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GIMEMA AML1310 TRIAL OF RISK-ADAPTED, MRD-DIRECTED THERAPY FOR YOUNG ADULTS WITH NEWLY DIAGNOSED ACUTE MYELOID LEUKEMIA

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Abstract:

We designed a trial in which post-remission therapy of young patients with de novo AML was decided combining cytogenetics/genetics and post-consolidation levels of minimal residual disease (MRD). After induction and consolidation, favorable-risk patients (FR) were to receive autologous stem cell transplant (AuSCT) and poor-risk patients (PR) allogeneic stem cell transplant (ASCT). Intermediate-risk patients (IR) were to receive AuSCT or ASCT depending on the post-consolidation levels of MRD. ASCT was to be delivered whatever the source of stem cells. Three hundred-61/500 patients (72%) achieved a CR, 342/361 completed the consolidation phase and were treatment allocated: 165 (48%) to ASCT (122 PR, 43 IR MRD-positive) plus 23 rescued after salvage therapy, for a total of 188 candidates; 150 (44%) to AuSCT (115 FR, 35 IR MRD-negative) plus 27 IR patients (8%) with no leukemia-associated phenotype, for a total of 177 candidates. Overall, 110/177 (62%) and 130/188 (71%) AuSCT or ASCT candidates received it, respectively. Two-year overall (OS) and disease-free survival (DFS) of the whole series was 56% and 54%, respectively. Two-year OS and DFS were 74% and 61% in the FR category, 42% and 45% in the PR category, 79% and 61% in the IR MRD-negative category, 70% and 67% in the IR MRD-positive category. In conclusion, AuSCT may still have a role in FR and IR MRD-negative categories. In the IR MRD-positive category, ASCT prolongs OS and DFS to equal those of the FR category. Using all the available sources of stem cells, ASCT was delivered to 71% of the candidates.

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1 *In memory of Francesco Lo Coco, a dear friend and a valuable colleague*

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5 **GIMEMA AML1310 TRIAL OF**
6 **RISK-ADAPTED, MRD-DIRECTED THERAPY FOR YOUNG ADULTS WITH**
7 **NEWLY DIAGNOSED ACUTE MYELOID LEUKEMIA**

8
9 *Running Title: Risk-adapted, MRD-driven therapy for AML*

10
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42 **Key Points**

43

- 44 1. A risk-adapted, MRD-driven transplant strategy is a feasible approach for the treatment of younger
45 adults with AML.
46 2. Pre-transplant MRD positivity should not be a contraindication to the delivery of allogeneic stem cell
47 transplant in younger adults with AML.

48

49 **Abstracts**

50 We designed a trial in which post-remission therapy of young patients with *de novo* AML was decided
51 combining cytogenetics/genetics and post-consolidation levels of minimal residual disease (MRD).
52 After induction and consolidation, favorable-risk patients (FR) were to receive autologous stem cell
53 transplant (AuSCT) and poor-risk patients (PR) allogeneic stem cell transplant (AlloSCT).
54 Intermediate-risk patients (IR) were to receive AuSCT or AlloSCT depending on the post-consolidation
55 levels of MRD. AlloSCT was to be delivered whatever the source of stem cells. Three hundred-61/500
56 patients (72%) achieved a CR, 342/361 completed the consolidation phase and were treatment
57 allocated: 165 (48%) to AlloSCT (122 PR, 43 IR MRD-positive) plus 23 rescued after salvage therapy,
58 for a total of 188 candidates; 150 (44%) to AuSCT (115 FR, 35 IR MRD-negative) plus 27 IR patients
59 (8%) with no leukemia-associated phenotype, for a total of 177 candidates. Overall, 110/177 (62%) and
60 130/188 (71%) AuSCT or AlloSCT candidates received it, respectively. Two-year overall (OS) and
61 disease-free survival (DFS) of the whole series was 56% and 54%, respectively. Two-year OS and DFS
62 were 74% and 61% in the FR category, 42% and 45% in the PR category, 79% and 61% in the IR
63 MRD-negative category, 70% and 67% in the IR MRD-positive category. In conclusion, AuSCT may
64 still have a role in FR and IR MRD-negative categories. In the IR MRD-positive category, AlloSCT
65 prolongs OS and DFS to equal those of the FR category. Using all the available sources of stem cells,
66 AlloSCT was delivered to 71% of the candidates. EudraCT number (2010-023809-36);
67 ClinicalTrials.Gov Identifier (NCT01452646).

68

69 Introduction

70
71 In spite of the continuously growing knowledge about the genetic and molecular landscape of acute
72 myeloid leukemia (AML),¹⁻⁶ the paradigm of treatment for young adults with AML is still largely
73 based on the “*one size fits all*” approach, with post-remission strategies still depending on donor-
74 availability rather than on the actual risk of disease relapse.⁷ In the short term, this has led to
75 satisfactory rates of complete remission (CR) (70-80%) but in the long term survival estimates are still
76 disappointing, with less than 30-40% of patients becoming long-term survivors.^{8,9}

77 Indeed, dealing with the high propensity for relapse and the considerable genetic heterogeneity of AML
78 requires either development of new agents or adoption of modern, risk-adapted therapeutic programs.
79 Risk-adapted approaches may consist in integrating pre-treatment prognosticators, such as cytogenetics
80 and molecular genetics, with post-treatment parameters, such as assessment of minimal (or measurable)
81 residual disease (MRD).¹⁰

82 Even though in AML cytogenetic is a historical and robust determinant of outcome, the modern
83 stratification of the patients in "favorable-" "intermediate-" or "adverse-risk" categories relies ever
84 more increasingly on the baseline molecular pattern.^{11,12} Based on this, favorable-risk patients achieve
85 OS and DFS rates of 50-60% at 3-5 years with standard chemotherapy while those with adverse-risk
86 show OS and DFS rates of 5-20% at 3-5 years if not submitted to allogeneic stem cell transplantation
87 (AlloSCT).^{7,11,13} Therefore, it appears that in favorable- and adverse-risk patients the sole
88 genetic/cytogenetic profile, regardless of the MRD levels, is helpful enough to guide decisions for the
89 delivery of AlloSCT in the post-remission phase. On the other hand, there are no accepted criteria to
90 direct the decision-making process after consolidation for patients in the intermediate-risk category: for
91 these patients, evaluation of the MRD status appears appropriate to extrapolate those at high (MRD
92 positive) or low (MRD negative) risk of relapse, for whom differentiated treatments may be adopted.

93 Although MRD assessment in AML is prognostic¹⁴⁻¹⁸ still less than 50% of relapses are detected by
94 MRD, thus the false negative rate is still high resulting in low specificity. Moreover, MRD is assessed
95 exploiting disparate flow cytometry or molecular protocols so that its use for treatment decisions in
96 AML is still at an early stage. Depending on the technical platforms and targets, a sensitivity of 10^{-3} to
97 10^{-6} is reported.¹⁹ In particular, we observed that the integrated evaluation of baseline prognosticators
98 and MRD improves risk-assessment and helps optimizing post-remission therapy.²⁰ In fact, directing
99 MRD-positive patients towards intensified therapy like AlloSCT while sparing those MRD-negative
100 the procedure-related morbidity and mortality, may be highly beneficial in terms of toxicity
101 minimization.¹⁰

102 Considering all the above, the GIMEMA (Gruppo Italiano Malattie EMatologiche dell'Adulto)
103 Foundation has developed a risk-adapted, MRD oriented, prospective clinical trial, the strategy of
104 which consisted in the prognostic integration of pre-treatment cytogenetics and genetics with post-
105 consolidation MRD, as detected by multiparametric flow-cytometry (MFC). Based on this strategy,
106 patients were to receive a post-consolidation autologous stem cell transplantation (AuSCT) or AlloSCT
107 respectively, depending on their risk profile. We report here the final analysis of this multicenter study.

