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Hematopoietic stem cell transplantation (HSCT) in children with juvenile myelomonocytic leukemia (JMML): results of the EWOG-MDS/EBMT trial

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Abstract

Allogeneic hematopoietic stem cell transplantation (HSCT) is the only proven curative therapy for juvenile myelomonocytic leukemia (JMML). We report the outcome of 100 children (67 males and 33 females) with JMML given unmanipulated HSCT after a preparative regimen including busulfan, cyclophosphamide and melphalan. Forty-eight and 52 children were transplanted from an HLA-identical relative or an unrelated donor (UD), respectively. Source of hematopoietic stem cells was bone marrow, peripheral blood and cord blood in 79, 14 and 7 children, respectively. Splenectomy had been performed before HSCT in 24 children. The 5-year cumulative incidence of transplant-related mortality and leukemia recurrence were 13% and 35%, respectively. Age greater than 4 years predicted an increased risk of disease recurrence. The 5-year probability of event-free survival for children given HSCT from either a relative or an UD was 55% and 49%, respectively (p=NS), median observation time of patients alive being 40 months (range 6-144 months). In multivariate analysis, age greater than 4 years and female sex predicted poorer outcome. Results of this study compare favorably with previously published reports. Disease recurrence remains the major cause of treatment failure. Outcome of UD-HSCT recipients is comparable to that of children transplanted from an HLA-identical sibling.

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Introduction

Juvenile myelomonocytic leukemia (JMML) is a rare hematopoietic malignancy of early childhood, representing 2-3% of all pediatric leukemias.[1-3] JMML usually runs an aggressive clinical course, median duration of survival for children left untreated being less than 12 months from diagnosis.[1-5] Some young children with JMML (mainly those diagnosed before 2 years of age) may experience a longer course, sometimes characterized by temporary clinical improvement in the absence of therapy.[2,6] Death usually occurs as the result of tumor cell infiltration of organs, leading to organ dysfunction, infection and bleeding.

Neither intensive nor moderate chemotherapy approaches have been demonstrated to consistently improve the outcome of children with JMML,[4-9] and allogeneic hematopoietic stem cell transplantation (HSCT) is presently the only curative treatment for this disorder. Different studies have reported that a significant proportion of children with JMML are cured by an allograft.[10-15] Leukemia recurrence represents the main cause of treatment failure in children with JMML given HSCT, relapse rate being as high as 50%.[13,14] Relapse occurs early, at a median of 4 months from transplantation and generally within the first year after the allograft.[10,11,15]

All studies published so far on the results of HSCT in children with JMML enrolled a limited number of patients treated with heterogeneous approaches. This fact significantly precluded the possibility of identifying patient-, disease- and transplant-related variables predicting the clinical course of a child with JMML treated with HSCT.

In this paper we analyse the outcome of 100 children with JMML given unmanipulated HSCT after an homogeneous preparative regimen consisting of three alkylating agents, namely busulfan, cyclophosphamide and melphalan.

Patients and Methods

Approval for the study was obtained from the Institutional Review Board of each single institution. Written informed consent was provided by the patients' parents, according to the Declaration of

Helsinki. The patients included in this study were diagnosed as affected by JMML according to previously published criteria.[16,17] One-hundred children transplanted since January 1993 through December 2002 in 29 centers from 7 countries and reported to the European Working Group on Myelodysplastic Syndrome in Childhood (EWOG-MDS) were evaluated. Data concerning patient- and disease-characteristics and transplant outcome were collected by standardized questionnaires for each child enrolled into this study. Submitted data were reviewed by one physician (CN) and computerized error checks were performed to ensure data quality.

Patient characteristics, preparative regimen, GVHD prophylaxis and supportive therapy

The characteristics of the 100 children (67 males and 33 females) and of the transplant procedure are detailed in Table I and II, respectively. Fourteen children (7 males and 7 females) had clinical evidence of neurofibromatosis type-1 (NF-1) in association with JMML.

The median age at presentation was 1.4 years, with a range comprised between 0.1 and 14 years. Forty-eight children were transplanted from an HLA-identical relative (sibling in 46 cases and phenotypically identical aunt or mother in one case each); the remaining 52 children were given the allograft from an unrelated donor (UD).

Successful cytogenetic analysis of malignant cells was available in all cases but one. For 81 patients, karyotype taken within 6 weeks prior to HSCT was available, for the remaining 18 children the karyotype obtained at time of diagnosis was used. Monosomy of chromosome 7 was the most frequent cytogenetic abnormality, being observed in 20 out of the 33 patients with an abnormal karyotype.

