy will be reported if they fulfill the SAE definition.
- A standard procedure for protocol therapy administration will not be reported as a SAE. Hospitalization or prolonged hospitalization for a complication of therapy administration will be reported as a SAE.
- The administration of blood or platelet transfusion. Hospitalization or prolonged hospitalization for a complication of such transfusion remains a reportable SAE.
- A procedure for protocol/disease-related investigations (eg, surgery, scans, endoscopy, sampling for laboratory tests, bone marrow sampling, pharmacokinetic or biomarker blood sampling). Hospitalization or prolonged hospitalization for a complication of such procedures remains a reportable SAE.
- Prolonged hospitalization for technical, practical, or social reasons in the absence of an AE.
- A procedure planned before entry into the study (must be documented in the CRF). Prolonged hospitalization for a complication considered to be at least possibly related to the study drug remains a reportable SAE.

19.4.3 Pregnancy

All initial reports of pregnancy must be reported to GIMEMA within 24 hours of their knowledge of the event. Abnormal pregnancy outcomes (eg, spontaneous abortion, stillbirth and congenital anomaly) are considered serious adverse events and must be reported as a SAE. Any subject who becomes pregnant during the study must discontinue further study treatment. Because the effect of the study drug on sperm is unknown, pregnancies in partners of male subjects included in the study will be reported by the study-site personnel within 24 hours of their knowledge of the event. Follow-up information regarding the outcome of the pregnancy and any postnatal sequelae in the infant will be required.

The Investigator, in case of death, has to communicate the event also to his local Ethics Committee. The Sponsor will forward information about SAEs to the Ethics Committee according to the new Pharmacovigilance legislation.
Information about SUSAR will be forwarded by GIMEMA directly into the Eudravigilance database.

Any question concerning SAE reporting can be directed to GIMEMA Safety Desk
Phone: +39 06 70390518
Fax: +39 06 70390540
e-mail: safety-desk@gimema.it

20 Quality assurance

20.1 Control of data consistency

Computerized and manual consistency checks will be performed on newly entered forms; queries will be issued in case of inconsistencies. Consistent forms will be validated by the Data Manager to be entered on the master database. Inconsistent forms will be kept "pending" until resolution of the inconsistencies.

20.2 On-site quality control

In order to censure that the study is conducted according to Good Clinical Practice (GCP), GIMEMA Data Center will send to every single center an Investigator's File, will organize Training Meetings in which principal investigators as well as collaborative investigators will be involved. In these meetings the following issues will be addressed:

1. Regulatory procedures.
2. Compulsory documents to be sent to the Data Center in order to be authorized to enroll patients.
3. Study documents archive system.
4. Patient information sheet and informed consent: how to approach the patient and where to archive the document.
5. Biological samples centralization system.
6. Patient selection criteria and registration procedure.

7. CRFs, queries management.
8. SAEs/SUSARs.
9. Main source documents to be sent to the Data Center.

During the first Training Meeting, different operative procedures will be distributed and explained, procedures concerning patient selection criteria and registration procedures, CRFs and SAEs/SUSARs. All these will be in the Investigator's File as well.

During the general GIMEMA Meetings, a report concerning the conduction of the study will be distributed. In this report, up to date data can be found concerning not only accrual but also SAEs/SUSARs, list of participating centers and particular situations that may have arisen during the conduction of the trial. This report constitutes an important working tool for the Investigator and is also an up to date report to be periodically presented to the Ethics Committee.

Furthermore, the statistical design and the precise accrual will be a method to select data that need to be verified.

20.3 Central review procedures

For many years the GIMEMA has worked with a biological sample centralization system for ALL patients enrolled in GIMEMA Studies. All samples are sent to a single Laboratory in Rome (within 24 hours). Afterwards, these samples are processed in order to distribute the different analyses to be made between laboratories that guarantee the use of internationally recognized standards and that can use and manage most advanced technologies. This type of organizations allows a highly defined standard of diagnosis, as well as a uniform diagnostic work-up for all enrolled cases, and a closely monitored census of the illness during the course of the study. Besides, this system provides the same standard for all patients, otherwise not possible.