108

109 **Patients and Methods**

110 *Patients*

111 Previously untreated patients with a diagnosis of *de novo* AML according to the WHO diagnostic
112 criteria²¹ were recruited to the GIMEMA AML1310 Study (*EudraCT number 2010-023809-36*;
113 *ClinicalTrials. Gov Identifier NCT01452646*) provided they met the criteria for eligibility (see
114 supplemental material). The study was approved by the ethics committees of the participating
115 Hospitals/Academic Institutions and was conducted in accordance with the Declaration of Helsinki. All
116 participants gave their informed consent.

117

118 *Study Design*

119 The main objective of the study was to verify whether the delivery of a post remission therapy, the
120 intensity of which was risk-driven, improved the outcome of adult patients with AML in terms of
121 increased anti-leukemic efficacy. The primary endpoint of the study was overall survival (OS) at 24
122 months from treatment start, for comparative purposes we included a historical control consisting of
123 patients recruited to the previous LAM99P GIMEMA trial.¹ Secondary endpoints were complete
124 remission (CR) or CR incomplete (CRi) rate after induction, disease free survival (DFS) and
125 cumulative incidence of relapse (CIR) from CR. Upfront evaluation included bone marrow (BM)
126 aspirate for morphology, cytogenetics, molecular genetics and MFC analysis. The baseline MFC
127 assessment was a necessary step, not only for diagnostic purposes, but also to identify leukemia
128 associated immunophenotypes (LAIP). Identification of baseline LAIPs was the essential requirement
129 for monitoring MRD after therapy; at the established time-point, BM MRD was determined by a high-
130 sensitivity 8-color MFC assay. Based on several retrospective validations in the context of former
131 EORTC/GIMEMA protocols,²² the threshold for discriminating MRD negative from MRD positive
132 cases was set at 3.5×10^{-4} residual leukemic cells and the selected time-point was the post-consolidation
133 phase, once the hematologic recovery was complete. Patients were studied at diagnosis for the presence
134 of RUNX1-RUNX1T1 or CBF β /MYH11 rearrangements, defining core binding factor (CBF)
135 leukemias, and for NPM1, FLT3 and c-KIT mutations. In CBF or NPM1 positive AML, MRD was
136 investigated as reported elsewhere.^{14,23,24} Molecular analysis, LAIPs assessment and post-consolidation
137 MRD determinations were centralized at Laboratorio di Diagnostica Integrata Oncoematologica
138 "OPPO", at Tor Vergata University Hospital of Rome, whereas conventional karyotype was carried out
139 at local institutions. Response to treatment was assessed on BM and peripheral blood, according to the
140 recommendations of an international working group.²⁵ Patients who did not achieve CR/CRi or PR
141 after the first induction course or CR/CRi after two induction courses were considered as treatment
142 failures. The AML1310 trial was designed at a time when ELN 2010/2017 and NCCN 2018
143 recommendations were not yet published. Therefore, when the trial regulatory path was concluded, we
144 started recruiting and stratifying patients according to contemporary classification, that was the NCCN
145 2009 version 1.²⁶ For the purpose of our study, 4 categories of risk were identified (Table 1): favorable-
146 (NCCN-FR) or poor-risk (NCCN-PR) patients, who were submitted to AuSCT or AlloSCT
147 respectively; intermediate-MRD negative (NCCN-IR-Neg) or positive (NCCN-IR-Pos) patients, who
148 were to receive AuSCT or AlloSCT, respectively. Moreover, we enucleated a fifth group of patients
149 belonging to the intermediate-risk category, in whom we failed to identify any LAIP (NCCN-IR-no-

150 LAIP category); these patients were allocated to the AuSCT post-consolidation option. AlloSCT and
151 AuSCT were to be performed within three months of the end of the consolidation course.

152

153 *Treatment*

154 Induction consisted of i.v. daunorubicin 50 mg/m² daily on days 1,3 and 5; i.v. etoposide 50 mg/m²
155 daily on days 1 to 5; i.v. cytarabine 100 mg/m² as a daily continuous infusion, days 1 to 10. All pts in
156 CR/CRi, after one-two induction cycles received one consolidation course consisting of i.v.
157 daunorubicin 50 mg/m² daily on days 4,5 and 6 and i.v. cytarabine 500 mg/m² every 12 hours on days
158 1 to 6. In patients belonging to NCCN-FR and NCCN-IR categories, peripheral blood stem cell
159 collection was attempted by initiating, on day 20 from the start of consolidation therapy, G-CSF until
160 completion of stem cell collection. In the case of failure to collect a sufficient number of peripheral
161 blood stem cells, BM was used as a source. In the case of poor BM harvest, instead of AuSCT, patients
162 were to receive a second consolidation course with high dose cytarabine (HDARAC). Post-
163 consolidation therapy was based on risk-allocation: NCCN-FR patients were to receive AuSCT;
164 NCCN-PR patients were to receive AlloSCT; NCCN-IR patients were to receive AuSCT or AlloSCT
165 depending on the levels of BM MRD as measured by MFC, after consolidation therapy. Allocation to
166 AlloSCT required the procedure to be performed whatever the source of stem cells (HLA-identical
167 sibling, HLA-identical unrelated donor, cord blood, HLA-haploidentical sibling). Salvage therapy
168 consisted of one or two courses of i.v. fludarabine 30 mg/m² daily, on days 1-5; cytarabine 2000 mg/m²
169 daily, on days 1-5; idarubicin 8 mg/m² daily, on days 1-3. Whatever the original NCCN risk category
170 of assignment, patients with resistant disease after 1-2 cycles of induction therapy were considered
171 poor-risk and allocated to the AlloSCT procedure once CR/CRi was achieved.

