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HIGH FEASIBILITY AND ANTILEUKEMIC EFFICACY OF FLUDARABINE, CYTARABINE AND IDARUBICIN (FLAI) INDUCTION FOLLOWED BY RISK-ORIENTED CONSOLIDATION: A CRITICAL REVIEW OF A TEN-YEAR, SINGLE-CENTRE EXPERIENCE IN YOUNGER, NON M3 AML PATIENTS.

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Abstract

One hundred-five consecutive AML patients with the same induction-consolidation program between 2004 and 2013 were retrospectively analysed. Median age was 47 years. The first induction course included fludarabine and high-dose cytarabine (Ara-C) plus idarubicin, with or without gemtuzumab-ozogamicin 3mg/sqm (FLAI-5). Patients achieving CR received a second course without fludarabine but with higher dose of idarubicin. Patients not achieving CR received an intensified second course. Patients not scheduled for early allogeneic bone marrow transplantation (HSCT) where planned to receive at least 2 courses of consolidation therapy with Ara-C. Our double induction strategy significantly differs from described fludarabine-containing regimens, as patients achieving CR receive a second course without fludarabine, to avoid excess toxicity, and Ara-c consolidation is administrated at the reduced cumulative dose of 8 g/sqm per cycle. Toxicity is a major concern in fludarabine containing induction, including the recent MRC AML15 FLAG-Ida arm, and, despite higher anti-leukemic efficacy, only a minority of patients is able to complete the full planned program.

In this paper we show that our therapeutic program is generally well tolerated, as most patients were able to receive subsequent therapy at full dose and in a timely manner, with a 30day mortality of 4.8%. The omission of fludarabine in the second course did not reduce efficacy, as a CR rate of 83% (88/105) was achieved and 3-year disease-free survival and overall survival were 49.6% and 50.9%, respectively.

Our experience shows that FLAI-5/Ara-C+Ida double induction followed by risk-oriented consolidation therapy can result in good overall outcome with acceptable toxicity.

Introduction

In the last three decades no effective new drugs have been introduced in the therapeutic armamentarium of non promyelocytic acute myeloid leukaemia (AML), with the exception of gemtuzumab-ozogamicin (GO), whose potential benefit for AML patients has not been completely elucidated.[1,2] Standard induction therapy for younger AML patients is still based on a combination of daunorubicin (Dnr) and cytarabine (Ara-C), and consolidation chemotherapy has not substantially changed.[3,4] The outcome has however improved and AML is now cured in 35 to 40%

of adult patients who are 60 years of age or younger.[5] The more widespread use of alternative donors has increased the feasibility of allogeneic stem cell transplantation (HSCT)[6-8], whereas better management of both infections and GvHD has reduced induction- and transplant-related toxicity, respectively.[9,10] Moreover, improved prognostic stratification due to the identification of molecular markers, particularly among cytogenetically normal patients [11], opened the way for a more accurate, risk-oriented therapeutic strategy, at least for consolidation therapy.[12-16]

The first studies designed to improve the standard 3+7 induction regimen failed to produce better results.[17-18] Following the observation that fludarabine (Flu) increases the concentration of Ara-C triphosphate (Ara-CTP), i.e., the active metabolite of Ara-C, in leukaemia cells[19], in 1991 our group started investigating induction strategies based on fludarabine-containing regimens in relapsed/refractory and high-risk patients.[20,21] We showed that a regimen including only one cycle of Flu, Ara-C, Idarubicin (Ida) and G-CSF (FLAG-Ida) was effective, well tolerated and improved the feasibility of stem cell transplantation in younger, untreated, de novo AML patients.[22] In 2004, we modified the original schedule by omitting G-CSF priming (FLAI-5), adding a second induction course with Ara-C and Ida (ARA-C + IDA) in order to increase efficacy, and we improved the risk-oriented consolidation.

In the recent MRC AML15, standard 3+7 with or without etoposide was compared to FLAG-Ida induction. CR rate after the first course, relapse risk and survival were better in the FLAG-Ida arm, however, due to higher myelosuppression, only a minority of patients were able to complete consolidation therapy.[23]

In the present study, which includes previously unpublished data on 105 consecutive, newly diagnosed and uniformly treated AML patients, we critically review adherence to our defined strategy, report toxicity and efficacy data, evaluate the role and the best timing of allogeneic transplantation and analyse the prognostic factors for DFS and OS, in the light of the renewed interest for fludarabine-containing induction.

Patients and methods

Patients' clinical features

One hundred-five consecutive AML patients who were diagnosed at our institutionbetween January 1st, 2004 and December 31st, 2013 and who were considered fit for intensive treatment, were retrospectively included in this study in an intention-to-treat analysis. A fludarabine-

containing induction regimen was scheduled for all younger AML patients (<60 years old) and for older patients in very good clinical conditions. Exclusion criteria were; poor performance status (ECOG >3 according WHO), severe heart disease, liver, lung or kidney impairment (except for AML-related conditions). Only two patients below 60 years of age were not considered fit for intensive treatment because of severe comorbidities. Full informed consent was obtained from all patients, according to the Helsinki Declaration. Median follow-up was 48 months (range 2-130 months). Median age at diagnosis was 47 years (range 17-72 years), including 7 patients who were over 60 (6.7%). The most frequent WHO 2008 diagnosis was AML with mutated NPM 1 (34/105, 33%); mean marrow blast percentage was 77%; mean leukocyte count at diagnosis was 29,000/mmc (range 500-404,000/mmc). Two (2%), 85 (81%) and 15 (14%) patients had good-, intermediate- and high-risk karyotype, respectively, according to the MRC classification[14]; cytogenetic analysis was not informative in 3 patients (3%). FLT3-ITD mutation was detected in 24 patients (24%).

