The effect of allogeneic stem cell transplantation on outcome in younger acute myeloid leukemia patients with minimal residual disease detected by flow cytometry at the end of post-remission chemotherapy

Edward Laane
Åsa Rangert Derolf
Elisabet Björklund
Joanna Mazur
Hele Everaus
Stefan Söderhäll
Magnus Björkholm
Anna Porwit-MacDonald

Minimal residual disease (MRD) levels were determined by multi-parameter flow cytometry in 45 younger adult patients (≤60 years old) with acute myeloid leukemia (AML) in complete remission. Data were collected after induction (MRD1; n=43) and/or at the end of post-remission chemotherapy or before stem cell transplantation (SCT)(MRD2; n=31). Patients with detectable MRD2 who underwent allogeneic or autologous SCT had significantly better 5-year relapse-free survival than patients not transplanted (80%, 53% and 0%, respectively p=0.003). Therefore allogeneic SCT should be considered in younger adult AML patients with detectable MRD at the end of post-remission chemotherapy. Autologous SCT may be an alternative for patients not eligible for allogeneic SCT.

Key words: acute myeloid leukemia, minimal residual disease, flow cytometry, immunophenotyping, stem cell transplantation.

Haematologica 2006; 91:833-836

From the Department of Pathology and Cytology (EL, EB, APM), Department of Medicine, Division of Hematology (ARD, MB), Childhood Cancer Research Unit, Astrid Lindgren Child Hospital (SS), Karolinska University Hospital Solna and Karolinska Institutet, Stockholm, Sweden; Department of Epidemiology, Institute of Mother and Child, Warsaw, Poland (JM); Hematology-Oncology Clinic, Tartu University, Tartu, Estonia (EL, HE).

Correspondence:
Anna Porwit-MacDonald, Department of Pathology and Cytology, Karolinska University Hospital, Radiumhemmet, SE 171 76 Stockholm, Sweden.
E-mail: anna.mac-donald@karolinska.se

With modern chemotherapy, complete remission (CR) may be achieved in up to 70% of younger adults (≤60 years old), with acute myeloid leukemia (AML), but long-term survival rates are as low as 25-40%.[1] Relapse of the disease is due to minimal residual disease (MRD) defined as the persistence of submicroscopic levels of leukemic cells in bone marrow with morphological CR. So far, MRD levels detected by multi-parameter flow cytometry have been investigated as a predictor of prognosis in adult AML in four studies, which have shown that the presence of MRD defines patient populations with a poor outcome.[2-5] However, the concept of adjusting post-remission treatment according to the levels of MRD has not been established. Furthermore, it has not yet been fully determined whether MRD predicts prognosis in AML patients treated by allogeneic or autologous stem cell transplantation (SCT). The aim of this study was to evaluate the prognostic significance of MRD in relation to SCT in younger adult AML patients.

Design and Methods

Patients

Between July 1994 and June 2001, 62 younger adults were diagnosed as having non-promyelocytic AML at Karolinska University Hospital, (Stockholm, Sweden). Of these 62 patients, 53 (85%) achieved morphological CR. Eight patients were excluded due to uninformative phenotypes (n=3), lack of sufficient clinical data (n=2) or flow cytometric information (n=3). Follow-up MRD information was available for 45 patients with morphological CR (85%), 23 males and 22 females. At diagnosis the following data were recorded [(median (range)]: age 47 (19-60) years, percentage of bone marrow blasts 71% (34-96%), total white blood cell (WBC) count 23 (0.5-237)×10^9/L, hemoglobin level 89 (44-128) g/L, and platelet count 50 (15-263)×10^9/L. The median follow-up time of surviving patients was 5 years (range 2-9 years). Most of the MRD data were collected during 1994-2000 and integrated in the European BIOMED-1 Concerted Action (BMH-CMT 94-1675).[6] The results were not available to the treating physicians. The study was conducted according to the Helsinki Declaration and was approved by the local ethics committee.