172

173 *Statistical analysis and sample size calculation*

174 The primary objective was the percentage of OS at two years. An estimated number of 213 subjects
175 was initially required to accomplish this primary objective. This sample size was to achieve a 90%
176 power to detect a difference of 10% between the null hypothesis that OS at two years is 50% and the
177 alternative hypothesis that OS is 60%, using a Single-Stage Phase II design with a 5% significance
178 level (based on data of the historic control group GIMEMA LAM99P).¹ Based on the historical control
179 group, we also considered that approximately 70% of the observed patients would have been classified
180 as IR, therefore allowing to reach the figure of 150 patients available for MRD driven treatment
181 allocation. However, after 173 subjects were enrolled, only 56 belonged to the IR category (32% vs
182 70% expected). Therefore, to reach the target of 150 subjects belonging to the IR category, an
183 amendment to the protocol was adopted in 2013 and the sample size was adjusted to 515 subjects to
184 recruit. The efficacy analysis was performed as per treatment received, including individuals who
185 commenced induction therapy and censoring patients at the time when they received a non-assigned
186 treatment. OS (time elapsed from treatment start to death) and DFS (time from CR to relapse or death
187 in remission) were calculated using the Kaplan-Meier product limit estimator. Differences in terms of
188 OS and DFS were evaluated by means of Log-Rank test in univariate analysis and by means of Cox
189 regression model in multivariate analysis, after assessment of proportionality of hazards. All variables
190 with a p-value less than 0.15 in univariate analysis were considered into the multivariate models. The
191 influence of the transplant (AuSCT and AlloSCT) on the survival outcome was evaluated in the Cox

192 model by means of a time-dependent covariate. Cumulative incidence of relapse (CIR) was estimated
193 by cumulative incidence curves using the proper non-parametric method. Patients' and disease
194 characteristics were summarized by means of cross-tabulations for categorical variables or by quintiles
195 for continuous variables. Differences between categorical variables or response rates in subgroups were
196 tested by the chi-squared or Fisher exact tests, as appropriate. Confidence intervals were calculated at
197 95% level and all tests were two-sided, accepting $p \leq 0.05$ as indicating a statistically significant
198 difference. All analyses were performed using the SAS (version 9.4) and R (R Foundation for
199 Statistical Computing, Vienna, Austria) system software. Study data were collected and managed using
200 the REDCap20 electronic data capture tools hosted at GIMEMA Foundation.

201

202 **Data Sharing Statement**

203 Individual participant data will not be shared.

204 **Results**

205 Between January 2012 and May 2015, 515 patients with de novo AML, seen at 55 GIMEMA
206 institutions, were registered to the trial. Fifteen patients did not commence induction because of pre-
207 therapy death, infections or ineligibility, 500 started treatment and were available for the analysis.
208 Demographic characteristics are summarized in Table 2 and 3. Median age was 49 (18-60.9) years and
209 52% were males. For 429 evaluable patients, cytogenetic distribution was favorable, intermediate and
210 poor in 11%, 73% and 16% respectively. Among 500 cases, RUNX1/RUNX1T1 was detected in 27
211 (5%) with 12 (44%) also c-KIT mutated; CBF β /MYH11 was positive in 37 (7%) with 4 (11%) also c-
212 KIT mutated; FLT3-ITD and NPM1 mutations were detected in 46 (9%) and in 107 (21%)
213 respectively. Finally, concomitant mutations of NPM1 and FLT3-ITD were observed in 80 cases
214 (16%). We found no instances of FLT3 mutations in CBF positive AML. Based on this data, patients'
215 distribution within the risk-categories was as follows: 138 (28%) were NCCN-FR, 127 (25%) NCCN-
216 IR, 47 (9%) NCCN-IR-no-LAIP, 188 (38%) NCCN-PR. Patients' disposition is illustrated in Figure 1.
217 After the first induction cycle, 333 (67%) and 21 (4%) patients achieved a CR and CRi, respectively. A
218 second induction course was delivered to 10 of 13 patients in PR, with seven entering CR. Therefore,
219 after one-two cycles of induction 361 (72%) patients obtained a CR: 88% in the NCCN-FR category,
220 65% and 69% in the NCCN-IR and NCCN-PR category respectively ($p < 0.001$). Eighty-four (17%)
221 patients had a refractory AML and 63 of them received a salvage therapy; 23 of these 63 (37%)
222 achieved a CR. Three-hundred-42/361 (95%) patients started the consolidation phase and were
223 treatment allocated: 177 (52%) to AuSCT [115 (65%) NCCN-FR, 35 (20%) NCCN-IR-Neg, 27 (15%)
224 NCCN-IR-no-LAIP] and 165 (48%) to AlloSCT [122 (74%) NCCN-PR, 43 (26%) NCCN-IR-Pos]. Of
225 the 177 AuSCT candidates, 110 (62%) were transplanted [78 (71%) NCCN-FR, 20 (18%) NCCN-IR-
226 Neg, 12 (11%) NCCN-IR-no-LAIP]. Of the 165 AlloSCT candidates, 110 (67%) were transplanted [78
227 (71%) NCCN-PR, 32 (29%) NCCN-IR-Pos]. If we include also the 23 patients who achieved a CR
228 after salvage therapy, the group of AlloSCT candidates enlarges to 188. Since 20 of these 23 patients
229 were given AlloSCT, the number of AlloSCT candidates who received it was 130/188 (71%). For the
230 78 patients belonging to the NCCN-PR category, the source of stem cells was a HLA-identical sibling
231 in 26, a HLA-identical unrelated donor in 34, umbilical cord blood in 1 and HLA-haploidentical sibling
232 in 17; for the 32 belonging to the NCCN-IR-Pos category, the source of stem cells was a HLA-identical
233 sibling in 12, a HLA-identical unrelated donor in 9, umbilical cord blood in 1 and HLA-haploidentical
234 sibling in 10. By physicians' decision, one patient belonging to NCCN-PR category received AuSCT
235 and one belonging to NCCN-FR category received AlloSCT.

236

237 *Overall survival, Disease Free Survival and Cumulative Incidence of Relapse*

238 OS and DFS rates at 24 months of our historical control were 49% (95%CI 47-52) and 55% (95% CI
239 52-59), respectively.¹ In the present trial, after a median follow-up of 28.8 months, 2-year OS was 56%
240 (95% CI 52-61) with a median duration of 38 months (Figure 2) and DFS was 54% (95% CI 49-60)
241 with a median duration of 32.4 months (Figure 2). The estimated OS at 24 months of 56% was less

242 than the alternative hypothesis of 60%. However, the upper value of 95% confidence interval included
 243 also the alternative hypothesis of 60% 2-year survival. Therefore, we considered the trial as not
 244 conclusive with regards to the primary endpoint. CIR, considering death in CR as a competing risk,
 245 was 33% (95% CI 28-38) (Figure 2). When splitting the survival analysis according to the identified
 246 categories of risk, 2-year OS was 42% (95% CI 36-50) for NCCN-PR patients, 58% (95% CI 50-68)
 247 for NCCN-IR patients, 74% (95% CI 67-82) for NCCN-FR patients and 50% (95% CI 37-67) for
 248 NCCN-IR-no LAIP patients ($p < 0.0001$) (Figure 3). Two-year DFS was 45% (95% CI 37-55) for
 249 NCCN-PR patients, 61% (95% CI 52-73) for NCCN-IR patients, 61% (95% CI 52-71) for NCCN-FR
 250 patients and 48% (95% CI 33-70) for those belonging to the NCCN-IR-no LAIP category ($p = 0.026$)
 251 (Figure 3). Using this risk-adapted approach, DFS duration of NCCN-FR and NCCN-IR was
 252 superimposable whereas the NCCN-IR-no LAIP one was the shortest. When we focused on the NCCN-
 253 IR patients, whose post-consolidation choice was MRD-driven, no significant differences were
 254 observed in terms of 2-year OS between those MRD negative [79% (95% CI 66-94)] and MRD
 255 positive [70% (95% CI 57-86)] ($p = 0.713$) (Figure 4). The same was observed regarding the 2-year
 256 DFS [MRD negative = 61% (95% CI 47-80); MRD positive = 67% (95% CI 53-83)] ($p = 0.773$) (Figure
 257 4). The multivariate analysis confirmed the independent role of risk category in affecting CR rate,
 258 duration of OS and DFS. The transplant procedure (AuSCT plus AlloSCT), analyzed as a time-
 259 dependent variable, affected independently duration of OS. Age affected independently duration of OS
 260 and DFS whereas WBCc achievement of CR (Table 2S).

