ALLEGATO 3

nilg
northen italy leukemia group


# STUDY PROTOCOL

A multicentric randomized trial in adult patients with acute myelogenous leukemia (AML), to compare:

**1**) a standard-dose versus high-dose remission induction regimen, and

**2**) an autologous blood stem cell transplantation versus an autologous blood stem cell-supported multicycle high-dose chemotherapy program, within a risk-oriented postremission strategy reserving allogeneic stem cell transplantation for high-risk cases.

**(Protocol NILG-AML 02/06 – Versione 5 del 16/01/2013)**

# 1 INDEX

# 2 ABBREVIATIONS

| | |
|---|---|
| ABMT | Autologous Bone Marrow Transplant |
| AE | Adverse Event |
| ALT | Alanine Aminotransferase |
| AML | Acute Myelogenous Leukemia |
| ANC | Absolute Neutrophil Count |
| APL | Aplasia |
| APTT | Activated Partial Thromboplastin Time |
| AR | Adverse Reaction |
| Ara-C | Arabinosyl-cytosine/Cytarabine |
| AST | Aspartate Aminotransferase |
| BM | Bone Marrow |
| BU-CY2 | Busulfan - Cyclophosphamide |
| CALGB | Cancer and Leukemia Group B |
| CBF | Core Binding Factor |
| CIR | Cumulative Incidence of Relapse |
| CNS | Central Nervous System |
| CR | Complete Remission |
| CRc | Complete cytogenetic Remission |
| CRF | Case Report Form |
| CRi | Complete Remission with incomplete hematological recovery |
| CRm | Complete molecular Remission |
| CRO | Contract Research Organization |
| CSA | Cyclosporin A |
| CTC-NCI | Common Toxicity Criteria from the National Cancer Institute |
| DFS | Disease-free Survival |
| DSMB | Data Safety Monitoring Board |
| EBMT | European Group for Blood and Marrow Transplantation |
| ECOG | Eastern Cooperative Oncology Group |
| EGIL | European Group for the Immunological Characterization of Leukemias |
| EKG | Electrocardiogram |
| FAB | French-American-British (classification system) |
| G-CSF | Granulocyte-Colony Stimulating Factor |
| GPC | Good Clinical Practice |
| GU | Gazzetta Ufficiale |
| GvHD | Graft versus Host Disease |
| HBV | Hepatitis B Virus |
| HCV | Hepatitis C Virus |
| HD | High Dose |
| HDS | High Dose Sequential |
| HIV | Human Immunodeficiency Virus |
| HLA | Human Leucocyte Antigen |
| HR | High Risk |
| ICE | Idarubicin-Cytarabine-Etoposide |
| IDR | Idarubicin |
| IEC | Independent Ethics Committee |
| IND | Indeterminate |
| IR | Intermediate Risk |

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|---|---|
| LDH | Lactate Dehydrogenase |
| MDR1 | Multidrug Resistance Type 1 |
| MDS | Myelodysplastic Syndrome |
| MRC | Medical Reseach Council |
| MS | Myeloid Sarcoma |
| MUD | Marrow Unrelated Donor |
| NILG | Northern Italy Leukemia Group |
| NR | Non Response |
| NYHA | New York Heart Association |
| O | Other |
| OS | Overall Survival |
| PB | Peripheral Blood |
| PT INR | Prothrombin Time International Normalized Ratio |
| QoL | Quality of Life |
| R1 | Random 1 |
| R2 | Random 2 |
| RAEB-2 | Refractory Anemia with Excess of Blasts, type 2 |
| RDE | Remote Data Entry |
| REC | Recurrence |
| RES | Resistance |
| SAE | Serious Adverse Event |
| SCT | Stem Cell Transplant |
| SR | Standard Risk |
| SWOG | South Western Oncology Group |
| TRD | Treatment-Related Death |
| U | Unknown |
| UNE | Unevaluable (karyotype) |
| UNM | Unassessable (metaphases) |
| VP16 | Etoposide |
| WBC | White Blood Cell |
| WHO | World Health Organization |

# 3 SYNOPSIS

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| **TITLE** | A phase III trial in adult acute myelogenous leukemia (AML) comparing **1**) standard-dose versus high-dose remission induction therapy and **2**), within a risk-oriented postremission strategy, an autologous blood stem cell transplantation versus an autologous blood stem cell-supported multicycle high-dose program (Protocol NILG-AML 02/06). |
| **PROTOCOL VERSION** | No. 4, dated 9 april 2010 |
| **PATIENTS** | Adult patients with AML and related conditions, as per inclusion/exclusion criteria. All patients aged 16+ years will be included in the AML Prospective Register even if excluded, for any reason and at any point, from the clinical trials. |
| **STUDY DESIGN** | **AML Prospective Register**<br>Multicentric prospective registration and outcome study for all patients aged 16+ years with a new diagnosis of AML and allied diseases, regardless of their eligibility to the NILG-AML 02/06 trials.<br>**NILG-AML 02/06 Trials**<br>Multicentric, prospective, double randomisation assessing in different risk groups the risks/benefits of different therapeutic strategies for remission induction and consolidation.<br>**Remission induction (R1)**<br>**Pre-R1 stratification of randomisation**<br>• by age ≤ vs. > 60 years<br>**Randomisation :** standard ICE chemotherapy vs sequential high-dose cytarabine-containing therapy, with appropriate supportive/prophylactic measures as per disease and treatment type, and with morphological, cytogenetic and molecular monitoring of remission in responsive patients.<br>**Remission consolidation (R2)**<br>Patients are divided into standard and high risk cases (SR, HR) on the basis of clinical presentation and cytogenetic study results (the latter only known after the remission induction phase):<br>▪ **SR:** favorable cytogenetics, or intermediate risk cytogenetics without any adverse clinical risk factor (FAB M0, 6, 7 or corresponding WHO category; undifferentiated, bilineal or biphenotypic acute leukemia; myeloid sarcoma; MDS-associated AML or secondary AML, high-risk MDS [RAEB-2], FLT3 mutation, complete remission after cycle 2, persistence of preexisting cytogenetic abnormality despite morphological CR; total white cell count >50 $x10^9$/L; hepatosplenomegaly);<br>▪ **HR:** all non-SR cases.<br>**Risk-oriented therapy**<br>• HR patients will be electively submitted to allogeneic stem cell transplantation (allo-SCT), whenever possible (related/unrelated donor/cord blood; ablative/non-ablative conditioning according to national and local protocols and guidelines).<br>• Provided sufficient blood stem cells were previously collected (≥$2x10^6$/kg CD34+ cells), SR patients and HR patients excluded from allo-SCT and aged 65 years or less will be randomized in R2.<br>• HR/SR patients unable to be randomized in R2 because of inadequate blood stem cell yield will receive intermediate-dose consolidation<br>**Pre-R2 stratification of randomisation**<br>– by risk class (SR, HR) at registration and number of mobilized CD34+ blood stem cells (highest single apheretic yield < versus ≥7 $x10^6$/kg).<br>**Randomisation :** myeloablative autologous blood stem cell transplantation vs non-myeloablative, multicycle, autologous blood stem cell-supported high-dose cytarabine-based therapy.<br><br>**Age-limited therapeutic procedures**: Patients aged 60-65 years eligible to Random 1 but presenting with significant comorbidity can receive a decreased-intensity regimen if randomized to the experimental arm. Patients aged >65 years are treated with age-adapted therapy, and are excluded from R1 and R2 and transplant procedures, and will be followed in the observational outcome study. |
| **OBJECTIVES** | To evaluate comparatively the risk/benefit ratios of the experimental treatments, according to the following objectives:<br>**Remission induction (R1)**<br>**Primary endpoint**<br>Complete remission (CR) rate after cycle 1<br>**Secondary efficacy outcome measures**<br>1. CR with incomplete hematology recovery (CRi)<br>2. Complete cytogenetic remission (CRc)<br>3. Treatment-related death (TRD)<br>4. Feasibility and efficacy of treatments in different age and risk groups |

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| | **Safety outcome measures**<br>Toxicity (clinical adverse events)<br>**Remission consolidation (R2)**<br>**Primary endpoint**<br>Length of remission (DFS, disease-free survival)<br>**Secondary efficacy outcome measures**<br>1. Overall survival (OS)<br>2. Remission duration and cumulative incidence of relapse (CIR)<br>3. Treatment-related death (TRD)<br>4. Feasibility and efficacy of treatments in different age and risk groups<br>5. Significance and clinical correlates of remission monitoring results<br>6. Quality of Life evaluation in long term survivors<br>**Safety outcome measures**<br>Toxicity (clinical adverse events)<br>**Outcome study**<br>Descriptive analyses of the patients not included in R1 and R2.<br>Prospective analysis of risk-oriented allogeneic stem cell transplantation strategies.<br>For patients in Random 1, explorative analyses of long-term overall survival and DFS and CIR (cumulative incidence of relapse) will be performed. |
| **SAMPLE SIZE** | ≥500 patients (see Statistical considerations). |
| **NUMBER OF CENTRES** | 16 participating in the Northern Italy Leukemia Group (NILG) network. |
| **STUDY POPULATION** | **AML REGISTER**<br>**Eligibility criteria**<br>1. Age 16+ years.<br>2. Diagnosis of untreated (or only hydroxyurea/cyclophosphamide) acute myelogenous leukemia (AML, including myeloid sarcoma and acute promyelocytic leukemia) or high-risk myelodysplasia (RAEB-2), either *de novo* or following an antecedent hematological disorder, or secondary to chemo-radiotherapy for other cancer.<br>3. Signed informed consent for the outcome research study.<br><br>**Remission induction (R1)**<br>**Inclusion criteria**<br>1. Age 16-65 years.<br>2. Diagnosis of untreated (or only hydroxyurea/cyclophosphamide) acute myelogenous leukemia (AML, including myeloid sarcoma) or high-risk myelodysplasia (RAEB-2), either *de novo* or following an antecedent hematological disorder, or secondary to chemo-radiotherapy for other cancer.<br>3. Adequate sampling for full cytological, cytochemical, cytogenetic and immunobiological disease characterization by revised FAB, EGIL and WHO criteria.<br>4. ECOG performance status 0-2 or reversible ECOG 3 score following intensive care of complications.<br>5. Signed informed consent.<br><br>**Exclusion criteria**<br>1. Diagnosis of acute promyelocytic leukemia.<br>2. Pre-existing, uncontrolled pathology such as cardiac disease (congestive/ischemic, acute myocardial infarction within the past 3 months, untreatable arrythmias, NYHA classes III and IV), severe liver disease with serum bilirubin >3 mg/dL and/or ALT >3 x upper normal limit (unless attributable to AML), kidney function impairment with serum creatinine >2 mg/dL (unless attributable to AML), and severe neuropsychiatric disorder that impairs the patient's ability to understand and sign the informed consent, or to cope with the intended treatment plan.<br>3. Known HIV positive serology.<br>4. Other active hematological or non-hematological cancers with life expectancy <1 year.<br>5. Pregnancy (fertile women will be advised not to become pregnant while on treatment; and male patients to adopt contraceptive methods).<br><br>**Remission consolidation (R2)**<br>**Inclusion criteria**<br>1. Confirmed CR status after Random 1.<br>2. Sufficient amount of autologous blood stem cells (≥2 x$10^6$/kg) following A8 consolidation/mobilization chemotherapy cycle no. 3.<br>3. Age ≤65 years.<br>4. Signed informed consent by the patient or parent/tutor in patients aged <18 years.<br>5. SR risk class or HR risk class if ineligible to allogeneic SCT. |

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| | 6. Functional echocardiography of the left ventricle with ejection fraction ≥50%.<br><br>**Exclusion criteria**<br>1. Unresolved hematological or extrahematological toxicity of CTC grade II or greater<br>2. Unresolved/unimproved bacterial or fungal infections from prior therapy, involving major anatomical sites (central nervous system, respiratory tract, gastrointestinal system, genito-urinary system, soft tissues and muscolo-skeletal structures) and requiring patient hospitalization with use of parenteral antimicrobial and antifungal drugs |
| **TREATMENT AND STUDY DRUGS** | **Remission induction (R1)**<br>**Standard arm**<br>1. Conventional-dose ICE chemotherapy (cycle 1, days 1-7: idarubicin, cytarabine, etoposide, G-CSF), followed by IC chemotherapy (cycle 2, days 1-7: idarubicin, cytarabine, G-CSF) in patients achieving an early response<br>2. Only in the case of documented refractoriness to ICE chemotherapy cycle 1, high-dose chemotherapy will be delivered as cycle 2 instead of IC. Treatment will include idarubicin with cyclosporin A to downmodulate the multidrug resistance type 1 mechanism, and high-dose sequential cytarabine 3 g/m$^2$/dose (2 g/m$^2$ in patients aged >60 to 65 years), and G-CSF (HDS-3/-2).<br>**Experimental arm**<br>1. High-dose sequential chemotherapy (HDS-2) (cycle 1, days 1-3 and 8-10: idarubicin, cytarabine 2 g/m$^2$/dose (1 g/m$^2$ optionally in "frail" patients aged 60-65 years, HDS-1), G-CSF), followed by IC chemotherapy (cycle 2, days 1-7: idarubicin, cytarabine, G-CSF) in patients achieving an early response<br>2. Only in the case of documented refractoriness to HDS-2, further high-dose chemotherapy will be delivered as cycle 2 instead of IC. Treatment will include high-dose cytarabine 3 g/m$^2$/dose (2 g/m$^2$ if aged >60 to 65 years), idarubicin with cyclosporin A to downmodulate the multidrug resistance type 1 mechanism, and G-CSF (HDS-3/-2), like in standard arm patients refractory to ICE chemotherapy.<br>Following induction and early IC consolidation, all patients in both treatment arms will receive chemotherapy cycle 3 (days 1-4: cytarabine at cumulative dose 8 g/m$^2$ and G-CSF) (A8) for autologous blood stem cell harvest and cryopreservation. Second randomisation will occur at this stage for all patients excluded from allogeneic stem cell transplantation and in whom sufficient blood stem cells were collected.<br>**Remission consolidation (R2)**<br>**Standard arm**<br>Autologous blood stem cell transplantation and G-CSF following high-dose busulphan-cyclophosphamide and no further therapy beyond that (i.e. observation). Reinfused blood stem cells will be 2-6 x10$^6$/kg CD34+ cells.<br>**Experimental arm**<br>Three consecutive, monthly high-dose cycles (days 1-6: idarubicin, cytarabine at cumulative dose 20 g/m$^2$) (A20), each followed by the reinfusion of 1-2x10$^6$/kg CD34+ autologous blood stem cells and G-CSF. Because a minimum of two A20 courses is considered adequate, total amount of reinfused blood stem cells will be 2-6 x10$^6$/kg like in the standard arm of the trial. Treatment is followed by observation.<br>**Risk-oriented postremission therapy:** patients not included in R2 will be enrolled in an outcome study, including analysis of allogeneic stem cell transplant strategies<br>**See above for age-limited therapeutic procedures** |
| **STATISTICAL METHODS** | The primary end-points for the calculation of sample size are the complete remission (CR) rate after cycle 1 for R1 and disease-free survival (DFS) for R2.<br>**Current clinical expectations**<br>Both the literature and prior data of NILG suggest a CR rate after cycle 1 of 80% (15% refractory) and 65% (30% refractory) for SR and HR patients, respectively.<br>It can be assumed a global CR rate of 420 CR out of 500 patients originally randomized in R1 (84%), an expected drop-out rate of 50% (relapse, toxicity, early allo-SCT in HR cases, lack of stem cell mobilization, unacceptance of the trial), and an expected DFS at 4 years of 60% and 25% in SR and HR patients, respectively.<br>**SAMPLE SIZE**<br>**Remission induction (R1)**<br>It will be necessary to accrue 250 patients per arm with the expectation of 38% relative risk reduction in favor of the experimental arm (i.e., 174 vs. 204 patients in CR after cycle 1) with an alfa error of 0.05 (two-tailed) and power 80%.<br>**Remission consolidation (R2)**<br>Two hundred ten patients can be expected to be randomized in R2 with the expectation of 32% relative risk reduction in favor of the experimental arm (i.e., 38 vs. 59 patients with 4-year DFS) with an alfa error of 0.05 (two-tailed) and power 80%. |
| **LENGTH OF STUDY** | Starting in january 2007, 5 years for patient enrolment and a minimum of 1 year of follow-up from date of randomisation of the last patient.Accordingly, study results should be amenable to final analysis in 2013. Patients enrolled in the outcome studies are followed-up annually to assess survival and |

