GIMEMA
Gruppo Italiano Malattie EMatologiche dell'Adulto

"A Phase II study with a sequential clofarabine-cyclophosphamide combination schedule as salvage therapy for refractory and relapsed acute lymphoblastic leukemia (ALL) in adult patients"

GIMEMA Protocol LAL1610
EudraCT number 2010-019742-12

17/01/12	Final Version 1.0

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

Study Responsabilities:

For GIMEMA Foundation:

Sponsor according to European Directives:	**GIMEMA Foundation, Rome**
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This protocol has been approved by GIMEMA Foundation. The following signatures documents this approval.

_____ _____
Prof. Franco Mandelli Date
GIMEMA Foundation Scientific Coordinator

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Prof. Sergio Amadori Date
AL Working Party Chairman

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Dr. Renato Bassan Date
Study Coordinator

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Prof. Giovanna Meloni Date
Study Co-coordinator

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 GIMEMA Foundation. 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

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1 Synopsis

"A Phase II study with a sequential clofarabine-cyclophosphamide combination schedule as salvage therapy for refractory and relapsed acute lymphoblastic leukemia (ALL) in adult patients. GIMEMA Protocol LAL1610. EudraCT number 2010-019742-12

Study Phase: II

Objectives:

The primary objective of this trial is to assess the activity - in terms of percentage of complete remission (CR) - of Clofarabine in combination with Cyclophosphamide in adult patients with refractory and relapsed (≤24 months from first CR) ALL.

The secondary objectives are:

- To assess the safety and tolerability of Clofarabine when used in combination with Cyclophosphamide. The chosen indicator of feasibility is incidence rate of severe toxic side effects as evaluated by means of the Common Toxicity Criteria (CTC) scale
- To assess minimal residual disease (MRD) status after treatment.
- To assess disease-free survival (DFS) at 1 year.
- Overall survival (OS) at 1 year.
- Cumulative incidence of relapse (CIR) at 1 year.
- DFS, OS and CIR in different risk groups.

Study design:

The proposed treatment schedule consists of a combination of Clofarabine plus Cyclophosphamide administered over 5 consecutive days (Treatment scheme). This is an open, nonrandomized prospective phase II trial aimed to evaluating the activity of this combination in terms of CR rate.

- **STEP 1.** All eligible patients will be screened for the availability of an HLA-matched or partially mismatched compatible HSCT donor, of both family related, or unrelated type (early activation required), including cord blood and haploidentical siblings.
  Moreover, pre-treatment investigation will include collection and storage of patients ALL cells for ALL diagnosis and subclassification, immunophenotype, MRD and molecular genetic analyses.

- **STEP 2**. Cycle 1 will be applied to all eligible patients once all enrolment criteria are confirmed.

- **STEP 3**. After cycle 1, response will be evaluated.

- **STEP 4**. After remission induction cycle 1, only responsive patients (CR or PR, see below for definitions) may be given cycle 2, according to the opinion of the responsible physician and with a minimum intercycle interval of 4 weeks from day 1 of cycle 1. All NR patients will be declared off study and will not be given a second course with the study combination. The suggested treatment following cycle 2 (or cycle 1 if cycle 2 is omitted) is HSCT.

Population:

Inclusion criteria:

- Age 18-60 years.
- ALL with B-/T precursor phenotype refractory to first line therapy.
- ALL with B-/T precursor phenotype with 1st isolated bone marrow relapse, occurring ≤24 months from the achievement of first CR, after chemotherapy or hematopoietic stem cell transplantation (HSCT) defined as follows:
  ≥5% leukemic blasts in the bone marrow not attributable to another cause (e.g. marrow regeneration); if there are no circulating blasts and the bone marrow contains 5-20% leukemic blasts, a repeat bone marrow performed at least one week later is necessary to confirm relapse.
- An ECOG performance status 0-2 or reversible ECOG 3 score following intensive care of complications.
- Adequate hepatic and renal function, unless considered due to organ leukemic involvement:
  - Serum creatinine <1.5 mg/dl; if serum creatinine >1.5 mg/dl, then the estimated glomerular filtration rate (GFR) must be >60 mL/min/1.73 m^2 as calculated by the Modification of Diet in Renal Disease equation where Predicted GFR (ml/min/1.73 m^2) = 186 x (Serum Creatinine)$^{-1.154}$ x (age in years)$^{-0.023}$ x (0.742 if patient is female), x (1.212) if patient is black.
  - Serum bilirubin ≤1.5 x upper limit of normal (ULN).
  - Aspartate transaminase (AST)/alanine transaminase (ALT) ≤2.5 x ULN.
  - Alkaline phosphatase ≤2.5 x ULN.
- Women of childbearing potential must have a negative serum pregnancy
- Signed written informed consent according to IGH/EU/GCP and national local laws.

Exclusion criteria:

- Prior exposure to Clofarabine or, in primary refractory patients, to Cyclophosphamide during the induction courses.
- Patients relapsed >24 months from first CR.
- 2nd or subsequent bone marrow relapse
- Philadelphia chromosome-positive (Ph+) ALL.
- Diagnosis of Burkitt-type/B-ALL, or B-/T-lymphoblastic lymphoma with <25% bone marrow involvement.
- Concurrent or isolated central nervous system (CNS) relapse.
- Pre-existing, uncontrolled pathology such as cardiac disease (congestive/ischemic, acute myocardial infarction within the past 3 months, untreatable arrhythmias, NYHA classes III and IV).
- Severe neurological or psychiatric disorder that impairs the patient's ability to understand and sign the informed consent, or to cope with the intended treatment plan.
- Active uncontrolled systemic fungal, bacterial, viral, or other infection (defined as exhibiting ongoing signs/symptoms related to the infection and without improvement, despite appropriate antibiotics or other treatment).
- HIV positive serology or active hepatitis infection.
- Concurrent diagnosis of active cancer requiring concurrent chemotherapy and/or radiotherapy, and/or with a life expectancy <1 year.
- Patients who are pregnant (women of childbearing potential must have a negative serum pregnancy test). Post-menopausal women must be amenorrhoic for at least 24 months to be considered of non-childbearing potential. Male and female patients must agree to employ an effective barrier method of birth control throughout the study and for up to 3 months following discontinuation of study drugs

Treatment:

Eligible patients will receive a maximum of two consecutive treatment cycles.

Treatment scheme

Patients will receive a maximum of two consecutive cycles of Clofarabine-Cyclophosphamide, at an intercycle interval of 28 days or greater, according to tolerability and clinical status.

Cycle 2 will be administered only to patients obtaining at least a partial response (PR) after cycle 1, defined as a reduction in bone marrow blasts from >50% to between 5-25%. Non-responsive (NR) patients after cycle 1 will go off-study. The post-remission treatment policy is free (HSCT is suggested), but will be registered for all cases.

Study drugs and schedule (cycle 1, cycle 2)

- CLOFARABINE 40 mg/m^2/day IV as a 1-hr infusion on days 1, 2, 3, 4, 5 (2-hrs before each dose of Cyclophosphamide).
- CYCLOPHOSPHAMIDE 400 mg/m^2/day IV as 1-hr infusion on days 1, 2, 3, 4, 5 (2-hrs after each dose of Clofarabine).

Patients will be strictly monitored for the potential occurrence of unacceptable toxicities to assess the need of Clofarabine dose-reduction, in case of grade IV toxicity (prolonged severe cytopenia, severe extrahematologic toxicity).

Concurrent medications:

- Allopurinol 300 mg PO/d or bd according to uric acid concentration.
- Paracetamol 1000 mg PO 30 min prior to Clofarabine.
- Prednisolone 40 mg/m^2/d IV/PO in two divided doses on days –1 to +5 (30 min prior to Clofarabine), then tapered.
- Prophylactic aciclovir, ciprofloxacin and cotrimoxazole from treatment day 1. Antifungal prophylaxis is commenced after day +5.
- Granulocyte colony-stimulating factor (G-CSF) from day +6 and until the absolute neutrophil count exceeds 1x10^9/l.

Sample size

This study is designed to evaluate the complete response rate (CR) of the Clofarabine and Cyclophosphamide combination.

In the proposal, to reject the null hypothesis that $p \leq 0.25$ vs. the alternative hypothesis that $p > 0.50$ with Type I error probability (α) equal to 0.05 and 85% power (1-β), a maximum of 27 evaluable patients has to be accrued.

In the first stage of the study, 10 evaluable patients will be enrolled and the trial will be terminated if 2 or fewer responses will be achieved; otherwise, 17 further evaluable patients will be enrolled in the second stage. If the total number of responses will be less than or equal to 10, the combination therapy will not be recommended for further studies. If the total number of CRs is at least 11, the treatment will be deemed worthy of further investigations. Calculations were implemented in PASS2008 using a Simon two stage (minimax) phase II study design.

Study duration:

2.6 years (18 months for patient enrolment + 1 month study therapy + 1 year follow-up).

2 Background and introduction

2.1 Adult ALL failing first-line therapy: treatment overview*

*relevant parts of this section were prepared after the manuscript "Management of Ph-negative ALL relapse / New drugs in Ph-negative ALL" by Hervé Dombret, Emmanuel Raffoux, Nicolas Boissel, from Department of Clinical Hematology, Hôpital Saint-Louis (AP-HP), Paris, France. Kindly provided by Hervè Dombret as a cooperation project of the EWALL (European Working Group for Adult ALL).

2.1.1 Background

Although as many as 80-90% of adult patients with ALL enter CR, the majority will subsequently relapse and only 30% to 40% survive 5 or more years, at which time they are considered cured. Survival depends on risk factors such as age, white cell count, time to CR, disease immunophenotype, cytogenetics and molecular abnormalities. Traditionally, these features are used to identify risk groups with variable incidence of relapse and then survival probabilities that range from less than 20% to greater than 50%.

2.1.2 Significance of relapse and retreatment strategy

Leukemic relapse in adult patients with ALL is a major determinant of outcome and an unresolved therapeutic issue. Results are unsatisfying and ALL in relapse is still regarded as an almost incurable disease.[Ref. 1-Ref. 4] Although a second CR (CR2) may be achieved in some patients, the rate of CR2 remains low and the median post-relapse survival short. It is generally accepted that no cure can be achieved with chemotherapy alone at this stage of the disease. The only curative approach is HSCT. In general, this requires achievement of a relatively durable CR2 with a rescue regimen that must be associated with as little toxicity as possible. In this context, relapses occurring after HSCT represent an even more problematic issue. Primary refractory ALL occurs in about 5% of cases and is possibly a worse clinical condition.

Four large reports have described the general outcome of adults with relapsed ALL (Table 1)[Ref. 5-Ref. 8] predominantly including patients treated in an attempt to reach CR2. Salvage treatments included vincristine/prednisone (VP), vincristine/doxorubicin/dexamethasone combinations with or without cyclophosphamide (VAD, CVAD, Hyper-CVAD), methotrexate and L-asparaginase containing regimens, lymphoma-like regimens, acute myeloid leukaemia (AML)-like regimens, intermediate- or high-dose cytarabine-based regimens with or without fludarabine and granulocyte colony-stimulating factor (IDAC, HDAC, FLAG, IDA-FLAG), or standard front-line ALL induction as used in newly-diagnosed patients.[Ref. 5, Ref. 6] A few patients even received immediate HSCT. Second CR rates ranged from 31% to 50%. Long-term post-relapse OS was uniformly very poor, ranging from 3% to 7% at five years. Multivariate analysis methodology varied among these studies, but, overall, the main identified prognostic covariates were age, first CR duration and hematological parameters at relapse (mainly WBC or circulating blast count). Specific prognosis of extramedullary relapse compared to isolated marrow relapse remains a debated issue.