261

262 *MFC and molecular integrated evaluation of MRD*

263 As an ancillary activity of the protocol, of 251 patients whose AML was characterized by the presence
 264 of a molecular marker useful for MRD assessment, we received 112 BM samples (RUNX-RUNX1=9,
 265 CBFB-MYH11=9 and NPM1=94) at the post-consolidation time-point. In 60 of these, we had the
 266 opportunity to combine the post-consolidation results of MFC and RT-qPCR MRD studies. This
 267 integrated analysis identified 4 categories of patients: double negative (MFCneg/PCRneg), double
 268 positive (MFCpos/PCRpos) and single positive (MFCpos/PCRneg or MFCneg/PCRpos). Patients who
 269 were double negative had a 2-year OS and DFS of 89% (95% CI 71-100) and 69% (95% CI 44-100),
 270 respectively. Patients who were MFCpos/PCRneg had a 2-year OS and DFS of 88% (95% CI 73-100)
 271 and 76% (95% CI 58-100), respectively. Patients who were MFCneg/PCRpos had a 2-year OS and
 272 DFS of 87% (95% CI 72-100) and 65% (95% CI 45-92), respectively. Finally, patients who were
 273 double positive had a 2-year OS and DFS of 55% (95% CI 34-87) and 22% (95% CI 9-58),
 274 respectively (Figure 5 A and B, $p = 0.037$ and 0.003 , respectively)).

275

276 *AuSCT versus HDARAC consolidation*

277 As per protocol, 19 patients (18 NCCN-FR and 1 NCCN-IR) received HDARAC, since they did not
 278 have enough stem cells collected. Figure 6 shows OS and DFS of these patients compared to those who
 279 were submitted to AuSCT. OS was 83% (95% CI 67-100) and 85% (95% CI 78-93), respectively
 280 ($p = 0.753$); DFS was 68% (95% CI 50-93) and 63% (95% CI 54-73), respectively ($p = 0.595$). Of these
 281 19 patients, 15 were NCCN-FR MFCneg/PCRneg, MFCpos/PCRneg or MFCneg/PCRpos, 3 NCCN-
 282 FR MFCpos/PCRpos and 1 was NCCN-IR MRD negative.

283

284 Discussion

285 The role of molecular and cytogenetic abnormalities in predicting response to therapy and survival in
286 patients with AML has been extensively documented.²⁴⁻²⁶ Indeed, genetic/cytogenetic abnormalities are
287 powerful prognosticators, so that obtaining information about their presence is essential for an optimal
288 decision-making process. The clinical implication is that, based on their genetic status, patients would
289 benefit from more or less aggressive post-consolidation strategy such as AuSCT and AlloSCT or, in a
290 more modern vision, from targeted new agents. However, prognostic models barely based on pre-
291 treatment covariates such as genetic status, have a limited predictive ability.^{11,29} This highlights the
292 need not only to expand further our knowledge about the genetic and molecular pattern of AML but
293 also highlights the potential role of “factors after diagnosis” such as MRD monitoring. Therefore,
294 integrating baseline factors and monitoring of MRD appears a promising tool to refine and possibly
295 customize our outcome prediction ability in AML. This philosophy was at the basis of the GIMEMA
296 AML1310 protocol in which, deviating from the classical “*one size fits all*” approach, we applied a
297 risk-adapted and MRD-driven approach. AlloSCT is generally recommended when the risk of relapse
298 exceeds 35%-40% if the procedure is not performed.^{7,10} In this view, NCCN-PR category represents a
299 priority and, in these patients, AlloSCT should be performed as soon as CR is achieved. However, a
300 HLA-identical sibling is available for less than 30% of the patients³⁰ and, in reality, even less than 30%
301 receive it, due to disease recurrence.¹¹ In our study, utilization of any available source of stem cells
302 resulted in 71% of AlloSCT candidates receiving it. Adoption of this strategy also translated in a 2-year
303 OS and DFS of 42% and 45% respectively for the NCCN-PR category (Figure 2). Such figures
304 compare very favorably with the two-year OS and DFS of 20%-30% currently reported for this
305 category.^{27,29} Based on our study design, patients belonging to the NCCN-FR category were given
306 AuSCT as a post-consolidation therapy. The role of AuSCT is controversial; in one randomized study it
307 provided better DFS and similar OS as conventional consolidation chemotherapy.³² In our NCCN-FR
308 category, 2-year OS and DFS were 74% and 61% respectively (Figure 3). We believe there is still a
309 role for AuSCT; indeed, this option has the advantage of sparing patients multiple courses of post-
310 consolidation chemotherapy (usually high/intermediate dose cytarabine). In fact, the recently revised
311 ELN classification suggests that limiting AuSCT to MRD negative AML might improve the results.¹¹
312 Based on our limited experience, high dose cytarabine might represent the choice for patients with very
313 “high quality” CR such as those NCCN-FR MFCneg/PCRneg (Figure 6). Management of patients
314 belonging to the NCCN-IR category is still controversial. For these patients, the relapse rate after
315 AuSCT can be as high as 50%-55%,¹⁰ so that this option appears as a suboptimal approach. Indeed,
316 AlloSCT is recommended for patients within this category. However, in selected patients with MRD
317 negative-CR there might still be a room for AuSCT.¹⁰ In the present study, we planned AlloSCT or
318 AuSCT for NCCN-IR patients, based on the level of MRD after the post-consolidation course. By
319 making this choice, we observed that the two-year OS and DFS were 58% and 61% respectively
320 (Figure 3). This figure compares very favorably with recent analyses showing, for these patients, a 2-
321 year OS and DFS of approximately 35% and 50%.^{27,29} Using this strategy, we also noted that the 2-year
322 DFS of NCCN-IR patients was prolonged to equal that of NCCN-FR patients (Figure 3). Finally,
323 within the NCCN-IR category, we focused on outcome as influenced by the post-consolidation MRD
324 status. By delivering AuSCT to NCCN-IR-Neg and AlloSCT to NCCN-IR-Pos patients, we observed