Diagnostic work up and risk assessment

Diagnostic work up was performed on the bone marrow (BM) samples of all patients upon admission. When a bone marrow aspirate could not be obtained, a bone marrow biopsy was performed and cytofluorimetric and molecular analyses were carried out on peripheral blood samples.

Immunophenotypic analysis was performed by analysing erythrocyte-lysed whole BM blasts with a broad panel of monoclonal antibodies to define the cellular lineage and to identify relevant antigen aberration patterns and the pathological leukaemia phenotype for future minimal residual disease assessment (MFC-MRD). MFC MRD positivity was defined as being at least 25 events /10,000 analysed cells.

A Q-banded chromosome study was performed on diagnostic BM samples using standard cytogenetic techniques. Karyotyping was carried out on QFQ-banded chromosomes and was reported using the ISCN-1995 nomenclature after analysing a minimum of 20 metaphases for samples with no clonal aberrations. The prognostic significance of karyotypic findings was defined according to the MRC criteria.[14]

The following molecular parameters were evaluated: FLT3-ITD, NPM1 gene mutation A, WT1 and BAALC gene expression. WT1 copy number was measured using the WT1 Profile Quant® Kit (European Leukemia Net) from Ipsogen (Marseille, France) in duplicate, and expressed as WT1

copy number per 10,000 ABL copies; a number of WT1 copies/10,000 ABL greater than 24,000 was considered hyper-expressed, whereas a cut-off of WT1 copies/10,000 ABL lower than 500 was chosen to define MRD negativity as per our published experience.[24,25] BAALC copy number was measured using the BAALC ProfileQuant® Kit (Marseille France); a number of BAALC copies/10,000 ABL greater than 1000 was considered over-expressed.[26] NPM1 mutation (NPM1-A mutation) was measured using Mutant Quant® Standard from Ipsogen (Marseille, France).[27] All Real-Time PCR were performed on DNA Engine 2 (Opticon®, MJ Research®). FLT3-ITD mutations were searched for adopting a PCR strategy and primers reported elsewhere, separating amplicons in a high resolution agarose 2% gel electrophoresis.[28]

Starting from 2007, molecular data (FLT3 and NPM1 mutational status) were integrated into the cytogenetic-based prognostic stratification, thus leading to the definition of a comprehensive risk score (CRS). Patients with either favourable karyotype or an NPM1 mutation (NPM1-mut) in the absence of an FLT3-ITD mutation were defined as low-risk, while those with high-risk karyotype, FLT3-ITD mutation in the absence of an NPM1 mutation, and secondary AML according to the WHO 2008 classification[29] were considered as high-risk. Patients who were not included in either the low- or high-risk groups were considered as intermediate-risk.

Induction and consolidation therapy

The first course of the two-phase induction program (induction I) included Fludarabine 30mg/m2 in a 30' infusion, followed by a 4h infusion of cytarabine 2 g/m2 4 hours later on days 1-5; Idarubicin 10mg/m2 was added subsequently in a 1h infusion on days 1, 3, and 5 (FLAI-5). Patients achieving haematological complete remission (CR) were given the second induction course consisting in five days of a 4h cytarabine 2 g/m2 infusion, followed by a 1h infusion of Idarubicin at the increased dose of 12mg/m2 on days 1, 3, and 5 (Ara-C+Ida).

Patients not achieving CR after FLAI-5 were treated with an intensified second induction course which included mitoxantrone 12 mg/m2/day on days 1–4, etoposide 100 mg/m2/ on days 1–4 and a 6-hour infusion of cytarabine 1 g/m2/day on days 1–4 (MEC). Patients in CR after MEC received a second, identical course and then underwent HSCT, if feasible, or proceeded with the standard consolidation program.

Until 2008, consolidation chemotherapy consisted in two courses of a 4 h infusion of Ara-C 2 g/m2 once daily for 4 consecutive days (HDAC). After 2008, a third HDAC cycle was added.

Between January, 2007 and August, 2010, GO was added to the therapy of all newly diagnosed patients at a dosage of 3 mg/sqm on day 6 of Induction I. Patients again received GO during alternated standard HDAC consolidation cycles, for a maximum of 2 further administrations.

HSCT from any available donor was scheduled in first remission for high-risk patients, for patients achieving CR after MEC, and for selected younger intermediate-risk patients. All patients who were considered eligible for HSCT in 1st CR but who, for any reason could not be immediately transplanted received HDAC consolidation until transplant.

Chemotherapy-based myeloablative conditioning regimens included thiotepa (THIO), busulfan (BU), and fludarabine (referred to as TBF herein) or BU-cyclophosphamide (CY). Regimens based on total body irradiation (TBI) included 9.9 to 12 Gy TBI in fractionated doses, with fludarabine (FLU-TBI) or cyclophosphamide (CY-TBI).[7,30]

Recipients of HLA-identical sibling grafts received cyclosporin A (CyA) + short-course methotrexate (MTX). Recipients of unrelated donor grafts received CyA + MTX + antithymocyte globulin (ATG; Thymoglobulin; Sanofi Aventis, France) 3.75 mg/kg on days −3 and −2 prior to transplantation. Umbilical cord blood recipients received CyA + mofetil-mycophenolate (MMF) and ATG. Recipients of haploidentical (HAPLO) grafts were given CyA from day 0, MMF from day +1, and CY 50 mg/kg on days +3 and +5.[31]

Outcome definitions and Statistical Analysis

Complete remission was defined as complete blood count recovery with marrow blasts <5% upon morphological assessment. Haematological relapse was defined as disease recurrence with at least 5% marrow blasts or recurrence of extra-medullary, biopsy-proven AML localization.

Disease free survival (DFS) was calculated from the date of CR determination to haematological relapse or last follow-up. Patients who underwent allo-BMT in first remission were censored at transplant date. When analyzing separately patients undergoing allo-BMT in first CR, DFS was calculated from the time of transplant to leukemia relapse or last follow-up.