Morphological diagnosis and cytogenetics

The diagnosis of AML was based on morphology and cytochemistry according to the French-American-British (FAB) classification:[7] three patients had M0, ten had M1, 9 had M2, eleven had M4, 10 had M5, one had M6, and there was one case of acute undifferentiated leukemia (AUL). All samples were reviewed and reclassified according to the World Health Organization (WHO) classification:[8] according to this reclassification, three had AML with recurrent genetic abnormalities (one AML with t(8;21)(q22;q22), one with inv(16)(p13q22) or t(16;16)(p13;q22) and one with 11q23 (*MLL*) abnormality), four had therapy-related AML, and 38 had AML not otherwise categorized (four with minimally differentiated AML, ten with AML without maturation, six with AML with maturation, eight with acute myelomonocytic leukemias, eight with acute monoblastic and monocytic leukemias, one with acute erythroid leukemia, and one with bilineage acute leukemia). Morphological CR was defined as less than 5% blasts without detectable Auer rods in bone marrow samples displaying ≥20% cellularity with the maturation of all hematopoietic cell lines in the bone marrow aspirate. Karyotypes (available for 43 patients) were classified into three groups essentially in accordance with the descriptions of Grimwade *et al.*:[9] (i) two patients with t(8;21) and inv(16) formed a group with a favorable prognosis; (ii) five patients with 5q-, near-tetraploid and complex aberrant karyotypes (i.e. three or more clonal chromosome aberrations) were considered as a group with an unfavorable prognosis; (iii) 36 patients with trisomy 8, t(9;22), other aberrations and normal karyotypes formed the group with an intermediate prognosis.

Treatment and outcome

Most patients (n=42) received ICE induction therapy: idarubicin (10 mg/m^2 infusion days 1-3), AraC (1000 mg/m^2 infusion twice daily days 1-4), and etoposide (100 mg/m^2 infusion days 1-5). Patients in CR were given two cycles of consolidation therapy consisting of idarubicin (10 mg/m^2 infusion days 1-2), AraC (1000 mg/m^2 infusion twice daily days 1-3), and etoposide (100mg/m^2 infusion days 1-4). The third cycle of consolidation consisted of AraC (1000 mg/m^2 infusion twice daily days 1-6). One patient with AUL was given ABCDV, which consisted of AraC, betamethasone, cyclophosphamide, doxorubicin and vincristine, and two patients were treated with mitoxantrone, etoposide and AraC (MEA).[10] Allogeneic SCT in the first CR was performed in 16 patients.[11] Autologous SCT as consolidation in the first CR was performed in 15 patients. Patients in CR with no sibling donor received total body irradiation and high-dose AraC followed by autologous-SCT.[12,13] Thirty patients achieved morphological CR after one induction course, 12 after two courses and 3 after three courses. MRD levels were determined at two time-points: at first morphological CR after induction treatment and at the end of post-remission chemotherapy or before SCT. A relapse was diagnosed in 17/45 patients who achieved morphological CR (38%) at a median of 10 months after achieving the remission (range 2-20 months). One patient developed a myelodysplastic syndrome (MDS). Relapse-free survival for patients who had achieved CR was determined as the duration from the date of the first CR to a censoring point, first relapse or death due to any cause. The median duration of relapse-free survival was 36 months (range 2-105 months). Overall survival was measured from the date of diagnosis to death or the censoring point. Twenty patients (44%) died and the median overall survival was 42 months (range 4-107 months). The cause of death was AML relapse in 13 patients, MDS in one patient, allogeneic transplant-related complications in three patients and non-leukemia related factors in three patients.