21 Ethical considerations

21.1 Patient protection

The responsible investigator will ensure that this study is conducted in agreement with either the Declaration of Helsinki (Tokyo, Venice, Hong Kong, Somerset West and Edinburgh amendments) or the laws and regulations of the country, whichever provides the greatest protection to the patient.
The protocol has been written, and the study will be conducted according to the ICH Harmonized Tripartite Guideline for Good Clinical Practice (ref: http://www.wma.net/en/30publications/10policies/b3/index.html). The protocol will be approved by the Local, Regional or National Ethics Committees.

21.2 Subject identification

The name of the patient will not be asked for nor recorded at the Data Center. A sequential identification number will be automatically attributed to each patient registered in the trial. This number will identify the patient and must be included on all case report forms.

21.3 Informed consent

All patients will be informed of the aims of the study, the possible AEs, the procedures and possible hazards to which he/she will be exposed, and the mechanism of treatment allocation. They will be informed as to the strict confidentiality of their patient data, but that their medical records may be reviewed for trial purposes by authorized individuals other than their treating physician. An example of a patient informed consent statement is given as an appendix to this protocol. It is the responsibility of the individual investigator to translate the enclosed informed consent document. The translated version should be dated and version controlled. The translated informed consent form is part of the documents to be submitted to the ethics committee for approval. The competent ethics committee for each institution must validate local informed consent documents before the centre can join the study. It is the responsibility of the Local Ethics Committee to guarantee that the translation is conforming to the ICH-GCP guidelines. It will be emphasized that the

participation is voluntary and that the patient is allowed to refuse further participation in the protocol whenever he/she wants. This will not prejudice the patient's subsequent care. Documented informed consent must be obtained for all patients included in the study before they are registered or randomized at the GIMEMA Data Center. This must be done in accordance with the national and local regulatory requirements. For European Union member states, the informed consent procedure must conform to the ICH guidelines on Good Clinical Practice.

22 Administrative responsibilities

22.1 The Study Coordinator

The Study Coordinator and co-Coordinator (in cooperation with the Data Center) will be responsible for writing the protocol, reviewing all case report forms and documenting their review on evaluation forms, discussing the contents of the reports with the Data Manager and the Statistician, and for publishing the study results. They will also generally be responsible for answering all clinical questions concerning eligibility, treatment, and the evaluation of the patients.

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22.2 The GIMEMA Data Center

The GIMEMA Data Center will be responsible for reviewing the protocol, collecting case report forms, controlling the quality of the reported data, and generating reports and analyses in cooperation with the Study Coordinator. All methodological questions should be addressed to the GIMEMA Data Center.

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23 Trial sponsorship and financing

The sponsor of the study is GIMEMA Foundation – Franco Mandelli Onlus.
GIMEMA has signed an agreement with Amgen for the supply of Blinatumomab free-of-charge and for a financial contribution to the study. This agreement is enclosed.
GIMEMA has signed an agreement with Bristol-Myers Squibb for the supply of Dasatinib free-of-charge. This agreement is enclosed.
Upon study completion, Dasatinib will be further provided free of charge by BMS until deemed of benefit for the patient.

24 Trial insurance

The GIMEMA insurance program covers unforeseen situation cased by the therapy in study. Problems associated to ongoing illnesses are obviously excluded, problems that could have been seen even if the patient did not participate to the study. The insurance also covers the civil responsibility of the monitor, of the Investigator and his collaborators.

25 Publication policy

Once the trial has been closed and the Writing Committee has presented the main study publication, any participating center may, eventually, use its own data (data generated in its own center) for educational purposes, publications and presentations. These may be sent to Sponsor for approval with a 15 days notice for abstracts, presentations or educational material and a 30 days notice for publications. The investigator is due to include sponsor's name in any final publication.

25.1 Authorship

The final publication of the trial results will be written by the Study Coordinators on the basis of the final analysis performed at the GIMEMA Data Center. A draft manuscript will be submitted by the study coordinator to the Data Center for review.
Authors of the manuscript will be the Study Coordinators, the investigators who have included more than 10% of the eligible patients in the trial (by order of inclusion), two members of the Data Center team. Unless further agreement is made with the Study Coordinators and the Data Center, all other participants or representatives of the Data Center who have contributed to the trial will be mentioned in the acknowledgment section of the manuscript.

25.2 Responsibility for publication

The manuscript will be sent to a major scientific journal after revision by the Study Coordinators.
The title of all manuscripts will include "GIMEMA", and all manuscripts will include an appropriate acknowledgment section, mentioning all investigators who have contributed to the trial, as well as supporting bodies.