Overall Survival (OS) was calculated from the date of diagnosis to death or last follow-up.

Continuous variables were compared using Student's T test or, where necessary, Wilcoxon's Rank test. Dichotomous variables were compared using the Chi-square test or, where necessary, Fisher's exact test.

A logistical-regression model was built for multivariate CR analysis, including only variables with a p value <0.100 in early univariate assessment.

Survival curves were built using the Kaplan Meier method, and univariate survival analysis was performed using the Log-rank test. For DFS evaluation in the whole cohort of patients and in the subgroup analysis for patients undergoing allo-BMT in CR1, a landmark analysis was performed at day 90, including all patients alive and achieving CR after one or two induction cycles.[32] A Cox Proportional Hazard Model was built for multivariate survival analysis, including only the variables that respected proportional risk assumption.[32]

All statistical analyses were performed using IBM SPSS® v22, Debian (Linux) version.

RESULTS

Toxicity and haematological recovery.

Ninety-eight of the 105 patients (93%) received at least 90% of the scheduled dose of theinduction I course. The main reasons for significant dose reduction were; age> 60 years (4 patients), disease-related acute renal failure (2 patients) and death before completion of the induction therapy (one patient). FLAI 5 was generally well tolerated. Thirty-day mortality was 4.8% (5/105). Three deaths were due to infectious complications, while two patients died of haemorrhagic events. Considering only patients receiving the full schedule, 30-day mortality was 3% (3/98). The main extra-haematological toxicity was mucositis (grade III-IV) in 4 patients which required total parenteral nutrition for a mean of 10 days (range 6-19 days). We did not observe any sinusoidal obstructive disease in either the GO-treated cohort or in other patients.

All CR patients achieved complete blood count recovery after induction I; median recovery time was 17 days both for neutrophils >500/mmc and for platelets >50,000/mmc (range 9-25 and 11-34 days, respectively). During induction I, patients were transfused with a median of 6 packed red blood cell units and 5 packed platelet units (range 1-21 and 1-19, respectively). Most patients (72%) experienced febrile neutropenia for a median of 5 days; intravenous antibiotics were given for a median of 14 days/patient. Nineteen patients (17%) had possible or probable invasive fungal infections, and intravenous antifungal therapy was delivered for a median of 10 days.

The median time from haematological recovery to start of the induction II course was 21 days (range 7-157 days); 12 patients (13.6%) of the 88 who were eligible for induction II waited for

more than 30 days and only 4 waited more than 40 days (4,5%). The main reason for the delay was documented invasive fungal infection (mainly pulmonary aspergillosis, requiring lobectomy in 2 patients).

The Induction II course was well tolerated without any serious extra-haematological toxicity; haematological recovery times were similar to those observed in Induction I.

The overall 60-day mortality was 7.6% (8/105). Of note, the three patients who died after day 30 but before day 60 did not achieve CR with FLAI-5, and two of them (66%) showed persistence of peripheral blasts at the time of death.

Complete Remission analysis

After completing the first induction course, 83 patients achieved CR (79.1% overall, 83% of evaluable patients), whereas 17 (16.2% overall, 17% of evaluable patients) did not respond. After the second induction course, the cumulative CR rate was 88/105 (83.1% overall, 91.7% of evaluable patients). The probability of achieving CR was significantly affected by NPM1 mutation, CRS, and WT1 expression levels at diagnosis ($p<0.03$, <0.03 and <0.05, respectively, Tab. I). Adding GO to FLAI-5 did not improve the CR rate.

The multivariate logistic regression model showed NPM1 mutation as the strongest predictor of CR probability ($p <0.001$, Tab. I). All NPM mutated patients except one achieved CR.

A detailed analysis of factors impacting CR probability is provided in Table I.

Among informative CR patients, MFC-MRD negativity was achieved in 27/69 patients after cycle 1 (39.1%) and in 38/63 after cycle 2 (60.3%), whereas WT1-based MRD negativity was achieved in 70.8% and 88.4% of patients after cycle 1 and cycle 2, respectively.

Consolidation chemotherapy

Fifty-five patients were considered eligible for the chemotherapy consolidation program. Twelve patients received two consolidation courses, 26 received 3 courses or more, whereas 9 and 8 patients received one or **no** consolidation courses, respectively. F**our** patients diagnosed with myeloid sarcoma and achieving CR received further treatment with radiotherapy alone. The main reasons the other 13 patients did not complete the consolidation program were; clinical conditions (n. 6), patient refusal (n. 7) and slow blood count recovery (n. 2). All patients

undergoing the full consolidation program received HDAC in a timely manner, with a median interval between each course of 1.1 months (range 0.9-1.4).

Overall, planned time and dose density was respected in 38/51 (75%) of patients eligible for full consolidation chemotherapy program.

HSCT

Forty-five of the 88 patients achieving CR were scheduled for HSCT in first CR. Thirty- three (73.3%) of these patients actually underwent HSCT after a median of 4 months from the start of FLAI-5 (range 1-10 months). Among these 33 patients, 18 proceeded to BMT immediately after induction II, 8 after one course of HDAC, 6 after two, and 1 patient after three courses. The reasons for not performing HSCT in eligible patients included disease relapse (n=2), infections (n=1), and lack of donor (n=9).

Nine patients received HSCT in second CR, one in third CR and one with persistent disease.

The source of stem cells was an HLA identical sibling in 16 patients, an unrelated matched donor in 9, a HAPLO sibling in 16, and cord blood in 3.

The number of patients undergoing HSCT has steadily increased over time due to the wider use of alternative donors.

Relapse rate and Disease Free Survival

Eighty-eight patients achieving CR were potentially evaluable for DFS analysis. One of them proceeded directly to HSCT shortly after the induction I course without any further therapy and was excluded from analysis.