Flow cytometry

Flow cytometric immunophenotyping was performed as described previously.[14] Data were acquired using a FACScan or FACS-Calibur flow cytometer, equipped with Lysis II or Cell Quest software programs from Becton Dickinson [(BD), Franklin Lakes, NJ, USA]. At diagnosis a total of 15×10^3 non-gated events were acquired. Based on immunophenotypes at diagnosis, phenotypic abnormalities were defined and used in the investigations of follow-up samples. Three-color monoclonal antibody combinations were tested in normal or reactive bone marrow samples for their sensitivity in detecting leukemia-associated immunophenotypes (LAIP) in the current study or by other groups.[2,4,15,16] At follow-up, at least 30×10^3 cells were analyzed in each tube. Live-gate analysis was used in five cases (a total of 100-500×10^3 analyzed cells) with a sensitivity of at least 0.015%. Data were analyzed using Paint-a-Gate Pro (BD) software (Figure 1). In most cases CD34/SSC or CD117/SSC gates were applied. Detectable MRD was defined as a distinct cluster of 15-20 dots. Sensitivity levels were determined as: (i) 0.1% if 30,000 events were acquired, (ii) 0.05% if 30,000 events were acquired in cases with highly aberrant LAIP such as co-expression of CD34 and CD7, CD14, CD56 or CD65 and CD34$^+$/CD15$^+$/HLA–DR$^-$ and (iii) 0.015% if the live-gate approach was used.[15]

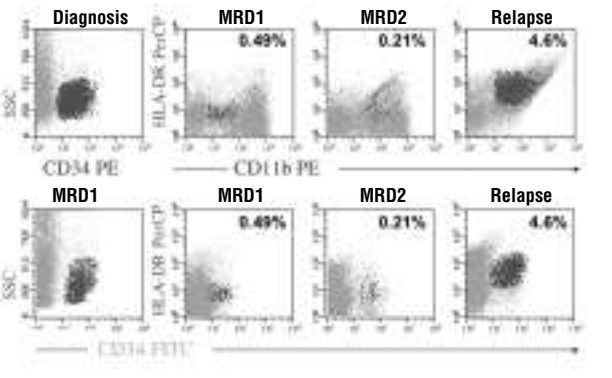

Figure 1. Example of sequential flow cytometry MRD analyses after induction (MRD1), at the end of post-remission treatment (MRD2) and before overt relapse in an AML patient who eventually relapsed. The three-color monoclonal antibody combination CD34 FITC/CD11b PE/HLA-DR PerCP was used in the follow-up samples. The aberrant phenotype relied on over-expression of CD11b and lower than normal expression of HLA-DR in CD34$^+$ cells. The positivity for CD11b and HLA-DR was similar at diagnosis and at incipient relapse (exemplified in the right upper and lower plots). The left upper plot shows CD34 positivity at diagnosis [CD34 PE/Side scatter (SSC) plot] and the left lower plot shows the gating strategy during follow-up (CD34 FITC/SSC plot). The frequencies of CD34$^+$/CD11b$^+$/HLA-DR^{dim} triple positive cells at indicated time-points expressed as percentages of total bone marrow events are shown in the upper and lower middle plots. The diagnostic flow cytometry panel included FITC-conjugated CD7, CD10, CD19, CD22 and CD34; PE-conjugated CD5, CD8, CD11b, CD19, CD38 and CD56; PerCP-conjugated CD3, CD4, CD20, CD45, HLA-DR, cytoplasmic CD3 and all IgG controls from Becton Dickinson; FITC-conjugated CD2, CD4, CD15, anti-MPO, anti-TdT, PE-conjugated CD2, CD13 and CD33 from DakoCytomation (DAKO, Glostrup, Denmark), PE-conjugated CD34 and CD117 from Immunotech (Marseille, France), PE-Cy5 conjugated CD13, CD14, CD19 and CD33 from Caltag Laboratories (San Francisco, CA, USA) and FITC-conjugated CD65 from An-der-Grub Biosearch (Vienna, Austria).

Statistical analyses

Dichotomous variables were compared between different groups using the χ^2 test and continuous variables by Student's t test. Spearman's rank correlation (r) was used to investigate correlations between continuous variables. Relapse-free survival and overall survival were estimated according to the Kaplan-Meier method and differences between groups were analyzed using the log-rank test. The Cox proportional hazard method was used for multivariate analysis. All calculations were performed using the SPSS 12.0 software (SPSS, Chicago, IL, USA). All reported *p* values are two-sided.

