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

Statistical Analysis Plan

17/01/12	Final Version 1.0

1 Trial Design

1.1 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.

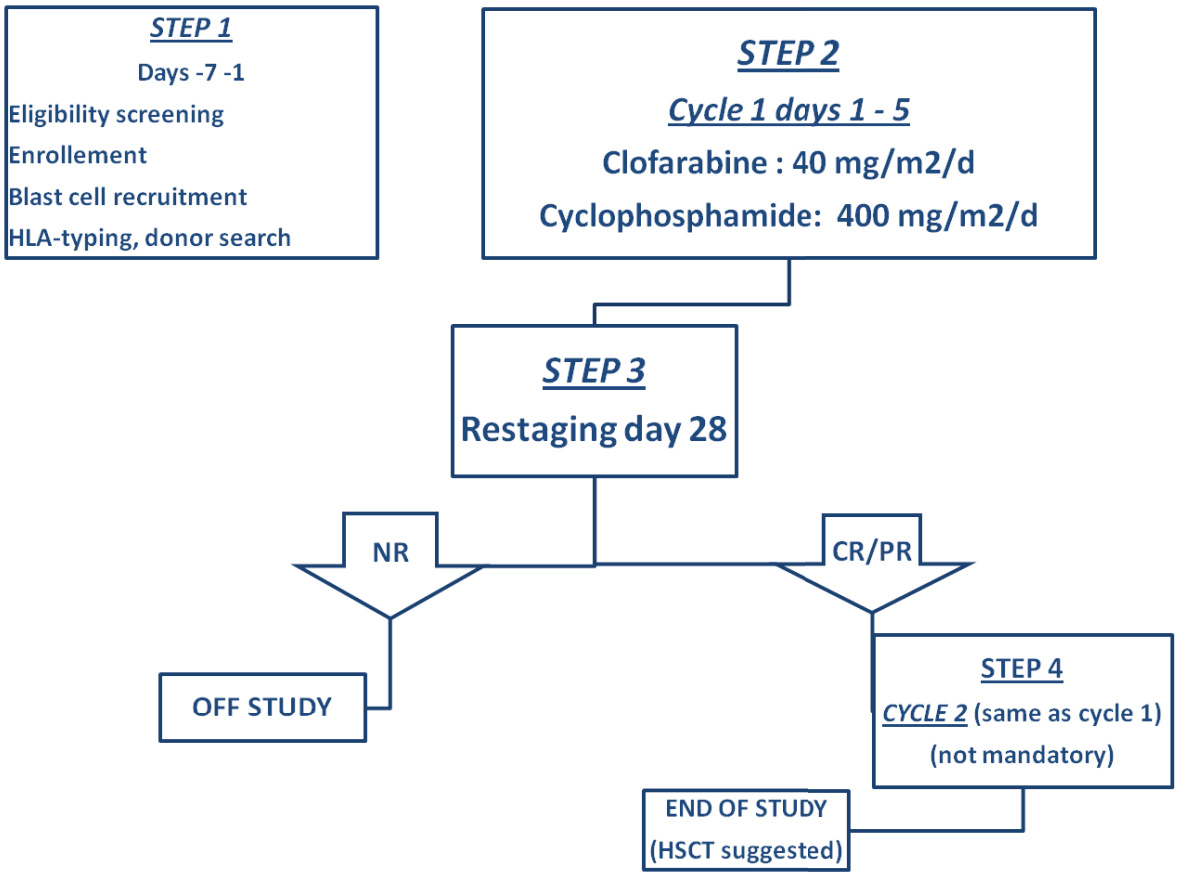
- **STEP 2**. Cycle 1 will be applied to all eligible patients once all enrolment criteria are confirmed.

- **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.

1.1.1 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



1.2 End-points

1.2.1 Primary

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

1.2.2 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.

1.3 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.

2 Criteria of evaluation

2.1 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).

2.2 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.

2.3 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.

3 Statistical considerations

3.1 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.

3.2 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.

3.3 Interim analysis

No interim analysis will be performed