325 no difference in terms of 2-year OS or DFS (Figure 4). The stratification role of MRD determination in
326 intermediate-risk patients has been recently suggested in a prospective survey of the NCRI-AML17
327 trial.³³ According to the authors, a MRD positive finding helps selecting patients who can benefit from
328 AlloSCT. An indirect confirmation of the importance of MRD determination in intermediate-risk
329 category was that our 47 NCCN-IR-no-LAIP patients who were submitted to AuSCT had the shortest
330 duration of 2-year OS and DFS (Figure 2). A reasonable explanation is that these patients harbored
331 significant post-chemotherapeutic levels of MRD, meaning that AlloSCT would have been the most
332 appropriate choice. Our results highlight the potent anti-leukemic effect exerted by AlloSCT in NCCN-
333 IR-Pos patients and the minimization of toxicity after AuSCT in NCCN-IR-Neg ones. This
334 interpretation can be extended to include the overall population we had under investigation; indeed,
335 generating the maximum anti-leukemic effort in high-risk patients (NCCN-PR + NCCN-IR-Pos) and
336 preserving from excess of toxicity those who are at low-risk (NCCN-FR + NCCN-IR-Neg) appears a
337 very plausible goal. In this view, the integration of different techniques for MRD monitoring may offer
338 the chance to improve even further our capability to discriminate prognostically discrete subsets of
339 patients, directing treatment more precisely. Combining MFC and RT-qPCR for cases carrying a
340 molecular signature, we demonstrated that double positive patients had the worst prognosis. For these
341 patients, a front-line intensified program appears a reasonable option (Figure 5). Although there is
342 evidence that AlloSCT is not able to reverse the unfavorable long-term impact of MRD positivity,³⁴⁻³⁷
343 we believe that a pre-transplant MRD positive status should not be a contraindication for performing
344 it.³⁸⁻⁴⁰ In the study by Walter and Araki, patients who were MRD positive before the transplant had an
345 outcome comparable to the one of patients with active disease. However, these studies were
346 retrospective, the patient population was heterogeneous in terms of age, conditioning regimens received
347 and there was a concentration of adverse karyotype and secondary AML in the group of MRD positive
348 patients. Our experience takes advantage of a prospective and homogeneous context in terms of therapy
349 delivered and risk-stratification. A recent, retrospective analysis of 547 patients enrolled in
350 HOVON/SAKK protocols indicates that, although all categories benefit from AlloSCT, the absolute
351 benefit was greater in pre-transplant MRD positive than MRD negative patients.⁴¹ Our present
352 experience adds a piece of information favoring the use of AlloSCT in MRD positive patients, and
353 future trials should possibly explore the prognostic role of different levels of pre-transplant MRD³⁸ and
354 the value of post-transplant maintenance.

355 In conclusion, we recognize that the study suffers from some intrinsic limitations due to the changes
356 occurring over the time (more modern biologic knowledge, new AML classifications and an ever more
357 frequent MRD monitoring) that make the historical control and the study population not fully
358 superimposable. However, this is one of the first attempts to apply a prospective program of risk-
359 adapted, MRD-driven therapy, integrating upfront genetics and post-consolidation MRD status, in
360 AML of adults. In the NCCN-FR category, AuSCT guarantees the same survival expectation as
361 multiple courses of cytarabine. In the NCCN-IR category, AlloSCT can be avoided if MRD is not
362 measurable; if MRD is positive, AlloSCT can prolong OS and raise the DFS duration to the level of
363 NCCN-FR patients. Finally, using all the available sources of stem cells, allowed AlloSCT to be
364 delivered to a large proportion of the candidates, emphasizing the feasibility of the trial transplant
365 policy.

366

367 **Authors' contribution**

368

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382 **Declaration of interests**

383

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545
546

547 **Table 1** Risk categories in which the patients were stratified

548 **1.NCCN Favorable-Risk (NCCN-FR)**

549 Inv(16)

550 t(8;21)

551 t(16;16)

552 RUNX1/RUNX1T1 without c-Kit mutations

553 CBF β /MYH11 without c-Kit mutations

554 NPM1 mutation without FLT3 mutations

555

556 **2.NCCN Intermediate-Risk (NCCN-IR) Post-consolidation MRD Negative**

557 Normal karyotype

558 +8 Only

559 t(9;11) only

560 other karyotypic abnormalities not listed as FR or PR

561 RUNX1/RUNX1T1 with c-Kit mutation

562 CBF β /MYH11 with c-Kit mutation

563 no NPM1 mutations

564 no FLT3-ITD mutations

565

566 **3.NCCN Intermediate-Risk (NCCN-IR) Post-consolidation MRD Positive**

567 As in 2 but with measurable MRD after the consolidation course

568 **4.NCCN Poor-Risk (NCCN-PR)**

569 Complex karyotype (≥ 3 abnormalities)

570 -5/5q-

571 -7/7q-

572 Abnormalities of 11q23, excluding t(9;11)

573 inv(3)

574 t(3;3)

575 t(6;9)

576 FLT3-ITD mutations

577

578 **5.NCCN Intermediate-Risk LAIP negative (NCCN-IR-no LAIP)**

579 Patients belonging to the intermediate-risk category in whom no leukemia associated

580 immunophenotype (LAIP) was identified, at diagnosis

581

582 **Table 2.** Patients demographics and clinico-biologic characteristics.

	Overall
No.	500
Median Age (range)	49 (18-60,9)
Sex	
Male no./total no. (%)	260(52)
Female no. (%)	240(48)
Median WBC (range)	14x10 ⁹ /L (0.16-352)
Cytogenetics Favorable risk no./total no. (%)	47(11)
Cytogenetics Intermediate risk no./total no. (%)	315(73)
Cytogenetics Poor risk no./total no. (%)	67(16)
RUNX1/RUNX1T1 no./total no. (%)	27(5)
RUNX1/RUNX1T1/c-KIT^{mut} no./total no. (%)	12/27 (44)
CBFβ/MYH11 no./total no. (%)	37(7)
CBFβ/MYH11/c-KIT^{mut} no./total no. (%)	4/37 (11)
FLT3-ITD^{mut} no./total no. (%)	46(9)
NPM1^{mut} no./total no. (%)	107(21)
NPM1^{mut}/FLT3-ITD^{mut} no./total no. (%)	80(16)
NCCN-FR no./total no. (%)	138(28)
NCCN-IR no./total no. (%)	127(25)
NCCN-IR-no LAIP no./total no. (%)	47(9)
NCCN-PR no./total no. (%)	188(38)

583 WBC = white blood cell count

584 NCCN-FR = National Comprehensive Cancer Network-Favorable Risk

585 NCCN-IR = National Comprehensive Cancer Network-Intermediate Risk

586 NCCN-IR-no LAIP = National Comprehensive Cancer Network-Intermediate Risk with no

587 Leukemia Associated Immuno Phenotype

588 NCCN-PR = National Comprehensive Cancer Network-Poor Risk

589

590 **Table 3.** Patients demographics and clinico-biologic characteristics according to treatment received.