Results and Discussion

Frequency of aberrant phenotypes

AML blasts from 93.5% of all patients (58/62) and 94% of those in patients in morphological CR (50/53) expressed LAIP at diagnosis. Aberrant phenotypes were divided into four groups: cross-lineage infidelity (n=32), asynchronous antigen expression (n=48), antigen over-expression (n=9) and lack of antigen expression (n=5). Blasts from 28 of 50 patients (56%) expressed highly aberrant phenotypes (mainly cross-lineage infidelity) and blasts from 22 patients (44%) had aberrant phenotypes, based mostly on maturation asynchrony (Table 1).[15,17,18] At diagnosis 13, 20, 7 and 5 patients expressed one, two, three and four LAIP, respectively.

MRD as a prognostic factor in AML

Prediction of survival by MRD. MRD was analyzed at the first time point in 43 patients in morphological CR and at the second

Table 1. Aberrant phenotypes used in MRD studies.

Aberrant phenotype	*No (%)*
Cross-lineage infidelity	32 (34)
CD2	5
CD7	14
CD19	5
CD56	8
Antigen over-expression	9 (10)
$CD34^{++}$	9
Antigen under-expression	5 (5)
CD33-	5
Asynchronous antigen expression	48 (51)
$CD34^{+}/CD38^{-}/My^{-}$	1
TdT^{+}/My^{-}	6
$CD34^{+}/CD11b+$	11
$(CD34^{+},CD117^{+})/HLA\text{-}DR^{-}/My^{-}$	5
$(CD34^{+},CD117^{+})/CD4^{+}/My^{-}$	5
$(CD34^{+},CD117^{+})/CD15^{++}/My^{-}$	15
$(CD34^{+},CD117^{+})/CD14^{+}$ or $CD65^{+}/My^{+}$	5

Overall, 94 aberrant phenotypes were detected in 45 patients. My: myeloid marker.

time point in 31 patients. MRD was evaluated at both time points in 30 patients. MRD was detectable in 32 (74%) patients at the first time-point and in 17 (55%) at the second time-point. The mean MRD levels were 0.23% (median 0.13%, range 0.01-2.48%) and 0.23% (median 0.06%, range 0.04-2.0%) at the first and second time-points, respectively. By Cox analysis, detectable MRD at the first time point did not predict either relapse-free or overall survival, but there was a trend for longer relapse-free survival in patients with no detectable MRD at the second time-point (p=0.061). Both relapse free survival and overall survival were longer in patients who underwent SCT than in the remainder (p<0.001 and p=0.001, respectively). However, by log-rank test patients with detectable MRD at the first and second assessments had significantly lower 5-year relapse-free survival rates than patients without detectable MRD [51% versus 90% for the first time-point (p= 0.044) and 42% versus 85% for the second time-point (p=0.039), respectively]. Overall survival was not significantly different between patients according to MRD status at the first or second time-points. The percentage of blasts in bone marrow at diagnosis, absolute WBC count, hemoglobin levels, platelet count, number of cycles to achieve morphological CR, and cytogenetic risk groups were not significant predictors of relapse-free or overall survival.

Prognostic significance of allogeneic SCT in relation to MRD status

Since SCT was the only significant prognostic factor in the studied group of AML patients we decided to investigate how this therapy influenced survival of MRD-positive and MRD-negative patients. For this analysis, the patients were divided into four categories: (a) no detectable MRD and allogeneic SCT; (b) detectable MRD and allogeneic SCT; (c) no detectable MRD and no allogeneic SCT; (d) detectable MRD and no allogeneic SCT. These groups had significantly different relapse-free survival rates based on MRD status at both the first and second time points (Figure 2A and B) and showed a trend for a difference in overall survival (p=0.069) based on the second MRD assessment. Patients with detectable MRD at the first and/or second time-points who did not undergo allogeneic SCT had the worst outcome (5-year relapse-free survival 24% and 20%, respectively; and overall survival 34% and 35%). MRD-positive