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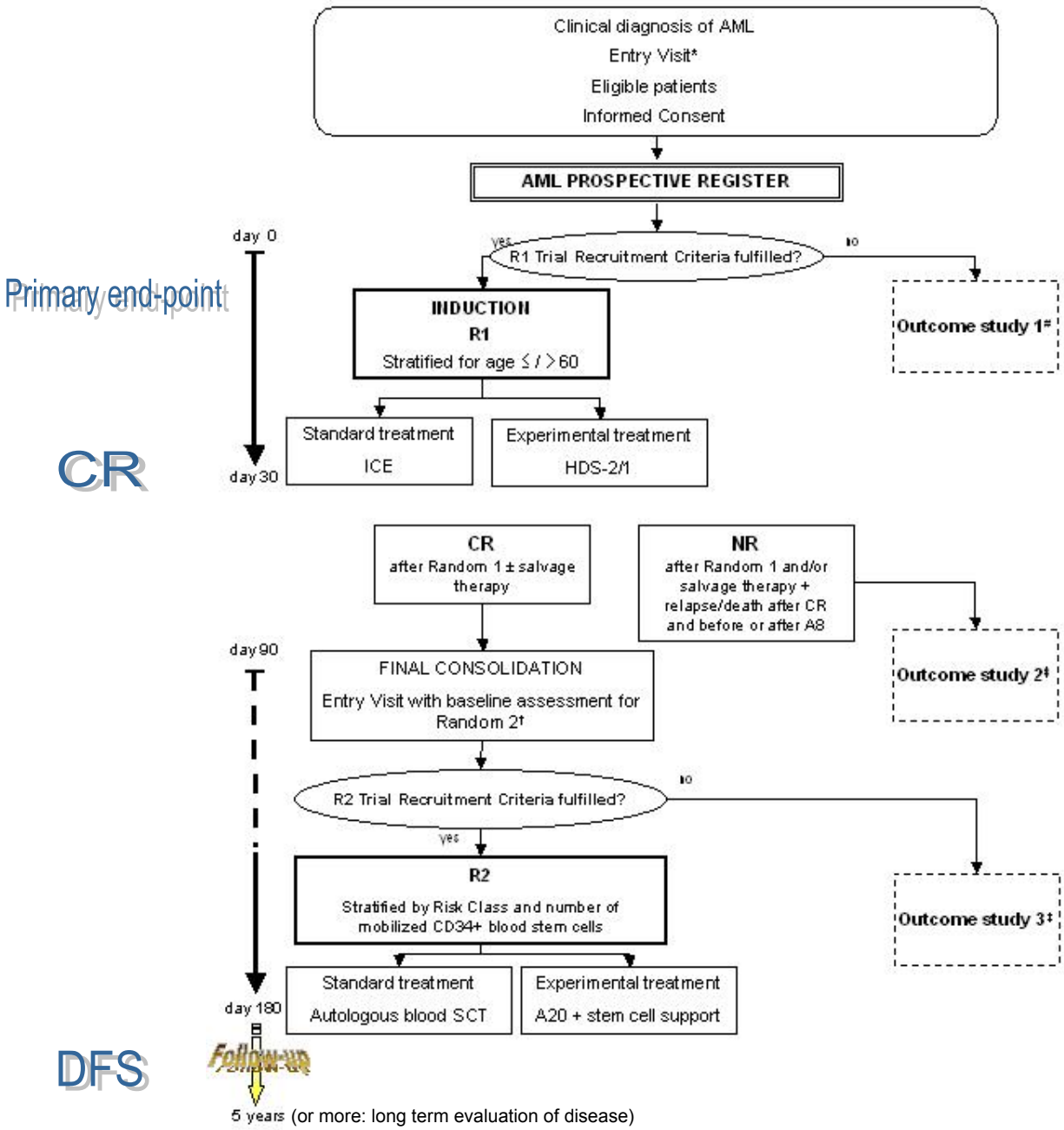
| | disease status. |

# 4 FLOW-CHART (Figure 1)




* screening tests in *section* **15.1**

# updated yearly.

† Evaluation of all relevant prognostic characteristics, including complete remission (CR), response to salvage therapy and remission status after A8.

$ All NR after a) ICE or HDS-1 for patient aged >65 yrs, b) ICE or HDS-2 and Salvage HDS-2/3 for patients aged ≤65 yrs and c) relapse/death in CR before or after A8. Updated on a yearly basis to assess survival and disease status.

‡ All patients excluded from Random 2, including prospective analysis of allogeneic transplantation strategies. Follow-up visits are performed yearly

# 5 Diagnostic Procedures and Clinical Conduct before/during Study

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| PRE-TREATMENT EVALUATION | • Bone marrow (BM) morphology (aspirate, core biopsy) and peripheral blood (PB) morphology according to FAB/WHO criteria; cytochemical reactions and immunophenotyping for AML diagnosis and subclassification; cytogenetics and selected molecular biology assays.<br>• Centralization and storage of diagnostic/biological material for research purposes (minimal residual disease, future studies) at prefixed time points.<br>• Medical history, physical examination, ECOG performance status, chest X-ray, ultrasound scan of abdomen for hepatosplenomegaly.<br>• Pregnancy test (when indicated).<br>• 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).<br>• Serology for HBV, HCV, and HIV infections.<br>• Cytology/biopsy of suspect sites of disease (CNS, pleural effusion, skin, etc.) to define the extent of extramedullary involvement.<br>• Blood group and HLA-DR analysis, the latter up to age 65. |
| TREATMENT AND CLINICAL MONITORING | **<u>During remission induction (cycle 1 ICE or HDS-2/-1 or cycle 2 HDS-3/-2)</u>**,<br>• Physical examination, ECOG performance status, full blood counts at least on alternate days until remission, serum biochemistry at least weekly. Continuous toxicity evaluation.<br>**<u>Assessment of response (after cycle 1 ICE or HDS-2/-1 or cycle 2 HDS-3/-2)</u>**<br>• On day 28 (or earlier/later as clinically indicated) from day 1 of chemotherapy: BM examination (morphology; cytogenetics if previously abnormal); PB examination.<br>**<u>Assessment of response (during postremission therapy)</u>**<br>• Physical examination, ECOG performance status, full blood counts at least on alternate days while in-hospital, serum biochemistry weekly. Toxicity evaluation.<br>• BM examination in the case of any abnormal physical or hematological finding suggesting AML regrowth.<br>• BM and PB sampling for residual disease evaluation (at time-points indicated in the protocol).<br>• Biological follow-up, i.e. analysis of PB/BM samples for selected gene rearrangements/anomalies (CBF-translocations, NPM mutation etc.) in CR patients at pre-fixed time points as per protocol specifications.<br>**<u>Clinical Follow-up for all CR patients</u> (3-monthly for 3 years from end of final consolidation, i.e. 90 days after R2 for standard arm and 30 days after third A20 for experimental arm; 6-monthly subsequently).**<br>• Physical examination, ECOG performance status, full blood count, serum biochemistry. Toxicity and Quality of Life evaluation.<br>• BM examination in the case of any abnormal physical or hematological finding suggesting AML regrowth.<br>• BM and PB sampling for residual disease evaluation (time-points indicated in the protocol). |

# 6 TREATMENT PLAN (Figure 2)

The aim of this clinical study in adult AML is to compare by risk category (1) the complete remission rate to ICE or HDS-2 chemotherapy and (2) the disease-free survival following consolidation with either a myeloablative autologous blood stem cell transplantation or blood stem cell supported cycles with high-dose cytarabine, reserving allogeneic stem cell transplantation for cases with high-risk characteristics. Patients have a diagnosis of AML (excluding promyelocytic) or high-risk myelodysplasia and are aged 16+ years (no upper age limit). All AML patients will be registered into an outcome research project, regardless eligibility to the therapeutic trial. The first flow-chart (**Figure 2a**) pertains to patients aged 16-65 years (the HDS-1 option is reserved to "frail" patients aged 60-65 years). Patients aged 66+ years are treated with age-adapted therapy, are off study if not in CR after cycle 1, and are excluded from randomisation and transplant procedures (**Figure 2b**).

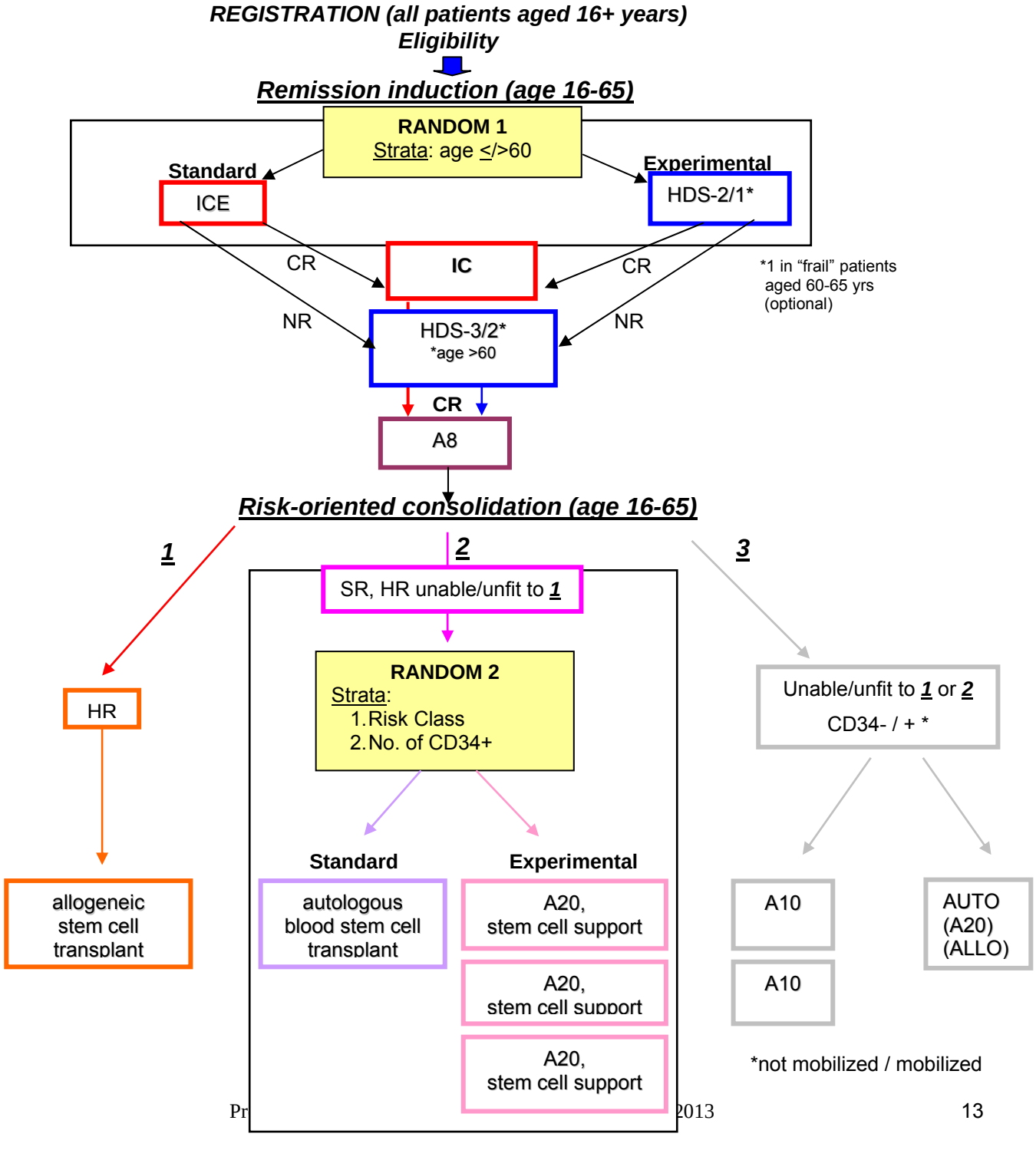


**Figure 2a**

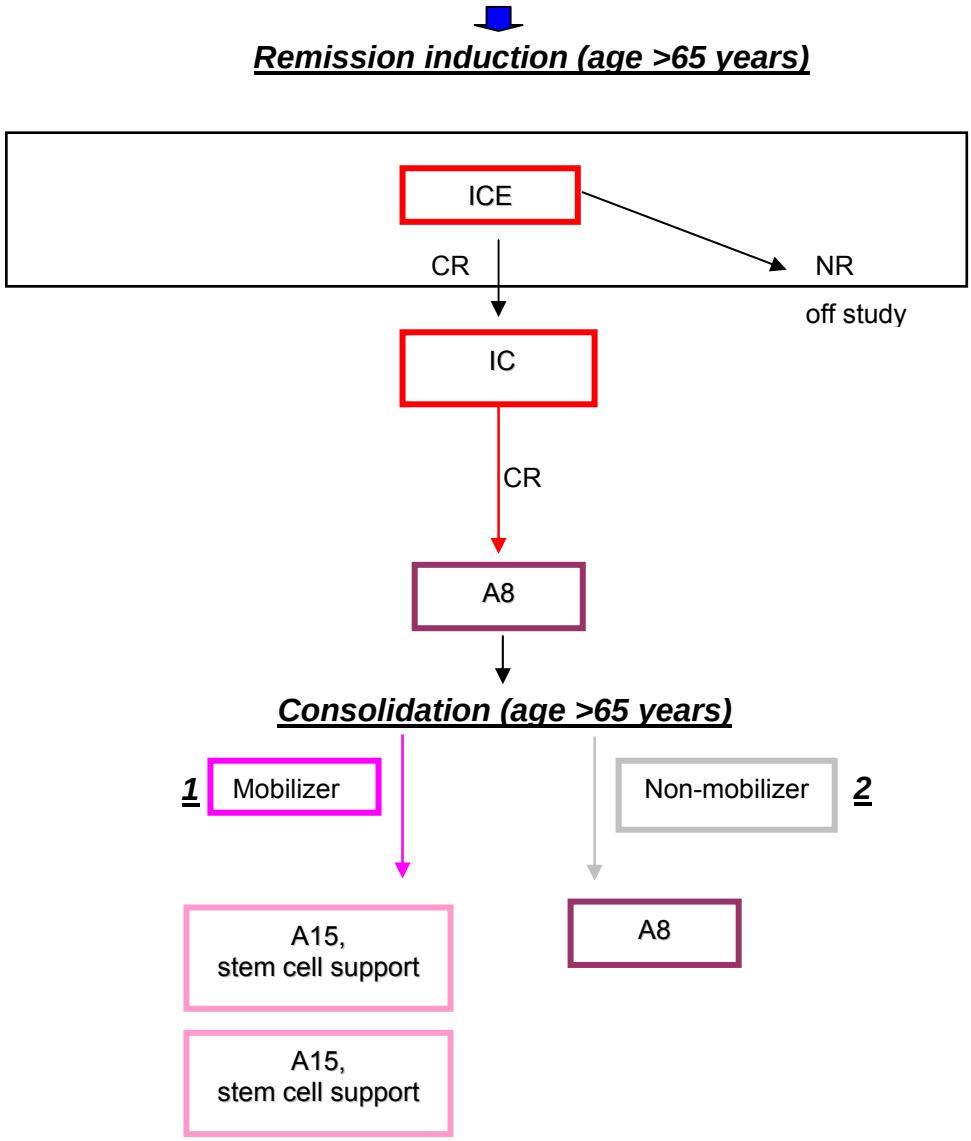

Remission induction (age >65 years)
ICE
CR
NR
off study
IC
CR
A8
Consolidation (age >65 years)
1
Mobilizer
Non-mobilizer
2
A15,
stem cell support
A8
A15,
stem cell support

**Figure 2b**

# 7 BACKGROUND AND RATIONALE

## *7.1 PROBLEMS IN THE MANAGEMENT OF ADULT AML*

The many problems inherent the management of adult AML are reviewed in **Appendix 1**. As shown, treatment is traditionally subdivided into the remission induction phase and the postremission consolidation phase. Results over the last two decades have remained consistently stable, with some differences among trials being explained by inclusion criteria, especially the upper age limit (from 50 to 65 years, with a pejorative prognostic effect from ageing) and the inclusion into study of AML related to myelodysplasia (MDS) or secondary to chemoradiotherapy or with higher incidence of adverse karyotype despite a *de novo* disease (with pejorative effects even with the application of intensive therapy and allogeneic SCT)[1-3]. Bearing in mind these important differences, most studies reported an initial CR rate of 60%-80% and a 3-5 year DFS rate of 25%-45%.