		AlloSCT	AuSCT	HDARAC	p
	n	131	111	19	
Median age		46.7	48.4	54.7	0.033
(range)		(18-60.9)	(18-60.8)	(27-59.5)	
Sex (%)	Male	66 (50)	59 (53)	10 (53)	0.909
	Female	65 (50)	52 (47)	9 (47)	
Median WBC		12.90x10 ⁹ /L	16.7x10 ⁹ /L	11.6x10 ⁹ /L	0.462
(range)		(0.16-352)	(0.90-186)	(1.24-102)	
Risk Category (%)	NCCN-FR	1 (1)	78 (71)	18 (95)	<0.001
	NCCN-IR	41 (32)	20 (18)	1 (5)	
	NCCN-PR	87 (66)	1 (1)	0 (0)	
	NCCN-IR-no LAIP	2 (1)	12 (10)	0 (0)	

591 AlloSCT = allogeneic stem cell transplant

592 AuSCT = autologous stem cell transplant

593 HDARAC = high dose cytosine arabinoside

594 WBC = white blood cell count

595 NCCN-FR = National Comprehensive Cancer Network-Favorable Risk

596 NCCN-IR = National Comprehensive Cancer Network-Intermediate Risk

597 NCCN-IR-no LAIP = National Comprehensive Cancer Network-Intermediate Risk with no

598 Leukemia Associated Immuno Phenotype

599 NCCN-PR = National Comprehensive Cancer Network-Poor Risk

600

601 **Figure Captions**

602

603 **Figure no. 1**

604 Consort diagram of patients' disposition

605

606 **Figure no. 2**

607 Overall Survival (A), Disease Free Survival (B) and Cumulative Incidence of Relapse (C) of the whole
608 patients' population

609

610 **Figure no. 3**

611 Overall (A) and Disease Free Survival (B) plotted by National Comprehensive Cancer Network
612 (NCCN) categories of risk

613

614 **Figure no. 4**

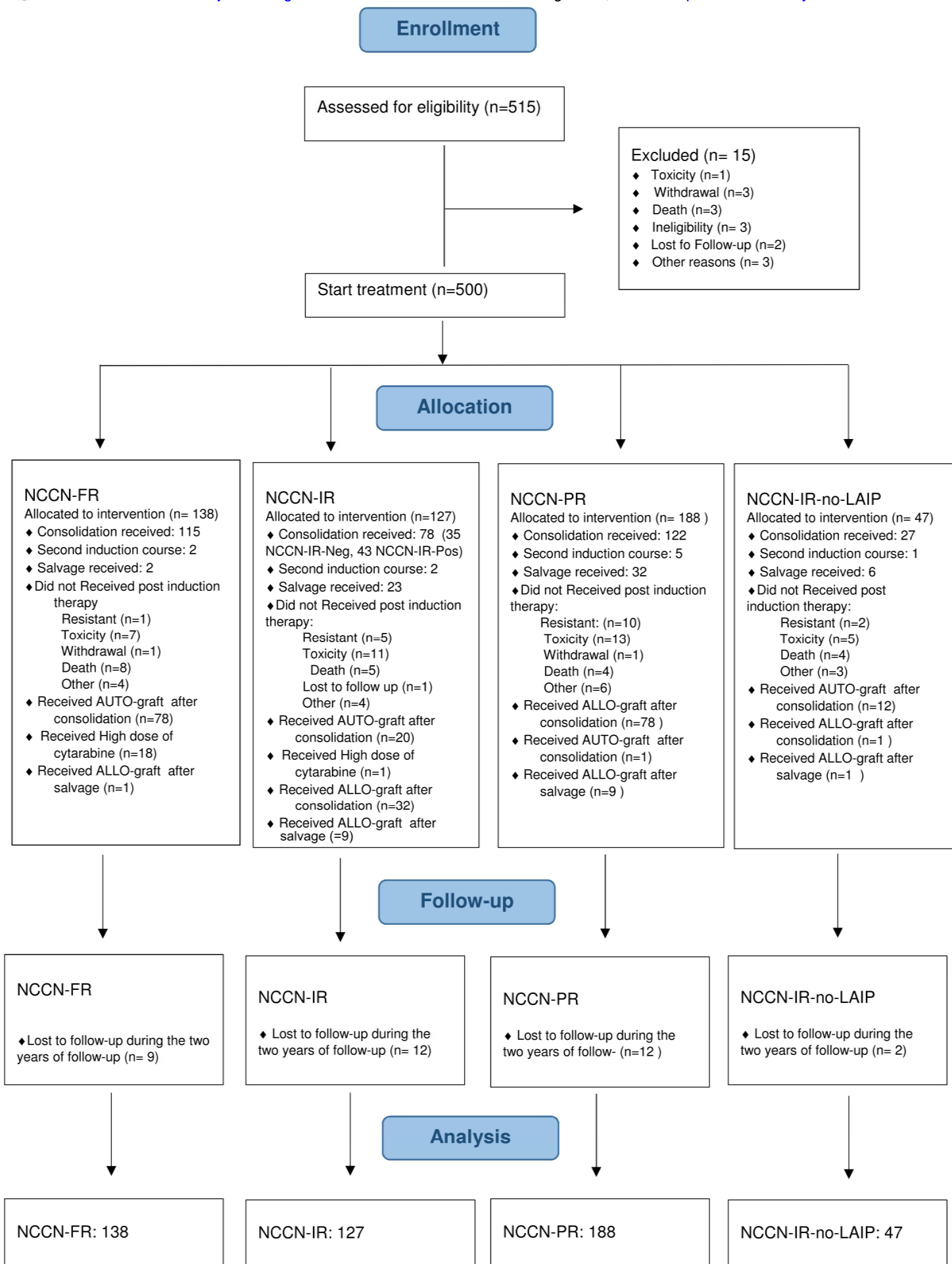
615 Overall (A) and Disease Free Survival (B) of National Comprehensive Cancer Network Intermediate
616 Risk (NCCN-IR) category, plotted by the status of Minimal Residual Disease (MRD) after
617 consolidation therapy