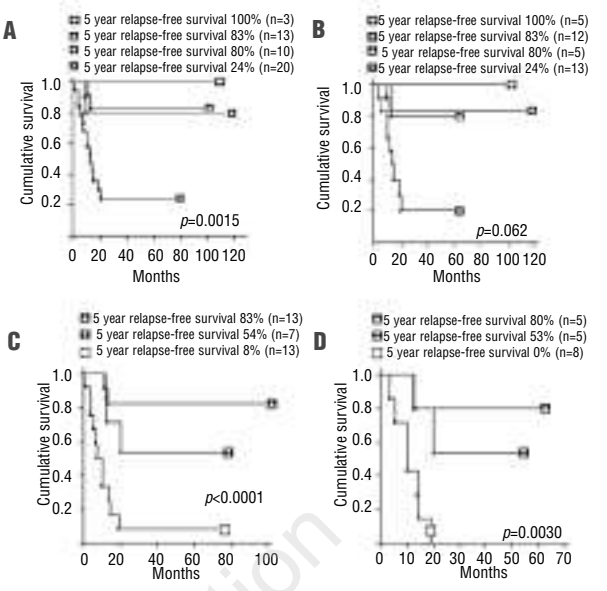

Figure 2. Relapse-free survival (RFS) of younger adult AML patients divided according to MRD status and type of post-remission treatment. MRD status was studied after induction treatment (A and C) and at the end of post-remission chemotherapy or before stem cell transplantation (B and D). A and B: AML patients 19-60 years old were divided in four groups: no detectable MRD (MRD^{-}) and allogeneic SCT (□); detectable MRD (MRD^{+}) and allogeneic SCT (□); MRD^{-} and no allogeneic SCT (□); and MRD^{+} and no allogeneic SCT (□). MRD^{+} patients who underwent allogeneic SCT had similar RFS to MRD^{-} patients who did not undergo allogeneic SCT. C and D: Only MRD^{+} patients were considered. The MRD^{+}/no allogeneic SCT group was divided into two subgroups: patients who underwent autologous SCT (□) and those who received only conventional chemotherapy (□). Patients with detectable MRD after induction treatment (C) or at the end of post-remission chemotherapy/before stem cell transplantation (D) subjected to allogeneic SCT (□) had significantly longer relapse-free survival than patients who received autologous SCT (□) or only conventional chemotherapy (□).

patients who underwent allogeneic SCT had similar cumulative relapse-free survival (Figure 2A and B) to patients who had no detectable MRD at the first and/or second time-point. Similar overall survival rates were also observed in both these groups (67% versus 70% for the first time-point and 75% versus 75% for the second time-point, respectively).

Prognostic significance of autologous SCT in relation to MRD status

During 1994-1997 eligible AML patients were included in an extended British-Italian-Swedish phase II trial of autologous SCT as consolidation treatment in first morphological CR.[12] The treatment results have been promising.[12,13] Since autologous SCT improved relapse-free and overall survival, we analyzed the potential impact of this management in the MRD-positive younger adult-AML patients in more detail. Indeed, patients with detectable MRD at the first and/or second time-points who underwent SCT had significantly better relapse-free survival (Figure 2C and D) than had patients in morphological CR who received only conventional post-induction chemotherapy. However, the comparison of relapse-free survival (Figure 2C and D) showed that MRD-positive patients who underwent allogeneic SCT had a significantly better prognosis than patients who underwent autologous SCT or received conventional chemotherapy. The 5-year overall survival rate was also significantly better in the allogeneic SCT recipients than in the autografted group or those who received conventional chemotherapy [67%, 46 % and 31% for the first time-point (p=0.021) and 75%, 53% and 25% for the second time-point (p=0.046), respectively].