The impact of HSCT in CR2 cannot be readily evaluated in these studies as donor versus no-donor analysis was done. In addition, a search for an alternative unrelated donor was often initiated only after a relapse had occurred. All studies, however, agreed to consider that HSCT in CR2 was the major factor for longer post-relapse survival, even if never exceeding 25%. A landmark analysis was performed in the UKALL/ECOG

study, after excluding patients transplanted in CR1 as well as those receiving a nonmyeloablative or mismatch transplant in CR2. 7 Long-term survival was estimated at 15% for autologous HSCT, at 16% for matched unrelated donor HSCT, at 23% for sibling donor HSCT, versus only 4% for chemotherapy. In the LALA study, post-HSCT survival was much longer when allogeneic HSCT was performed after CR2 achievement than directly or after CR2 induction failure.[Ref. 6]

Table 1. Outcome of adult patients with ALL in first relapse (various salvage treatments).

Reference (year)	Patients (N)	Patients treated intensively (N)	Second CR rate	Estimated OS from relapse	Main prognostic factors
Thomas et al. (1999)	314 *	314	31%	3% at 5 years	Age PB blast count CR1 duration
Tavernier et al. (2007)	421 *	373	50%	7% at 5 years	WBC Platelet count
Fielding et al. (2007)	609 *	NA	NA	7% at 5 years	Age CR1 duration
Vives et al. (2008)	198 *	183	42%	5% at 5 years	Age CR1 duration Female gender BM involvement CNS involvement

*including Ph+ ALL patients; PB: peripheral blood; WBC: white blood cell count; BM: bone marrow: CNS: central nervous system; NA: not available.

2.1.3 Salvage chemotherapy studies

Recent studies aimed at prospectively evaluating the role of different specific chemotherapy regimens in the setting of relapsed or refractory ALL are listed in Table 2.[Ref. 9-Ref. 22] Patient numbers were usually relatively low and none of these studies used a control reference arm. There is actually no standard chemotherapy regimen for patients with relapsed ALL. The two largest studies are from the MDACC using the Hyper-CVAD regimen,[Ref. 10] and from the GIMEMA using the ALL-97 protocol.[Ref. 16] The Hyper-CVAD regimen consists of a sequence of repeated bolus of cyclophosphamide at the dosage of 300 mg/m2 combined with doxorubicin, vincristine, dexamethasone (Hyper-CVAD) alternating with high-dose methotrexate and cytarabine (M/A). The GIMEMA ALL-R97 salvage chemotherapy was an AML-like regimen consisting of HDAC (3 g/m2/d from day 1 to day 5) with a single high-dose idarubicin (40 mg/m2 on day 3). The majority of the 135 patients enrolled were either in first relapse (99 patients) or refractory to first-line induction therapy (28 patients). The proportion of patients with Ph-positive ALL was 16%. The overall CR rate was 55%, but the 3-year DFS and OS were estimated at 16% and 10% only, respectively. Adverse cytogenetics other than the t(9;22) translocation and duration of first CR were the two factors predictive of a worse outcome. Forty-five % of the patients who achieved CR actually received an allogeneic HSCT in persistent CR (N = 34 patients). Due to the high incidence of post-transplant relapse incidence and of the transplant-related mortality (TRM), the benefit associated with HSCT was not statistically significant. Notably, the availability of a HLA-identical sibling was the only favorable factor identified for a better post-relapse outcome in a Spanish study.[Ref. 13] Finally, a very recent study from the MDACC has reviewed the outcome of adults with ALL after second rather than first

salvage treatment.[Ref. 23] A large cohort of 288 patients was analyzed. Overall, the CR rate was only 18% and the median OS only 3 months. Only 22 patients (8%) were able to undergo an allogeneic HSCT and their survival was only 18% at one year.

Table 2. Chemotherapy studies in adult patients with relapsed/refractory ALL.

Reference (year)	Salvage chemotherapy	Patients (N)	Second CR rate	Estimated DFS from CR2	Estimated OS from relapse
Mazza et al. (1996)	HDAC-MTZ	34 *†	17%	Median, 2.4 mos	Median, 4.5 mos (for 1st salvage)
Koller et al. (1997)	HDAC-MTZ ± GM-CSF	64 *†	38%	Median, 4.6 mos (for 1st salvage)	Median, 4.6 mos
Koller et al. (1997)	Hyper-CVAD	66 *†	44%	Median, 12 mos (for 1st salvage)	Median, 9.7 mos
Giona et al. (1997)	IDA-IDAC-PDN	61 *†	56%	16% at 3 years	10% at 3 years
Montillo et al. (1997)	FLAG	12 *	83%	Median, 13.5 ws	Median , 16 ws
Martino et al. (1999)	VDS-MTZ-CPM-IDAC-PDS-MTX	45 *	74%	Median, 4.6 mos	Median, 5.7 mos
Rosen et al. (2000)	HDAC-MTZ	31 *†	23%	NA	Median, 4 mos
Reman et al. (2004)	AMSA-IDAC-VP16	40	40%	Median, 3.2 mos 12% at 3 years	Median, 5.4 mos
Camera et al. (2004)	IDA-IDAC-PDN	135 *†	55%	Median, 5 mos 16% at 3 years	Median, 6.4 mos 10% at 3 years
Di Bona et al. (2005)	MTZ-MTX-VCR-HD-PDN	36*	61%	Median, 5.2 mos	Median, 7.6 mos
Specchia et al. (2005)	FLAG-IDA	23 *	39%	Median, 6 mos	Median, 4.5 mos
Yavuz et al. (2006)	FLAG-IDA	22 *†	42%	NA	Median, 3 mos
Candoni et al. (2006)	DXM-AraC	25 *†	80%	NA	39% at 1 year
Giebel et al. (2006)	FLAM	50 *†	50%	15% at 2 years	12% at 2 years
Tedeschi et al. (2007)	IDA-AraC	25 *	44%	Median, 6 mos	Median, 8 mos

*including Ph+ ALL patients; †: included second and subsequent relapses; IDA: idarubicin; PDN: prednisone; PDN: prednisolone (HD, high-dose); AraC: cytarabine; IDAC: intermediate-dose cytarabine; HDAC: high-dose cytarabine; MTZ: mitoxantrone; DXM: daunoxome; CPM: cyclophosphamide; VDS: vindesine; MTX: methotrexate; AMSA: amsacrine; VP16: etoposide; GM-CSF: granulocyte macrophage colony-stimulating factor; Hyper-CVAD regimen: combined hyperfractionated CPM, vincristine, adriamycine, and dexamethasone; FLAG regimen: combined fludarabine, cytarabine, and granulocyte colony-stimulating factor; FLAM regimen: combined fludarabine, cytarabine, and MTZ; NA: not available; Giona et al. and Camera et al. reported an analysis of the same Italian study, in 1997 and 2004, respectively.

The update of a recent Italian study from the Northern Italy Leukaemia Group (NILG)[Ref. 24] confirms these figures and the need for improved treatment strategies (Table 3, Figure 1). After the closure of the mitoxantrone/high-dose methotrexate study,[Ref. 23] most relapsing patients in the NILG trial were retreated with a sequential schedule including high-dose cytarabine (3 g/m2/bd on days 1-2 and 8-9) plus idarubicin (17.5 mg/m2 on days 3 and 10), with or without cyclosporine A to down-modulate MDR1 activity when present.[Ref.

[26, Ref. 27] Despite early encouraging results, the long-term analysis of results (Figure 1) did not show an improved outcome compared to literature data (Table 2).

Table 3.

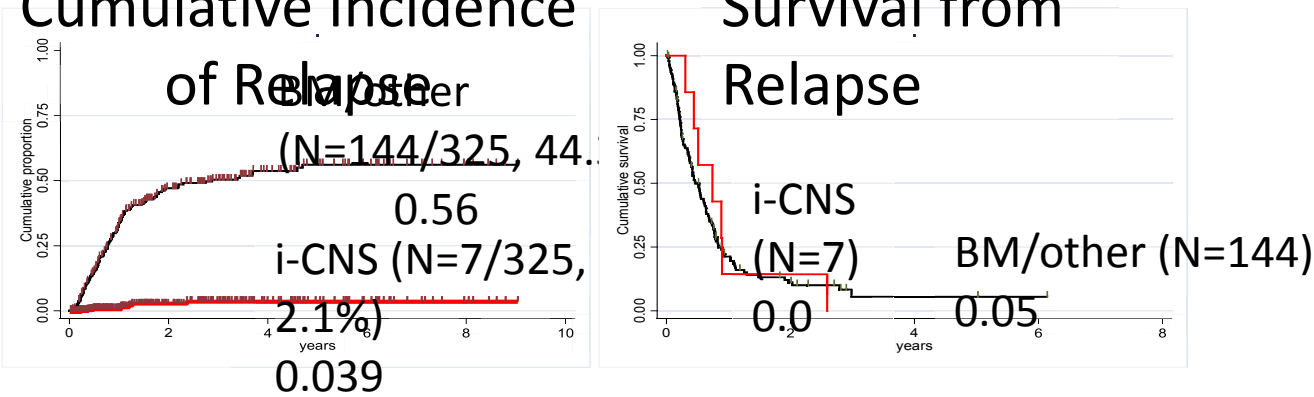
Incidence of Relapse by Site and Disease Subtype

Update on 409 patients

Subset	No.	CR no.	Relapse BM/other/ combined	Relapse i-CNS
BCP Ph-	289	241	98 (34.2%)	3 (1.8%)
BCP Ph+	99	87	39 (44.8%)	0 (0%)
TCP	91	77	37 (48%)	4 (5.1%)
LBL	30 24 (T), 6 (B)	28 22 (T), 6 (B)	5 (17.8%)	0 (0%)
TOTAL	409	353	149 (42.2%)	7 (1.9%)

BCP, B-cell precursor; TCP, T-cell precursor; LBL, lymphoblastic lymphoma
Relapse: BM, bone marrow; i-CNS, isolated central nervous system

Figure 1.



2.1.4 Post-transplant relapse

Relapse after allogeneic HSCT remains, even up to the present days, a frequent clinical problem in adults with ALL as it is associated with a most dismal prognosis likelihood. The individual management of these patients is guided by age, time between HSCT and relapse, associated graft-versus-host disease or infection, and hematopoietic chimerism. Later relapses may benefit from standard ALL reinduction followed, in younger patients, by a second transplant from an alternative donor whenever possible. Earlier relapses in patients with

an established mixed hematopoietic chimerism may be treated with more or less intensive salvage chemotherapy, including new drugs, followed by donor lymphocyte infusions. [Ref. 27]

2.1.5 Summary and current expectations

In conclusion, leukemic relapse, as well as the rare primary refractory cases, still represent in adult ALL a primary unmet clinical need. First hematological relapse is indeed the major therapeutic challenge in adult ALL as it affects at least 50% of all patients entering CR (80-90% probability), while refractory ALL accounts for about 5% of cases. Most importantly, survival following relapse is short and very few of these patients (<10%) can achieve cure.