The Group Chairman, the Study Coordinators and the Data Center must approve all publications, abstracts and presentations based on patients included in this study. This is applicable to any individual patient registered/randomized in the trial, or any subgroup of the trial patients. Such a publication cannot include any comparisons between randomized treatment arms nor an analysis of any of the study end-points unless the final results of the trial have already been published by the Study Coordinators.

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Appendix B: WHO performance status scale

Grade Performance scale

0	Able to carry out all normal activity without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out light work.
2	Ambulatory and capable of all self-care but unable to carry out any work; up and about more than 50% of waking hours.
3	Capable of only limited self-care; confined to bed or chair more than 50% of waking hours
4	Completely disabled; cannot carry on any self-care; totally confined to bed or chair.

Appendix C: Common Terminology Criteria for Adverse Events

In this study, adverse events and/or adverse drug reactions will be recorded according to the **Common Terminology Criteria for Adverse Events (CTCAE), version 4.0.** This reference is used as the standard grading scale for each single Adverse Event term.

The full version of the document (version 4.0.) is available on the NCI website (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcaev4.pdf)

Appendix D: World Medical Association Declaration of Helsinki

Ethical Principles for Medical Research Involving Human Subjects

World Medical Association
Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and amended by the:
29th WMA General Assembly, Tokyo, Japan, October 1975
35th WMA General Assembly, Venice, Italy, October 1983
41st WMA General Assembly, Hong Kong, September 1989
48th WMA General Assembly, SomersetWest, Republic of South Africa, October 1996
52nd WMA General Assembly, Edinburgh, Scotland, October 2000
53rd WMA General Assembly,Washington, DC, USA, October 2002 (Note of Clarification added)
55th WMA General Assembly, Tokyo, Japan, October 2004 (Note of Clarification added)
59th WMA General Assembly, Seoul, Republic of Korea, October 2008
64th WMA General Assembly, Fortaleza, Brazil, October 2013

Preamble
1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data. The Declaration is intended to be read as a whole and each of its constituent paragraphs should be applied with consideration of all other relevant paragraphs.
2. Consistent with the mandate of the WMA, the Declaration is addressed primarily to physicians. The WMA encourages others who are involved in medical research involving human subjects to adopt these principles.

General Principles
3. The Declaration of Geneva of the WMA binds the physician with the words, "*The health of my patient will be my first consideration*", and the International Code of Medical Ethics declares that, "*A physician shall act in the patient's best interest when providing medical care*".
4. It is the duty of the physician to promote and safeguard the health, well-being and rights of patients, including those who are involved in medical research. The physician's knowledge and conscience are dedicated to the fulfilment of this duty.
5. Medical progress is based on research that ultimately must include studies involving human subjects.
6. The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best proven interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.
7. Medical research is subject to ethical standards that promote and ensure respect for all human subjects and protect their health and rights.
8. While the primary purpose of medical research is to generate new knowledge, this goal can never take precedence over the rights and interests of individual research subjects.
9. It is the duty of physicians who are involved in medical research to protect the life, health, dignity, integrity, right to selfdetermination, privacy, and confidentiality of personal information of research subjects. The responsibility for the protection of research subjects must always rest with the physician or other health care professionals and never with the research subjects, even though they have given consent.
10. Physicians must consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.
11. Medical research should be conducted in a manner that minimises possible harm to the environment.
12. Medical research involving human subjects must be conducted only by individuals with the appropriate ethics and scientific education, training and qualifications. Research on patients or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional.
13. Groups that are underrepresented in medical research should be provided appropriate access to participation in research.
14. Physicians who combine medical research with medical care should involve their patients in research only to the extent that this is justified by its potential preventive, diagnostic or therapeutic value and if the

physician has good reason to believe that participation in the research study will not adversely affect the health of the patients who serve as research subjects.
15. Appropriate compensation and treatment for subjects who are harmed as a result of participating in research must be ensured.

Risks, Burdens and Benefits
16. In medical practice and in medical research, most interventions involve risks and burdens. Medical research involving human subjects may only be conducted if the importance of the objective outweighs the risks and burdens to the research subjects.
17. All medical research involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and groups involved in the research in comparison with foreseeable benefits to them and to other individuals or groups affected by the condition under investigation. Measures to minimise the risks must be implemented. The risks must be continuously monitored, assessed and documented by the researcher.
18. Physicians may not be involved in a research study involving human subjects unless they are confident that the risks have been adequately assessed and can be satisfactorily managed. When the risks are found to outweigh the potential benefits or when there is conclusive proof of definitive outcomes, physicians must assess whether to continue, modify or immediately stop the study.