## *7.2 DO PROGNOSIS - TO -TREATMENT RELATIONSHIPS EXIST?*

It is evident how prognostic variables can significantly affect outcome. These facts were reviewed in detail (**Appendix 1**) and will not be mentioned here. One crucial question is whether prognostic factors can be used to modify therapeutic choices in individual patients. This requires:

- (1) the recognition of reliable risk factors;
- (2) the availability of different treatments that are more or less indicated for different risk classes.

Presently, it can be concluded, with regard to the questions raised, that:

- (1) different risk groups can be recognized on account of the cytogenetic risk profile of leukemic cells (plus other selected factors);
- (2) different therapeutic options could be preferably employed in different prognostic groups, in view of either an increased therapeutic efficacy or, when equivalence is demonstrated/expected, of a reduced toxicity.

These issues are reviewed in detail below (*sections* **7.3**, **7.4**) and represent the bulk of evidence supporting the present clinical trial.

## *7.3 RISK CLASSES IN ADULT AML: CYTOGENETICS AND OTHER*

The risk is herein defined as the cumulative prognostic effect exerted by all (known) prognostic factors in a given patient. Although terminology is largely arbitrary, in looking at published adult AML trials, standard/good risk (SR) patients do usually have CR rates >80% and DFS rates $\geq$50%, whereas in the high-risk (HR) group corresponding figures are $\leq$50% and $\leq$25%, respectively. Intermediate risk (IR) cases share an intermediate outcome. The three risk classes (where “intermediate” is sometimes substituded by “standard” when “good” is concurrently used to define the best risk group) are nowadays identifiable mainly through the cytogenetic study on AML blasts, as convincingly shown by MRC, ECOG-SWOG and CALGB studies and widely accepted (**Appendix 1**)[4].

Clearly, cytogenetics is not the only important factor. A fault of a purely cytogenetic risk classification is that standard/good risk cases represents only a minority of all cases, and that the large (two thirds) IR group with intermediate DFS of about 40% lacks a precise prognostic definition. Thus efforts are devoted to identify additional prognostic indicators. Apart from some useful clinical parameters (WBC counts, age, multidrug resistance proteins etc.), insights into disease genetics are highly informative. For instance (**Appendix 1**), FLT-3 mutations (either internal tandem duplications or point mutations) in cases with normal/IR cytogenetics can have a worsening prognostic effect, decreasing variably the expected CR and DFS rates, the outcome of FLT-3+ IR cytogenetic cases being more similar to that of HR cases[5-10]. Similar examples from overexpression or, on the contrary, downregulation of other genes/proteins (such as BAALC, CEBPA, bcl-2/bax, MLL, NPM etc.) were produced in a few years, to show the influence in IR group of several genetic alterations that normally go undetected unless a specific molecular assay is performed[11-22]. A comprehensive approach to analyse the genetic disturbance in AML can be taken with the microchips technique, studying at one time the expression of thousands of genes. These studies document a strict correlation between gene profiling results, AML subtype, and underlying cytogenetic anomaly[23-27]. Again, the adoption of this technique in the IR group allowed to detect subsets at significantly better or worse prognosis.

## 7.4 *TREATING AML: DIFFERENT OPTIONS VS. RISK CLASS*

### *7.4.1 CR induction*

Standard CR induction is attempted with a "3+7" or "DAT"-like regimen. CR figures reported above refer to the results achievable with these regimens, with or without minor variations represented by days/dosage of conventional cytarabine, type of anthracyclinic drug, additional use of etoposide/6-mercaptopurine/G-CSF. These regimens are not effective in about 10%-20% of the patients, the risk of failure correlating directly with the risk class (see *section* **7.3**).
Thus attempts have been initiated to overcome resistance by using more effective drugs frontline. An ideal candidate is HD-Ara-C (high-dose cytarabine: $\geq$1 g/m$^2$/dose). Several large national groups performed or are carrying out controlled clinical trials with HD-Ara-C in induction (**Appendix 1**)[28-31], others have extensively used the drug in open studies, with sophisticated mathematical models to validate its usefulness[32] or showing a positive effect even in unfavorable cytogenetic risk groups[33]. In a meta-analytical review so far published in abstract form only (Kern W and Estey EH: *HD-Ara-C in induction therapy of AML: meta-analysis of three trials involving 1,691 randomized patients*. Blood 2002; 100: 198a), HD-Ara-C did not improve CR. However, no data were provided as regards different cytogenetic and/or clinical risk classes, and it transpires (the studies reviewed were the three older ones) that most of the observed toxicity could be abscribed to the 3 g/m$^2$ regimen and to the lack of G-CSF support. G-CSF was later shown to decrease significantly neutropenia and neutropenic complications in HD-Ara-C-treated patients[34]. More importantly, both the meta-analysis, the cited studies and others[35] found that HD-Ara-C in induction, whether improving or not CR rate, led to a significant prolongation of DFS and had a statistically positive impact on overall survival too. Interestingly, when a timed-sequential induction schedule was used, with a tight sequence on days 1 and 8, results were improved too[36]. At the present, further experience needs to be collected with HD-Ara-C in induction, particularly as regards its effects in different risk subsets and exploiting further the concept of timed-sequential therapy (as in the salvage protocol previously used, **Appendix 2**).
There are other drugs to be considered as part of an induction regimen, namely fludarabine (to increase intracellular Ara-CTP retention following exposure to cytarabine), and the calycheamycin-conjugated anti-CD33 monoclonal antiboby. As of september 2005, neither agent is licensed for use in first-line therapy of adult AML in Italy (**Appendix 1**).

### *7.4.2 CR induction: prior NILG data*

In NILG AML study 01/00 (**Appendix 2**) patients were stratified according to cytogenetics (SR, IR, HR) plus selected clinical features, FLT-3 mutations, and response to induction cycle 1. On the basis of additional risk factors, IR cases were incorporated into SR and HR groups, respectively. This distinction proved effective, allowing to separate early SR cases (one third of the total; CR rate after ICE 90.5%) from HR ones (CR 75.5%, P=0.002). A more detailed analysis of outcome in relation to discrete clinico-cytogenetic risk groups is given below (**7.4.4**).

### *7.4.3 Refractory AML*

Persistence of AML following standard-type induction regimens is reported in 10%-20% of the cases (**Appendix 1**). One question concerns the definition of refractoriness. In the past, refractory AML was defined after the failure of two identical standard induction courses. However, the likelihood of entering CR on repeating the same chemotherapy course or excluding intermediate/high-dose Ara-C is only 26.4%-38%[37,38]. Meanwhile, studies on refractory and relapsed disease showed response rates in the order of 40%-50% by using a variety of HD-Ara-C-containing regimens, suggesting a benefit from this treatment as compared to a second conventional course. These observations constitute an obvious background to explore this drug earlier on (**7.4.1**), i.e as second cycle in nonresponsive cases. This issue was developed by NILG in a formal study strategy.

### *7.4.4 Refractory AML: prior NILG data*

In the previous NILG trial, ICE-unresponsive patients were transferred to a high-dose, cytarabine-based salvage. Observed results met study expectations, the salvage rate being about 50%. Another important observation concerned the outcome of different risk categories (**Appendix 2**). As shown, the efficacy of the

salvage cycle was not affected by cytogenetic or clinical risk factors. On the contrary, if one looks at CR rates after ICE, the risk of refractory AML increased progressively (from ≤10% to 43%) with the worsening of the clinico-cytogenetic risk profile.
Because ICE-refractory cases are the same salvaged by the high-dose regimen, it may extrapolate that an earlier use of this schedule (as cycle 1) would increase the early CR rate in a sizable proportion of cases, i.e. half of those refractory to ICE. For instance, in the cytogenetic IR group, 22/40 (55%) more patients would enter CR, and a further 9/20 (45%) in the HR group. Because an early CR is a recognized favorable prognostic factor for both DFS and overall survival, and because of the reported positive effect on long-term outcome from an early HD-Ara-C application (**7.4.1**), these are seminal observations for the the induction trial considered in the current study.

### *7.4.5 Postremission therapy: choice and toxicity issues*

Conceptually, the discussion as to the best postremission regimen revolves around the same lines as induction problems and has been examined in detail (**Appendix 1**). Some important issues concern toxicity and peculiar aspects of the reviewed treatments:

- (1) Chemotherapy. Attendant risks are mostly limited to few weeks following drug exposure and essentially tuned on the intensity (i.e. drug types and dosages) and number (i.e. repetitive toxic phases) of chemotherapy cycles. Short-term toxicity: mainly mucositis and neutropenic complications; long-term toxicity: negligible in well performing cases; cardiotoxicity possible after high cumulative doses of anthracyclines; fertility is usually preserved. Short-term toxicity from myelosuppression associated with the use of HD-Ara-C is the worst (**Appendix 2**), with an expected remissional death rate of 8% on the average.
- (2) Autologous bone marrow/blood stem cell transplantation. Short-term toxicity is higher than with chemotherapy; the risk of remissional death can be as high as 10% but seems to be reducible with the use of G-CSF-mobilized blood stem cells (there is no definitive proof for that). One study focused on the relationship between number of CD34+ cells and risk of posttransplantation relapse, documenting an increased risk for patients able to mobilize in a single apheresis (i.e. the highest single yield of 1-6 aphereses) ≥7 x$10^6$/kg CD34+ cells[39]. Long-term toxicity can be substantial following exposure to total body irradiation and/or high-dose alkylating agents or etoposide (endocrine and gonadal damage inducing sterility in virtually all cases, veno-occlusive disease of the liver using busulfan, cataracts using TBI, aseptic bone necrosis, second cancers including leukemias etc.) (**Appendix 1**)[40-46].
- (3) Allogeneic stem cell transplantation. The toxicity arising from immune reactions between the graft and host (globally indicated as GVHD, acute and chronic) and from the necessary immunosuppressive medications is additive to that from the high-dose conditioning regimen and may create the most dangerous conditions for developing life-threatening complications. Treatment-related death may occur in up to 20% of the cases, with ample variations depending on the age limit considered and other disease- and procedure-related factors (**Appendix 1**). A recent retrospective Registry study emphasized the risk of procedural deaths by unrelated-donor SCT, the leukemia-free survival being no better than that achieved with autotransplantation in comparable patient groups, despite the reduced risk of relapse conferred by MUD-SCT[47]. One important issue may regard the general impact of different SCT strategies (from related/unrelated/haploidentical donor, or cord blood) in an unselected patient population including a large fraction of cases above the age threshold most usually considered for the procedure (<45-55 years). This kind of registration study is a current objective of EBMT.

### *7.4.6 Postremission therapy and toxicity: prior NILG data*

Toxicity issues in postremission therapy were tackled in NILG AML trial 01/00 (**Appendix 2**). In this study, one major objective was to keep HD-Ara-C-related toxicity as low as possible by adopting, for patients selected for repetitive HD-Ara-C-based courses, an autologous blood stem cell (plus G-CSF) support following each of the three planned cycles, in order to reduce the risk of prolonged marrow hypoplasia and pancytopenic death. This strategy proved effective, allowing rapid blood count recovery and early discharge from the hospital. Among the first 96 evaluable patients receiving a total of 169 A20 cycles (i.e. a course with cumulative Ara-C 20 g/$m^2$ plus IDR), no death was registered during the in-hospital stay, compared with 8% in the historical experience. Multiple HD-ara-C courses were administered at rather short intervals to unselected patients in first CR, with no or very little acute hematological grade III-IV toxicity.

### *7.4.7 Postremission therapy: options and results by risk class*

As reviewed (**Appendix 1**), the issue as to the best therapy for CR patients has attracted much interest and stimulated the inception of randomized clinical trials over the past 25 years. One accepted conclusion was that allogeneic stem cell transplant is the preferred option for HR cases, in whom the risk of relapse with any other treatment modality is so high as to offset the higher toxicity burden of transplantation.
Much of the remaining debate concerns:

- (1) toxicity problems (see *sections* **7.4.5, 7.4.6**);
- (2) the fact that, because of nearly superimposable results in older studies (or frankly superimposable results as found in newer studies and meta-analytical reviews)[41,43,48-52], there is often no clearcut therapeutic advantage for ABMT/SCT over chemotherapy in SR/IR classes.

This issue is further compounded by:

- (1) the widespread use of peripheral blood-derived stem cells in recent years (that may reduce significantly the risk of mortality during an autotransplant);
- (2) the counterbalancing evidence that a small amount of autologous stem cell support may allow to administer safely repetitive HD-Ara-C courses to unselected patients (see *section* **7.4.6**, **Appendix 2**);
- (3) the fact that most randomized trials included patients with an upper age limit of 45-55 years, thereby excluding the bulk of older age groups, for whom it may be even more difficult to apply (and then compare in terms of both efficacy and toxicity) the enlisted treatment procedures.

Interestingly, the benefit from repetitive HD-Ara-C cycles was confirmed in recent publications from CALGB and other groups, not only in patients with CBF-mutated type AML and favorable-risk cytogenetics but also in patients with normal cytogenetics i.e. in a large proportion of the IR group[35,53-58].

### *7.4.8 Postremission therapy: options and results by risk class in prior NILG study*

In NILG study 01/00 a flexible postremission approach was adopted (**Appendix 2**). Although the study is not yet amenable to final analysis, interim evaluations confirmed allogeneic SCT as the preferred treatment option for HR cases wheres the SR category was successfully managed by adopting three consecutive A20 courses with autologous stem cell support. The main conclusion after a maximum follow-up period of >4 years, is that SR patients (comprising IR cytogenetics without any additional clinical risk factor) obtaining a CR can plateau at approximately 50% in the DFS analysis, with a modest detrimental effect exerted by an age >55 years. It was demonstrated that older age patients tolerate as well as younger ones the three consecutive A20 cycles, to confirm the value of the stem cell support in a traditionally difficult-to-treat patient population. According to the data, 80% of CR patients would be able to mobilize enough autologous CD34+ blood stem cells as to support all three HD-Ara-C cycles or, alternatively, an autografting procedure.

## *7.5 SUMMARY ON CURRENT INDICATIONS AND PROBLEMS IN THE MANAGEMENT OF ADULT AML*

In the year 2005, given the data generated by the international experience as well as by NILG in AML study 01/00 (more that 400 patients enrolled), there are some statements that may support the design of a new clinical trial. Some of the conclusions stem directly from the issues insofar reviewed. For others, new data will be presented.

### *7.5.1 How to select patients and disease subtypes*

Selecting an upper age limit is crucial for the design and the interpretation of a clinical trial. Patients known as “older” adults, i.e. those above 55 years of age pose specific therapeutic questions (prevalence of unfavorable prognostic factors such as poor-risk cytogenetics, multidrug resistant phenotypes, incidence of MDS-AML) and can exhibit a reduced therapeutic tolerance. On the other hand, AML peaks at this age and above, and many older patients are nowadays very fit and healthy and tolerate well (semi)intensive treatments if well supported. Prior NILG experience demonstrated no major difference in response and toxic complications between patients aged <55 years and those aged 55-68 years. Presently, there is no objective reason to exclude *a priori* a fit patient up to an age of 70 from a therapeutic trial in adult AML.
With regard to disease subtype, patients with AML following MDS or secondary to chemoradiotherapy for other cancers constitute a peculiar clinico-prognostic subtype. However there is no evidence that their treatment should differ from that of *de novo* AML (provided they are clearly recognized as HR).

For both older adults (56-70 years) and/or those with MDS/secondary-AML, subgroup analyses can be easily obtained, to assess both treatment feasibility and efficacy. The exclusion of these cases would cause a significant study bias (artificially improving results) and has no theoretical basis, except for the induction of an increased risk of treatment failure.
Specific disease subtypes to be included into study are all FAB/WHO AML variants and HR MDS that is prognostically indistinguishable from AML (**Appendix 3**), excluding only acute promyelocytic leukemia.

### *7.5.2 How to induce CR*

CR should be induced with one chemotherapy course. This would be extremely cost-effective, reducing in-hospital stay, need for intravenous medications, psychological distress, and above all it would improve survival and DFS. Standard induction is inadequate for many patients with any one or more HR characteristics, and the majority of the patients present with at least one HR feature. New regimens must be designed and tested, using good supportive measures and assessing results in comparable risk subsets. The interest on anthracyclines is now reduced compared to previous years, with idarubicin remaining an outstanding drug within this type of products. HD-Ara-C is candidate to substitute for standard-dose cytarabine in two/three-drug combinations. Studies were incepted worldwide. Optimal schedule and dosage (1 vs. 2 vs. 3 g/m$^2$) are unknown, particularly in older age groups (>60 years) where the dosing of 3 g/m$^2$ does not seem applicable because of the toxic side effects with high death rate observed in one consolidation trial from CALGB, whereas 1 or 2 g/m$^2$ were (and are) normally used in induction without clearcut differences in outcome (past SWOG and ongoing AMLCG trials). Other drugs are of interest but are not licensed as first line therapy in Italy and therefore will not be reviewed.
Patients with refractory AML are best treated with an alternative salvage regimen rather than with the same induction course. The salvage schedule may contain drugs able to influence positively the response to anthracyclines (MDR1 inhibitors, or liposome-encapsulated anthracyclines) and cytarabine (fludarabine), or both, and increased-dose cytarabine (i.e. 3 g instead of 1-2 g/m$^2$/dose).