Even if associated with relatively poor results, allogeneic HSCT remains the only hope of long-term cure in these patients. The development of novel HSCT procedures, as well as HSCT using alternative stem cell sources, must thus be encouraged in this clinical setting. Prior to HSCT, the achievement of an "as good and as durable as possible" CR2 should be privileged. Even if there is no standard chemotherapy regimen that may be recommended, the strategy is generally stratified on the duration of first CR. In late relapsing patients (>12-18 months from date of prior CR), it may still be reasonable to use standard ALL induction and consolidation as salvage therapy without transplantation. In early relapsing patients (<12 months), high dose-intensive protocols need to be used. Improvement of response rates through the incorporation of new agents in salvage combinations is advisable and is under clinical investigation by several groups.

2.1.6 New drugs

New treatments that might improve the clinical outcome of ALL patients are emerging. These treatments have been or are currently tested in patients with relapsed or refractory disease. These include mainly new nucleoside analogs, such as Clofarabine and others, new formulations of existing chemotherapeutic agents, and monoclonal antibodies against leukemia-associated antigens. Except for Clofarabine, that represents the focus of the current protocol, other investigational and new agents will not be considered in detail.

2.2 Drug in study

2.2.1 Introduction

Clofarabine (Clolar™ or Evoltra™, Genzyme) is a deoxyadenosine analog currently approved for the treatment of children with ALL who had not responded to or had relapsed following at least two prior regimens. The active 5'-triphosphate form acts to terminate DNA chain elongation and inhibit DNA repair through incorporation into the DNA chain. It also inhibits ribonucleotide reductase with reduction of dNTP pools, and induces apoptosis through direct and indirect action on mitochondria by releasing pro-apoptotic factors.[Ref. 28, Ref. 29]

2.2.2 Clinical studies

In a first Phase 1 paediatric trial, the maximum tolerated dose was established at 52 mg/m2/d for 5 days. Dose-limiting toxicities were reversible liver toxicity and skin rash.[Ref. 31] In the subsequent pivotal Phase 2 trial, that included 61 children with refractory or relapsed ALL treated with single-agent Clofarabine at this dose level for 5 days, responses included 12 complete and 6 partial remissions.[Ref. 32] Responses were observed in both B-lineage and T-lineage subsets. Clofarabine is now tested in combination with more conventional chemotherapy, in refractory/relapsed ALL patients. In an ongoing US pediatric study, Clofarabine has been combined to Cyclophosphamide and Etoposide, with the aim of interfering with the repair of DNA damages caused by these two agents.[Ref. 33, Ref. 35] In addition, in 2007 Karp et al.[Ref. 34] published a highly valuable clinical-laboratory study on a novel sequential combination of Clofarabine followed by Cyclophosphamide for adults with refractory acute leukemia. The core study concept was that rapid DNA repair of DNA interstrand cross links induced by Cyclophosphamide could be prevented by prior administration of Clofarabine, through inhibition of ribonucleotide reductase and DNA synthesis. It was anticipated that the synergistic combinations of the pharmacological effects from both drugs would eventually lead to increased apoptosis and cell killing, optimizing treatment results. For this purpose, a phase I trial was carried out, to assess the maximum tolerated dose (MTD) of Clofarabine followed by fractionated Cyclophosphamide. The protocol design is reported in Table 4.

Table 4. Sequential Clofarabine-Cyclophosphamide protocol (dose level 1).

Drugs*	Dose/route	Sequence		d0	d1	d2	d3		d8	d9	d10
Clofarabine	20 mg/m^2/iv.**	2-hr before Cyclophosphamide			X	X	X		X	X	X
Cyclophosphamide	200 mg/m^2/iv.	after Clofarabine (d1)		X	X						
	400 mg/m^2/iv.	after Clofarabine				X	X		X	X	X

*planned dose escalation: Clofarabine up to 50 mg/m^2/d; Cyclophosphamide up to 800 mg/m^2/d
**dose level 0 = 10 mg/m^2/d

The study included 18 total subject with acute leukemia, of whom 6 with highly refractory ALL (age range 27-67 years). The schedule proved effective yet toxic in the first 6 patients treated, especially with regard to hepatic dysfunction (4 cases with grade 3) and marrow aplasia longer than 42 days (4 cases), in association with sepsis (n=2), ischemic colitis (n=1) and fungal pneumonia (n=4) which caused the death of 2 of the patients (33%). Induction of tumor lysis syndrome was observed in some cases. For these reasons, the Clofarabine dose was de-escalated to level 0 i.e. 10 mg/m2/d. Twelve further patients were treated accordingly, with a significantly reduced toxicity (only 1 with marrow aplasia >42 days, 2 induction deaths, i.e. 17%). With regard to outcome, 2/3 patients with ALL achieved a CR with dose level 1, as did 1/3 with dose level 0. The overall CR rate was 66.6% (4/6), but the results suggested poor regimen activity in ALL at dose level 0. In ex

vivo experiments performed on leukemic blast cells obtained from the patients, increased DNA damage was observed with Clofarabine and Cyclophosphamide compared to Cyclophosphamide alone in 12 of the 13 patients tested. Toxicity data (same dose level) suggested a reduction in chemotherapeutic intensity (cumulative Clofarabine dose 120 mg/m^2 over 6 days, cumulative Cyclophosphamide dose 2400 mg/m2 over 7 days; overall length of treatment schedule 11 days). In early 2010 some additional new data concerning the use of Clofarabine with and without other associated drugs in both pediatric and adult ALL failing first line therapy became available (Table 5). [Ref. 35-Ref. 42] The information collectively suggests that the optimal drug dosage is 40 mg/m^2/d for 5 consecutive days, which is also the maximum tolerable dose when Clofarabine is used in combination with other drugs. In adults, the same dose is feasible in association with Cytarabine at 1 g/m^2 for 5 days, but this regimen appears poorly active. Instead, the combination with Cyclophosphamide pioneered by Vitale et al.[Ref. 30] appears promising and worthy of further clinical testing.

Table 5. Recent pediatric and adult studies with Clofarabine for relapsed and refractory ALL.

Study (year)	N. of patients	Clofarabine dose and schedule; other drugs	CR rate, n. (%)	Notes
Pediatric patients				
Hijiya (2009)	20	40 mg/m^2/d; plus Etoposide 100 mg/m^2/d, and Cyclophosphamide 440 mg/m^2/d	9 (45%)	MTD of combination defined
Locatelli (2009)	25	40 mg/m^2/d; plus Etoposide 150 mg/m^2/d, and Cyclophosphamide 400 mg/m^2/d	14 (56%)	CR rate 76% in B-lineage vs. 12% in T-lineage; no therapy-related death; reversible toxicities
Steinherz (2010)	12 (ALL, AML)	30[1] or 40[2] mg/m^2/d; Topotecan, Thiotepa, and Vinorelbine	1/6 (17%)[1], 5/6 (83%)[2]	Clofarabine MTD 40 mg/m^2/d
Adult patients				
Kantarjian (2003)	12	40 mg/m^2/d dd 1-5	2 (17%)	Severe reversible liver dysfunction (15%-25%)
Faderl (2005)	2	40 mg/m^2/d dd 2-6; plus Cytarabine 1 g/m^2/d dd 1-5	0 (0%)	Feasibility with Cytarabine confirmed
McGregor (2009)	3	52 mg/m^2/d	2 (67%) with blast cell disappearance	Patients could proceed to HSCT
Vitale (2009)	2 (Ph+)	40 mg/m^2/d dd 2-6; plus Cyclophosphamide 400 mg/m^2/d dd 1-5	2	Hematological recovery after 16-21 days; 1-2 log reduction of BCR-ABL transcript; no significant toxicity
Advani (2009)	36	40 mg/m^2/d dd 1-5; plus Cytarabine 1 g/m^2/d dd 1-5	6 (17%)	Regimen not active; 10 early deaths

MTD, maximum tolerated dose

2.2.3 Toxicity profile

In the studies insofar quoted, Clofarabine MTD was established for both adults and children (40 mg/m^2/d in standard schedules of 5 days), main side effects were gastrointestinal toxicity, fever, skin rash, hand-foot

syndrome and transient liver enzymes/bilirubin elevation, but no neurotoxicity was evidenced. Systemic inflammatory-response-like syndromes eventually associated with capillary leak were observed in some children. Prolonged myelotoxicity with bone marrow aplasia lasting >42 days may be an effect of drug associations particularly with the higher cumulative Cyclophosphamide dosing, like in Karp study [Ref. 34], i.e. 200 mg/m^2 on days 0 to 1, and 400 mg/m^2 on days 2 and 3 and 8 to 10 (total 2.400 mg/m^2), or may be related to the rather unusual length of the retreatment protocol with two chemotherapy blocks one week apart. Limiting liver toxicity with the occurrence of symptomatic sinusoidal obstruction syndrome led investigators to amend the US study in association with Cyclophosphamide/Etoposide, excluding patients with prior HSCT, viral hepatitis and/or cirrhosis, or elevated baseline conjugated bilirubin levels. [Ref. 31]

3 Objectives of the trial

3.1 General objectives

3.1.1 Primary objective

The primary objective of this trial is to assess the activity - in terms of percentage of CR - of Clofarabine in combination with Cyclophosphamide in adult patients with refractory and relapsed (≤24 months from first CR) ALL.

3.1.2 Secondary objectives

- To assess the safety and tolerability of Clofarabine when used in combination with Cyclophosphamide. The chosen indicator of feasibility is incidence rate of severe toxic side effects as evaluated by means of the Common Toxicity Criteria (CTC) scale.
- To assess MRD status after treatment.
- To assess DFS at 1 year.
- OS at 1 year.
- CIR at 1 year.
- DFS, OS and CIR in different risk groups.

4 Patient selection criteria

To be eligible, all patients must satisfy all required inclusion criteria and simultaneously meet no exclusion criterion.

4.1 Inclusion criteria

- Age 18-60 years.
- ALL with B-/T precursor phenotype refractory to first line therapy.
- ALL with B-/T precursor phenotype at 1st isolated bone marrow relapse, occurring ≤24 months from the achievement of first CR, after chemotherapy or HSCT defined as follows: ≥5% leukemic blasts in the bone marrow not attributable to another cause (e.g. marrow regeneration); if there are no circulating blasts and the bone marrow contain 5-20% leukemic blasts, a repeat bone marrow performed at least one week later is necessary to confirm relapse.
- An ECOG performance status 0-2 or a reversible ECOG 3 score following intensive care of complications.
- Adequate hepatic and renal function, unless considered due to organ leukemic involvement:
    - Serum creatinine <1.5 mg/dL; if serum creatinine >1.5 mg/dL, then the estimated glomerular filtration rate (GFR) must be >60 mL/min/1.73 m^2 as calculated by the Modification of Diet in Renal Disease equation where Predicted GFR (ml/min/1.73 m^2) = 186 x (Serum Creatinine)$^{-1.154}$ x (age in years)$^{-0.023}$ x (0.742 if patient is female) x (1.212) if patient is black.
    - Serum bilirubin ≤1.5 x upper limit of normal (ULN).
    - Aspartate transaminase (AST)/alanine transaminase (ALT) ≤2.5 x ULN.
- Alkaline phosphatase ≤2.5 x ULN.
- Signed informed consent.
- Women of childbearing potential must have a negative serum pregnancy

4.2 Exclusion Criteria

- Prior exposure to Clofarabine or, in primary refractory patients, to Cyclophosphamide during the induction courses.
- Patients relapsed >24 months from first CR.
- 2nd or subsequent bone marrow relapse.
- Ph+ ALL.
- Diagnosis of Burkitt-type/B-ALL, or B-/T-lymphoblastic lymphoma with <25% bone marrow involvement.
- Concurrent or isolated CNS relapse.