Vulnerable Groups and Individuals
19. Some groups and individuals are particularly vulnerable and may have an increased likelihood of being wronged or of incurring additional harm. All vulnerable groups and individuals should receive specifically considered protection.
20. Medical research with a vulnerable group is only justified if the research is responsive to the health needs or priorities of this group and the research cannot be carried out in a non vulnerable group. In addition, this group should stand to benefit from the knowledge, practices or interventions that result from the research.

Scientific Requirements and Research Protocols
21. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.
22. The design and performance of each research study involving human subjects must be clearly described and justified in a research protocol. The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, sponsors, institutional affiliations, potential conflicts of interest, incentives for subjects and information regarding provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study. In clinical trials, the protocol must also describe appropriate arrangements for post-trial provisions.

Research Ethics Committees
23. The research protocol must be submitted for consideration, comment, guidance and approval to the concerned research ethics committee before the study begins. This committee must be transparent in its functioning, must be independent of the researcher, the sponsor and any other undue influence and must be duly qualified. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and standards but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in this Declaration. The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee, especially information about any serious adverse events. No amendment to the protocol may be made without consideration and approval by the committee. After the end of the study, the researchers must submit a final report to the committee containing a summary of the study's findings and conclusions.

Privacy and Confidentiality
24. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information.

Informed Consent

25. Participation by individuals capable of giving informed consent as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no individual capable of giving informed consent may be enrolled in a research study unless he or she freely agrees.
26. In medical research involving human subjects capable of giving informed consent, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, post-study provisions and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information. After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed. All medical research subjects should be given the option of being informed about the general outcome and results of the study.
27. When seeking informed consent for participation in a research study the physician must be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent must be sought by an appropriately qualified individual who is completely independent of this relationship.
28. For a potential research subject who is incapable of giving informed consent, the physician must seek informed consent from the legally authorized representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the group represented by the potential subject, the research cannot instead be performed with persons capable of providing informed consent, and the research entails only minimal risk and minimal burden.
29. When a potential research subject who is deemed incapable of giving informed consent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorized representative. The potential subject's dissent should be respected.
30. Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious patients, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research group. In such circumstances the physician must seek informed consent from the legally authorized representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research must be obtained as soon as possible from the subject or a legally authorized representative.
31. The physician must fully inform the patient which aspects of their care are related to the research. The refusal of a patient to participate in a study or the patient's decision to withdraw from the study must never adversely affect the patient-physician relationship.
32. For medical research using identifiable human material or data, such as research on material or data contained in biobanks or similar repositories, physicians must seek informed consent for its collection, storage and/or reuse. There may be exceptional situations where consent would be impossible or impracticable to obtain for such research. In such situations the research may be done only after consideration and approval of a research ethics committee.

Use of Placebo
33. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best proven intervention(s), except in the following circumstances:
Where no proven intervention exists, the use of placebo, or no intervention, is acceptable; or
Where for compelling and scientifically sound methodological reasons the use of any intervention less effective than the best proven one, the use of placebo, or no intervention is necessary to determine the efficacy or safety of an intervention and the patients who receive any intervention less effective than the best proven one, placebo, or no intervention will not be subject to additional risks of serious or irreversible harm as a result of not receiving the best proven intervention. Extreme care must be taken to avoid abuse of this option.

Post-Trial Provisions

34. In advance of a clinical trial, sponsors, researchers and host country governments should make provisions for post-trial access for all participants who still need an intervention identified as beneficial in the trial. This information must also be disclosed to participants during the informed consent process.

Research Registration and Publication and Dissemination of Results

35. Every research study involving human subjects must be registered in a publicly accessible database before recruitment of the first subject.