Splenectomy before transplantation had been performed in 24 children.

In order to evaluate the impact of therapy before transplantation on post-transplant outcome, patients were subdivided into 2 groups according to the different kind of therapy received before transplantation: group 1 comprised 84 children given either no treatment (43 patients, 12 of whom splenectomized), or differentiative therapy (i.e. cis-retinoic acid, 1 patient who subsequently

received splenectomy) or low-intensity chemotherapy (i.e. 6-thioguanine, 6-mercaptopurine, low-dose cytarabine, etc., 40 patients, 9 of whom splenectomized); group 2 consisted of 16 children (2 of whom splenectomized) receiving schemes of chemotherapy similar to those adopted for children with acute myelogenous leukemia (AML).

The preparative regimen was based on the use of busulfan (16-20 mg/Kg given orally over 4 consecutive days), cyclophosphamide (60 mg/Kg/day for 2 consecutive days) and melphalan (140 mg/m^2 in single dose).[18] In 25 children transplanted in Italian Centers, a pharmacokinetic (PK) study of busulfan was performed. In detail, PK study was performed on the first administration of busulfan and the dosage adjusted starting from the fifth administration in order to maintain plasmatic levels between 500 and 800 ng/mL.[19]

Most children (35/48, 73%) given the allograft from an HLA-identical relative received cyclosporine-A (Cs-A, 1-3 mg/Kg/day) alone to prevent occurrence of graft-versus-host disease (GVHD). The combination of Cs-A, short term methotrexate (MTX, 15 mg/m^2 on day +1 and 10 mg/m^2 on days +3, +6 and +11) and either anti-thymocyte globulin (ATG) or, in a few cases, the monoclonal antibody Campath1-G was employed in the majority of patients transplanted from an unrelated volunteer.

Supportive therapy, as well as prophylaxis and treatment of infections, were similar among centers participating in this study. Human Cytomegalovirus (HCMV) serological status was studied before transplantation in donor/recipient pairs (see Table II for details). In all patients, HCMV infection was monitored and treated according to previously reported strategies.[20]

HLA-typing

HLA-A, -B antigen serological typing and a low-resolution generic DRB1 oligotyping were available for all donor and recipient pairs. Unrelated donors were located through networks of national and international bone marrow and cord blood donor registries. In all unrelated donor-recipient pairs, as well as when the donor was a relative other than an HLA-identical sibling, histocompatibility was determined by serology for HLA-A and -B antigens and by high-resolution

DNA typing for DRB1 antigen. All children transplanted from an unrelated volunteer were either identical or had 1 antigenic/allelic disparity with their donor.

Definitions

Acute GVHD was diagnosed and graded by investigators at each transplant Center according to previously reported criteria.[21] All patients surviving more than 10 days after transplant were considered at risk for developing acute GVHD. Children alive at day +100 post-transplant with sustained donor engraftment were considered to be evaluable for chronic GVHD, which was classified according to previously reported criteria.[22] Tissue biopsy samples were obtained to confirm diagnosis of GVHD, whenever clinically indicated and feasible. Treatment of both acute and chronic GVHD was administered according to the protocols in use at each single institution.

Myeloid engraftment was defined as the first of 3 consecutive days when neutrophil count was higher than $0.5x10^9$/L and platelet engraftment as the first of 7 consecutive days with an unsupported platelet count higher than $50x10^9$/L. Patients who did not engraft, as well as those with transient engraftment of donor cells, were considered to have graft failure.

Statistical analysis

Analysis used July 1, 2003 as the reference date, i.e., the day at which all centers locked data on patient outcomes.

Overall survival (OS) was defined as the probability of survival, irrespective of disease state, at any point in time; patients alive at their last follow-up were censored, while only death was considered as an event. Event-free survival (EFS) was defined as the probability of being alive, disease-free and with complete donor chimerism at any time point; death, relapse, rejection and graft failure were considered as events, while patients alive and disease free with donor engraftment at their last follow-up were censored. Both these probabilities were analyzed by the Kaplan-Meier method and comparisons between probabilities in different patient groups were performed using the log-rank test.[23]

Relapse incidence (RI) was defined as the probability of having a relapse before time *t*; death without experiencing a relapse was considered a competing event. On the contrary, transplant-related mortality (TRM) was defined as the probability of dying without previous occurrence of a relapse, which was the competing event. Both these probabilities were estimated as cumulative incidence curves, as previously described.[24-26]

Also the probabilities of acute and chronic GVHD and those of neutrophil and platelet engraftment were estimated as cumulative incidence. For acute GVHD analysis, relapse, death and either rejection or graft failure were treated as competing events, while patients alive and relapse-free at day +100 without having experienced acute GVHD were censored. For chronic GVHD, only patients surviving in remission and with donor engraftment for at least 100 days were evaluated. Also in this case, relapse, death and either rejection or graft failure were considered competing events, data being censored at time of last follow-up for patients who did not experience chronic GVHD.