- Pre-existing, uncontrolled pathology such as cardiac disease (congestive/ischemic, acute myocardial infarction within the past 3 months, untreatable arrhythmias, NYHA classes III and IV).
- Severe neurological or psychiatric disorder that impairs the patient's ability to understand and sign the informed consent, or to cope with the intended treatment plan.
- Active uncontrolled systemic fungal, bacterial, viral, or other infection (defined as exhibiting ongoing signs/symptoms related to the infection and without improvement, despite appropriate antibiotics or other treatment).
- HIV positive serology or active hepatitis infection.
- Concurrent diagnosis of active cancer requiring concurrent chemotherapy and/or radiotherapy, and/or with life expectancy <1 year.
- Patients who are pregnant (women of childbearing potential must have a negative serum pregnancy test). Post-menopausal women must be amenorrhoic for at least 24 months to be considered of non-childbearing potential. Male and female patients must agree to employ an effective barrier method of birth control throughout the study and for up to 3 months following discontinuation of study drugs. (see paragraph 8.4)

5 Trial Design

5.1 General design

The proposed treatment schedule consists of a combination of Clofarabine plus Cyclophosphamide administered over 5 consecutive days (Treatment scheme). This is an open, nonrandomized prospective phase II trial aimed to evaluating the activity of this combination in terms of CR rate.

- **STEP 1.** All eligible patients will be screened for the availability of an HLA-matched or partially mismatched compatible HSCT donor, of both family related, or unrelated type (early activation required), including cord blood and haploidentical siblings.
  Moreover, pre-treatment investigation will include collection and storage of patients ALL cells for ALL diagnosis and subclassification, immunophenotype, MRD and molecular genetic analyses.

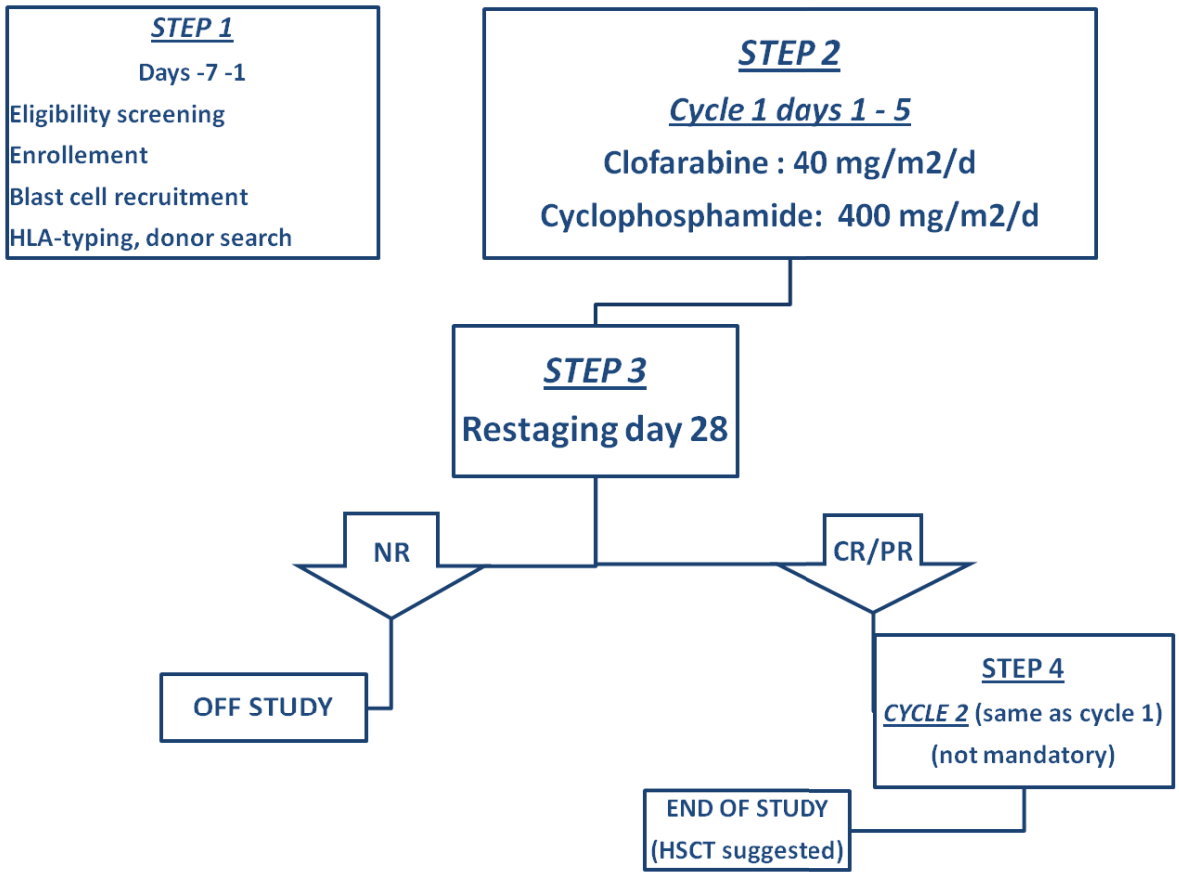
- **STEP 2**. Cycle 1 will be applied to all eligible patients once all enrolment criteria are confirmed.

- **STEP 3**. After cycle 1, response will be evaluated.

- **STEP 4**. After remission induction cycle 1, only responsive patients (CR or PR, see below for definitions) may be given cycle 2, according to the opinion of the responsible physician and with a minimum intercycle interval of 4 weeks from day 1 of cycle 1. All NR patients will be declared off study and will not be given a second course with the study combination. The suggested treatment following cycle 2 (or cycle 1 if cycle 2 is omitted) is HSCT.

5.1.1 Treatment scheme

Patients will receive a maximum of two consecutive cycles of Clofarabine-Cyclophosphamide, at an intercycle interval of 28 days or greater, according to tolerability and clinical status.
Cycle 2 will be administered only to patients obtaining at least a partial response (PR) after cycle 1, defined as a reduction in bone marrow blasts from >50% to between 5-25%. Non-responsive (NR) patients after cycle 1 will go off-study. The post-remission treatment policy is free (HSCT is suggested), but will be registered for all cases



5.2 End-points

5.2.1 Primary

The primary endpoint of the study is the rate of patients in CR after induction therapy.

5.2.2 Secondary

Secondary study endpoints are:

- Toxicity of grade 2 or greater according to CTCAE version 4.0 (Appendix C).
- Analysis of MRD response in remission patients. The MRD response will be assessed by flow cytometry (and by molecular biology when possible) evaluating ALL-associated immunophenotypes in BM samples taken after cycle 1 and 2, in correspondence of the morphological analysis of CR (see Summary Table 8.4 for details). A major MRD response is defined by a decrease of the leukemic clone to less than 0.1% compared to baseline, while a complete MRD response is obtained when the abnormal phenotype is no longer detectable with a sensitivity level of 10^{-3} to 10^{-4}.
- DFS at 1 year, defined as the time interval between the evaluation of CR and relapse of the disease or death in first CR; patients still alive, in first CR, will be censored at the time of the last follow-up. In this case, the DFS curve will be truncated at 1 year.
- OS at 1 year, defined as the time interval between inclusion and death for any cause. Patients still alive will be censored at the time of the last follow-up. In this case, the OS curve will be truncated at 1 year.
- CIR at 1 year; it will be calculated from the date of achievement of the first CR, using the cumulative incidence method, considering death in CR as a competing risk. Patients still alive, without a date of relapse, will be censored at the time of the last follow-up. In this case, the CIR curve will be truncated at 1 year.
- DFS, OS and CIR in two different risk groups: VHR (very high risk) includes relapses within 6 months from the date of the CR achievement; HR (high risk) includes relapses after 6 months from the date of the CR achievement.

5.3 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 adverse events and risks of the protocol outweighed the benefits that are hypothesized to be related to therapy.

RISKS IN PARTICIPATING IN THE STUDY

Toxicity

Refractory or relapsed ALL in adult patients is to be considered a medical emergency which is fatal within some days or a few weeks if left untreated and that requires rapid initiation of salvage chemotherapy. The risk of developing life-threatening complications is high due to the disease itself and may be temporarily exacerbated by treatment, which must be aggressive enough in an attempt to revert the course of this highly malignant clinical condition. The risk of chemotherapy resistance is also high due to prior exposure to several active antineoplastic drugs at high cumulative doses. For these reasons, and also because responsive patients may still receive a HSCT with curative intent (unlike advanced solid tumors patients, most of them being rather fit at the time of disease progression), highly intensive retreatment schedules are normally adopted. Therefore, high-degree toxicity is expected and it has become customary (see Introductory part) to accept an early mortality rate up to 20%, provided the study schedule can induce a 50% or greater CR rate, given an estimated probability of response from the literature of 40% ± 10%. Thus, trial participation entails a relatively high risk of treatment-related toxicity, associated to the development of several complications. The most frequent are infections, such as septicemia with/without organ involvement, especially pneumonia, from both Gram+ and Gram- germs. Invasive fungal infections from *Candida* and *Aspergillums* are also frequently observed. This is favored by the prolonged marrow hypoplasia with neutropenia <0.5 x10^9/L and the immunosuppressive properties of Clofarabine (and to the prior treatment). The infectious risk is counteracted by an appropriate policy of antimicrobial surveillance and prophylaxis, plus broad spectrum empirical antibiotic therapy at the onset of febrile neutropenia and/or other sign of infection, associated with the use of granulocyte colony-stimulating factor (G-CSF) to accelerate granulocyte recovery. Subsequent febrile episodes are reinvestigated and treated with antibiogram-guided therapy and/or antifungals. Severe anemia <8 g/dl and thrombocytopenia <20 x 10^9/L are also anticipated, mandating for transfusional support until recovery of the bone marrow function. Other expected toxicities include hepatic and metabolic dysfunctions, to be managed with general supportive measures (albumin, plasma infusions), severe gastrointestinal mucositis requiring parenteral nutrition and tumor lysis syndrome with kidney function impairment, preventable by the use of allopurinol/rasburicase and hyperhydration at study entry. Clofarabine-related cytokine release syndrome is treated by antinflammatory drugs such as paracetamol and steroids. Patients are treated at selected clinical Units by dedicated medical and nonmedical personnel highly skilled in the conduct of clinical studies employing intensive antileukemic treatment and management of attending toxicities. The real-time collection and evaluation of all toxic side-effects associated with the study protocol is a primary study endpoint and will guide the decision to reduce treatment intensity in accordance.

Psychosocial distress

Trial participation can entail psychological distress for patients beyond that caused by the illness itself: patients may experience depression, stress, uncertainty as a result of trial participation, loneliness, donor dependence and fear.

Trial participation can also be a social burden for patients, straining on relationships with partners and on other social contacts.

Benefits to participating patients

- Expected tumor remission, the likelihood of prolonging life and the possibility of undergoing a HSCT (a potential life-saving approach).
- A longer symptom-free period.
- Benefits to future patients and science. At least 30 patients nationwide would benefit annually from the experimental treatment, should it prove effective.

6 Therapeutic regimens, expected toxicity, dose modifications

6.1 Drug information

6.1.1 Clofarabine

6.1.1.1 Formulation

Clofarabine is a concentrate for solution for infusion and consists of Clofarabine in a concentration of 1 mg/ml formulated in unbuffered normal saline (comprised of Water for Injections USP/Ph Eur and Sodium Chloride USP/Ph Eur at 0.9%) with a pH range of 4.5-7.5. The container/closure system is a 20mL Ph Eur Type I flint glass vial with a 20 mm polypropylene flip-off cap with aluminum overseal.