Three-year DFS was 49.6% (median 35 months) in all patients; DFS duration was significantly influenced by CRS, secondary disease, NPM1 mutation and age at diagnosis below 45 years ($p < 0.001$, < 0.001, < 0.03 and < 0.05, respectively).

Low-risk patients had a 3-year DFS of 75.5% (median not reached), intermediate patients had a 3-year DFS of 51.7% (median 63 months), whereas high-risk patients had a 3-year DFS of 0% (median 7 months, Fig.1 a). Patients with secondary AML had a poor outcome, with a median DFS of only 7 months.

NPM1 mutation conferred a significantly lower probability of relapse regardless of the simultaneous presence of the FLT3-ITD mutation (Fig. 1b). The presence of FLT3-ITD per se did not

significantly impact DFS duration (Fig. 1c), as much as overexpression of BAALC or WT1. However, in the absence of NPM1 mutation, FLT-3-positive patients showed very poor outcome (Fig. 1d).

Adding GO to induction and consolidation therapy did not significantly impact on DFS duration.

Multivariate Cox proportional Hazard showed that CRS was the only independent predictor of longer DFS ($p<0.001$) in the whole cohort.

Among CR patients, MRD evaluation was a strong predictor of DFS duration: patients achieving MFC-MRD negativity after cycle 2 had a 3-year DFS of 79.8% (median not reached), compared to 18.8% for patients with MFC MRD positivity (median 13 months, $p <0.001$).

Among the 55 patients who did not receive front-line BMT, 3-year DFS was 48.2% (median 35 months). No patient showed disease progression during consolidation therapy. Patients receiving at least two consolidation cycles had a 3-year DFS of 59% (median not reached), compared to 22.9% (median 8 months) for patients receiving only one or no consolidation ($p<0.001$, data not shown). The outcome further improved if a third consolidation cycle was added (3-year DFS 73.4%, median not reached, $p <0.001$, Table II).The interval between haematological recovery after induction I and the beginning of induction II did not significantly influence DFS probability. Three-year DFS was 75.5%, 49.4% and 0% in low-, intermediate- and high-risk patients, respectively (median not reached, 35 months and 6 months, respectively, $p <0.001$, Fig. 2).

Multivariate DFS analysis confirmed CRS and number of consolidation cycles as being significant, independent predictors of longer DFS ($p <0.02$).

All 26 relapsed patients underwent re-induction therapy. Seventeen of them achieved second CR (65%), whereas 7 did not respond and 2 patients died of infections. Patients with good, intermediate and high CRS had a 2^{nd} CR rate of 100% (3/3), 71% (10/14) and 44% (4/9), respectively. Eleven out of 17 2^{nd} CR patients were able to proceed to BMT (65%), while the main reason for not performing BMT was lack of donor (5 patients).

Eleven out of 33 patients transplanted in first CR relapsed (33%). Median DFS in these 11 patients was not reached, 3-year DFS was 53.9%. None of the variables we analysed significantly impacted DFS duration in these patients (Tab. II).

Four patients (36.3%) and 1 (50%) patient relapsed after HSCT performed in second or subsequent CR, respectively.

Among all transplanted patients, five died due to transplant-related complications, with estimated three-year non-relapse mortality (NRM) of 17.5%. Twenty-eight out of 44 transplanted patients

(63.6 %) are alive and disease-free at the time of analysis, after a median follow-up of 44 months (range 2–128).

Overall Survival

Whole cohort of patients. After a median follow-up of 51 months, 48 patients died (45.7%), with a 3-year OS of 50.9% (median 38 months). In univariate analysis, OS was significantly affected by CRS, karyotype and secondary disease (p <0.001, <0.003 and <0.001, respectively). Low- and intermediate-risk patients had a relatively good outcome, with a 3-year OS of 77.9% and 61.4%, respectively (median not reached in both groups), compared to high-risk patients who had 3-year OS of 18.3% (median 11 months, Figure 3a).

Age above 45 years did not significantly impact on OS, whereas leukocytosis above 30,000 WBC/mmc only had a borderline impact on OS (p=0.059). If considered separately, none of the molecular variables significantly impacted on OS (data not shown).

Multivariate Cox proportional Hazard model showed that the only independent predictor of long OS was CRS (p<0.001).

Patients not transplanted in first CR. Eighteen of 55 (32.7%) patients who were not scheduled for early BMT died, with a 3-year OS of 68.2%, (median not reached) (Tab. II). OS significantly improved if the whole consolidation program was administered, with patients receiving an additional third cycle showing a 3-year OS of 100% (median not reached), compared to 43.6% (median 23 months) for patients receiving 2 or fewer consolidation cycles (p<0.05, Tab. II).

Low-risk patients had a 3-year OS of 94.1% (median not reached), whereas patients with intermediate or poor risk had a 3-year OS of 72.6% (median not reached) and 11.1% (median 10 months), respectively (p<0.001, Tab.3). Time from haematological recovery after cycle 1 to administration of cycle 2 did not significantly influence the OS rate.

Both CRS and number of administered consolidation cycles retained their prognostic value in multivariate Cox model (p<0.02 and <0.03, respectively, Tab. II).

Among CR patients, MRD evaluation was a strong predictor of longer survival: patients achieving MFC MRD negativity after cycle 2 had a 3-year DFS of 80.9% (median not reached), compared to 48.8% for patients with MFC MRD positivity (median 34 months, p <0.03).

Patients transplanted in first CR. Among the 33 patients who received BMT in first remission, 13 died, 9 due to disease relapse and 4 of transplant-related toxicity. Patients receiving more than one consolidation cycle while waiting for BMT had a significantly higher probability of death as compared to patients who proceeded directly to BMT or received only one consolidation course (3-year OS 49.1 and 0%, respectively, p <0.05, Tab. II). CRS, BMT year and time from haematological recovery after cycle 1 to administration of cycle 2 did not significantly influence the OS **rate** (Tab. II).