### *7.5.3 How to assess CR*

Morphological CR is a sound prerequisite to improve survival (**Appendix 1**). However, a number of studies have confirmed a predictive role for additional investigations such as the kinetics of blast cells clearance[59] and the persistance of submicroscopic disease, either in the form of cytogenetic lesion at time of morphological CR[60] or of gene rearrangements/abnormal expression in cases with an assessable anomaly[61-64] or of AML-associated immunophenotype[65,66].Cases with detectable minimal residual disease (MRD) have a significantly increased risk of relapse within weeks/months from a positive assay, and should perhaps be considered for increased-intensity therapy regardless the initial clinico-cytogenetic risk class. This concept is already well established in acute lymphoblastic leukemia. More recently, gene transcripts were identified (WT1, PRAME) that could allow for the monitoring of MRD in all CR patients, or even in a large number of cases with normal cytogenetics (NPM). The need for these studies was underlined in a reappraisal of the definition of CR by an international *ad hoc* Committee[67]. This evaluation includes also an early assessment of the clearing of blast cells from the bone marrrow and the peripheral blood.

### *7.5.4 How to define risk classes*

Cytogenetic analysis is the baseline for risk class assignment. In cases with favorable cytogenetics i.e. t(8;21) or inv(16), the role of additional abnormalities is as yet undefined. Some studies confirmed a dominant prognostic significance of CBF mutations over additional chromosome markers while others including ourselves have shown discrepant results. Also, the favorable prognosis of these AML variants declines sensibly in the presence of leukocytosis (variable cut-offs found in different studies)[68] or when the underlying molecular anomaly is not cleared off rapidly by chemotherapy (see *section* **7.5.3**).
Unfortunately many patients lack a cytogenetic AML marker or have no influential cytogenetics. Actually they constitute the vast majority, i.e. the IR group as they share an intermediate prognosis that often poses a therapeutic dilemma among different treatment modalities. As shown in **Appendix 1**, additional features can be used to dissect further their prognostic heterogeneity. The study of concurrent gene alterations (FLT-3, BAALC, CEBPA, NPM and others) appears particularly indicated to test the biological aggressivness of the diseases and/or to monitor treatment response. Lastly, several cellular mechanisms of drug resistance are implicated in refractory or relapsing AML. The study of these alterations may be an additional tool to subclassify more precisely patients with IR or normal cytogenetics. Gene expression profile studies may

ultimately lead to a more refined prognostic subclassification. Collection and storing of diagnostic samples is indicated for this and future studies.

### *7.5.5 How to consolidate CR*

Postremission therapy should take into account patient risk class, age and general performance scale. The risk of relapse is highly variable as is tolerance to treatment and risk of life-threatening complications. Patients at reasonable "risk" of cure by chemotherapy should be identified and treated in this way, although there is little consensus on what exactly the term "chemotherapy" implies apart from at least one HD-Ara-C-containing cycle or preferably more than one (see CALGB studies), and whether an autograft is truly superior or not. Age may play a central role in treatment choices, as older patients may be selectively dismissed from transplant-oriented programs and included in chemotherapy arms.
Risk-adapted consolidation is not possible in IR patients unless additional risk criteria are considered. By doing so, some IR cases would fall into the SR category but the majority will display a combined HR prognostic profile. For HR subsets, allografting is the treatment of choice. Source of allogeneic stem cells and grafting procedures are only in part standardized in clinical trials, being mostly related to transplant programs activated at single centers. Unrelated donor and cord blood transplants are increasingly considered in this setting, widening the access to the procedure for the patients that need it[69,70].
There is uncertainty about the relative merits of high-dose chemotherapy and autografting (with marrow or blood stem cells) in IR and HR cases too, especially when the relevant issue of remissional deaths is looked at, although some trials showed a trend to lower relapse rates with autotransplantation.
In general, the risk of remissional death is more challenging with allografting (and more so using unrelated and/or mismatched donors), with marrow-derived autografting and –whatever the therapy- in older patients. Autografting with blood stem cells reduces greatly acute marrow toxicity, but still there is no direct evidence for this to be better than marrow grafting or intensive chemotherapy. With an autograft, long-term toxicity with irreversible gonadal damage and other is a rule. Chemotherapy only may instead preserve fertility and therefore has to be seriously considered in younger patients, at least if adequate for risk class.

## *7.6 EXPERIMENTAL STUDY PROPOSAL*

With the reviewed background (**7.1- 7.5**, **Appendix 1**, **Appendix 2**), the current proposal is to activate a randomized phase III study (**Figures 1, 2**) comparing

**a**) two different induction schedules (CR phase) and

**b**) two different consolidation schedules (postremissional phase),

whitin a flexible risk-oriented approach where an allogeneic SCT is the preferential therapeutic option for HR patients.
The patient population is that of adults with AML, with age limit ≤ 65 years for Random 1 and 2, and no exclusion except for a diagnosis of acute promyelocytic leukemia. In order to evalute the early exclusion rate caused by inelegibility to the study particularly in the older age group (**10.1)** all patients aged ≥16 years and including acute promyelocytic leukemia will be registered in a general outcome research study.
Initial risk stratification for Random 1 is by (1) age, whereas final risk stratification for Random 2 is by (1) risk class and (2) number of mobilized CD34+ blood stem cells, with a cut-off threshold of < vs ≥7 x$10^6$/kg in the single apheresis with the highest yield, to balance the frequency of high yield (i.e. higher risk) patients in the two arms.

### *7.6.1 Random 1: Induction of CR*

With regard to CR induction, the proposal is to compare the standard induction (ICE) adopted in prior NILG study with an HD-Ara-C-based induction, which is similar to the salvage regimen used in prior protocol. ICE is clearly inadequate in several IR/HR cases, in whom refractory rates >10% and up to 40% are seen depending on exact risk subset. The high-dose regimen could rescue one half of all ICE-refractory cases, hence its use frontline could enhance early response rate while sparing additive toxicity and long hospitalization from a second induction, with a globally positive effect on long-term survival. Both efficacy and toxicity will be compared, also by risk class. Response will be evaluated at day 28.
Notably, apart from Ara-C dosing and time schedule, the cumulative idarubicin dosage (36 mg/$m^2$) is identical in the two induction regimens, as is G-CSF administrative schedule (from day 11).
In the high-dose regimen, to keep toxicity low in a patient population with an expected median age >50 years, the cytarabine concentration will be reduced from 3 g/$m^2$/dose as in reference regimen, to 2 g/$m^2$/dose, with a further reduction to 1 g/$m^2$/dose only in the light of stringent medical reasons, in "frail"

patients aged 60-65 years (ie. eligible patients in whom, according to the responsible physician, comorbidity such as infection, cardiovascular, metabolic, respiratory and neurologic diseases can substantially increase the risk of early induction death using cytarabine at 2 g/m$^2$). Also, older patients (>65 years) are not randomised and will continue study only if entering CR after the first course (**Figure 2b**). Because of the time necessary to obtain the cytogenetic study results in a multicentric trial, it is not possible to randomize IR/HR cytogenetic risk groups only. On the other hand, based on our previous estimates, the proportion of cases with favorable cytogenetics t(8;21) and inv(16) will be <10%, whilst toxicity from this arm is expected manageable and HD-Ara-C is highly effective in these AML subsets.
A second induction course will be necessary in patients unresponsive to either treatment arm. This will consist of a second high-dose cycle with cytarabine 3 g/m$^2$/dose (2 g/m$^2$ in patients ≥60 years of age) plus short-course cyclosporin A (CSA) to downmodulate resistance by MDR1 or other CSA-sensitive multidrug resistance mechanism. Selected, refractory patients with known HLA/DR-identical donor could be considered for immediate allogeneic SCT according to the judgement of the responsible physician.
All responders after initial randomisation (cycle 1) will be given conventional-dose induction cycle 2. Induction therapy will be completed after 8 weeks. Response evaluation will include bone marrow and peripheral blood morphology, cytogenetics and molecuar biology assays for MRD monitoring in cases with an assessable genetic lesions as per study specifications.

### *7.6.2 Random 2: Postremission consolidation*

All CR patients with SR features and aged ≤65 years, excluded by design from SCT, and all those with HR features unable to proceed to SCT within the given time schedule (**7.6.4**), will be considered for second randomisation, on the basis of prior experience in a phase II NILG study (**Appendix 2**). The co-administration of autologous blood stem cells increased the ability to deliver multiple HD-Ara-C courses, resulting in a positive therapeutic response (DFS ≥50% at 4 years) of the SR group, which included a large number of patients aged >55 years and/or with IR cytogenetics, allowing for the first time to deal safely with the concept of HD-Ara-C dose intensity. The real value of this treatment needs to be validated in a controlled clinical trial, adopting a control arm of known efficacy and toxicity. Therefore, patients with SR AML in CR will be randomized between a myeloablative autologous blood stem cell transplantation using a standard reference regimen like BU-CY2 (busulfan-cyclophosphamide) and three consecutive HD-Ara-C-based, autologous blood stem cell-supported cycles (experimental arm based on open phase II NILG-AML 01/00 study: effective therapy in SR group; negligible mortality and long-term toxicity). The postremission phase includes a first consolidation/mobilization cycle with intermediate-dose cytarabine and G-CSF, followed by harvest and cryopreservation of fixed amounts of CD34+ cells (3x 1-2x10$^6$/kg, plus a back up ≥1x10$^6$/kg). Patients will then receive the assigned consolidation therapy. Notably all randomized/treated patients will eventually be reinfused with comparable amounts of CD34+ cells (total 2-6x10$^6$/kg, either as single infusion or 3 separate infusions, depending on treatment arm), and there will be a comparable proportion of patients with high yield of ≥7 x10$^6$/kg CD34+ cells in both arms.

### *7.6.3 Non-Random 2 patients: Allogeneic SCT*

Because postremission therapy will be risk-oriented, fit patients up to 65 years of age with HR disease and an identified donor are eligible to allogeneic SCT. If no HLA-matched sibling donor is available, Centers will declare what type of alternative stem cells are sought for (MUD, cord blood, haploidentical). This strategy will be registered. Thereafter, SCT will be performed according to the transplant protocols of individual treatment centers, after chemotherapy cycle 3 at the latest. A timely decision about transplantation is necessary for the correct conduct of the trial. In this regard all HR patients without a suitable stem cell source (i.e. with no definite proof that a family/volunteer/cord blood/haploidentical source has been indentified) at the indicated timepoint will have to be randomised (**7.6.2**).

### *7.6.4 Non-Random 2 patients: Other cases*

All patients aged ≤65 years unable to be randomised because of an insufficient CD34+ cell yield can be consolidated with 1-2 courses of intermediate/high-dose cytarabine as in the previous study.
All other patients aged ≤65 years excluded from the random but mobilizing adequate CD34+ cells and having a left ventricle ejection fraction ≥50%, can be offered the standard consolidation arm (i.e. autotransplantation). Should a similar patient display a reduced left ventricular function or other medical controindication to myeloablative autotransplantation, final consolidation therapy with the experimental study arm will be allowed (**Appendix 2**). Alternatively, all these patients can be considered for allogeneic SCT as final consolidation therapy.

# 8 STUDY DESIGN

## *8.1 REGISTER OF AML PATIENTS*

All observed patients with an age of 16+ years and newly diagnosed, untreated acute myelogenous leukemia (acute promyelocytic leukemia included), either *de novo* or secondary to chemoradio-therapy or myelodysplasia-associated AML, or with high risk myelodysplasia (WHO/FAB classification), or related conditions according to diagnostic guidelines (**Appendix 3**) will be included in the "AML Prospective Register". Only the patients satisfying the inclusion/exclusion criteria for Random 1 and Random 2 will be enrolled into these trials, while the excluded patients will be enrolled in a separate outcome research study, together with any patient who is labeled 'off-study' during one of the randomised trials.

## *8.2 TRIAL DESIGN*

In this phase III multicentric randomized trial, we shall evaluate separately the efficacy and toxicity of two induction chemotherapy regimens (Random 1 – R1) and of two consolidation therapies (Random 2 - R2):

**Remission induction (R1):** a standard-dose conventional induction chemotherapy regimen (ICE) versus a high-dose sequential chemotherapy regimen (HDS-2), and

**Remission consolidation (R2):** a standard final consolidation therapy with a permanently myeloablative autologous blood stem cell transplantation versus non-ablative multicycle high-dose cytarabine-based consolidation with an autologous blood stem cell support.

For **R1**: eligible patients aged ≤65 will be stratified according to an age ≤ vs >60 years.

1. All patients will receive homogeneous supportive care and will be homogeneously analyzed for response at the same timepoints from induction day 1 (BM and PB morphology, submicroscopic disease evaluation with cytogenetics and gene expression analysis).
2. Patients refractory to induction cycle 1 in either randomisation arm will be homogeneously re-treated with a salvage regimen (HDS-3/-2), including cyclosporin A to reduce the risk of resistance to idarubicin, plus increased-dose cytarabine in those aged 60 years and less. An accepted alternative option is an immediate allogeneic stem cell transplantation. The latter will be considered on an individual basis.
3. Once a complete remission is induced, risk class is determined for each patient (SR, HR by mixed clinico-cytogenetic risk criteria). Meanwhile, a consolidation/blood stem cell mobilization cycle is administered. Blood stem cells are collected and cryopreserved.

For **R2:** eligible patients aged ≤65 will be stratified by risk class (*section* **12**), determined subsequent to the the start of chemotherapy, and CD34+ cell mobilization capacity. Patients undergoing R2 are not allowed to receive any different therapy including SCT at any time while in CR1.

1. Final consolidation therapy is risk-oriented.
2. HR patients with a compatible HLA identical donor are submitted to allogeneic SCT, the donor (sibling, unrelated, cord blood, haploidentical) having been previously identified as soon as CR is achieved. In HR patients without sibling donor, a search for an unrelated volunteer donor is initiated as soon as CR is documented and the HR status is confirmed. If a suitable donor is not found at the end of early consolidation (cycle 3), the patient must be considered for randomisation.
3. SR patients and HR patients without donor are randomised, provided they meet all eligibility criteria.
4. SR patients could be considered for allogeneic SCT outside the trial, provided this choice clearly represents the willing of both the patient and the physician in charge. Patient age may be critical for this choice, as the published evidence in favor of SCT in this risk class is for subjects aged <46 years[51]. These patients are however labeled off-study.
5. Final consolidation therapy for patients unable to participate into Random 2 because not mobilizing sufficient blood stem cells will be with intermediate/high-dose cytarabine courses or allogeneic SCT.
6. Final consolidation therapy for SR/HR patients mobilizing sufficient blood stem cells but unwilling to participate into Random 2 and already excluded from allogeneic SCT will be the standard treatment arm (autografting), or the experimental treatment arm in the case of contraindications to autografting (i.e. an abnormal heart function etc.).

Patients unresponsive to induction cycles 1-2 or relapsing at any time during the study are considered off-study and will be treated according to the specifications of the participating Institution or other salvage protocol activated at NILG sites.

# 9 STUDY OBJECTIVES

## *9.1 PRIMARY END-POINTS*

**Remission induction (R1)**

To compare the risk/benefit ratio in terms of Complete Remission (CR) rate of a conventional induction regimen (ICE) versus a sequential high-dose induction program with HD-Ara-C (HDS-2/-1).

**Remission consolidation (R2)**

To compare the risk/benefit ratio in terms of Disease-Free Survival (DFS) of a standard autologous blood stem cell transplantation versus a multicycle HD-Ara-C-based autologous blood stem cell-supported consolidation program.