Finally, for neutrophil and platelet engraftment competing events were relapse, death or rejection before engraftment.

All results were expressed as 5-year probability or 5-year cumulative incidence (%) and 95% confidence interval (95% CI).

A univariate analysis of EFS, RI and TRM was performed for the whole study population considering the following variables: patient and donor age, sex mismatch, interval diagnosis-HSCT, leukocyte, monocyte and platelet count at diagnosis, HbF percentage corrected for patient age at diagnosis, karyotype, leukocyte count and bone marrow blast percentage at HSCT, NF1, spleen size or splenectomy prior to HSCT, pre-transplant treatment, type of donor, stem cell source, infused cell dose, recipient and donor HCMV serology, type of GVHD prophylaxis, busulfan PK study, development of grade II-IV acute GVHD and development of chronic GVHD.

For this purpose, continuous variables were categorised as follows: each variable was first divided into four categories at approximately the 25^{th}, 50^{th}, and 75^{th} percentiles. If the relative event rates (ratio of the observed number of events to the expected number of events in a category, assuming no variation across categories) in two or more adjacent categories (and the mean times-to-event) were not substantially different, these categories were grouped. If no clear pattern was observed for the primary outcome, the median was taken as cut point.[27]

For multivariate analyses, the Cox proportional hazard regression model was used, including in the models all the variables with $P < 0.1$ in univariate analysis.[28,29]

Chi-square test was used to compare differences in percentages.

All *P* values were 2-sided and values less than 0.05 were considered statistically significant. *P* values greater than 0.1 were reported as non-significant (N.S.), whereas those between 0.05 and 0.1 were reported in detail.

The SAS package (SAS Institute, Cary, NC) and NCSS 2001 (Number Cruncher Statistical Systems, Kaysville, UT) were used for the analysis of the data.

Results

Engraftment and GVHD occurrence

Information on kinetics of myeloid recovery was available for all children included in this study. Three patients failed to engraft; 2 of them had received an UD HSCT. Two more patients, both transplanted from an UD, presented a secondary marrow failure, 27 and 39 days after HSCT, respectively. No other factor was associated with the occurrence of either primary or secondary graft failure. In children with sustained engraftment of donor cells, the median time to achieve neutrophil recovery was 18 days (range 8-44). In the Cox analysis on the whole population, the use of cord blood as stem cell source and the absence of grade II-IV acute GVHD were factors associated with a delayed neutrophil engraftment ($p = 0.0015$, RR = 0.16, 95% CI: 0.051-0.49 and $p = 0.017$, RR = 0.58, 95% CI: 0.38-0.91, respectively).

The median time to obtain a self-sustained platelet count higher than 50 x 10^9/L was 30 days (range 11-148). From the Cox model, we found that the most adverse factors for platelet recovery in the overall population were a platelet count at diagnosis < 100 x 10^9/L and the use of cord blood as stem cell source ($p = 0.02$, RR = 0.54, 95% CI: 0.32-0.91 and $p = 0.0065$, RR = 0,21, 95% CI: 0.07-0.65, respectively).

Grade II to IV acute GVHD developed in 40 patients. The cumulative incidence at day 100 of grade II-IV acute GVHD was 40% (31-51), whereas that of grade III-IV acute GVHD was 17% (11-26) (see also Figure 1). Children given HSCT from an HLA-compatible family donor had a cumulative incidence of grade II-IV acute GVHD comparable to that of patients transplanted from an unrelated volunteer (46% vs. 35%, respectively, P = N.S.). No patient- or transplant-related variable was significantly associated with the development of grade II-IV acute GVHD in a multivariate model.