6.1.1.2 Preparation, dosage and administration

Clofarabine concentrate for solution for infusion should be filtered using a 0.2 micron filter and diluted to a final concentration between 0.15 mg/mL and 0.4 mg/mL with 0.9% sodium chloride injection USP or European Pharmacopeia (EP) normal saline (NS), or 5% dextrose injection (D5W) USP or EP prior to infusion. **The diluted sterile concentrate should be used straight away or within 24 hours if stored in a refrigerator (at 2 to 8°C)**

The dose per protocol (mg/m2) will be administered as a 1-hour intravenous infusion on days 1 through 5 during induction therapy. To prevent drug incompatibilities, no other medications should be infused concurrently through the same iv lines as Clofarabine.

For supportive care and concomitant treatments see paragraph 6.5.

6.1.1.3 Side effects

Clofarabine is potent antineoplastic agent with potentially significant hematological and non-hematological adverse reaction. Among the risks associated with the treatment of patients with Clofarabine are those related to the myelosuppressive effects of Clofarabine.

Suppression of the bone marrow appears to be dose dependent. Because of the pre-existing immunocompromised condition, and prolonged neutropenia that can result from treatment with Clofarabine, patients are at increased risk for severe opportunistic infections, including sepsis. These events may lead to death.

Infections are frequent. Post-baseline infections were generally grade 3 and included *cellulitis, bacteremia, Herpes simplex, Herpes zoster, clostridium colitis* and *pneumonia*. There is a smaller incidence of grade 4 infections; these infections include *bacterial sepsis, enterococcal infection, Escherichia sepsis, fungal infection, fungal sepsis, pneumonia fungal, septic shock, sepsis, Staphylococcal bacteremia, Staphylococcal sepsis, systemic mycosis and varicella*.

Tumor lysis syndrome secondary to rapid destruction of peripheral leukemic cells; systemic inflammatory response syndrome (SIRS) and capillary leak syndrome are risks known to occur with Clofarabine treatment.

Patients undergoing treatment with Clofarabine should be evaluated and monitored for signs and symptoms of tumor lysis syndrome and cytokine release (e.g. tachypnoea, tachycardia, hypotension, pulmonary edema) that could develop into SIRS/capillary leak syndrome or organ dysfunction. Patients should receive IV fluids. The use of prophylactic steroids (e.g., 100 mg/m 2 hydrocortisone on days 1 through 3) may be of benefit in preventing signs or symptoms of SIRS or capillary leak syndrome (see "Appendix C").

Gastrointestinal effects of chemotherapy, such as *vomiting and diarrhea*, may result in *dehydration* and its sequelae, including *hypotension*. Thus, adequate hydration should be instituted in patients who experience vomiting, diarrhea and/or tumor lysis syndrome.

Hepatobiliary risks of Clofarabine are *elevated transaminases (AST and ALT) and elevated bilirubin*, *jaundice and hepatomegaly*.

Respiratory events, vomiting, headache, pyrexia, nausea, and febrile neutropenia are known as adverse effects of Clofarabine.

Patients with cardiac disease and those taking medicinal products known to affect blood pressure or cardiac function should be closely monitored during treatment with Clofarabine.

Careful hematological monitoring during therapy is important, and hepatic and renal function should be assessed prior to and during treatment with Clofarabine because of Clofarabine's predominantly renal excretion and because the liver is a target organ for Clofarabine toxicity. The respiratory status and blood pressure should be closely monitored during infusion of Clofarabine.

6.1.1.4 Storage and stability

Vials containing undiluted Clofarabine concentrate for solution for infusion should be stored at controlled room temperature (15 to 25 °C). Shelf-life studies of intact vials are 36 month.

6.1.2 Cyclophosphamide

6.1.2.1 Formulation

Cyclophosphamide is a nitrogen mustard alkylating agent, from the oxazophorines group. Cyclophosphamide for injection, USP is a sterile white powder containing Cyclophosphamide monohydrate, with the molecular formula C7H15Cl2N2O2P•H2O and a molecular weight of 279.1.

The chemical name for Cyclophosphamide is 2-[bis(2-chloroethyl)amino]tetrahydro-2H-1,3,2-oxazaphosphorine 2-oxide monohydrate.

6.1.2.2 Preparation and administration

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. Add the diluent to the vial and shake it vigorously to dissolve. If the powder fails to dissolve immediately and completely, it is advisable to allow the vial to stand for a few minutes. Use the quantity of diluent shown below to constitute the product. Cyclophosphamide should be prepared for parenteral use by adding 0.9% sterile sodium chloride solution. Solutions of cyclophosphamide may be injected intravenously without further dilution or may be infused following further dilution: Dextrose Injection, USP (5% dextrose), Dextrose and Sodium Chloride Injection, USP (5% dextrose and 0.9% sterile sodium chloride), 5% Dextrose and Ringer's Injection.

6.1.2.3 Side-effects

Bone marrow suppression, chemotherapy-induced nausea and vomiting, stomach ache, diarrhea, darkening of the skin/nails, alopecia, changes in color and texture of the hair, and lethargy. Hemorrhagic cystitis is a frequent complication, but this is prevented by adequate fluid intake and Mesna (sodium 2-mercaptoethane sulfonate). Interstitial pulmonary fibrosis, signs of nephrotoxicity, suppression of the gonads sometimes irreversible may occur.

6.1.3 Stability of constituted parenteral solutions

Cyclophosphamide (prepared for either direct injection or infusion) is chemically and physically stable for 24 hrs at room temperature or for six days in the refrigerator; it does not contain any antimicrobial preservative and thus care must be taken to assure the sterility of prepared solutions.

6.1.4 General information

Preparation for Administration

Both Clofarabine and Cyclophosphamide are prepared as sterile solutions for intravenous (IV) administration.

Dosage and administration

Clofarabine 40 mg/m^2/d is infused IV over 1 hr.

Cyclophosphamide 400 mg/m2/d is infused IV over 1 hr.

6.2 Dose and schedule

Clofarabine 40 mg/m^2/d is infused IV over 1 hr on each of 5 consecutive days (dd 1-5). The drug is infused 2 hrs prior to the start of Cyclophosphamide, on each of the 5 treatment days. Start-end time of Clofarabine infusion are 9-10 a.m., respectively.

Cyclophosphamide 400 mg/m^2/d is infused over 1 hr on each of 5 consecutive days (dd 1-5). The drug is infused 2 hrs after the end of Clofarabine, on each of the 5 treatment days. Start and end time of Cyclophosphamide infusion are 12 a.m.-1 p.m., respectively. The study regimen is reported in Table 6.

Table 6. Study regimen.

Drugs*	Dose/route	Sequence	d1	d2	d3	d4	d5
Clofarabine	40 mg/m^2/IV over 1 hr (start time 9 a.m.)	1st	X	X	X	X	X
Cyclophosphamide	400 mg/m^2/IV over 1 hr (start time 12 a.m.)	2nd, 2-hr after Clofarabine end	X	X	X	X	X

6.3 Treatment duration and second chemotherapy cycle

The duration of a chemotherapy course is 5 days. There will be one or two total Clofarabine-Cyclophosphamide courses, at an intercycle interval of at least 28 days or greater, as clinically indicated; the second course is applicable to CR/PR patients only, and this will also depend on the clinical status of the patients according to the indications of the treating physician. NR patients will not be given course 2 and will be declared off study.

6.4 Dose and schedule modifications

No schedule/dose modification is permitted unless strictly necessary. Acute, unexpected severe adverse events (SAE) in relation to the study drugs will be managed with total/partial drug withdrawal according to the opinion of the treating physician and will be immediately reported to Gimema Safety desk with the SAE report form.

Dose reduction for patients experiencing non-hematological toxicities:

if a patient develops a single clinically significant infection or severe toxicity (grade 3 in accord to the US National Cancer Institute (NCI) and Common Toxicity Criteria (CTC)), Clofarabine treatment should be delayed until the toxicity/infection resolves. *Nausea and vomiting, skin rash and anorexia are excluded from these cases*. Thereafter, treatment may be reinitiated at the full dose. In the case of an additional clinically significant toxicity/infection, Clofarabine treatment should be withheld until the toxicity/infection is clinically controlled and may be reinstituted with a 25% dose reduction. Any further severe toxicity/infection that does not recover within 8 weeks (from day 1 first cycle) or a life threatening or disabling toxicity (US NCI CTC Grade 4 toxicity) should qualify for withdrawal from treatment with Clofarabine.
No drug reduction is considered for Cyclophosphamide, unless evidence of excess toxicity from this treatment is obtained during progression of the study.

6.5 Concomitant treatments

6.5.1 Supportive care

Pretreatment hydration and prevention of tumor lysis syndrome before each Clofarabine-Cyclophosphamide cycle is accomplished as follows.

- Hyperhydration-alcalinization with normal saline/5% glucose solution (50/50, 2000 mL/d or more) plus1/6 M Na2HCO3 solution (500 ml/d), to warrant a daily urinary output >2 L and a normal kidney function (varying the IV fluid amount as necessary), with IV furosemide 20-40 mg bd/tid to avoid weight gain >1 kg. Add allopurinol 300-600 mg/d for uric acid concentration <9 mg/dl. Use IV urate-oxydase (Fasturtec) if hyperuricemia >8 mg/dl.
- The above preparative regimen to start at least 24 hours before chemotherapy, depending on degree of metabolic impairment, and to continue until WBC count is <10 $x10^9$/L or as necessary. In patients with kidney failure, chemotherapy is delayed until the creatinine concentration is <1.5 mg/dl and the uric acid <6 mg/dl.
- Patients with blood counts >50-100 $x10^9$/L or fast blast cell increase can be managed for 1-3 days with leucapheresis and low-dose corticosteroids only.
- All IV drugs are administered through a central venous access. Prior to any chemotherapy agent, give antiemetics such as granisetron, ondansetron or analogs.

6.5.2 Other concomitant therapies

Corticosteroids are administered along with Clofarabine-Cyclophosphamide starting from day 0, with the dual aim of reducing the incidence of Clofarabine inflammatory reaction and of improving the degree of antileukemic activity. To this end, prednisolone is administered either orally or IV at 20 mg/m^2/bd from day 0 to day 5, and then it is either discontinued or tapered.

Paracetamol 1000 mg may be administered per os (PO) 30' prior to each Clofarabine dose to prevent Clofarabine-related acute reactions (facultative). Do not administer paracetamol in case of altered liver function tests.

CNS prophylaxis will be administered to all patients owing to high-risk of neuromeningeal spread of ALL at relapse, with intrathecal Methotrexate 15 mg on day 2 of each Clofarabine-Cyclophosphamide course (provided the platelet count is >40 x10^9/L).

6.5.3 Prevention of infectious complications

The use of Clofarabine and Cyclophosphamide puts the patients at high risk of developing serious infectious complications, mandating for adequate antiinfectious surveillance and prevention. All patients **must** receive prophylactic medications like those indicated below and **cannot** be included in trials with placebo or other drugs whose activity is not yet proven.

1 **Antibacterial prophylaxis** with a quinolone (e.g. levofloxacin 500 mg/d PO or ciprofloxacin 500 mg/bd PO) from day 1 of chemotherapy and until resolution of the neutropenia (>1.0 x10^9/L).

2 **Antifungal prophylaxis** with itraconazole 300 mg/d, or fluconazole (400 mg/d PO), or caspopofungin/posaconazole from completion of chemotherapy and until resolution of the neutropenia (>1.0 x10^9/L).