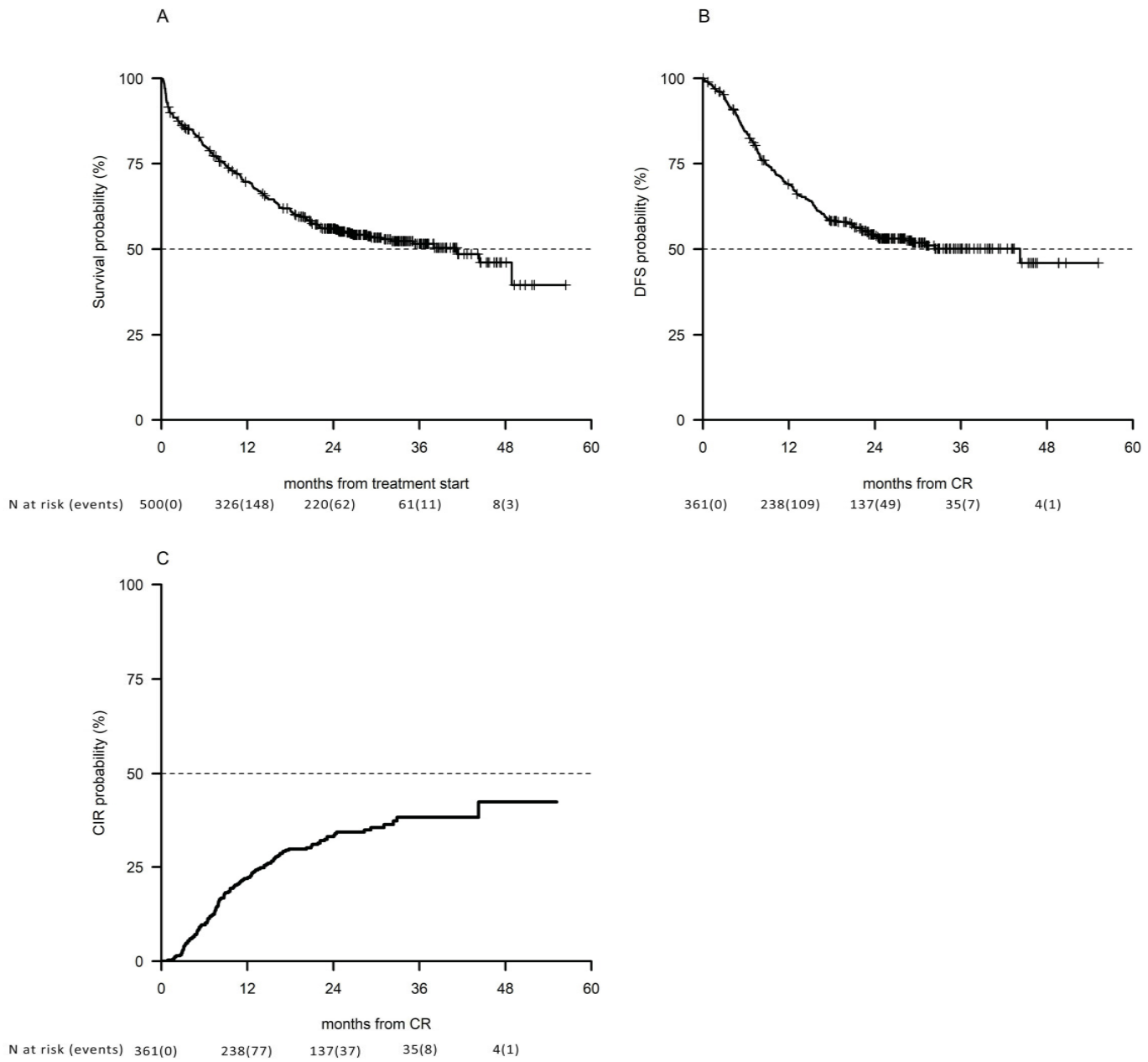
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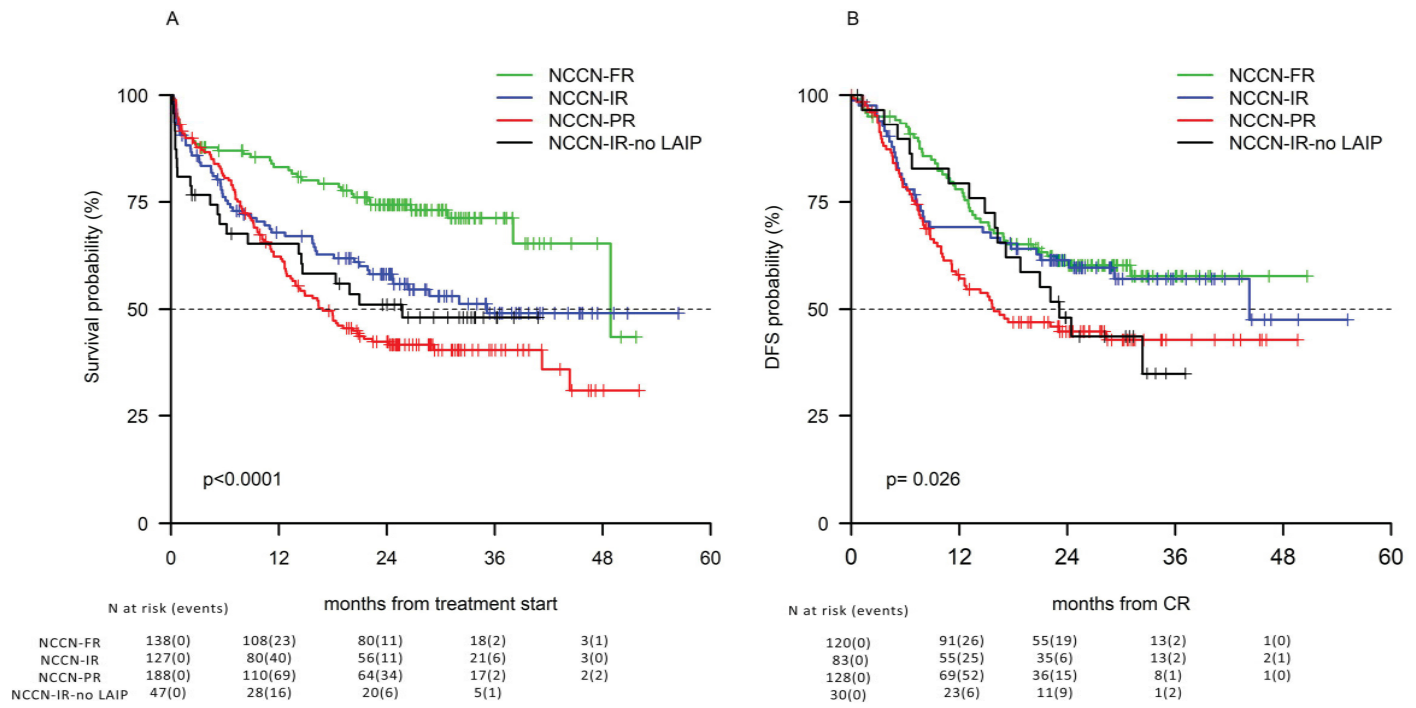
619 **Figure no. 5** Overall (A) and Disease Free Survival (B) of 60 patients whose Minimal Residual
620 Disease (MRD) was analyzed integrating multiparametric flow cytometry (MFC) and RT-qPCR (PCR).