Thirteen (15%) out of the 86 patients at risk developed chronic GVHD, which was limited in 6 cases and extensive in the remaining 7 patients. The overall cumulative incidence of chronic GVHD

was 17% (10-28). Children given HSCT from an HLA-compatible family donor had a cumulative incidence of chronic GVHD similar to that of patients transplanted from an unrelated volunteer (17% vs. 16%, respectively, P = N.S.). From the multivariate analysis performed using the Cox model, we found that previous grade II-IV acute GVHD was the only statistically significant risk factor for the occurrence of chronic GVHD (P = 0.016, RR = 4.96, 95% CI: 1.35-18.2).

Transplant-related mortality

Thirteen patients died for transplant-related causes, 5-year cumulative incidence of TRM being 13% (8-22) (Figure 2). The 5-year cumulative incidence of TRM for patients transplanted from either an HLA-identical sibling or an unrelated volunteer was 10% (5-24) and 16% (8-30), respectively (P=NS, see Figure 3). The median time to treatment-related death was 2.7 months (range 1-16). Table III lists probabilities of TRM, RI, and EFS not adjusted for differences in factors that influence transplant outcome. In univariate analysis, we found that patients transplanted from a female donor had a statistically higher probability of dying for transplant-related causes; all other variables did not have any impact on the probability of death due to transplant complications, possibly because of the limited number of events. None of the variables considered influenced TRM in multivariate analysis (see also Table IV).

Hepatic veno-occlusive disease occurred in 11 patients (5 of whom transplanted from an unrelated volunteer), but, fortunately, it was not fatal in any of them. Three of these 11 patients relapsed, the remaining 8 still being alive and in complete remission.

Relapse incidence

Thirty-four patients had hematological relapse after transplantation, at a median time of 6 months (range 2-36) after the allograft. Twenty-one children died due to disease progression at a median of 11 months after transplantation (range 2-65). Five-year cumulative incidence of relapse was 35% (Figure 2), with no significant difference between patients transplanted from either a relative or an unrelated donor (see also Table III for details).

In univariable analysis (Table III), the following features were associated with increased RI: female sex, age at diagnosis greater than 4 years, high percentage of HbF and blast percentage in the bone marrow at time of transplantation greater than 20%. From the Cox model, we found that only age at diagnosis greater than 4 years remained a predictive variable for an increased risk of relapse (see Table IV and Figure 4A for details).

Survival and leukemia-free survival

Overall, 66 children remain alive after HSCT, the 5-year Kaplan-Meier estimate of survival being 64% (54-74) (see also Figure 2).

Fifty-three patients are alive in first complete remission after HSCT, with a median observation time of 40 months (range 6-144). The 5-year cumulative probability of EFS after the first allograft is 52% (42-62) (see also Figure 2) for the whole cohort of patients studied, being 55% (41-70) and 49% (35-63) for patients given HSCT from either a relative or an unrelated donor, respectively (P=NS, Figure 3). Six patients are alive with disease and 7 patients are alive in hematological remission after a second allograft, which was performed in a total of 15 cases. Five of these 15 patients given a second transplant died because of further disease recurrence and 3 died due to transplant-related complications. In 10 out of the 15 children who received a second transplant the same donor used in the first HSCT was employed and total body irradiation was added as part of the preparative regimen in 8 out of these 15 patients. Moreover, less intensive GVHD prophylaxis was adopted in order to exploit a graft-versus-leukemia (GVL) effect; this choice resulted in the occurrence of grade II-IV acute GVHD in 8 of the 15 patients given a second allograft. The median follow-up of the 7 patients who are alive and disease free after the second HSCT is 2.3 years (range 0.4-5.4).

Univariate analysis of factors related to patient, disease and transplant influencing EFS showed that male sex and both age at diagnosis and age at transplantation younger than 4 years were associated with a better outcome. Both age at diagnosis and patient gender remained significant in

multivariate analysis (see also Table IV and Figures 4B and 5 for further details). All other variables did not have any impact on the probability of EFS. In particular, no significant differences in terms of EFS, RI and TRM were observed between children given AML-type chemotherapy or less intensive treatment (see Table III for details). Also, neither splenectomy prior to HSCT nor spleen size at time of transplantation influenced the outcome (Figure 6). Of particular interest are the data on cytogenetics: patients with monosomy 7 had an outcome comparable to that of children with either a normal karyotype or other cytogenetics abnormalities (see also Table III and Figure 7).

Finally, the probability of EFS of the 14 patients with NF1 was lower, although not statistically significant, than that of children who did not have NF-1 (see Table III for details).