3 ***P. Carinii* prophylaxis** for 3 months in CR patients, starting after the first cycle, with co-trimoxazole 800+160 mg/bd PO on alternate days (three times weekly).

4 **Antiviral prophylaxis** for 3 months in CR patients, starting after the first cycle, with acyclovir 400 mg/bd PO.

5 Serial determinations of the **immunoglobulin** (Ig) plasma levels, with Ig administration (0.4 g/kg IV) when total gammaglobulin concentration is <0.5 g/dl. To be performed monthly, starting from CR onwards.

Serial determinations of peripheral blood CMV Ag/CMV DNA, for pre-emptive therapy with anti-CMV drugs (Ganciclovir, Foscarnet) in cases showing a rising title. To be performed monthly, starting from CR onwards.

Serial (weekly) determinations of peripheral blood galactomannan antigen, for early detection of *Aspergillus* spp. infection with additional CT scans and therapeutic intervention.

6.5.4 Treatment of infectious complications

Established infections will be managed empirically with IV broad spectrum antibiotic combinations initially and according to institutional/national guidelines for neutropenic leukemia patients (usually associating a third generation cephalosporin or piperacillin/tazobactam with an aminoglycoside). Second-line therapy (vancomycin, carbapenems) is instituted after daily clinical and laboratory reassessment as needed, as is antifungal treatment with liposomal amphotericin B or other drugs (Posaconazole, Voriconazole).

6.5.5 Transfusion policy

During the periods of pancytopenia, patients are transfused with packed filtered red cell to maintain the Hb concentration >8-9 g/dl and with platelet concentrates to maintain the platelet count >10-20 $x10^9$/l. Patients should be transfused always in case of clinical symptoms of bleeding; in case of documented infections or fever, platelet counts should be maintained $>20^9$/l. Otherwise, keep platelet counts $>10^9$/l.

6.5.6 Use of G-CSF

To speed myeloid cell recovery from drug-induced marrow hypoplasia, recombinant human G-CSF is routinely used from day 6 at 5 mcg/kg/d given subcutaneously (SC) until the absolute neutrophil counts exceed 1.0 $x10^9$/l.

Table 7. Summary of concomitant medications.

DRUGS	DOSE/ROUTE	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6+
Clofarabine	40 mg/m^2/d IV (over 1 hr)		X	X	X	X	X	
Cyclophosphamide	400 mg/m^2/d IV (over 1 hr), 2 hrs after Clofarabine		X	X	X	X	X	
CONCOMITANT MEDICATIONS								
Methotrexate	15 mg IT			X				
Prednisolone	20 mg/m^2/bd PO/IV	X	X	X	X	X	X	taper
Paracetamol ***(facultative)***	1000 mg PO 30 min prior to Clofarabine		X	X	X	X	X	

G-CSF	5 mcg/kg/d SC		X, to neutrophils >1.0x10^9/L
Itraconazole/ Fluconazole	300 mg/d PO 400 mg/d PO		X
Aciclovir	400 mg/bd PO		daily for 3 months
Ciprofloxacin	500 mg/bd PO		daily to neutrophils >1.0 x10^9/L
Cotrimoxazole	800/160 mg/bd PO (Monday/Thursday)		twice weekly for 3 months to neutrophils >1.0 x10^9/L

7 Drug supply

7.1 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 availability and accountability for the duration of the trial.

Clofarabine will be supplied free of charge by the Genzyme Corporation through Penn Pharma in the UK. Penn Pharma will be supplying participating centres directly.

7.2.2 Packaging, dispensing and storage of Clofarabine

Packaging and labelling will be in accordance with GMP.

Clofarabine will be supplied in kits containing 4 vials per kit. The vials should be stored at room temperature (between +15°C and +25°C). The diluted sterile concentrate should be used straight away or within 24 hours if stored in a refrigerator (at 2° to 8°C)

Each carton and vial are labelled with a single panel label which complies with Annex 13 of the EC guide to Good Manufacturing Practices and national legislation to meet the requirements of the participating country. The site will be responsible for completing individual patients' details on each label.

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 Drug reconciliation procedures

The drug formulation, dose, number of bottles/capsules dispensed, received and returned must be recorded.

The investigator must maintain an accurate record of the shipment and dispensing of the study drug in a drug accountability ledger, a copy of which must be given to the GIMEMA at the end of the study. An accurate record of the date and amount of study drug dispensed to each patient must be available for inspection at any time. All drug supplies are to be used only for this protocol and not for any other purpose.

The investigator must not destroy any drug labels, or any partly used or unused drug supply. At the conclusion of the study and, as appropriate during the course of the study, the investigator will receive appropriate instructions concerning returns.

The contact for the Genzyme will be:
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8 Clinical evaluation, laboratory tests and follow-up

8.1 Before treatment starts

The following investigations must be performed between days -7 and 0 in all patients in whom the study eligibility is confirmed:

- Bone marrow (BM) morphology and peripheral blood (PB) morphology according to the EGIL/WHO criteria. Immunophenotyping for ALL diagnosis and subclassification. Cytogenetics and selected molecular biology assays.
- All BM/PB study samples will be received and processed centrally at the GIMEMA ALL study center in Rome. Collection and storage of relevant study material for MRD (immunophenotype, molecular biology) and other studies will be organized. The type of samples, timing of collection and shipment instructions are detailed below in **Appendix E**.
- Medical history, physical examination, ECOG performance status, chest X-ray.
- Full blood counts with differentials, complete biochemical profile including liver and kidney function tests, LDH, albumin and serum protein profile, electrolytes (Na, K, Cl, Ca), coagulation tests (APTT, PT INR, fibrinogen).
- Serology for HBV, HCV, CMV, EBV and HIV infections.
- Diagnostic lumbar puncture to exclude CNS involvement and cytology/biopsy of suspect other sites of disease (pleural effusion, skin, etc.) to define the extent of extramedullary involvement.
- Blood group and HLA-DR leukocyte profile of patient and siblings.

- One serum pregnancy tests

8.2 During treatment

Cycles 1 and 2

The following investigations must be performed in all patients undergoing treatment (cycle 1 and cycle 2):

- Physical examination, full blood counts at least on alternate days until discharge, serum biochemistry bi-weekly.
- Coagulation tests (APTT, PT INR, Fibrinogen).
- On day 2 to hematological recovery: physical examination and hematology (full blood counts with differentials) on alternate days; serum biochemistry (BUN, urea, creatinine, uric acid, albumin, total protein, total bilirubin, fractionated bilirubin, alkaline phosphatase, AST, ALT, LDH, electrolytes) and Coagulation tests (APTT, PT INR, Fibrinogen); toxicity evaluation every day.
- On day 28 (or earlier/later as dictated by clinical conditions and blood count): BM and PB examination samples (morphology, MRD response) will be sent for evaluation to the GIMEMA study central reference laboratory.
- One serum pregnancy test every 4 weeks

8.3 Follow-up

The following investigations must be performed in all patients after treatment during the clinical follow-up (2-monthly for 1 year):

- Physical examination, ECOG performance status, full blood count, serum biochemistry. Toxicity evaluation.
- BM examination in the case of any abnormal physical or hematological finding suggesting ALL relapse. Samples will be sent for evaluation to the GIMEMA study central.
- Coagulation tests (APTT, PT INR, fibrinogen).
- Two urine pregnancy tests after 4 an 12 weeks of the end of the study
- One serum pregnancy test after 8 weeks of the end of the study

8.4 Methods of birth control and pregnancies tests

Adults subjects of childbearing potential (ACBP) must:

- Understand the potential teratogenic risk to the unborn child and the need for effective contraception.
- Be capable of complying with effective contraceptive measures.
- Be informed and understand the potential consequences of pregnancy and the need to notify her study doctor immediately if there is a risk of pregnancy.

- Understand the need to commence the study treatment as soon as study drug is dispensed following a negative pregnancy test.
- Understand the need and accepts to undergo pregnancy testing based on the frequency outlined in this protocol.

ACBP enrolled in this protocol must agree to use two reliable forms of contraception simultaneously or to practice complete abstinence from heterosexual contact during the following time periods related to this study: 1) while participating in the study; 2) in case of dose interruptions; and 3) for at least 3 weeks after study treatment discontinuation.

The two methods of reliable contraception must include one highly effective method and one additional effective (barrier) method. ACBP must be referred to a qualified provider of contraceptive methods if needed. The following are examples of highly effective and additional effective methods of contraception:

- Highly effective methods:
  - Intrauterine device (IUD)
  - Hormonal (birth control pills, injections, implants)
  - Tubal ligation
  - Partner's vasectomy
- Additional effective methods:
  - Male condom
  - Diaphragm
  - Cervical Cap

Pregnancy tests

pre-menopausal women who are not surgically sterile must agree to have pregnancy tests:

1. One serum test within 48 hrs prior treatment start ,
2. One serum test every 4 weeks during treatment period (two tests total) ,
3. From study discontinuation respectively: one urine pregnancy test with a minimum sensitivity of at least 25 mIU/mL will be carried out after 4 weeks, one serum test after 8 weeks and the last urine pregnancy test with a minimum sensitivity of at least 25 mIU/mL will be carried out after 12 weeks.

8.5 Summary table

Time (days from starting CHT)	-7 to 0	Cycles 1 and 2: from day +2 to hematological recovery	Cycles 1 and 2: day + 28 (or earlier/later if necessary)	Follow-up: every 2 months for 1 year;
Informed consent	*			
Eligibility (Inclusion/Exclusion) criteria	*			
General medical history/present medical conditions	*			
Present disease leukemia history	*			
Physical examination	*	**On alternate days**	*	*
ECOG Performance status	*			*
Chest X-ray/ECG & visit/ Echocardiogram	*			
Hematology (full blood counts with differentials)	*	**On alternate days**	*	*
Serum biochemistry (BUN, urea, creatinine, uric acid, albumin, total protein, total bilirubin, fractionated bilirubin, alkaline phosphatase, AST, ALT ,LDH, electrolytes)	*	**Twice a week**		*
Coagulation tests (APTT, PT INR, fibrinogen)	*	**Twice a week**	*	*
Serology HBV/HCV/CMV/EBV/HIV	*			
Blood group and HLA-DR leukocyte profile of patients and siblings	*			
Serum Pregnancy test	*		*	*** (8 week)**
Urine Pregnancy test				*** 4 and 12 weeks**
BM and PB cytomorphology•	*		*	**If any suspect of relapse**
BM immunophenotype•	*		*** (for MRD)**	
BM cytogenetics	*		*	
BM molecular studies•	*		*** (for MRD)**	
Rachicentesis/Citology/biopsy of suspect sites of disease	*			
Toxicity evaluation		**Every day**	*	*

•Samples sent to the GIMEMA study central.

8.6 Biological samples conservation: timelines and procedures

8.6.1 Sample 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 at defined timepoints (days +28 of cycles 1 and 2); 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 E**".

Samples will be stored (cryopreserved in DMSO, dry pellet or pellet in GTC) at the central reference laboratory (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.

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.

8.6.2 Methods

8.6.2.1 Morphology

Smears of bone marrow and peripheral blood will be analyzed with the standard May-Grünwald and Giemsa stain. The smears will be performed centrally in the laboratory of Ematologia, Azienda Policlinico "Umberto I", Dipartimento Biotecnologie Cellulari ed Ematologia, Università "Sapienza" di Roma

8.6.2.2 Immunophenotype

The evaluation of immunophenotype will be done through cell suspension of marrow blood samples or through peripheral blood 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.