In summary, we demonstrated that the prognostic significance of MRD differs depending on the therapy applied. This explains why MRD was not identified as a significant predictor of prognosis in the younger adults with AML. Although our series of patients was limited, our data strongly indicate that patients in morphological CR who have detectable MRD remaining after post-remission chemotherapy have a worse prognosis and may benefit from allogeneic SCT in first CR. Furthermore, our data suggest that if allogeneic SCT is not feasible, autologous SCT could be considered as an alternative approach. Finally, AML patients in whom MRD is not detectable after induction treatment and who remain MRD-negative at the end of post-remission chemotherapy have long-term relapse-free survival and overall survival similar to allogeneic SCT recipients. These patients may, therefore, not require transplantation procedures that are associated with relatively high treatment-related morbidity and mortality. Therefore MRD analysis by flow-cytometry may be used for refining the selection of therapeutic strategies and improving clinical outcome in individual patients. Key issues in flow cytometric monitoring of MRD in AML are the sensitivity and specificity of the method. Highly sensitive-LAIP together with the live-gate approach, have a maximum sensitivity of 0.001-0.01%.[3,18] This level of sensitivity may be difficult to achieve with other antibody combinations, due to very small cell populations with similar phenotypes that may be present in regenerating bone marrow.[16,18] Data from previously published studies, as well as our study, indicate that MRD levels above 0.1% define the population of patients with the highest risk of relapse.[3,4,18,19] However, MRD levels between 0.01-0.1% may also define AML patients with an increased risk of relapse.[3] In our study, LAIP were identified in 94% of patients. By improving the panel of antibodies and the application of more fluorochromes, flow cytometry could be used for all AML patients.[16] The limitation of this approach may be a lower sensitivity due to the increased application of less aberrant LAIP. Another important way of improving the sensitivity and applicability of the flow cytometric method is to add CD45 into a four-color MRD assessment. Via CD45 gating, the blast cell population covers a separate area and does not overlap with other bone marrow cell populations. Furthermore, a recent study showed that CD45 gating improves the sensitivity of flow cytometry-based MRD monitoring by one log.[20] To confirm these single institution results and to further improve response-adapted management of patients with AML, large prospective international studies are needed in which MRD levels are thoroughly followed and used to allocate patients to different predefined therapies.

EL: collection, organization and analysis of all data, manuscript writing; ARD: collection and analysis of clinical data, manuscript writing; EB: collection and analysis of flow cytometry data, review of manuscript; JM: statistical analysis and data interpretation; HE: data interpretation and review of manuscript; SS and MB: project planning and design, data interpretation, review of manuscript; APM: project planning and design, data analysis, data interpretation, writing of manuscript. All authors approved the final version of the manuscript. Cytogenetic analyses were performed at the Department of Clinical Genetics, Karolinska University Hospital Solna. We thank Yrsa Bringensparr, Britt Lundh, Shalah Tarahumi and Margareta Waern for excellent technical assistance and Lewis Edgel for linguistic revision of the manuscript.

Supported by grants from the Swedish Cancer Society, Karolinska Institutet, the Stockholm County Council, and the Kristjan Jaak Stipendiumid, Tallinn, Estonia (EL) Preliminary results of this study were presented at the 10th Congress of European Hematology Association, Stockholm, Sweden, June 2-5, 2005.

Manuscript received November 29, 2005. Accepted April 10, 2006.