In the whole cohort of patients achieving CR after induction, front-line BMT did not significantly improve outcome: in landmark analysis, 3-year OS was 42.3% and 68.2% in patients receiving or not BMT in 1st CR (p = n.s, Fig 3b). The 3-year OS of intermediate-risk patients was 47.4% and 72.6% for those receiving or not BMT in 1st CR, respectively (median not reached, p= n.s., Fig 3c). On the contrary, high-risk patients benefited from early BMT: 3-year OS was 45.5% and 11.1% in patients receiving or not BMT in 1st CR, respectively (median OS was 34 and 11 months, respectively, p<0.001, Fig. 3d).

Only two favourable-risk patients underwent BMT in first CR, however both died due to non relapse mortality thus no comparison with non transplanted, low-risk patients could be made.

Discussion

A critical review of our ten-year experience with a large unselected cohort of younger patients shows that FLAI-5-based double induction followed by risk-oriented consolidation therapy can result in good overall outcome with low toxicity. It also confirms the importance of dose intensity and suggests that integrating clinical, cytogenetic and molecular parameters may optimize the application of HSCT. The vast majority of younger patients actually receive an intensive program of treatment, with some patients still dying of infection or haemorrhage before starting or completing induction.

Our study was not aimed at comparing different induction or consolidation regimens. Some of these issues were prospectively evaluated by the recent randomized AML15 MRC trial in which investigators showed that FLAG-Ida achieved more CRs than daunorubicin + cytarabine (3+7).[23] Similar conclusions, although on a smaller series of patients, had been drawn in an Italian randomized study comparing FLAG-Ida and Idarubicin, etoposide and Ara-C.[33] Furthermore, we

recently retrospectively compared fludarabine-containing and conventional induction regimens in NPM-mut AML patients and showed that FLAI-5 produced significantly higher CR rates compared to 3+7 (86% vs 69% with FLAI-5 and 3+7, respectively, p 0.021) and achieved longer DFS at 24 months ($p < 0.001$).[34] The response rate in the present study is identical to what we previously published[23], and similar data in a smaller cohort of patients have been reported in a phase II study evaluating the efficacy and the safety profile of FLAI plus GO as the induction regimen in younger, untreated CD33 positive AML patients.[35] Our intensive approach is made up of a double induction, with a variable second course depending on the residual blasts after the first induction, in order to minimize toxicity. The observation that CR rates did not significantly increase after the second course (79% vs. 84% after induction I and induction II courses, respectively) suggests that the FLAI-5 regimen alone displays high anti-leukaemia activity, with CR rates over 90% if we exclude patients who died before response assessment. The high anti-leukaemia efficiency of double induction can also be inferred by MRD analysis showing that MFC-MRD negativity was achieved in nearly 40% and 60% of patients after FLAI-5 and Ida-Ara-C, respectively. The high anti-leukaemia activity of FLAI-5 induction is coupled with no excess of deaths in the first 30 days after diagnosis (5%), as well as with short neutrophil and platelet recovery, and low rates of non haematological complications and severe infections. G-CSF was only administered to patients experiencing severe sepsis or uncomplicated fever in the late neutropenia period.We chose not to include G-CSF in the induction schedule as a priming agent, because of the controversial results regarding its efficacy, or to shorten the duration of neutropenia since recovery after FLAI-5 and ARA-C+IDA is quite short. The low rate of infection-related deaths reflects the general improvement in the treatment of infections. Of note, none of the patients in CR after FLAI-5 died of therapy-related complications during the subsequent courses. Further deaths occurring within 60 days from diagnosis were related to refractory disease. Toxicity and haematological recovery after the first and the second induction phases were comparable and most patients were able to receive subsequent therapies at full dose and in a timely manner. In the MRC trial in which two identical FLAG-Ida courses were delivered, Burnett *et al* reported that the second induction course was followed by prolonged severe neutropenia and thrombocytopenia with a negative impact on the timing and dose intensity of further therapies.[23] Our observation that approx. 94% of patients actually received more than 90% of the intended FLAI-5 dose (98/105), and that 89.2% of patients received more than 90% of the drug dosage that was planned for the second induction course (74/83) confirms the good feasibility of our induction program. The median overall Idarubicin dosage administered

in the induction phases is higher compared to what was planned in the MRC trial (66 mg / sqm vs. 48 mg/sqm) and rather similar to the dose of 72 mg/sqm indicated by earlier reports as the optimal dose.[36,37]

Patients in our series who completed the whole program of therapy at the scheduled times displayed the best outcomes, i.e. a 56.7% 3-year DFS for patients receiving at least two consolidation cycles, as observed in the larger MRC experience. However, only a minority of MRC patients received the defined program of chemotherapy in time, probably as a consequence of the profound myelosuppression caused by the double fludarabine-containing induction. In our experience, a significant higher proportion of patients was able to complete the full planned consolidation chemotherapy (38/51, 75%). Adding a low GO dose to FLAI-5 had no impact on CR rate, DFS or OS, unlike what was recently reported by some large prospective trials in younger[2] and older patients.[1,38] Our study however was not designed to compare the efficacy of an induction schedule with or without GO, and the low number of patients receiving GO did not allow us to carry out a significant retrospective comparison.

We found that the NPM gene mutation was associated with a higher CR rate and longer DFS, as previously reported.[16] In our study, the cytogenetic-based risk assessment showed less prognostic value than the comprehensive cytogenetic and molecular risk group. This may be due to the large prevalence of intermediate-risk cytogenetic whose prognostic value is specified by molecular data such as NPM and FLT3 status, as already reported. In addition, clinical factors such as secondary disease and leukocytosis still maintain an important prognostic role.