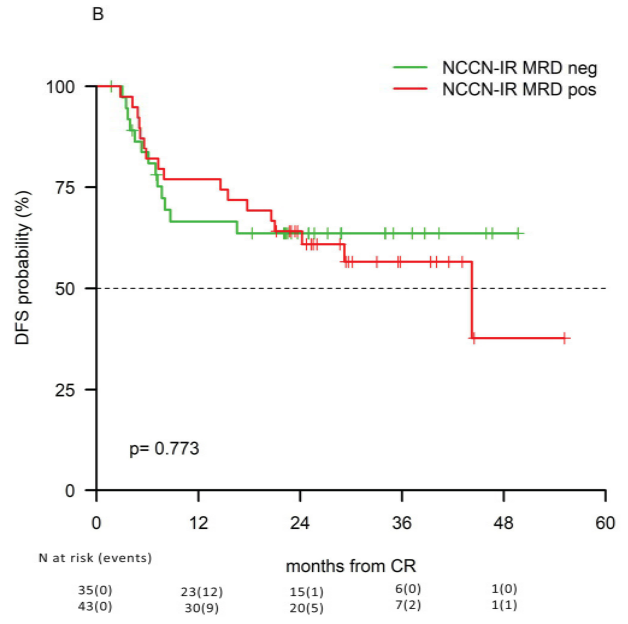
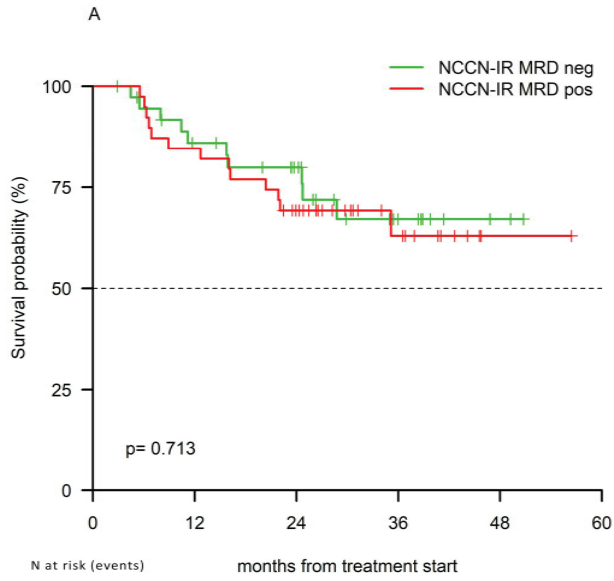
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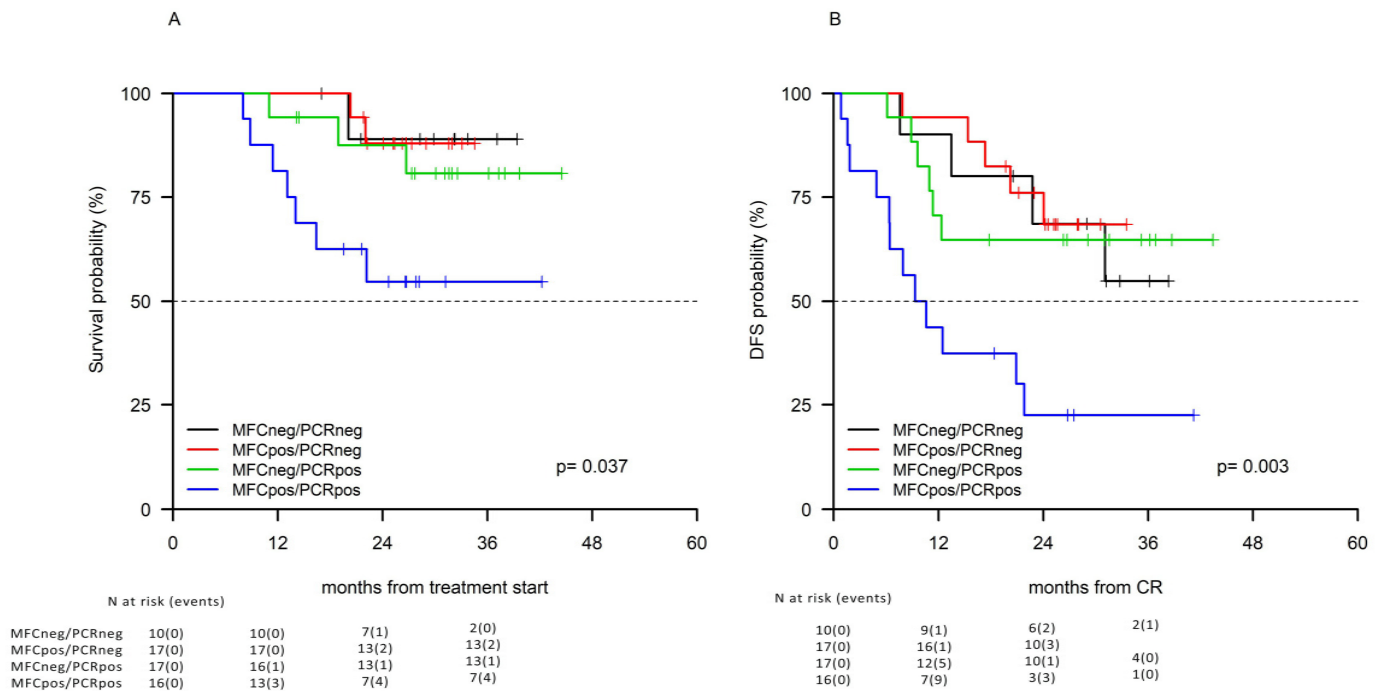
622 **Figure no. 6** Overall (A) and Disease Free Survival (B) of the 19 patients who received high dose of
623 cytarabine versus those who received autologous stem cell transplant (AuSCT).

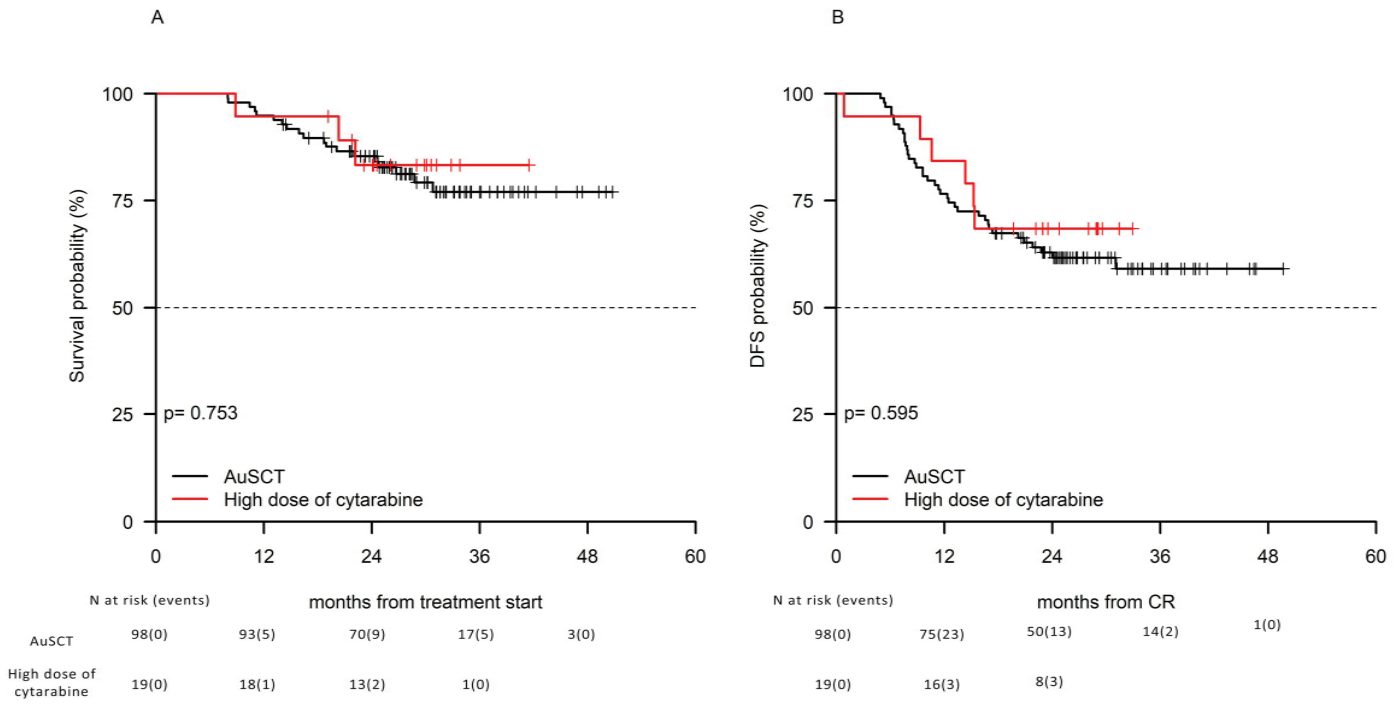














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GIMEMA AML1310 TRIAL OF RISK-ADAPTED, MRD-DIRECTED THERAPY FOR YOUNG ADULTS WITH NEWLY DIAGNOSED ACUTE MYELOID LEUKEMIA

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