## *9.2 SECONDARY END-POINTS*

**Remission induction (R1)**

1. CR with incomplete hematology recovery (CRi)
2. Complete cytogenetic remission (CRc)
3. Treatment-related death (TRD)
4. Feasibility and efficacy of the treatments in different age and risk groups

**Remission consolidation (R2)**

1. Overall survival (OS)
2. Remission duration and cumulative incidence of relapse (CIR)
3. Treatment-related death (TRD)
4. Feasibility and efficacy of the treatments in different age and risk groups
5. Significance and clinical correlates of remission monitoring results
6. Quality of Life evaluation in long term survivors

## *9.3 SAFETY END-POINTS*

**Remission induction (R1)**

Toxicity (clinical adverse events)

**Remission consolidation (R2)**

Toxicity (clinical adverse events)

## *9.4 OUTCOME STUDY OBJECTIVE AND END-POINTS*

Patients excluded for any reason and at any time from R1 or R2 and patients off-study for R1 and R2 (e.g., NR, relapse) will be included in a prospective, observational outcome study (see **Figure 1**) to describe the full clinical course of the disease at three different time phases:

a) Timepoint #1: patients included in the Register and not randomised in R1
b) Timepoint #2 and #3: included patients not randomized in R2. At this stage, a detailed analysis of transplantation strategies intended for all eligible patients will be included.

For each Outcome Study a minimal set of data about clinical characteristics and therapy will be collected annually along with information of vital status.

# 10 STUDY POPULATION

## *10.1 ELIGIBILITY CRITERIA FOR OUTCOME RESEARCH*

**Eligibility criteria**

1. Age 16+ years (no upper age limit if patient satisfies other study entry criteria).
2. Diagnosis of untreated (or only hydroxyurea/cyclophosphamide) acute myelogenous leukemia (AML, including myeloid sarcoma and acute promyelocytic leukemia) or high-risk myelodysplasia (RAEB-2), either *de novo* or following an antecedent hematological disorder, or secondary to chemo-radiotherapy for other cancer.
3. Signed informed consent for the outcome research study.

## *10.2 ELIGIBILITY CRITERIA FOR R1*

**Inclusion criteria**

1. A diagnosis of untreated AML or high-risk MDS is required. Pretreatment with hydroxyurea or cyclophosphamide in patients presenting with hyperleukocytosis is allowed. All diagnostic procedures need to be performed on freshly obtained bone marrow (BM) and peripheral blood (PB) samples. BM core biopsy is mandatory for investigational purposes (centralized evaluation of NPM mutation) and in selected instances (hypoplastic aspirate, MDS).
2. The diagnosis must be one of:
   - *de novo* AML and variants thereof (including rare diagnostic subsets),
   - secondary AML,
   - MDS-related AML or high-risk MDS (RAEB-2 by WHO criteria),
   - primary myeloid sarcoma (MS)

   Diagnostic subsets are detailed in **Appendix 3**, according to FAB, EGIL and WHO classifications, to include morphology, cytochemistry, immunophenotyping, cytogenetics, molecular biology, and storage of representative diagnostic material. For diagnosis confirmation, diagnostic reports wil be reviewed centrally at the NILG Central Unit (USC Ematologia, Ospedali Riuniti, Bergamo), with or without supplemental tests on BM/PB as necessary.
   Detailed indications on patient registration and diagnostic work-up including the forwarding of samples and diagnostic reports are given in **Appendix 4**.
3. Age 16-65 years.
4. ECOG performance status 0-2, unless a performance of 3 is unequivocally caused by the disease itself and not by preexisting comorbidity, and is considered and/or documented to be reversible following the application of antileukemic therapy and appropriate supportive measures.
5. Informed consent signed by the patient or by a parent/tutor in patients aged <18 years.

**Exclusion criteria**

1. Diagnosis of acute promyelocytic leukemia.
2. Pre-existing, uncontrolled pathology such as heart failure (congestive/ischaemic, acute myocardial infarction within the past 3 months, untreatable arrythmias, NYHA classes III and IV), severe liver disease with serum bilirubin >3 mg/dL and/or ALT >3 x upper normal limit (unless attributable to AML), kidney function impairment with serum creatinine >2 mg/dL (unless attributable to AML), and severe neuropsychiatric disorder that impairs the patient's ability to understand and sign the informed consent, or to cope with the intended treatment plan.
   N.B. For altered liver and kidney function tests, eligibility criteria can be reassessed at 24-96 hours, following the institution of adequate supportive measures.
3. Pre-existing HIV positive serology (i.e. already known before enrolment). If HIV positivity is detected after enrolment, the patient is sent off study.
4. A history of cancer that is not in a remission phase following surgery and/or radiotherapy and/or chemotherapy, with life expectancy <1 year.
5. Pregnancy declared by the patient herself, unless a decision is taken with the patient to induce a therapeutic aborption in order to carry on with AML therapy. A pregnancy test is performed at diagnosis but does not preclude the enrolment into study. Fertile patients will be advised to adopt contraceptive methods while on treatment.

## *10.3 ELIGIBILITY CRITERA FOR R2*

**Inclusion criteria**

1. Confirmed CR status after Random 1 (check of BM and PB morphology)
2. Sufficient amount of autologous blood stem cells ($\geq$2 x$10^6$/kg) following A8 consolidation/mobilization chemotherapy cycle no. 3
3. Age $\leq$65 years
4. Signed informed consent by the patient or by parent/tutor in patients aged <18 years
5. SR risk class or HR risk class unable to proceed to allogeneic SCT
6. Functional echocardiography of the left ventricle with ejection fraction $\geq$50%

**Exclusion criteria**

1. Unresolved hematological or extrahematological toxicity of CTC-NCI (Common Toxicity Criteria from the National Cancer Institute) grade II or greater
2. Unresolved/unimproved bacterial or fungal infections from prior therapy, involving major anatomical sites (central nervous system, respiratory tract, gastrointestinal system, genito-urinary system, soft tissues and muscolo-skeletal structures) and requiring patient hospitalization with use of parenteral antimicrobial and antifungal drugs

# 11 STUDY TREATMENTS

## *11.1 REMISSION INDUCTION (R1)*

### *11.1.1 General indications*

The duration of each cycle is 28 days. The first induction course is followed by early consolidation in patients entering CR at cycle 1. Cycle 1 R1(ICE or HDS-2/-1) to cycle 2 (IC) interval is delayed in CR patients until the ANC (absolute neutrophil count, $x10^9$/L) is >1.0. This rule does not apply to patients unresponsive to ICE. In patients not achieving CR after induction cycle 1, a salvage cycle will be given as cycle 2 only up to an age of 65 years.

### *11.1.2 Drug dosage reductions*

In the unlikely event of proved/suspected drug-related toxicity of grade IV, drug dosage can be reduced according to the opinion of the physician. HD-Ara-C is stopped and resumed in subsequent cycles at 50% of the planned dosage in patients who devolop acute neurological toxicity of grade >2; if neurological toxicity reappears, the drug is withdrawn and replaced by conventional-dose cytarabine (100 mg/$m^2$/dose). The indications for replacing HD-Ara-C 2 g/$m^2$ with 1 g/$m^2$ in “frail” patients aged 60-65 years are given below (Random 1, HDS-2 regimen).

### *11.1.3 Preparative regimen and leucapheresis/hydroxyurea/cyclophosphamide*

- 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 fuction (varying the i.v. fluid amount as necessary), with i.v. frusemide 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 i.v. urate-oxydase (Fasturtec) if hyperuricemia >8 mg/dL.
- The above preparative regimen to start 24-72 hours before chemotherapy, depending on degree of metabolic impairment, and to continue until WBC count is <10 $x10^9$/L and/or the end of chemotherapy. 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 initially with leucapheres and/or oral hydroxyurea 1-3 g daily for 2-4 days or iv. Cyclophosphamide max. 60 mg/kg.
- All i.v. drugs are administered through a central venous access. Prior to any chemotherapy agent, give anti-emetics such as granisetron, ondansetron or analogs.
- In patients receiving HD-Ara-C, start corticosteroid eye drops, to be given every 4-6 hours and to be continued until completion of treatment cycle.
- Refer to above indications also for preparative regimen of subsequent induction/postinduction cycles, with appropriate reduction of fluid amount in accordance with patient status, serum uric acid and creatinine concentration, and absence of detectable disease/leukocytosis.

## *11.2 PROPHYLAXIS*

### *11.2.1 Anti-infectious prophylaxis and transfusions*

AML patients are at the greatest risk of developing serious infectious complications during/after treatment, hence it is mandatory to treat them prophylactically with medications able to reduce this risk significantly, or to control the infectious complication once it has developed. Likewise, transfusions with blood procuts need to be routinely administered to support the patients during profound bone marrow aplasia phases. Detailed

indications about supportive care measures for this study including remission induction and postremission consolidation phases are given in **Appendix 5**. During periods of pancytopenia, transfuse patients 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 x$10^9$/l.

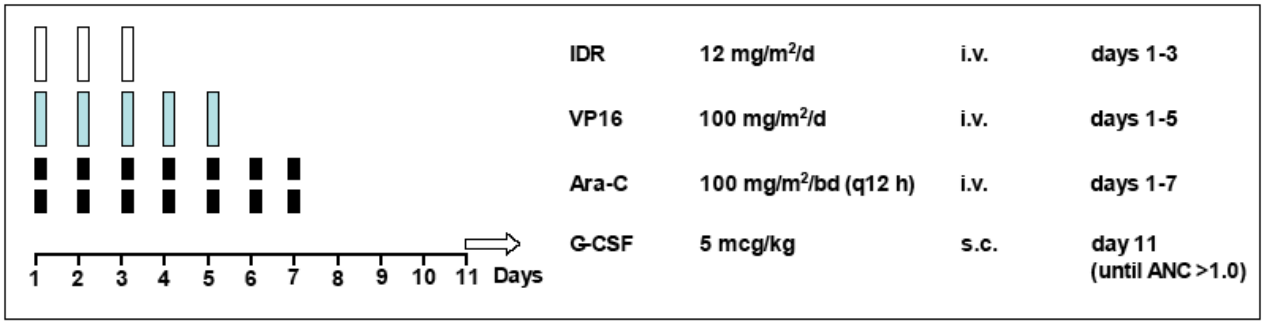
### *11.2.2 Standard arm: ICE*

Induction chemotherapy in standard treatment arm is with ICE cycle followed by IC early consolidation if response is achieved early, and followed by HDS-3 salvage if response is not achieved after ICE (**Figure 1a**). In patients aged >65 years, no further study treatment is given if CR is not documented after the first course (**Figure 1b**).

### *11.2.3 Experimental arm: HDS-2/1*

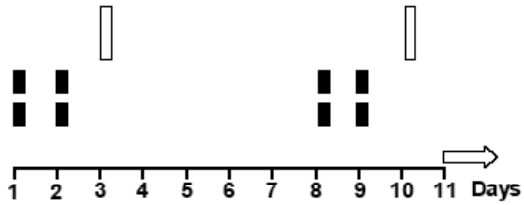
Induction chemotherapy in experimental treatment arm is with HDS-2 cycle followed by early consolidation with IC if response is achieved early, or by HDS-3/-2 salvage if response is not achieved after HDS-2 (**Figure 2a**). The figures added to HDS signifies the HD-Ara-C dosing of each high-dose sequential regimen, i.e. 2 or 3 g/$m^2$. Note the further dose reduction to 1 g/$m^2$ in "frail" patients aged 60-65 years (HDS-1). The latter group is identifiable through the evaluation of concurrent comorbidity (any type, conferring a very high risk of life-threatening complications with cytarabine 2 g/$m^2$ according to the opinion of the reponsible physician). Fit patients aged 60-65 years are given the HDS-2 regimen.

**RANDOM 1, standard arm: ICE cycle 1**




| Drug | Dosing | Route | Infusion time | Days |
|---|---|---|---|---|
| Idarubicin (IDR) | 12 mg/m²/d | i.v. | 30' | 1-3 |
| Etoposide (VP16) | 100 mg/m²/d | i.v. | 60' | 1-5 |
| Cytarabine (Ara-C) | 100 mg/m²/bd (q12 h) | i.v. | 30' | 1-7 |
| Filgrastim/ lenograstim (G-CSF) | 5 mcg/kg/d | s.c. | | 11 to ANC >1.0 |

**RANDOM 1, experimental arm: HDS-2/-1* cycle 1**




| | | | |
|---|---|---|---|
| IDR | 18 mg/m²/d | i.v. | days 3, 10 |
| Ara-C | 2* g/m²/bd (q12 h)<br>*1 if >65 yrs/"frail" 60-65 yrs (option) | i.v. | days 1, 2, 8, 9 |
| G-CSF | 5 mcg/kg | s.c | day 11<br>(until ANC >1.0) |

| Drug | Dosing | Route | Infusion time | Days |
|---|---|---|---|---|
| Idarubicin (IDR) | 18 mg/m²/d | i.v. | 30' | 3, 10 |
| Cytarabine (Ara-C) | 2 g/m²/bd (q12 h)<br>***1 if age >65 years, or "frail" aged 60-65 years (option)** | i.v. | 180' | 1, 2, 8, 9 |
| Filgrastim/ lenograstim (G-CSF) | 5 mcg/kg/d | s.c. | | 11 to ANC >1.0 |

### *11.2.4 Response evaluation following ICE or HDS-2/-1*

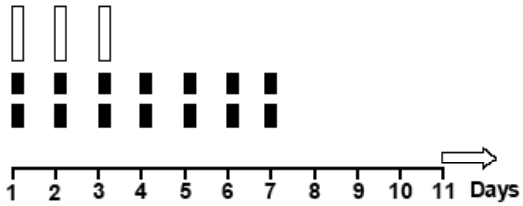
On (approximately) day 28 from start of ICE or HDS-2/-1, the peripheral blood and bone marrow will be examined for response assessment. The variable timing for this analysis depends also on patient status, the occurrence of treatment-related complications and the likelihood that some patients especially in elderly/MDS-type groups may have a more protracted course of blood cytopenia. See *section **13*** and **Appendix 4** for definition of response, required tests and their timing.

## *11.3 EARLY CONSOLIDATION*

### *11.3.1 Responsive patients: IC cycle 2 as early consolidation*

As stated, all patients with a documented CR status following ICE cycle 1 or HDS-2/-1 cycle 1 will receive the early consolidation cycle IC.

**IC cycle 2**




| | | | |
|---|---|---|---|
| IDR | 10 mg/m²/d | i.v. | days 1-3 |
| Ara-C | 100 mg/m²/bd (q12 h) | i.v. | days 1-7 |
| G-CSF | 5 mcg/kg | s.c | day 11<br>(until ANC >1.0) |

| Drug | Dosing | Route | Infusion time | Days |
|---|---|---|---|---|
| Idarubicin (IDR) | 10 mg/m²/d | i.v. | 30' | 1-3 |
| Cytarabine (Ara-C) | 100 mg/m²/bd (q12 h) | i.v. | 30' | 1-7 |
| Filgrastim/ lenograstim (G-CSF) | 5 mcg/kg/d | s.c. | | 11 to ANC >1.0 |

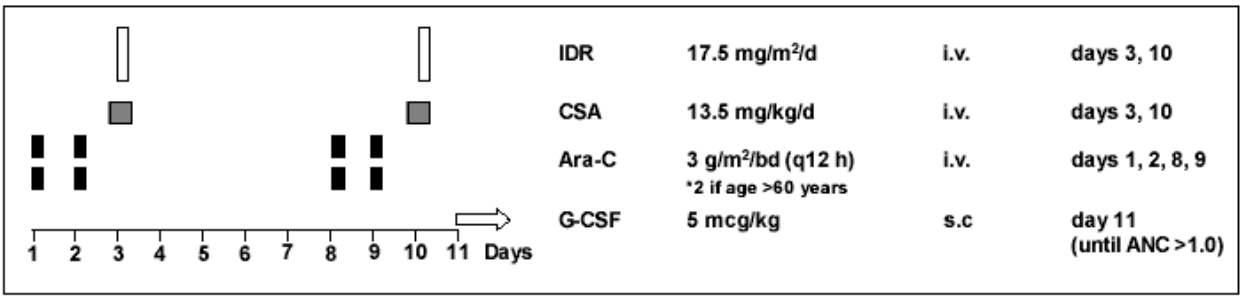
## *11.4 SALVAGE THERAPY*

### *11.4.1 Unresponsive patients: HDS-3/-2 cycle 2*

Patients unresponsive to ICE cycle 1 or HDS-2/-1 cycle 1 will be considered for a salvage attempt using a modified high-dose sequential cycle, with cytarabine increased from 2 to 3 g/m$^2$ and cyclosporin A added to dowmodulate potential multidrug resistance mechanisms (HDS-3). The cytarabine dose remains 2 g/m$^2$ in patients aged >60 years. Refractory patients aged >65 years are not considered for this type of salvage and are sent off study (**Figure 2b**).

**N.B.** Alternatively to HDS-3/-2 induction cycle 2, refractory patients can be considered for immediate transfer to allogeneic SCT procedure. This option is evaluated on a individual basis and may be best suitable for those aged <55 years and/or with only partial residual marrow disease (hypocellular, <50% blast cell content) and/or slow proliferative rate, whereas it is discouraged in the remainder.