Lastly, we attempted to retrospectively evaluate the role and the optimal timing of HSCT in the various prognostic risk groups. HSCT was performed in 41 patients (70% front-line and 30% in later phases of the disease). In the majority of patients with indication for early HSCT, the transplant was performed after the second induction and before the second consolidation course. Transplant-related mortality (TRM) was low but progressively increased when transplant was performed after more than 1 consolidation course. On the contrary, TRM was not significantly different when transplants were performed in first or subsequent CR and was not affected by the type of transplant or type of donor. Our experience seems to indicate that the benefit of first CR transplant is clear for patients in the high-risk group, but not for intermediate-risk patients. In this second group of patients HSCT reduced the relapse rate but did not significantly increase the OS due to the high likelihood of undergoing transplant in second CR with similar DFS.

In the last few years, 15 AML patients included in the present study received HSCT from

haploidentical donors. This procedure has the advantage of broadening the range of potential donors and reducing the time of the search, thus allowing the physicians to expand the "risk-oriented consolidation program". In conclusion, since molecularly-oriented drugs and cellular immunotherapy approaches are currently not available for every-day practice, younger AML patients might benefit from a fludarabine-Ara-C- and Idarubicin-containing induction course followed by a second similar regimen without fludarabine but with an increased dose of anthracycline in patients achieving CR. Consolidation should consist of three courses of HDAC for a cumulative dose of 24 g/sqm, with close attention to time and dose intensity. A dynamic prognostic work-up including not only clinical, cytogenetic and molecular data at diagnosis but also an evaluation of response to therapy, including MRD analysis, can identify patients who are likely to benefit from early HSCT.[5,39,40] Our experience shows that the most reasonable strategy is to transplant, in first CR, high-risk patients as well as those who do not achieve MRD negative CR after two induction courses, regardless of baseline risk. Other patients should be carefully followed-up with FCM or WT1/NPM MRD analysis[25,41,42] and possibly rescued with HSCT in second CR.[43]

Financial relationships and conflicts of interest

The authors declare that they have no conflicts of interest to disclose.