8.6.2.3 Cytogenetics

Analysis will be performed in the local laboratory

8.6.2.4 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. 51). 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. At diagnosis, in all cases, the technique named "Multiplex-RT-PCR" will be used as it allows screening different transcriptions at the same time (Ref. 43). The molecular analysis during the MRD monitoring will be performed through the Quantitative QRT- PCR system (Ref. 44, 45).

8.6.3 Shipping instructions

See **Appendix E**

9 Criteria of evaluation

9.1 Evaluation of response

Complete remission (CR)

Disappearance of any clinical and laboratoristic sign of ALL. The patient must be transfusion-free with neutrophils >1.0 $x10^9$/L and platelets >100 $x10^9$/L. BM examination must show absence or reduction of blast cell content (≤5%, none of which obviously leukemic), with cellularity in the normal or slightly hypocellular range and with evidence of trilineage hemopoiesis. BM is examined on day 28 from the start of chemotherapy cycle 1, or later as clinically indicated in ill/cytopenic patients and after cycle 2 in patients with CR/PR proceeding to this treatment. Bone marrow morphology is evaluated at each study site by an expert hemato-morphologist. It is required that two marrow slides are centralized for review. Bone marrow core biopsy is not mandatory, but may be helpful in selected cases.

Complete remission with incomplete blood count recovery (CRi)

Patients with CR marrow morphology but peripheral blood counts below the ranges given above (neutrophils <1.0 $x10^9$/L or platelets <100 $x10^9$/L).

CRi is considered as a positive treatment response, but is evaluated separately.

The sum of CR and CRi after cycle 1 represents the chosen efficacy indicator.

Partial Remission (PR)

Is defined by a bone marrow blast reduction from >50% to between 5 - 25%.

Treatment failure

Treatment failure may be due to one of the following causes and needs to be registered accordingly after the induction cycle(s) and at subsequent relapse.

- Non-responsive ALL (NR). Survived >7 days from the end of treatment, with persistent ALL in the PB and/or BM (> 25%, BM examined).
- Aplasia. Died after >7 days from the end of induction chemotherapy (day + 5), with cytopenic/aplastic BM (BM examined).
- Indeterminate. Died after <7 days from the end of induction chemotherapy (day + 5), or after >7 days with no PB blasts/undetermined BM, or did not complete chemotherapy. The "word" indeterminate here refers to the underlying ALL. The proximate cause of death may be known (ie infection etc).

9.2 Evaluation of events after achieving CR

Recurrence. Any of the following:

- Reappearance of ALL blasts in the PB.
- ≥5% leukemic blasts in the BM not attributable to another cause (e.g. marrow regeneration). If there are no circulating blasts and the BM contains 5-20% leukemic blasts, a repeat BM performed at least a week later is necessary to confirm the relapse.
- Reappearance of extramedullary disease.

Because surveillance BM is not recommended as routine, relapse is usually detected while investigating:

- unexplained or worsening cytopenia at follow-up visits;
- sudden-onset leukocytosis;
- systemic symptoms, such as malaise and fever, etc.

Treatment-related death (TRD)

Mortality due to treatment-related complications in CR patients.

9.3 Evaluation of toxicity

Treatment-related toxicity will be evaluated through CTC-NCI criteria for both hematological and extra-hematological toxicity (**Appendix C**). Only toxicities of grade 2 and greater will be collected for toxicity analysis.

Toxicity analysis for protocol amendment will focus on incidence and severity of:

- Hematological toxicity, reflected by an absolute duration of severe neutropenia <0.5 x10^9/L of 35 days or greater from chemotherapy day 1 of cycle 1; and/or an absolute duration of

thrombocytopenia <20 x10^9/L of 35 days or greater from the same time point. Both neutropenia and/or thrombocytopenia of this kind are associated to significant infectious episodes and/or hemorrhage.

- Extra-hematological toxicity, reflected by any CTC grade 3+ adverse event affecting major organs and tissues like the cardio-respiratory system, gastrointestinal tract, liver, kidney, central nervous system, the endocrine system and coagulation.

The principal investigator and co-investigators will continuously and closely monitor drugs toxicity and occurrence of potential AEs. Upon the enrolment of the first ten patients and in collaboration with the GIMEMA Safety Desk they will decide on whether to enrol any further patients or not.

10 Statistical considerations

10.1 Sample size

This study is designed to evaluate the complete response rate (CR) of the Clofarabine and Cyclophosphamide combination.

In the proposal, to reject the null hypothesis that $p \leq 0.25$ vs. the alternative hypothesis that $p > 0.50$ with Type I error probability (α) equal to 0.05 and 85% power (1-β), a maximum of 27 evaluable patients has to be accrued. In the first stage of the study, 10 evaluable patients will be enrolled and the trial will be terminated if 2 or fewer responses will be achieved; otherwise, 17 further evaluable patients will be enrolled in the second stage. If the total number of responses will be less than or equal to 10, the combination therapy will not be recommended for further studies. If the total number of CRs is at least 11, the treatment 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 (CR) 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); in case of relevant non-compliance to treatment and/or impossibility to evaluate response, a Per-Protocol analysis will also be performed, together with an analysis of non-compliance/non-evaluation.

All adverse events will be tabulated. All reported toxicities will be correlated with clinical outcome.

Patients' and disease characteristics will be summarized by cross-tabulations for categorical variables or by quantiles for continuous variables.

Differences in terms of categorical variables or response rates in subgroups will be evaluated by non-parametric tests (Chi-Squared and Fisher Exact) in univariate analysis and using logistic regression in multivariate analysis.

Survival distributions (OS and DFS) will be estimated using the Kaplan-Meier Product Limit estimator. Subgroups comparisons will be performed for descriptive purposes.
Differences in terms of OS and DFS will be evaluated by means of Log-Rank test in univariate analysis and by means of Cox regression model in multivariate analysis, after assessment of proportionality of hazards.
Cumulative incidence curves (e.g. for relapse rate) will be estimated using the proper non-parametric method. The Gray test will be applied for significance tests on cumulative incidence curves.

All analyses will be performed using the SAS system software (version 9.1.3) All tests will be two-sided, accepting $p \leq 0.05$ as indicating a statistically significant difference.

10.3 Interim analysis

No interim analysis will be performed

11 Quality of life assessment

Quality of life will not be assessed in this study.

12 Economic evaluation

No economic evaluation will be performed in this study.

13 Pharmacokinetic evaluation

No pharmacokinetic evaluation will be performed in this study

14 Translational research

No translational research will be directly performed in this study. However, stored biological material from study patients, who will be asked to sign a specific consent form, could be used in the future for such studies to obtain new insights into the mechanisms of response (or resistance) of ALL cells to Clofarabine and study combination.

15 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 copy of the favorable opinion of their local or national (whichever is applicable) ethics committee mentioning the documents that have been reviewed (including the 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, (according to all national requirements), informed consent, clearly mentioning the version number and the date.
- List of co-investigators who are authorized to work on this study.

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

16 Patients' registration procedure

Patients' registration will only be accepted from authorized investigators (see "Authorization procedure"). A patient can be registered after verification of the 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.

17 Forms and procedures for collecting data

17.1 Case report forms and schedule for completion

A web system data entry will be used for this trial. Severe Adverse Events (SAE)/Suspected Unexpected Serious Adverse Reaction (SUSAR) report forms must nonetheless be sent by fax to the Data Center.

All forms must be dated and signed by the responsible investigator or one of his/her authorized staff members.

17.2 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 in. The 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.

18 Reporting adverse events

18.1 Definitions

An **Adverse Event (AE)** is defined as any untoward medical occurrence in a patient or clinical investigation subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of an investigational medicinal product. 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 (GCP – European directive 2001/20/EC)

An **Adverse Reaction (AR)** is all untoward and unintended responses to an investigational medicinal product related to any dose administered *(GCP – European directive 2001/20/EC)*.

A **SAE)/Serious Adverse Reaction (SAR)** is defined as any untoward medical occurrence or effect that at any dose:

- results in death,
- is life threatening,
- requires hospitalization or prolongation (of),
- results in persistent or significant disability/ incapacity,
- is a congenital anomaly or birth defect or
- constitutes an important medical event (GCP – European directive 2001/20/EC).

Any AE is considered a SAE if it is associated with clinical signs
or symptoms judged by the investigator to have a significant clinical impact.
Events not considered to be SAEs are:

- all deaths due to disease progression;
- hospitalizations which:
  a) were planned before entry into the clinical study,
  b) are for elective treatment of a condition unrelated to the studied indication or its treatment,
  c) occur on an emergency outpatient basis and do not result in admission (unless fulfilling other criteria above),
  d) are part of the normal treatment or monitoring of the studied indication and are not associated with any deterioration in condition;
- all expected toxicities of study medication (infection, bleeding complication, well-known organ toxicity as indicated in the section *"Expected toxicities*" of the protocol, hematological toxicities), unless the event is life threatening or results in death.

An **Unexpected Adverse Reaction (UAR)** is an adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g., Investigators' Brochure). *(GCP – European directive 2001/20/EC)*.

18.2 Reporting procedure

18.2.1 Non-serious adverse events and non-serious adverse reactions

All AE and AR occurring during the treatment period must be recorded as indicated in the protocol.

18.2.2 Serious adverse events or Suspected Unexpected Serious Adverse Reactions

All SAE/SUSAR, related or not to the protocol treatment, must be reported to the GIMEMA Safety Desk.

This applies to all SAEs that occur during the study, from informed consent signature date up to 30 days after the last protocol treatment administration and at any time if they are suspected of being related to the study medication.

This must be documented on a SAE form completed in English and supplied by fax within 24 hours of the initial observation of the event. The investigator will decide if these events are related to the protocol treatment (i.e. unrelated, likely related, and not assessable) and the decision will be recorded on the SAE form, if deemed necessary by the investigator. The report must be as complete as possible, including details of the current illness and of the SAE, and an assessment of the possible causal relationship between the event and the investigational product. The information not available at the time of the initial report must be documented on a follow-up form.

The assessment of causality is made by the investigator using the following definitions:

RELATIONSHIP to treatment	**DESCRIPTION**
UNRELATED	There is no evidence of any causal relationship
UNLIKELY	There is little evidence to suggest there is a causal relationship (e.g. the event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (e.g. the patient's clinical condition, other concomitant treatments).
POSSIBLE	There is some evidence to suggest a causal relationship (e.g. because the event occurs within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (e.g. the patient's clinical condition, other concomitant treatments).
PROBABLE	There is evidence to suggest a causal relationship and the influence of other factors is unlikely.
DEFINITELY	There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.
NOT ASSESSABLE	There is insufficient or incomplete evidence to make a clinical judgment of the causal relationship.

The SAE/SUSAR should be documented on the SAE/SUSAR report form and a detailed description of the event should be enclosed.

PLEASE FAX THE REPORT TO:

GIMEMA Safety Desk:

Fax No. +39 06 70390540

The Investigator, in case of death, has to communicate the event to the GIMEMA Safety Desk, to his local Ethic Committee and has to provide any further information regarding the event

The Sponsor will forward SAE/SAUSAR reports to the Regulatory Authorities, Principal Investigator and Ethics Committee within seven days of receipt in case of death or a life-threatening event and within fifteen days in any other case.

The GIMEMA Safety Desk must report all SAEs and SUSARS (and follow-up reports) in a timely manner to allow company to report these events to other appropriate regulatory authorities within the designated timelines for expedited reporting.