**HDS-3/-2* cycle 2**




| Drug | Dosing | Route | Infusion time (start time) | Days |
|---|---|---|---|---|
| Idarubicin (IDR) | 17.5 mg/m$^2$/d | i.v. | 30' (12.00) | 3, 10 |
| Cyclosporin A (CSA) | 6 mg/kg<br>7.5 mg/kg | i.v. | 60' (8.00)<br>11 h (9.00) | 3, 10 |
| Cytarabine (Ara-C) | 3 g/m$^2$/bd (q12 h)<br>*2 if age >60 years | i.v. | 180' | 1, 2, 8, 9 |
| Filgrastim/ lenograstim (G-CSF) | 5 mcg/kg/d | s.c. | - | 11 to ANC >1.0 |

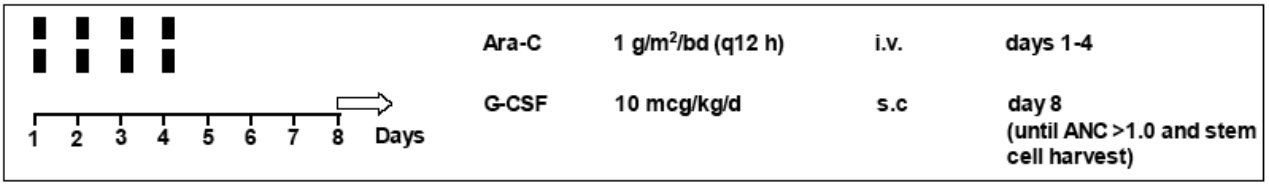
### *11.4.2 Response evaluation following HDS-3/-2*

On (approximately) day 28 from start of HDS-3/-2, the peripheral blood and bone marrow will be examined for response assessment. The variable timing for this analysis depends also on patient status, treatment-related complications and the likelihood that some patients especially in elderly/MDS-type groups may have a more protracted course of myelotoxicity from chemotherapy. See *section **13*** and **Appendix 4** for definition of response, required tests and their exact timing.

## *11.5 CONSOLIDATION/STEM CELL MOBILIZATION CYCLE A8*

After cycle 2 (early consolidation or salvage), all CR patients are to undergo the intermediate-dose cycle A8, which is intended for both early consolidation and stem cell mobilization and harvest.

**A8 cycle 3** (also used as final consolidation for nonmobilizers aged >65 years*)




| Drug | Dosing | Route | Infusion time | Days |
|---|---|---|---|---|
| Cytarabine (Ara-C) | 1 g/m²/bd (q12 h) | i.v. | 120' | 1-4 |
| Filgrastim/ lenograstim (G-CSF) | 5 mcg/kg/bd (q12 h) ***5 mcg/kg/d if used as final consolidation >65 years** | s.c. | | 8 to ANC >1.0 and stem cell harvest |

Stem cell harvest is attempted approximately 12-14 days after the end of chemotherapy, when the WBC count increases and autologous CD34+ cells can be enumerated (collect when >30/microL). The aim is to collect enough CD34+ to support three consecutive A20 cycles (a minimum of two is accepted) or an autologous SCT, depending on randomisation arm.
All patients will be reinfused with a comparable amount of autologous stem cells, i.e. a total of 2-6 $x10^6$/kg CD34+ cells. If possible, an additional amount $\geq$1 $x10^6$/kg should be collected (to support retreatment at relapse or to serve as a back-up). CD34+ cells will be cryopreserved in two/three separate bags, each containing 1-2 $x10^6$/kg CD34+ cells (a minimum of two bags i.e. 2 $x10^6$/kg CD34+ cells are needed to confirm eligibility to Random 2), plus the additional bag if obtainable. Poor- (<2 $x10^6$/kg CD34+ cells) and non-mobilizers are excluded from this randomisation .
This same A8 course is adopted as final consolidation step in patients older than 65 years who did not mobilize enough CD34+ autologous blood stem cells, with appropriate reduction of G-CSF schedule (**Figure 2b**)

## *11.6 FINAL CONSOLIDATION*

### *11.6.1 Age groups, application, timing*

All CR patients aged 16-65 years are treated as stated according to risk class and CD34+ cell mobilization capacity (**Figure 2a**). The criteria used to define risk classes are reported in *section **12***. Of patients aged >65 years, only those entering CR after the first induction cycle (Random 1) are eligible to receive postinduction therapy, however within a nonrandomized study design. These latter patients will be offered 1-2 HD-Ara-C-containing cycles A15 (mobilizers) (**11.8.4**) or alternatively one intermediate-dose cytarabine A8 course (**11.5**). The planned therapy for this advanced age group is shown in **Figure 2b**.
The planned intercycle interval (day 1 to day 1) of all chemotherapy cycles is 30 days, provided hematological criteria for CR are satisfied (see *section* **13.1**) If these criteria are not met, patients are reevaluated bi-weekly until therapy can be applied safely.

## *11.7 REMISSION CONSOLIDATION (R2)*

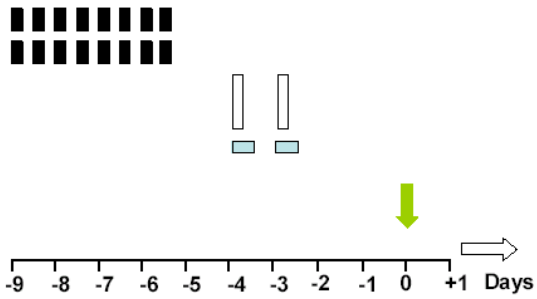
### *11.7.1 Standard arm: autologous SCT consolidation*

Autologous SCT with peripheral blood stem cells is performed following myeloablative treatment with BU-CY2 regimen. BU-CY2 is a standard autologous SCT conditioning schedule widely employed in adult AML studies particularly in European countries. To enhance compliance and efficacy of this therapy, the use of intravenous BU is recommended and is therefore referred to in the accompanying schema. Use of oral BU is possible, in this case at 1 mg/kg/qds for 4 consecutive days, as usual. The BUCY2 therapy will be administered provided the ANC and platelets exceed 1.0 and 100 $x10^9$/L, respectively, and no toxicity of grade II or greater persists after the preceeding chemotherapy cycle A8. Standard antiepileptic medication with sodium valproate is normally used along with BU administration.

### *11.7.2 Experimental arm: repetitive, supported A20 cycles*

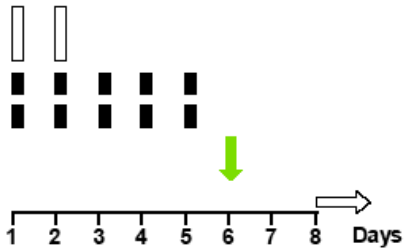
Experimental therapy consists of three (at least two) consecutive A20 cycles given at monthly intervals provided ANC and platelets exceed 1 and 100 $x10^9$/L, respectively, and no toxicity of grade II or greater persists after the preceeding chemotherapy cycle. When below the threshold given, blood counts are to be checked bi-weekly until compatible with the administration of the new cycle. As a major goal of this trial is to demonstrate a possible therapeutic effect from dose-intensive cytarabine in sensitive AML subsets, treatment must be delivered according to stated rules and without undue delay. While the intended number of cycles is three, this treatment is also applicable to patients mobilizing CD34+ cells for two such courses only (*section **11.5***). Each A20 cycle is followed by the reinfusion of 1-2 $x10^6$/kg CD34+ cells, and by G-CSF.

**RANDOM 2, standard arm: Autologous SCT**




| Drug | Dosing | Route | Infusion time (start time) | Days |
|---|---|---|---|---|
| Busulfan (BU) | 0.8 mg/kg/qds (every 6 h) | i.v. | 120' | -9 to -6 |
| Cyclophosphamide (CY) | 60 mg/kg/d | i.v. | 60' | -4 to -3 |
| MESNA | 1.5 g | i.v. | 30' (30' before and 3 h after CY, x3) | -4 to -3 |
| Autologous blood stem cells (Auto) | 2-6 $x10^6$/kg CD34+ | i.v. | | 0 |
| Filgrastim/ lenograstim (G-CSF) | 5 mcg/kg/d | s.c. | | +1 to ANC >1.0 |

**RANDOM 2, experimental arm: A20 cycle (monthly x3)**




| | | | |
|---|---|---|---|
| IDR | 8 mg/m²/d | i.v. | days 1, 2 |
| Ara-C | 2 g/m²/bd (q12 h) | i.v. | days 1-5 |
| Auto | 1-2 x10⁶/kg | i.v. | day 6 |
| G-CSF | 5 mcg/kg | s.c | day 8 (until ANC >1.0) |

| Drug | Dosing | Route | Infusion time | Days |
|---|---|---|---|---|
| Idarubicin (IDR) | 8 mg/m²/d | i.v. | 30' | 1, 2 |
| Cytarabine (Ara-C) | 2 g/m²/bd (q12 h) | i.v. | 180' | 1-5 |
| Autologous blood stem cells (Auto) | 1-2 x10⁶/kg CD34+ | i.v. | | 6 |
| Filgrastim/ lenograstim (G-CSF) | 5 mcg/kg/d | s.c. | | 8 to ANC >1.0 |

## 11.8 *FINAL CONSOLIDATION FOR PATIENTS EXCLUDED FROM RANDOM 2*

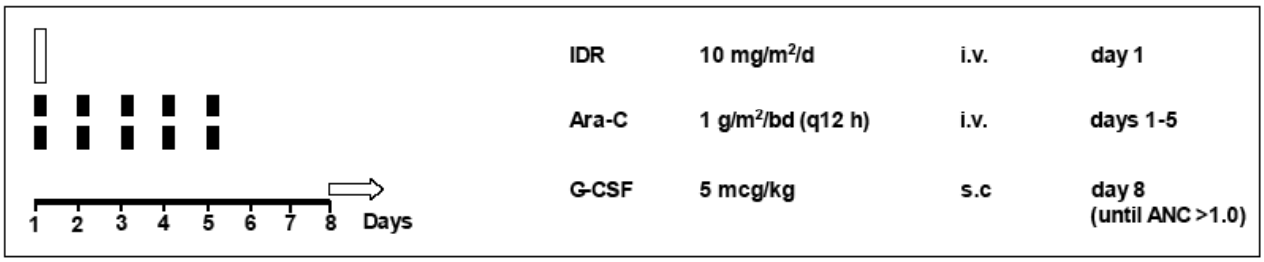
### *11.8.1 Elective allogeneic SCT in HR patients: timing and pretransplantation therapy*

For patients aged up to 65 years with HR AML, a search for a donor should be initiated as soon as possible. For these cases, an allogeneic SCT is the indicated consolidative option. Acceptable stem cell sources are both the bone marrow and peripheral blood of related/unrelated donors, haploidentical donors, as well as the cord blood, the latter only if the total amount of available nucleated cells is >2.5x$10^7$/kg (a count >3.5 is preferable, and cord blood transplant using two different cord blood units is also allowed). The SCT procedure can be either a myeloablative or a reduced-intesity one (age >55 years), according to institutional guidelines and other patient characteristics (comorbidity). Initiation and typology of donor search will be registered for all eligible patients.
SCT should be performed after and no later than consolidation/mobilization cycle A8. However, as logistics and patient risk class may impose a different time schedule, SCT can be either anticipated or postponed, the latter only if an donor has been identified but SCT is not yet possible following A8. Thus, in case of a planned off-therapy interval >6 weeks from A8 to SCT, further chemotherapy should be given to HR patients waiting for SCT (i.e. with a suitable donor already identified) in order to minimize the risk of pretransplantation relapse. This will be in the form of A20 or A10 cycle(s), depending on whether autologous peripheral blood stem cells were mobilized or not.
HR patients without an identified related or unrelated donor at completion of A8 chemotherapy must be considered for inclusion into the second randomisation, because of the low probability of finding a donor at this stage and the need to give further intensive consolidation therapy. No patient entered onto second randomisation will ever proceed to allogeneic SCT while in CR1.
In this trial SR patients should not be offered an allogeneic SCT in first CR, unless this is specifically required by a patient and is supported by the physician in charge. A SR patient wishing to undergo an allogeneic SCT in first CR cannot sign the informed consent for Random 2 and is labeled off-study.
Allogeneic SCT is also a suitable therapeutic alternative for SR patients unable to be randomised in R2 because unable to mobilize sufficient autologous stem cells.

### *11.8.2 Intermediate-intensity consolidation*

Patients excluded from allogeneic SCT and from postremission consolidation Random 2 because unable to mobilize CD34+ stem cells can be offered semi-intesive consolidation. Depending on general and hematological tolerance profile, one or two A10 cycles will be administered at a minimum interval of one month, provided toxicity of grade II or greater has resolved following the A8 cycle and the ANC is >1 x$10^9$/L.

**A10 cycle**




| Drug | Dosing | Route | Infusion time | Days |
|---|---|---|---|---|
| Idarubicin (IDR) | 10 mg/m²/d | i.v. | 30' | 1 |
| Cytarabine (Ara-C) | 1 g/m²/bd (q12 h) | i.v. | 120' | 1-5 |
| Filgrastim/ lenograstim (G-CSF) | 5 mcg/kg/d | s.c. | | 8 to ANC >1.0 |

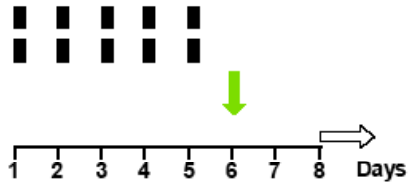
### *11.8.3 Postremission consolidation for patients not signing for Random 2*

Eligible patients not signing for Random 2 and not mobilizing CD34+ cells after A8 are treated as in *section* **11.8.2** with intermediate-dose Ara-C-based cycle(s) or allogeneic SCT.
Eligible patients not signing for Random 2 but mobilizing ≥2 x$10^6$/kg CD34+ cells after A8 should be treated according to the standard consolidation arm (autotransplantation, *section* **11.7.1**) or alternatively considered for allogeneic SCT. Patients with contraindications to autografting can be treated with stem cell-supported HD-Ara-C-containing cycles like in the experimental study arm (*section* **11.7.2**). This latter option must be discussed individually and may apply especially to younger patients wishing to preserve fertility, and to older patients for whom severe toxicity from BU-CY2 is anticipated due to prior treatment complications and/or comorbidity and/or age itself.

### *11.8.4 Postremission consolidation for patients aged >65 years*

Older patients in CR are eligible to nonrandomized consolidation with intermediate/high-dose cytarabine (A15) plus autologous blood stem cell support (mobilizers) or unsupported intermediate-dose cytarabine (A8) (**Figure 2b**). Two A15 or one A8 cycles are planned, respectively. A8 is similar to that employed for early consolidation/mobilization (**11.5**) and is therefore not shown again, except for G-CSF reduced to 5 mcg/kg/d. A15 is derived from A20 (**11.7.2**) differing for the lack of an anthracycline and a reduced cytarabine dosage, to limit further the risk of treatment-related toxicity in this patient population.

**A15 cycle**




| | | | |
|---|---|---|---|
| **Ara-C** | **1.5 g/m$^2$/bd (q12 h)** | **i.v.** | **days 1-5** |
| **Auto** | **1-2 x10$^6$/kg** | **i.v.** | **day 6** |
| **G-CSF** | **5 mcg/kg** | **s.c** | **day 8 (until ANC >1.0)** |

| Drug | Dosing | Route | Infusion time | Days |
|---|---|---|---|---|
| Cytarabine (Ara-C) | 1.5 g/m$^2$/bd (q12 h) | i.v. | 180' | 1-5 |
| Autologous blood stem cells (Auto) | 1-2 x10$^6$/kg CD34+ | i.v. | | 6 |
| Filgrastim/ lenograstim (G-CSF) | 5 mcg/kg/d | s.c. | | 8 to ANC >1.0 |

# 12 DEFINITION AND EVALUATION OF RISK CLASS

## *12.1 EVALUATION OF RISK CLASS*

The risk class is evaluated on the basis of cytogenetics plus selected clinico-diagnostic characteristics, the latter to allow sub-classification of IR cytogenetic group. Patients with SR cytogenetics are automatically included into SR group unless a concurrent HR cytogenetic anomaly is detected or if CR is not achieved following induction cycle 1. Patients with HR cytogenetics are automatically included into HR group. Patients with IR cytogenetics are evaluated for additional clinical risk factors allowing to subclassify them into SR (none present) or HR (any one present).