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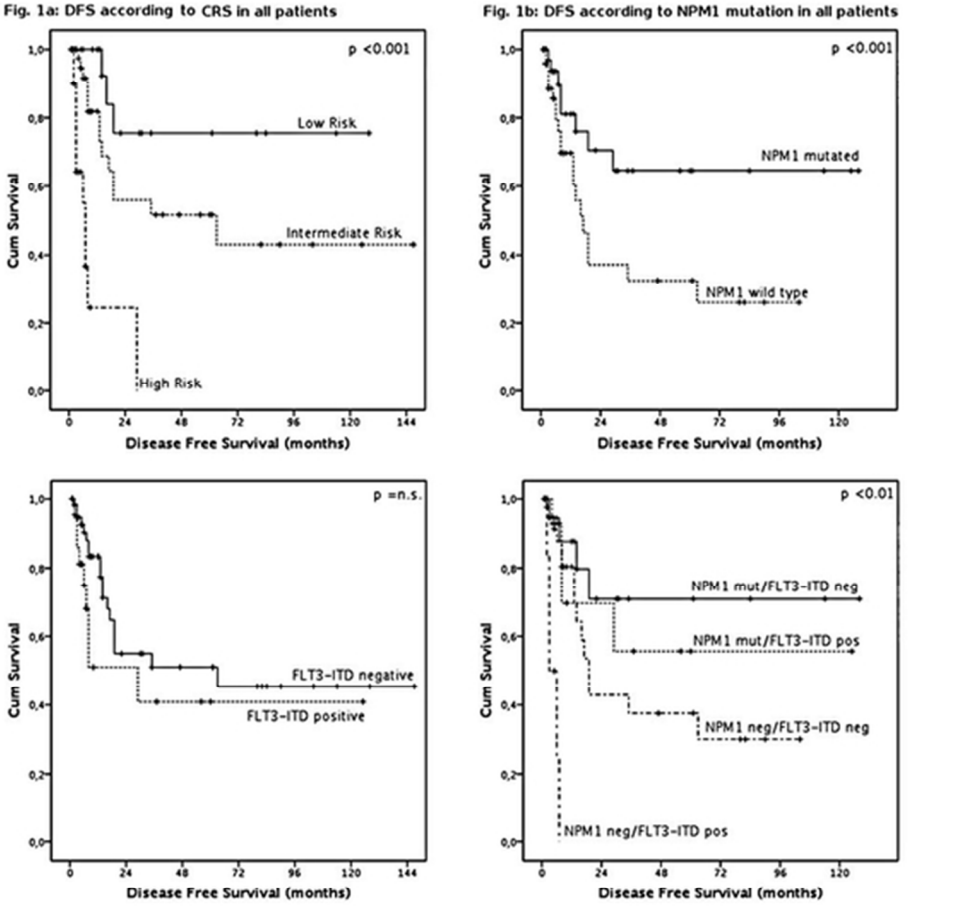
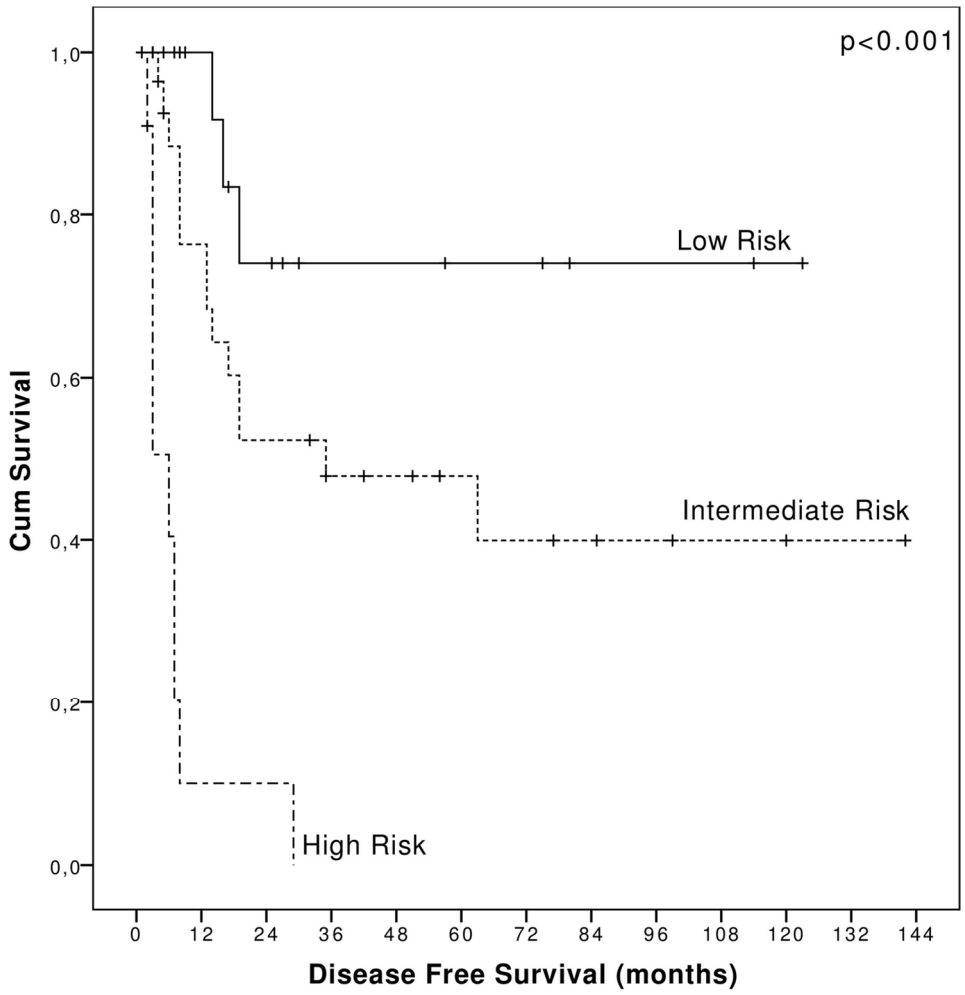
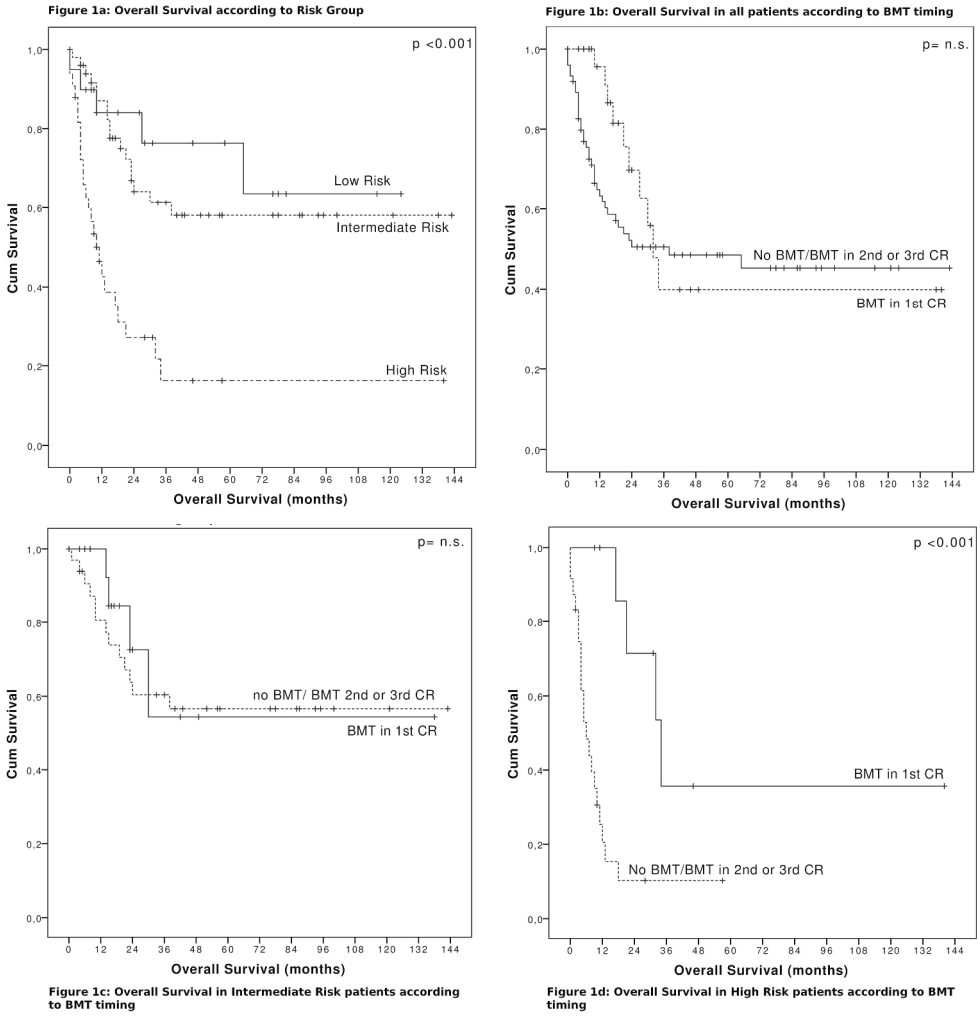


DFS according to CRS and molecular status
24x24mm (600 x 600 DPI)



DFS in patients not undergoing allogeneic BMT in first CR, according to CRS
63x66mm (600 x 600 DPI)



OS according to CRS
141x147mm (600 x 600 DPI)