References

1. Stone RM. Treatment of acute myeloid leukemia: state-of-the-art and future directions. Semin Hematol 2002;39 Suppl 2:4-10.
2. Venditti A, Buccisano F, Del Poeta G, Maurillo L, Tamburini A, Cox C, et al. Level of minimal residual disease after consolidation therapy predicts outcome in acute myeloid leukemia. Blood 2000;96:3948-52.
3. San Miguel JF, Vidriales MB, Lopez-Berges C, Diaz-Mediavilla J, Gutierrez N, Canizo C, et al. Early immunophenotypical evaluation of minimal residual disease in acute myeloid leukemia identifies different patient risk groups and may contribute to postinduction treatment stratification. Blood 2001;98:1746-51.
4. Feller N, van der Pol MA, van Stijn A, Weijers GW, Westra AH, Evertse BW, et al. MRD parameters using immunophenotypic detection methods are highly reliable in predicting survival in acute myeloid leukaemia. Leukemia 2004;18:1380-90.
5. Kern W, Voskova D, Schoch C, Hiddemann W, Schnittger S, Haferlach T. Determination of relapse risk based on assessment of minimal residual disease during complete remission by multiparameter flow cytometry in unselected patients with acute myeloid leukemia. Blood 2004;104:3078-85.
6. San Miguel JF, Ciudad J, Vidriales MB, Orfao A, Lucio P, Porwit-MacDonald A, et al. Immunophenotypical detection of minimal residual disease in acute leukemia. Crit Rev Oncol Hematol 1999;32:175-85.
7. Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DA, Gralnick HR, et al. Proposals for the classification of the acute leukaemias. French-American-British (FAB) co-operative group. Br J Haematol 1976;33:451-8.
8. Jaffe ES, Harris NL, Stein H, Vardiman JW, editors. World Health Organization Classification of Tumours. Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues. Lyon: IARC Press. 2001.
9. Grimwade D, Walker H, Oliver F, Wheatley K, Harrison C, Harrison G, et al. The importance of diagnostic cytogenetics on outcome in AML: analysis of 1,612 patients entered into the MRC AML 10 trial. The Medical Research Council Adult and Children's Leukaemia Working Parties. Blood 1998;92:2322-33.
10. Bjorkholm M, Liliemark J, Gahrton G, Grimfors G, Gruber A, Hast R, et al. Mitoxantrone, etoposide and ara-C vs doxorubicin-DNA, ara-C, thioguanine, vincristine and prednisolone in the treatment of patients with acute myelocytic leukaemia. A randomized comparison. Eur J Haematol 1995;55:19-23.
11. Ringden O, Ruutu T, Remberger M, Nikoskelainen J, Volin L, Vindelov L, et al. A randomized trial comparing busulfan with total body irradiation as conditioning in allogeneic marrow transplant recipients with leukemia: a report from the Nordic Bone Marrow Transplantation Group. Blood 1994;83:2723-30.
12. Rohatiner AZS, Bassan R, Bjorkholm M, Rule S, Newland AC, Raimondi R, et al. High-dose treatment (HDT) with peripheral blood progenitor cell (PBPC) support as consolidation of first remission in younger patients (PTS.) with acute myelogenous leukemia (AML). Blood 1998; 92 Suppl 1:293a[abstract].
13. Smith ML, Spinelli O, Rambaldi A, Bassan R, Di F, Bainton N, et al. High-dose treatment (cytosine arabinoside and total body irradiation) with stem cell support as consolidation of first remission in younger patients with acute myeloid leukemia. Ann Hematol 2004;83 Suppl 1:S32[abstract].
14. Bjorklund E, Mazur J, Soderhall S, Porwit-MacDonald A. Flow cytometric follow-up of minimal residual disease in bone marrow gives prognostic information in children with acute lymphoblastic leukemia. Leukemia 2003;17:138-48.
15. Macedo A, Orfao A, Ciudad J, Gonzalez M, Vidriales B, Lopez-Berges MC, et al. Phenotypic analysis of CD34 subpopulations in normal human bone marrow and its application for the detection of minimal residual disease. Leukemia 1995;9:1896-901.
16. Kern W, Danhauser-Riedl S, Ratei R, Schnittger S, Schoch C, Kolb HJ, et al. Detection of minimal residual disease in unselected patients with acute myeloid leukemia using multiparameter flow cytometry for definition of leukemia-associated immunophenotypes and determination of their frequencies in normal bone marrow. Haematologica 2003; 88:646-53.
17. San-Miguel JF, Vidriales MB, Orfao A. Immunological evaluation of minimal residual disease (MRD) in acute myeloid leukaemia (AML). Best Pract Res Clin Haematol 2002; 15:105-18.
18. Coustan-Smith E, Ribeiro RC, Rubnitz JE, Razzouk BI, Pui CH, Pounds S, et al. Clinical significance of residual disease during treatment in childhood acute myeloid leukaemia. Br J Haematol 2003;123:243-52.
19. Sievers EL, Lange BJ, Alonzo TA, Gerbing RB, Bernstein ID, Smith FO, et al. Immunophenotypic evidence of leukemia after induction therapy predicts relapse: results from a prospective Children's Cancer Group study of 252 patients with acute myeloid leukemia. Blood 2003;101:3398-406.
20. Kern W, Voskova D, Schnittger S, Schoch C, Hiddemann W, Haferlach T. Four-fold staining including CD45 gating improves the sensitivity of multiparameter flow cytometric assessment of minimal residual disease in patients with acute myeloid leukemia. Hematol J 2004;5:410-8.