## *12.2 CYTOGENETIC RISK CLASS*

Cytogenetic risk classes are defined by combining the data of three major studies: MRC, ECOG-SWOG and CALGB (**Appendix 1**). The highest risk score will be adopted for cases belonging to different risk categories in any of the three classifications. This model is at some variance with prior risk stratification (**Appendix 2**), putting some IR cytogenetics in the HR category (in view of CALGB study, not available at time of previous NILG trial). The use of molecular biology to detect CBF type mutations and MLL mutations is accepted as an alternative means to diagnose the corresponding chromosome translocations.

1. **Standard risk (SR)**. Low risk abnormalities without any concurrent high-risk abnormality. Low risk abnormalities include t(8;21) and/or AML1/ETO fusion gene, inv(16) and/or CBFB-MYH11 fusion gene, t(16;16) and del(16q).
2. **Intermediate risk (IR)**. Normal cytogenetics or intermediate risk anomalies without any concurrent high-risk abnormality. Intermediate risk abnormalities include +6, +11, +13, +22, del(12p), t(9;11), -Y.
3. **High risk (HR)**. High risk anomalies, with or withour concurrent SR/IR abnormalities. High risk anomalies include -5/del(5q), -7/del(7q), t(11;19)/t(11q23) and MLL gene rearrangements, t(9;22), abn 3q,9q,11q,12p,20q,21q,17p, iso(17q), +8, +21, t(3;3), t(3;5); inv(3), t(6;9), t(6;11), and complex karyotype with $\geq$3 unrelated clonal markers.
4. **Other (O)**. Any other cytogenetic abnormality not classified as SR/IR/HR will be registered separately. For therapeutic purposes, these cases will be treated as the HR group.
5. **Unknown (U)**. Any case with unknown cytogenetics. This category includes cases with no assessable metaphase (UNM) as well as those in whom karyotype could not be evaluated (UNE). For therapeutic purposes, these cases will be included into the HR group.

## *12.3 DEFINITION OF RISK CLASS*

### *12.3.1 Standard Risk (SR)*

1. **SR cytogenetics and/or molecular biology**, no concurrent HR cytogenetic anomaly, CR achieved after induction cycle 1 (all criteria to be satisfied).
2. **IR/normal cytogenetics without any of the following:**
    - FAB subtype M0, 6 or 7, WHO undifferentiated, bilineal, biphenotypic acute leukemia; prior MDS; HR MDS; secondary AML; primary myeloid sarcoma (MS); extramedullary AML (CNS, “chloroma”-like lesions etc.);
    - WBC count >50 x$10^9$/L;
    - hepato (lower liver edge >2 cm from costal margin) and/or splenomegaly (spleen $\geq$1 cm from costal margin, confirmed by ultrasound scan with longitudinal axis >12 cm);
    - FLT-3 mutation;
    - persistence of cytogenetic alterations at time of morphological CR after ICE/HDS-2/-1 cycle 1;
    - CR achieved after HDS-3/-2 cycle 2.

### *12.3.2 High Risk (HR)*

1. **HR cytogenetics and/or molecular biology.**
2. **IR/normal cytogenetics with any of the following:**
    - FAB subtype M0, 6 or 7, WHO undifferentiated, bilineal, biphenotypic acute leukemia; prior MDS; HR MDS; secondary AML; primary myeloid sarcoma; extramedullary AML (CNS, "chloroma"-like lesions etc.);
    - WBC count >50 $x10^9$/L;
    - hepato (lower liver edge >2 cm from costal margin) and/or splenomegaly (spleen ≥1 cm from costal margin, confirmed by ultrasound scan with longitudinal axis >12 cm);
    - FLT-3 mutation;
    - persistence of cytogenetic alterations at time of morphological CR after ICE/HDS-2/-1 cycle 1;
    - CR achieved after HDS-3/-2 cycle 2.
3. **O/U cytogenetics.** These patients are classified as HR because of the numerical predominance of the HR subsets as a whole, which makes statistically more likely their belonging to the HR rather than the SR category.

# 13 DEFINITION AND EVALUATION OF TREATMENT RESPONSE

## *13.1 COMPLETE REMISSION (CR)*

Disappearance of any clinical and laboratoristic sign of AML, including extramedullary AML if previously detectable. The patient must be transfusion-free with ANC >1.0 $x10^9$/L and platelets >100 $x10^9$/L. BM examination must show reduction of blast cell content (<5%, none of which obviously leukemic i.e. no Auer rods etc.), with cellularity in the normal or slightly hypocellular range and with evidence of trilineage hemopoiesis. BM is examined on day 28 from start of chemotherapy cycle 1 or 2, or later as clinically indicated in ill/cytopenic patients.

## *13.2 DISEASE-FREE-SURVIVAL (DFS)*

The time elapsed between the dates of CR and recurrence or death in CR from any cause.

## *13.3 COMPLETE REMISSION WITH INCOMPLETE HEMATOLOGICAL RECOVERY (CRi)*

All stated criteria for CR are met except for blood counts that remain below the thresholds given in *section **13.1***. This may be seen in patients with prior history of MDS and in the elderly, reflecting an insufficient marrow reserve or an underlying functional dysplastic state, or simply it reflects incomplete treatment response. CRi must be distinguished from CR.

## *13.4 COMPLETE CYTOGENETIC AND MOLECULAR REMISSION (CRc, CRm)*

A morphological and clinical CR plus the disappearance of previously detectable cytogenetic abnormality(ies) (CRc) and/or molecular anbnormality(ies) (CRm).

## *13.5 TREATMENT FAILURE*

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

- Resistant AML (**RES**). Survived ≥7 days from end of induction chemotherapy, with persistent AML in PB and/or BM (BM examined).
- Aplasia (**APL**). Died ≥7 days from end of induction chemotherapy, with cytopenic/ aplastic BM (BM examined).
- Indeterminate (**IND**). Died <7 days from end of induction chemotherapy, or >7 days with no PB blasts/undetermined BM, or did not complete chemotherapy. The “word” indeterminate here refers to the underlying AML. The proximate cause of death may be known (i.e. infection etc).
- Recurrence (**REC**). Reappearance of AML blasts in PB and/or BM (≥5%), or reappearance of extramedullary disease. Isolated dysplastic changes are considered relapse. Because surveillance BM is not recommended as routine, relapse is usually detected while investigating:
  (1) unexplained or worsening cytopenia at follow-up visits,
  (2) sudden-onset leukocytosis, or
  (3) systemic symptoms such as malaise and fever etc.

## *13.6 OVERALL SURVIVAL (OS)*

The time elapsed between the dates of entry onto trial and death from any cause.

## *13.7 REMISSION DURATION AND CUMULATIVE INCIDENCE OF RELAPSE (CIR)*

Defined for CR patients only and measuring the interval from date of CR to relapse. Because CR deaths are excluded from this calculation, it is better reported as a cumulative incidence of relapse graph.

## *13.8 TREATMENT-RELATED DEATH (TRD)*

Mortality due to treatment-related complications in CR patients undergoing at least one postremission cycle.

# 14 DEFINITION AND EVALUATION OF TOXIC COMPLICATIONS

## *14.1 TOXICITY EVALUATION*

Treatment related toxicity will be evaluated through CTC-NCI criteria for both hematological and extrahematological toxicity (**Appendix 6**). Safety and tolerability of experimental treatments will be captured in specific CRFs. Safety assessments will include the collection of specific information on adverse events and serious adverse events.

## *14.2 ADVERSE EVENTS AND REACTIONS*

Information about all adverse events (AE) and adverse reactions (AR), whether volunteered by the patient, discovered by investigator questioning, or detected through physical examination, laboratory test or other means, will be collected and recorded as appropriate according to the indications below . The following definitions will apply:

- AE: any undesirable and unfavourable sign, symptom or medical condition occurring after starting study protocol
- AR: any undesirable and unfavourable sign, symptom or medical condition related to a protocol drug
- Serious AE (SAE): AE resulting:
  - in death (excluding death from refractory/relapsed AML); or
  - life threatening; or
  - requiring hospitalisation or prolongation of existing hospitalisation (excluding hospitalisation due to febrile neutropenia); or
  - resulting in persistent or significant disability or incapacity;
  - or resulting in a congenital anomaly or birth defect.

### *14.2.1 Notification of adverse events and reactions*

All adverse events will be recorded on CRFs. Events that are included in the study endpoints, will be recorded on the CRFs for endpoints. All unexspected serious adverse events, possibly or likely related to trial treatment will be reported in the “Unexspected Serious Adverse Events Report Form” and will to be submitted immediately (within 24 hours) by FAX (0872570206) to the Coordinating Center Consorzio Mario Negri Sud (Figure 3). The Coordinating Centre, in turn, will notify the unexpected serious adverse events, according to current laws and regulations, to all the competent regulatory and institutional bodies.

The flow chart presented in **Figure 3** depicts the modality for the reporting of SAE.

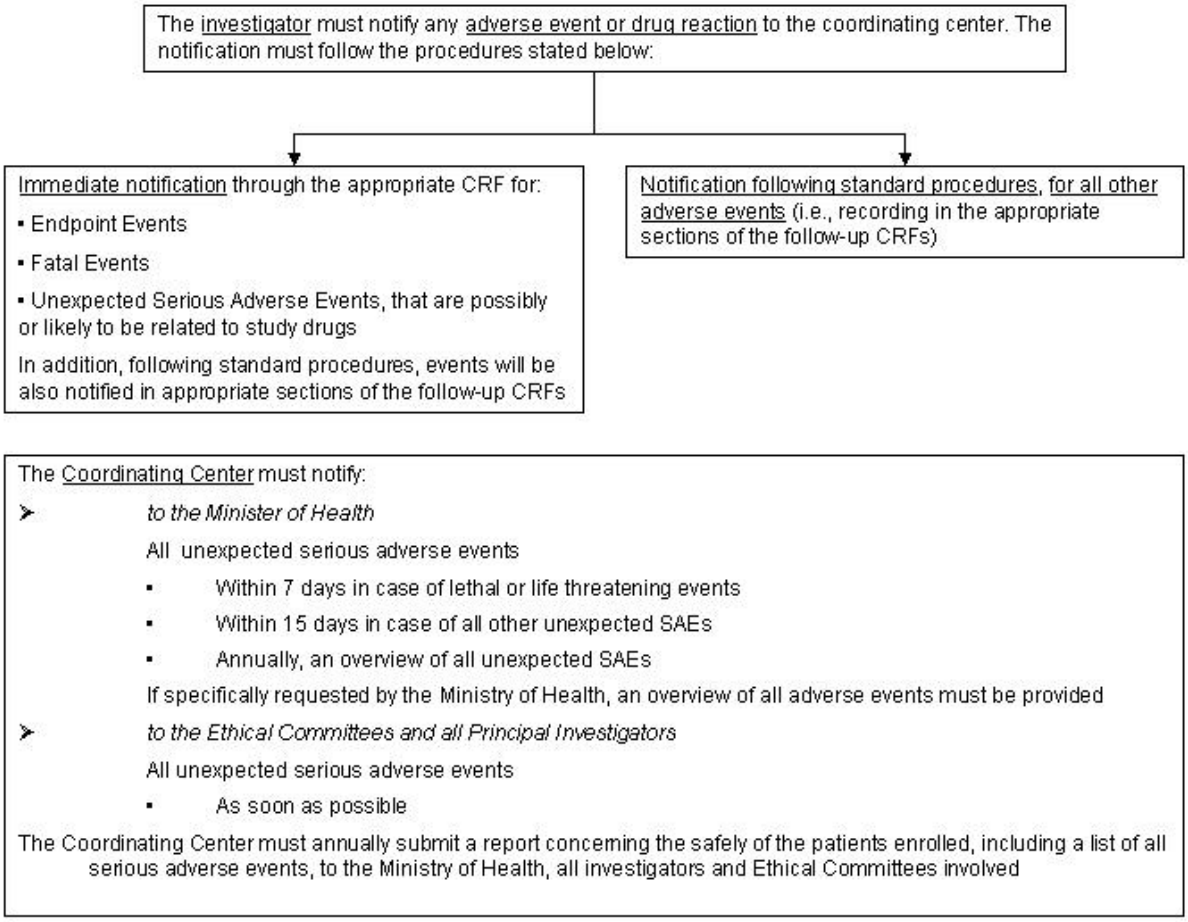



**Figure 3. Notification of relevant Events**

# 15 PRE-ENROLMENT AND ON STUDY ASSESSMENT

## *15.1 PRE-ENROLMENT INVESTIGATIONS*

All patients to be screened for eligibility and enrolment onto study:

- Diagnostic study (BM aspirate; trephine when hypocellular, or required for the diagnosis and the NPM study; PB: cytochemical reactions, immunophenotyping, cytogenetics and selected molecular biology assays according to FAB/EGIL/WHO criteria (**Appendix 3**)
- Storage of diagnostic/biological material for research purposes (minimal residual disease, cell banking, future studies) (**Appendix 4**)
- Medical history, physical examination, ECOG performance status, chest X-ray, EKG, ultrasound scan of abdomen for hepatosplenomegaly
- Pregnancy test (in fertile women)
- 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, and HIV infections
- Cytology/biopsy of clinically suspicious sites of disease (CNS, pleural effusion, skin, etc.) to define the extent of extramedullary involvement
- Blood group and HLA-DR analysis (the latter up to an age of 65 years)

## *15.2 ON STUDY MONITORING*

### *15.2.1 During treatment*

- Physical examination and ECOG performance status before chemotherapy; full blood counts before treatment and daily or at least on alternate days while in-hospital and cytopenic; serum biochemistry weekly. Toxicity evaluation.
- BM examination in the case of any abnormal physical or hematological finding suggesting AML regrowth
- BM and PB sampling for residual disease evaluation (at time-points indicated in the protocol) (**Appendix 4**)
- Enumeration of autologous blood stem cells at time of their collection and cryopreservation after “A8” chemotherapy cycle.

### *15.2.2 Response assessment (after 1st cycle; after 2nd cycle in refractory patients)*

- On day 28 (or earlier/later as clinically indicated) from day 1 of chemotherapy: BM examination (morphology; cytogenetics if previously abnormal); PB examination
- Physical examination and ECOG performance status after chemotherapy; full blood counts and serum biochemistry after treatment and before discharge. Toxicity evaluation

### *15.2.3 Post-therapy follow-up*

- Physical examination, ECOG performance status, full blood count, serum biochemistry at every follow-up access. Toxicity evaluation. Quality of Life evaluation.
- BM examination in the case of any abnormal physical or hematological finding suggesting AML regrowth
- BM and PB sampling for residual disease evaluation (time-points indicated in the protocol) (**Appendix 4**)

Follow-up visits and tests must be performed:

- every 3 months during the first 3 years from end of final consolidation,
- every 6 months thereafter,
- when required by the Coordinating Institution.

Pregnancies and their outcome must be recorded and reported at any time during active follow-up.

# 16 EFFICACY ASSESSMENTS

The occurrence of any clinical event included in the primary end-points will be notified using specific CRFs, to be forwarded to the Coordinating Centre together with all relevant documentation:

1. CR: written bone marrow morphology report or report indicating regression of AML in extramedullary sites if originally present; plus copy of an automated full blood cell count with white cell differentials
2. Recurrence: written bone marrow report or report indicating extramedullary site of relapse; plus copy of an automated full blood cell count with white cell differentials
3. Death: no particular documentation is required other than correct and timely compilation of corresponding CRF

Each event will be independently evaluated by two evaluators. Disagreement between the two evaluators will be addressed by the Chairman of the Committee.

# 17 SAFETY ASSESSMENTS

Safety assessments will consist of monitoring and recording of pre-defined safety and tolerability end-points, all serious adverse events, and regular measurements of vital signs. Information on any adverse event, including side effects of study treatments, will be collected in the patient‘s source document and CRF. In addition, information on unexpected serious adverse events will also be reported in the "Unexpected Serious Adverse Events Report Form", and notified to the Coordinating Centre within 24 hours of learning of its occurrence.
Specific tables containing information on safety aspects of (theoretically) high-risk patients will be periodically reviewed by the Data Safety Monitoring Board (DSMB).

# 18 INSURANCE POLICY AND FINANCING

The study is sponsored, managed and conducted by the Northern Italy Leukemia Group (NILG). NILG represents clinicians that, in routine practice, bear responsibility for the care of AML patients, according to the Sistema Sanitario Nazionale (National Health System). Clinicians will work according to Decreto Ministeriale of 17/12/2000, published on GU n. 43 at 22/02/2005, that regulates non-commercial clinical trials, and has been imposed to improve clinical practice. Hence, the patients will be treated according to best clinical standards within their rights as citizens under the Sistema Sanitario Nazionale. Therefore, there is no need for any other additional insurance policy, other than the existing one in the Public Care.
Financing will be sought to secure organisational and coordinational aspects of the trial only. No financial rewarding is available or foreseen for any of the participating centers.