TABLE I: CR analysis

		Death 30 gg (%)	Death 60gg (%)	CR after FLAI 5 (%)	p (univ.)	p (multiv.)	CR after II cycle (%)	p (univ.)	p (multiv.)
ALL PATIENTS	**Total: 105**	**5/105 (4.8)**	**9/105 (8.6)**	**83/105 (79.1) NR: 17 (16.2)**	**-**	**-**	**88/105 (83.8) NR: 8 (7.6)**	**-**	**-**
Age	< 45 yrs	1/45 (2)	3/45 (7)	37/45 (82)	1.000	-	41/45 (91)	0.073	0.131
	> 45 yrs	4/60 (7)	8/60 (10)	46/60 (77)			47/60 (78)		
Karyotype	Favorable/Intermediate	3/87 (3)	4/87 (5)	73/87 (84)	0.049	0.230	77/87 (89)	0.084	0.123
	High Risk	1/15 (7)	3/15 (20)	9/15 (60)			9/15 (60)		
NPM	Wild type	3/64 (5)	5/64 (8)	46/64 (72)	0.008	0.008	50/64 (78)	0.024	0.003
	Mutated	1/35 (3)	1/35 (3)	33/35 (94)			34/35 (97)		
FLT3	Wild type	4/77 (5)	5/77 (7)	60/77 (78)	0.754	-	64/77 (83)	0.447	-
	FLT3 ITD	0/24 (0)	1/24 (4)	21/24 (88)			22/24 (92)		
Disease Onset	De novo	3/90 (3)	5/90 (6)	75/90 (83)	0.043	0.145	80/90 (89)	0.063	0.192
	Secondary	2/15 (13)	4/15 (27)	8/15 (53)			8/15 (53)		
WBC at diagnosis	<30.000/mmc	1/54 (2)	2/54 (4)	46/54 (85)	1.000	-	50/54 (93)	0.075	0.008
	>30.000/mmc	4/50 (8)	7/50 (14)	36/50 (72)			37/50 (74)		
Risk Group	Good	1/20 (5)	1/20 (5)	19/20 (95)	0.015	0.641	19/20 (95)	0.025	0.602
	Intermediate	1/51 (2)	1/51 (2)	42/51 (82)			47/51 (92)		
	Poor	3/33 (9)	6/33 (18)	21/33 (64)			21/33 (64)		
Mylotarg	FLAI	4/80 (5)	6/80 (8)	64/80 (80)	0.547	-	69/80 (86)	0.529	-
	MY-FLAI	1/25 (4)	3/25 (12)	19/25 (76)			19/25 (76)		
WT1atdiagnosis	<24000	3/79 (4)	5/79 (6)	65/79 (82)	0.130	0.151	69/79 (87)	0.041	0.144
	>24000	1/16 (6)	1/16 (6)	10/16 (63)			11/16 (69)		
BAALC at diagnosis	<1000	3/72 (4)	5/72 (7)	55/72 (76)	0.284	-	60/72 (83)	1.000	-
	>1000	1/18 (6)	1/18 (6)	16/18 (89)			16/18 (89)		
Sex	Male	2/59 (4)	6/59 (10)	49/59 (83)	0.425	-	51/59 (86)	0.292	-
	Female	3/46 (7)	3/46 (7)	34/46 (74)			37/46 (80)		
Marrow Blasts	<30%	0/12 (0)	1/12 (8)	10/12 (83)	0.295	-	10/12 (83)	1.000	-
	>30%	5/89 (6)	7/89 (8)	70/89 (79)			75/89 (84)		

TABLE II: DFS and OS analysis according to consolidation therapy

		Relapse (%)	Median DFS (months)	3-year DFS (%)	p (univ.)	p (multiv.)	Deaths (%)	Median OS (months)	3-year OS (%)	p (univ.)	p (multiv.)
NO BMT in 1st CR#	**Total: 55**	**26/55 (47)**	**35***	**48.2**	**-**	**-**	**18/55 (34)**	**NR**	**68.2**	**-**	**-**
Number of Consolidation	3 or more	5/26 (19)	NR	73.3	0.000	0.018	2/26 (8)	NR	100	0.000	0.031
	2 or less	21/29 (72)	13	30.3			16/29 (55)	21	45		
Time from recovery to 2nd Induction	20 days or less	8/25 (32)	NR	57.5	0.132	0.790	7/25 (28)	NR	75.4	0.514	-
	21 days or more	15/26 (58)	29	43.7			10/26 (39)	NR	60.9		
Comprehensive Risk Score	Good	3/17 (18)	NR	75.5	0.000	0.011	2/17 (12)	NR	94.1	0.000	0.005
	Intermediate	14/29 (48)	35	49.4			8/29 (28)	NR	72.6		
	Poor	9/9 (100)	6	0			8/9 (89)	10	11.1		
BMT in 1st CR	**Total: 33**	**11/33 (33)**	**NR**	**53.9**	**-**	**-**	**13/33 (39)**	**32**	**43.3**	**-**	**-**
Time from recovery to 2nd Induction	20 days or less	5/18 (28)	30	45.9	0.773	-	6/18 (33)	32	29.3	0.679	-
	21 days or more	4/11 (36)	NR	51.9			5/11 (45)	32	51.1		
Number of Consolidation	1 or none	9/26 (34)	NR	55.8	0.976	-	9/26 (35)	34	49.1	0.042	0.049
	2 or more	2/7 (29)	18	50			4/7 (57)	27	0		
BMT Year	Before 2010	6/12 (50)	30	45.9	0.773	-	8/12 (66)	23	33.3	0.292	-
	2010 or after	5/21 (24)	NR	51.9			5/21 (24)	NR	51.4		
Comprehensive Risk Score	Good	0/2 (0)	NR	100	0.698	-	2/2 (100)	10	0	0.126	0.898
	Intermediate	7/19 (37)	NR	56.3			6/19 (32)	30	47.4		
	Poor	4/12 (33)	NR	51.9			5/12 (42)	34	45.5		

#For dose intensity analysis only patients surviving induction therapy and achieving CR are included..
*For Patients not undergoing BMT in first remission, DFS is censored at transplant time were applicable.