36. Researchers, authors, sponsors, editors and publishers all have ethical obligations with regard to the publication and dissemination of the results of research. Researchers have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. All parties should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results must be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest must be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

Unproven Interventions in Clinical Practice

37. In the treatment of an individual patient, where proven interventions do not exist or other known interventions have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorised representative, may use an unproven intervention if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. This intervention should subsequently be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information must be recorded

Appendix E: Management of toxicity

Management of pleural effusion

Fluid retention events were typically managed by supportive care measures. Subjects who develop symptoms suggestive of pleural effusion such as dyspnea or dry cough should be evaluated by chest X-ray. Dasatinib should be interrupted if pleural effusion grade >2 occurs until the toxicity decrease to grade ≤1. Corticosteroids should be given in association with diuretics. If pleural effusion grade >1 and <2 occurs, Dasatinib at a lower dosage (100 mg QD or 80 mg QD) should be administered in association with low doses of corticosteroid and possible diuretics. In all cases, a chest X-ray should be repeated previous to Dasatinib is reinitiated or increased at the initial dose. Severe pleural effusion may require thoracentesis and oxygen therapy.

Appendix F: Timepoints and procedures for collection and mailing of biological samples

1) Collection of biological samples and sequence of drawings shipment

Biological samples (**BM and PB**) are centralized at different time points during therapy:

- at **diagnosis** before Dasatinib, in order to characterize patients in a homogenous way:
  - BM sample for cytogenetics (in heparin without any phenol or in medium for cytogenetics)
  - BM (at least 2 test-tubes containing NA citrate + 1 test-tube with EDTA)
  - PB sample (at least 2 test-tubes with NA Citrate, if WBC <10 x 10^9/L send at least 20 ml in Na Citrate) + 2 test-tubes with EDTA
- at days +22, +45 o 57 from starting treatment with Dasatinib:
  - BM (at least 2 test-tubes containing NA citrate)
  - PB sample (at least 2 test-tubes with NA Citrate, if WBC <10 x 10^9/L send at least 20 ml in Na Citrate)
- **at day +85 (evaluation of response)** (either when the patient will be considered in CHR, or when he/she will be considered resistant/in progression) and at the **end of treatment** (when the patient will go off treatment protocol):
  - BM (at least 2 test-tubes containing NA citrate + 1 test-tube with EDTA)
  - PB sample (at least 2 test-tubes with NA Citrate, if WBC <10 x 10^9/L send at least 20 ml in Na Citrate)
  - In case of CMR, repeat a centralized bone marrow after 2 weeks
- **during follow-up time points (for time points, see paragraph 15.1):**
  - BM (at least two test-tubes containing NA citrate + 1 test-tube with EDTA)
  - PB sample (at least 2 test-tubes with NA Citrate, if WBC <10 x 10^9/L send at least 20 ml Na Citrate + 1 test-tube with EDTA)
- **in the event of relapse or in resistant patients**
  - BM sample for cytogenetics (in heparin without any phenol or in medium for cytogenetics)
  - BM (at least one test-tube containing NA citrate + 1 test-tube with EDTA)
  - PB sample (at least 2 test-tubes with NA Citrate, if WBC <10 x 10^9/L send at least 20 ml in Na Citrate + 2 test-tubes with EDTA)

2) Biological samples shipment instructions

The instructions for shipping biological samples are as follows:

a. The sample collections **must be done on the same day** it is shipped. Plastic and non-glass test-tubes should be used (do not send the syringe).

b. Air Sea Postal-Pack code *555* displays should be used to ship these biological samples. Most GIMEMA Centers already have them available. These displays can be used more than once and, thus, they will be resent to the participating Centers on a regular basis. If a given Center has run out of provisions, please, contact the Central Lab as soon as possible.

c. In order to actually ship the samples, please contact the courier through the "green number" and ask for EXPRESS shipment (**GIMEMA client code will be provided at site opening**). Costs will be charged to the recipient. Please, do not forget to keep the code given by the courier which will be used to track the shipment whenever necessary.

d. Fresh samples must be sent at room temperature; previously stored material (cryopreserved in DMSO or pellet in GTC) should be shipped in "dry ice". In case of doubts, please, contact the Central Lab.

e. Biological samples shipment can be done from Monday to Thursday.

f. Samples must be shipped to:

Dr. Antonella Vitale
Laboratorio di Centralizzazione
Ematologia, Azienda Policlinico "Umberto I", Dipartimento Biotecnologie Cellulari ed Ematologia, "Sapienza" Università di Roma, via Benevento 6 - 00161 Roma

Please, inform the Central Lab whenever you ship samples either by phone, e-mail or fax:

- Phone: +39 06 4416 39 826;
- E-mail: lab_centralizzazione@bce.uniroma1.it
  vitale@bce.uniroma1.it
- Fax: +39 06 442419844