# 19 STATISTICAL ASPECTS

## *19.1 RANDOMISATION PROCEDURES*

The randomisation procedure is centralized at the Coordinating Center. The criteria for stratification of randomisation in R1 and R2 are specified in *section* **8.2**. The randomisation will be performed using appropriate software applying the biased coin design.

## *19.2 SAMPLE SIZE CALCULATIONS*

The primary end-points for the calculation of sample size are the complete remission (CR) rate after cycle 1 for Random 1 and disease-free survival (DFS) for Random 2. Both the literature and prior data of NILG suggest an HR classification of 67%, and a CR rate after cycle 1 of 80% (15% refractory) and 65% (30% refractory) for SR and HR patients, respectively, with a DFS at 4 years of 60% and 25%, respectively. It is assumed that one half of HR patients refractory to standard induction are effectively rescued in the experimental arm, with two thirds of all cases belonging to HR subsets.

**Random 1 (Figure 4)**
It will be necessary to accrue 250 patients per arm with the expectation of 38% relative risk reduction in favor of the experimental arm (i.e., 174 vs. 228 patients with CR after cycle 1) with an alfa error of 0.05 (two-tailed) and power 80%.

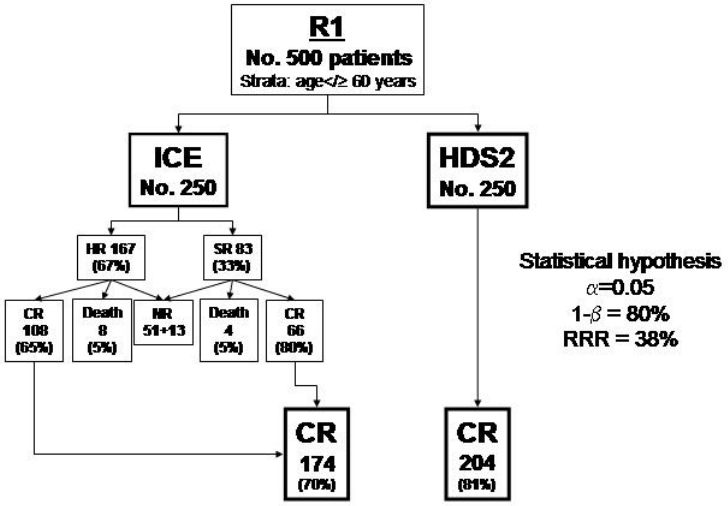



**Figure 4**

**Random 2 (Figure 5)**
It can be assumed a global CR rate of 420 CR out of 500 patients originally randomized in R1 (84%), an expected drop-out rate of 50% (relapse, toxicity, early allo-SCT in HR cases, lack of stem cell mobilization, unacceptance of the trial), and an expected DFS at 4 years of 60% and 25% in SR and HR patients. Two hundred ten patients can be expected to be randomized in R2 with the expectation of 32% relative risk reduction in favor of the experimental arm (i.e., 38 vs. 59 patients with 4-year DFS CR) with an alfa error of 0.05 (two-tailed) and power 80%.

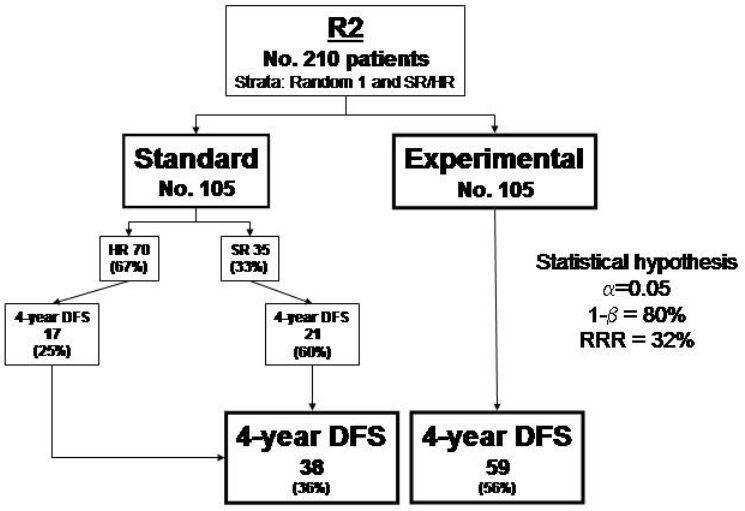



**Figure 5**

## *19.3 MAIN ANALYSES*

Statistical analyses will be carried out according to the intention-to-treat principle.
To evaluate:

- comparability of experimental groups at baseline: Student t-test and analysis of variance for normally distributed continuous variables, and the corresponding non parametric-test (Wilcoxon test, Mann-Whitney U test, and analysis of variance for non parametric data) in the case of abnormal distribution;
- primary end point for R1: $\chi^2$ and Mantel-Haenszel stratification for categorical variables, and multiple logistic regression for the simultaneous adjustment of the many variables;
- primary end point for R2: plots of the Kaplan-Meier estimates of the survival curves will be presented. Treatment efficacy will also be evalueted according to baseline prognostic factors by using a Cox proportional hazards model with terms for therapy will be fitted to the data will be performed.

Parameter estimates with their respective 95% confidence interval will be presented.
The effect of experimental treatments on secondary outcome measures will be evaluated as a "scientific exercise" to gain some insight into the long-term efficacy and safety profile of experimental treatments.
The main analysis will be performed at the end of the study, after 1 year of follow-up from the randomization of last patient.
All patients agreeing and signing the ad hoc consent for the additional long term observation will continue to be followed up in order to collect data for the evaluation of the long term outcome disease and survival, at least until 3 year or more from the end of their consolidation therapy.

## *19.4 SUBGROUP ANALYSES*

In the framework of multivariable models allowing for possible unbalance for relevant prognostic covariates, test of interaction (consisting in a model including each of these variables, treatment and their interaction with treatment) will be carried out at the 5% significance level, being in the context of exploratory analyses.

## *19.5 ADDITIONAL ANALYSIS*

**Quality of Life (QoL) and long-term toxicity evaluation**

Patients undergoing Random 2 treated with either autologous SCT or stem-cell supported chemotherapy courses will be asked to participate into a QoL-assessment at 1 and 3 year from the end of their particular therapy, respectively **(Appendix 7)**[71-73]**.**

The specific aims are:

1) to measure the QoL, as defined by SF-36;
2) to determine the difference between treatment arms concerning the initial and late impact of therapy on QoL;
3) to determine whether demographic characteristics (such as age at diagnosis, sex), clinical status, and treatment complications (organ damage and or toxicity) are associated with and modify the QoL outcome.

To evaluate QoL differences between the different experimental schemes, unadjusted and multivariable adjusted models, that incorporate demographic characteristics, clinical status and treatment complications will be fitted to the data.

## *19.6 INTERIM ANALYSES*

Efficacy in terms of CR for Random 1 and DFS for Random 2 will be monitored using the sequential procedure of Peto. There will be one interim analysis to assess efficacy, scheduled at approximately ½ of expected NR and deaths for Random 1 (see Figure Y) and ½ of expected events for Random 2, respectively.

Safety aspects will be monitored. No formal boundaries will be proposed to monitor safety, but clear, consistent, and persistent evidence of net harm that overwhelms any benefit will be made apparent to the DSMB. A recommendation by the DSMB to stop the trial will be based on the pattern of treatment effect across all end-points, as well as the overall benefit/risk ratio of tested treatments.

# 20 PROTOCOL AMENDMENTS

Changes to the protocol (except for minor administrative changes) can be made only in the form of amendments, which must be reviewed and approved by the Steering Committee.

Based upon their review of the interim study data, the DSMB will have the authority to recommend amendments to the protocol. Prior to implementation, all amendments will be reviewed and approved by the local health authorities and Independent Ethics Committee (IEC) as required.

# 21 DATA MANAGEMENT

## *21.1 DATA-COLLECTION*

Investigators must enter the information required by the protocol into the electronic Patient Data Collection Forms (eCRFs). The CRFs will be electronically forwarded to the study data management center. One print-out version of the CRF will be retained at the investigational site. Once the CRFs are received by the data management center, their receipt will be recorded, and they will be forwarded to the responsible data management staff for processing.

At the time of interim analysis and study closure analysis, documentation supporting the primary endpoints will be forwarded to the data management center for adjudication by the Endpoint Committee.

## *21.2 DATABASE MANAGEMENT AND QUALITY CONTROL*

Database management and quality control for this study are under the responsibility of the Coordinating Center.

At the Coordinating Center, an expert personel will review the eCRFs for completeness and accuracy. Errors, omissions or questions will be entered on data query forms, which will be returned to the investigational site for resolution. After the investigator response is received at the data management center, the resolutions will be entered into the database. A copy of the signed data query form will be kept with the print-out of the eCRFs. Quality control audits of all key safety and efficacy data in the database will be made at designated times during the study.

When the database has been declared to be complete and accurate, the database will be locked and unblinded.

## *21.3 DATA TRANSMISSION AND PROTECTION*

The study will use remote data-entry (RDE) on electronic case report forms (eCRFs) that will be entered, transmitted and stored electronically. A print-out of the compiled eCRFs will be stored at the investigational center and at the Coordinating Center, to be used as a backup copy. Electronic signatures are required together with combined identification codes/passwords before access is granted to the computerized system and at the start of a data entry session.

To guarantee the secrecy of the data, but also to avoid manipulation and loss of data, precautionary action (hardware and software) are taken.
In particular:
At the Coordinating Center:

1. Access to data collected from the participating centers is reserved only to authorized members of Coordinating Center
2. The data-collection network is protected by a firewall
3. The internet connection is encrypted with a digital certificate (SSL technology)
4. The database is located on a server that is protected with a password, that is changed periodically
5. Access to the database is protected with a password and is only accessible by responsible persons of Coordinating Center
6. Periodical back-ups will guarantee secure copies, to allow retrieval of both stored data and the data-collection system
7. The patient is registered and identifiable with a code, to guarantee anonymity

At the participating center:

1. Each center will receive a digital certificate and a ”username” and a ”password” for each one of the investigators appointed by the PI of the center. Only these investigators will be authorized to enter data on the eCRFs
2. The investigators or research nurse can only enter and view data concerning their own patients

**Property of data and publication policy**
All data generated from this study are the property of The Northern Italy leukemia Group (NILG). Analysis and publication of these data will be the responsability of the Steering Committee in conjunction with the Scientific and Coordinating Office. The parties agree to submit all manuscripts and abstracts to all other involved parties 30 days prior to submission.

# 22 STUDY COMMITTEES

**STEERING COMMITTEE**
Renato Bassan (Chairman), Giuseppe Rossi, Enrico Pogliani, Filippo Marmont, Fabio Ciceri and Alessandro Rambaldi.

Besides the Steering Committee which acts as the Sponsor of the trial, and has the full responsibility for the planning, conduction, analysis, publication of the study protocol and results, the following Committees are established.

**ENDPOINT COMMITTEE**
Renato Bassan (Chairman), Tamara Intermesoli, Erika Borlenghi
The Endpoint Committee members will be independent and will not have direct contact with patients randomized into this study. Thus Tamara Intermesoli and Erika Borlenghi will be excluded from the evaluation of data coming from their Centers. Operationally, only in case of disagreement will the Chairman express his final opinion. The main roles and responsibilities of the Endpoint Committee are:

- To agree on definitions for the primary endpoints and on standard procedures for assessing these endpoints.
- To validate blindly the events recorded and reported by the Investigators as end-points of the study.

The decisions of the Endpoint Committee will be used for health authority submissions and publications.

**DATA AND SAFETY MONITORING BOARD (DSMB)**

Mario Cazzola (chairman) Federico Caligaris-Cappio, Gianni Tognoni, Silvano Manzoni
The roles and responsibilities defined by the same DSMB include specifically:

- To monitor safety of the whole study population through periodical analyses
- To monitor efficacy: one interim analysis is during the study

The interim efficacy and safety analyses will be performed semi-blinded (i.e., R1: treatments A vs B and R2: C vs D). The DSMB statistician will possess a copy of the treatment codes for unblinding purposes if deemed necessary by the DSMB.
The study may be amended, or stopped early, or a treatment arm may be discontinued should any of these be deemed necessary based upon DSMB recommendations.
The Chairman of the DSMB will discuss such recommendations with the Chairman of the Steering Committee. Any significant amendment (including recommended discontinuation) to the study will be notified as appropriate to competent authorities.

**SCIENTIFIC COMMITTEE**
Daniele Mattei, Massimo Bernardi, Michela Tassara, G Gianfaldoni, Francesco Mannelli, Irene Cavattoni, Martino Introna, Tiziano Barbui.

**SCIENTIFIC AND COORDINATING OFFICE**
Roberto Marchioli (Coordinator), Arianna Masciulli, Rosamaria Marfisi, Marco Scarano, Riccardo Cavazzina, Anne Rutjes, Valeria D'Eramo, Francesco Marchioli, Anna Polidoro, Barbara Ferri, Alessandra Carobbio, Elena Oldani, Federica Delaini.

# 23 SETTING OF THE STUDY

All centers involved in this study are involved in The Northern Italy Leukemia Group (NILG) network.

# 24 ETHICS AND GOOD CLINICAL PRACTICE

The last revision of the Helsinki Declaration (Appendix 8) as well as the previsions of the Oviedo Declaration provide the general framework for the ethical conduct of the study.
The study protocol is designed to ensure adherence to Good Clinical Practice (GPC) principles and procedures, as described in the following documents and accepted, with their signature, by the Investigators:
1. ICH Harmonized Tripartite Guidelines for Good Clinical Practice 1996.
2. Directive 91/507/EEC, The Rules Governing Medicinal Products in the European Community.
3. US 21 Code of Federal Regulations dealing with clinical studies (including parts 50 and 56 concerning informed consent and IRB regulations).

## *24.1 INDEPENDENT ETHICS COMMITTEE*

Before implementing this study, the protocol, the proposed informed consent form and other information to subjects, must be reviewed by a properly constituted Independent Ethics Committee (IEC). A signed and dated statement that the protocol and informed consent have been approved by the IEC must be given to the Coordinating Center before study initiation. Any amendment to the protocol, other than administrative ones, must be approved by this Committee.

## *24.2 INFORMED CONSENT*

The Coordinating Center will supply a proposed informed consent forms (for Register, Randomisation 1, Randomisation 2, for the additional observational follow-up), which are part of the protocol and comply to regulatory requirements which must be approved by the IEC together with the protocol.
Modified versions of the informed consent forms proposed by individual Investigators and approved by their IEC must be forwarded (together with the documentation of protocol approval) to the Coordinating Center.

## *24.3 PRIVACY RULES FOR PROTECTED HEALTH INFORMATION*

According to the Italian legislation (which complies with and implements European Union regulations), participating patients must be duly informed, and give their explicit signed agreement, on the way their rights to the confidentiality of personal data are duly respected.

# 25 MONITORING PROCEDURES

The Steering Committee of the trial has delegated the GCP monitoring aspects of the study to the Consorzio Mario Negri Sud that will act as a Contract Research Organization (CRO). The responsibilities and the operational procedures of related activity are set-out in the ad hoc contract.

## *25.1 RECORDING OF DATA AND RETENTION OF DOCUMENTS*

Essential documents, as listed below, must be retained by the investigator for as long as needed to comply with national and international regulations (generally 2 years after discontinuing clinical development or 15 years after study closure).
The Investigator agrees to adhere to the document retention procedures by signing the protocol. Essential documents include:

1. IEC approvals for the study protocol and all amendments
2. Source documents and laboratory records
3. Print-out of eCRF
4. Patients' informed consent forms
5. Any other pertinent study document

## *25.2 AUDITING PROCEDURES*

Inspections by Regulatory Authorities during the study, and/or after its completion, could be expected and are welcome.

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# 27 ATTACHMENTS

***Appendix 1: "General Aspects of AML Therapy in Adults"***

***Appendix 2: "NILG Data in AML"***

***Appendix 3: "AML Diagnosis"***

***Appendix 4: "AML Cell Sampling and Response Monitoring"***

***Appendix 5: "Management of Infections in AML Patients"***

***Appendix 6: "NCI/NIH Common Toxicity Criteria"***

***Appendix 7: "Questionario sullo Stato di Salute SF-36"***

***CRF (Case Report Form)***

***Informazioni per il paziente riguardo il trattamento dei dati sensibili e consenso informato per il Registro Leucemie Acute nell'adulto.***

***Informazioni per il paziente ed il medico curante e consenso informato per Random 1 e per Random 2***