ANY QUESTION CONCERNING SAE OR SADR REPORTING CAN BE DIRECTED TO

GIMEMA Safety Desk

Phone: +39 06 70390518

Fax: +39 06 70390540

e-mail: safety-desk@gimema.it

ALL FORMS MUST BE DATED AND SIGNED BY THE RESPONSIBLE INVESTIGATOR OR ONE OF HIS/HER AUTHORIZED STAFF MEMBERS.

19 Quality assurance

19.1 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.

19.2 On-site quality control

In order to censure that the study is conducted according to Good Clinical Practice, the 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. Compulsory documents to be sent to the Data Center in order to be authorized to enrol patients.
2. Study documents archive system.
3. Patients' information sheet and informed consent: how to approach the patient and where to archive the document.
4. Biological samples centralization system.
5. Patients' selection criteria and registration procedure.
6. CRFs, queries management.
7. SAEs/SUSARs.
8. 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 patients' selection criteria and registration procedures, CRFs and SAEs/SUSARs. All these will also be in the Investigator's File.

During the general GIMEMA meetings, a report concerning the conduct 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 conduct 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.

19.3 Central review procedures

For many years the GIMEMA has worked with a biological sample centralization system for over 1500 ALL patients enrolled in the GIMEMA studies. All samples are sent to a single laboratory in Rome (within 24 hours). Thereafter, the samples are processed in order to perform the different analyses in the dedicated 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 disease during the course of the study. Besides, this system provides the same standard of care for all patients, otherwise not possible. This also enables adequate monitoring of MRD, when applicable.

20 Ethical considerations

20.1 Patients' 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.

20.2 Subject identification

The name of the patient will not be asked for nor will it be 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.

20.3 Informed consent

All patients will be informed of the aims of the study, the possible AE, 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(s). 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 the 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 the local informed consent documents before the centre can join the study. It is the responsibility of the Local Ethical Committee to guarantee that the translation is conform 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. This implies that "the written informed consent form should be signed and personally dated by the patient or by the patient's legally acceptable representative".

21 Administrative responsibilities

21.1 The Study Coordinator

The Study Coordinator(s) (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. The Study Coordinator(s) will also be responsible for answering all clinical questions concerning eligibility, treatment and evaluation of the patients.

Study coordinator:

Dr. Renato Bassan

Study co-coordinator:

Prof. Giovanna Meloni

21.2 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(s). All methodological questions should be addressed to the GIMEMA Data Center.

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22 Trial sponsorship and financing

The sponsor of the study is GIMEMA Foundation

23 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 encountered even if the patient had not participated to the study. The insurance also covers the civil responsibility of the monitor, of the investigator and his/her collaborators.

24 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 day notice for abstracts, presentations or educational material and a 30 day notice for publications. The investigator is due to include the sponsor's name in any final publication.

24.1 Authorship

The final publication of the trial results will be written by the Study Coordinator 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 Coordinator, 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 Coordinator and the Data Center, all other participants or representatives of the Data Centrr who have contributed to the trial will be mentioned in the acknowledgment section of the manuscript.

24.2 Responsibility for publication

The manuscript will be sent to a major scientific journal after revision by the coordinator of the trial.

The title of all manuscripts will include the "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 Coordinator and the Data Center must approve all publications, abstracts and presentations based on the 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 Coordinator.

Appendix A: References

Ref. 1 Welborn J. Impact of re-induction regimens for relapsed and refractory acute lymphoblastic leukemia in adults. Am J Hematol. 1994; 45: 341–344.

Ref. 2 Bassan R, Lerede T, Barbui T. Strategies for the treatment of recurrent acute lymphoblastic leukemia in adults. Haematologica. 1996; 81: 20–36.

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Ref. 4 Garcia-Manero G, Thomas DA. Salvage therapy for refractory or relapsed acute lymphocytic leukemia. Hematol Oncol Clin North Am. 2001; 15: 163–205.

Ref. 5 Thomas DA, Kantarjian H, Smith TL, et al. Primary refractory and relapsed adult acute lymphoblastic leukemia: characteristics, treatment results, and prognosis with salvage therapy. Cancer. 1999; 86: 1216-1230.

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Ref. 8 Vives S, Oriol A, Tormo M, et al. Otcome of 198 adults after relapse of acute lymphoblastic leukemia (ALL) included in 4 consecutive first line PETHEMA trials. Haematologica. 2008; 93 (Suppl 1); abstract n°0012.

Ref. 9 Mazza JJ, Leong T, Rowe JM, Wiernick PH, Cassileth PA. Treatment of adult patients with acute lymphocytic leukemia in relapse. Leuk Lymphoma. 1996; 20: 317-319.

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Appendix B: ECOG Performance status

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

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

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

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, Somerset West, Republic of South Africa, October 1996
52nd WMA General Assembly, Edinburgh, Scotland, October 2000
53th WMA General Assembly, Washington 2002 (Note of Clarification on paragraph 29 added)
55th WMA General Assembly, Tokyo 2004 (Note of Clarification on Paragraph 30 added)
59th WMA General Assembly, Seoul, October 2008

A. INTRODUCTION

9. 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 not be applied without consideration of all other relevant paragraphs.

10. Although the Declaration is addressed primarily to physicians, the WMA encourages other participants in medical research involving human subjects to adopt these principles.

11. It is the duty of the physician to promote and safeguard the health of patients, including those who are involved in medical research. The physician's knowledge and conscience are dedicated to the fulfillment of this duty.

12. 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."

13. Medical progress is based on research that ultimately must include studies involving human subjects. Populations that are underrepresented in medical research should be provided appropriate access to participation in research.

14. In medical research involving human subjects, the well-being of the individual research subject must take precedence over all other interests.

15. 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 current interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.

16. In medical practice and in medical research, most interventions involve risks and burdens.

17. Medical research is subject to ethical standards that promote respect for all human subjects and protect their health and rights. Some research populations are particularly vulnerable and need special protection. These include those who cannot give or refuse consent for themselves and those who may be vulnerable to coercion or undue influence.

18. Physicians should 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.

B. BASIC PRINCIPLES FOR ALL MEDICAL RESEARCH

19. It is the duty of physicians who participate in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects.

20. 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.

21. Appropriate caution must be exercised in the conduct of medical research that may harm the environment.

22. The design and performance of each research study involving human subjects must be clearly described 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, other potential conflicts of interest, incentives for subjects and provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study. The protocol should describe arrangements for post-study access by study subjects to interventions identified as beneficial in the study or access to other appropriate care or benefits.

23. The research protocol must be submitted for consideration, comment, guidance and approval to a research ethics committee before the study begins. This committee must be independent of the researcher, the sponsor and any other undue influence. 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 change to the protocol may be made without consideration and approval by the committee.

24. Medical research involving human subjects must be conducted only by individuals with the appropriate scientific training and qualifications. Research on patients or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional. The responsibility for the protection of research subjects must always rest with the physician or other health care professional and never the research subjects, even though they have given consent.

25. Medical research involving a disadvantaged or vulnerable population or community is only justified if the research is responsive to the health needs and priorities of this population or community and if there is a reasonable likelihood that this population or community stands to benefit from the results of the research.

26. Every medical research study involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and communities involved in the research in comparison with foreseeable benefits to them and to other individuals or communities affected by the condition under investigation.

27. Every clinical trial must be registered in a publicly accessible database before recruitment of the first subject.

28. Physicians may not participate in a research study involving human subjects unless they are confident that the risks involved have been adequately assessed and can be satisfactorily managed. Physicians must immediately stop a study when the risks are found to outweigh the potential benefits or when there is conclusive proof of positive and beneficial results.

29. Medical research involving human subjects may only be conducted if the importance of the objective outweighs the inherent risks and burdens to the research subjects.

30. Participation by competent individuals as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no competent individual may be enrolled in a research study unless he or she freely agrees.

31. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information and to minimize the impact of the study on their physical, mental and social integrity.

32. In medical research involving competent human subjects, 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, 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.

33. For medical research using identifiable human material or data, physicians must normally seek consent for the collection, analysis, storage and/or reuse. There may be situations where consent would be impossible or impractical to obtain for such research or would pose a threat to the validity of the research. In such situations the research may be done only after consideration and approval of a research ethics committee.

34. When seeking informed consent for participation in a research study the physician should 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 should be sought by an appropriately qualified individual who is completely independent of this relationship.

35. For a potential research subject who is incompetent, 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 population represented by the potential subject, the research cannot instead be performed with competent persons, and the research entails only minimal risk and minimal burden.

36. When a potential research subject who is deemed incompetent 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.

37. 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 population. In such circumstances the physician should 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 should be obtained as soon as possible from the subject or a legally authorized representative.

38. Authors, editors and publishers all have ethical obligations with regard to the publication of the results of research. Authors 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. They should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results should be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest should be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

C. ADDITIONAL PRINCIPLES FOR MEDICAL RESEARCH COMBINED WITH MEDICAL CARE

39. The physician may combine medical research with medical care only to the extent that the research 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.

40. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best current proven intervention, except in the following circumstances:

- The use of placebo, or no treatment, is acceptable in studies where no current proven intervention exists; or
- Where for compelling and scientifically sound methodological reasons the use of placebo is necessary to determine the efficacy or safety of an intervention and the patients who receive placebo or no treatment will not be subject to any risk of serious or irreversible harm. Extreme care must be taken to avoid abuse of this option.

41. At the conclusion of the study, patients entered into the study are entitled to be informed about the outcome of the study and to share any benefits that result from it, for example, access to interventions identified as beneficial in the study or to other appropriate care or benefits.

42. The physician must fully inform the patient which aspects of the 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 interfere with the patient-physician relationship.

In the treatment of a patient, where proven interventions do not exist or have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorized representative, may use an unproven intervention if in the physician's judgment it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, this intervention should be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information should be recorded and, where appropriate, made publicly available.

Appendix E: Time points 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 of relapse/refractory ALL**:
  - BM unstained slides (from 2 to 4)
  - BM (at least two 5 ml test-tubes containing Na citrate)
  - PB sample (at least two 5 ml test-tubes containing Na citrate; if WBC <10 x 10^9/L send at least four 5 ml test-tubes in Na citrate)
- at **days +28 of Cycles 1 and 2**:
  - BM unstained slides (from 2 to 4)
  - BM (at least two 5 ml test-tubes containing Na citrate)
  - PB sample (at least two 5 ml test-tubes containing Na citrate; if WBC <10 x 10^9/L send at least four 5 ml test-tubes in Na citrate)
- at **time of subsequent relapse/progression**:
  - BM unstained slides (from 2 to 4)
  - BM (at least two 5 ml test-tubes containing Na citrate)
  - PB sample (at least two 5 ml test-tubes containing Na citrate; if WBC <10 x 10^9/L send at least four 5 ml test-tubes in Na citrate)

2) Biological samples shipment instructions

Instructions for shipping of the biological samples are as follows:

a. The biological sample collection must be done on the same day of the shipment. Plastic and non-glass test-tubes should be used (do not send syringes).

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 ship the samples, please, contact TNT through the "**number 199803868**" and ask for TNT 10:00 EXPRESS shipment (**GIMEMA client code 1624953**). Costs will be charged to the recipient. Please, do not forget to keep the code given by TNT 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, Università "Sapienza"

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Phone: +39 06 4416 39 826;

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E-mail: centralizzazione@libero.it

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Please, inform the Central Lab whenever you ship samples, by phone fax or mail.