Discussion

This is the largest study reported so far on children with JMML, treated in the context of a prospective clinical trial with the same preparative regimen. With the follow-up now available, our data support the conclusion that allogeneic HSCT may cure approximately 50% of patients with JMML, disease recurrence being the major cause of treatment failure. The probability of being alive and disease-free of children enrolled in this study compares favourably with that of many previously published reports on HSCT in children with JMML.[10-14] In particular, the overall probability of EFS of the 48 children transplanted from an HLA-identical relative is 55%, a value better than that (38%) reported in the retrospective analysis published by our EWOG-MDS group on 24 children given the allograft from a family donor.[11] Likewise, the EFS probability of 49% at 5 years after the allograft we have obtained in children transplanted from an unrelated volunteer is higher than that reported by the recent retrospective analysis of the National Marrow Donor Program in 46 children (24% at 2 years after transplantation).[14]

The choice of adopting a preparative regimen consisting of 3 alkylating agents was based on a preliminary study demonstrating the safety of this therapy[18] and on the fact that a retrospective analysis of the EWOG-MDS group showed that a myeloablative therapy including busulfan was associated with a better EFS and a lower relapse incidence in comparison to regimens employing total body irradiation.[11] Furthermore, we reasoned that avoiding radiotherapy could have the advantage of reducing the risk of severe radiation-induced growth retardation,[30] endocrine and neuropsychological sequels,[31-33] and secondary malignancies.[34,35] The results of this study confirm that the preparative regimen is safe, as the cumulative incidence of TRM was only 13%, with no significant difference between recipients of either HLA-identical sibling or UD transplant.

Also in terms of ultimate outcome, our results seem to indicate that using UDs offers minimal or possibly no significant disadvantage as compared to employing an HLA-identical sibling. These data are in agreement with previously published studies in children with acute leukemia, where the probability of EFS in recipients of sibling HSCT was reported to be comparable to that of children

given an UD allograft in the most recent years, thus suggesting the possibility of applying the same indications for HSCT independently of the type of donor available, i.e. an HLA-identical sibling or an HLA-matched UD.[36,37] Several factors may have contributed to the favourable outcome of our children transplanted from an UD. The possibility of selecting the donor using high-resolution molecular typing of HLA loci has been suggested to be potentially able to decrease the risk of graft-failure, GVHD and TRM.[38,39]A learning and experience effect in handling recipients of UD HSCT, as well as optimization of the strategies of both prevention and treatment of GVHD, are also variables which could have contributed to the improved outcome.

Our results confirm the conclusion, already reported in previously published studies,[11-15] that relapse is the major cause of treatment failure in patients with JMML undergoing allogeneic HSCT. Relapse occurred in one third of our patients after a relatively short time from the allograft, median time from HSCT being 6 months with only 2 patients having relapsed later than 18 months after transplantation. Disease progression was also the most frequent cause of death. Previously published studies have found that older age,[11,15] increased HbF[14] and abnormal karyotype[15] are patient-specific risk factors for relapse after transplantation and that occurrence of chronic GVHD protects from the risk of disease relapse.[14] Age above 2 years at diagnosis and high percentage of HbF at diagnosis have been found to predict short survival also in studies analysing the natural history of the disease in patients with JMML not given HSCT.[2,5,6] In univariate analysis, we found that older age, female sex, increased percentage of HbF and blast percentage in the bone marrow greater than 20% predicted the occurrence of leukemia relapse. However, only the former of these 4 variables remained significant in multivariate analysis.

Despite the usually aggressive re-emergence of the malignant clone and the short time interval between first and second HSCT, a substantial number of our children (7 out of the 15 who were given a second allograft) have achieved a second sustained hematological remission thanks to a second transplant. It is reasonable to hypothesize that less intensive GVHD prophylaxis could have contributed to the sustained remission after the second allograft in these patients, by better

preserving GVL effect. This finding is in agreement with previously published reports[40,41] and indicates that leukemia relapse does not necessarily means a desperate prognosis and that a second transplant should be considered as an option to be offered to every patients in good clinical conditions.

Despite the delayed hematological recovery, the ultimate outcome of cord blood transplant recipients was comparable to that of children given HSCT using either bone marrow or peripheral blood progenitors, thus providing further support to previously published studies which reported similar probabilities of EFS in children with malignancies transplanted with either placental blood or bone marrow-derived hematopoietic stem cells.[42,43] The advantages of using cord blood are mainly represented by the prompt availability of this source of hematopoietic progenitors, which shortens the time needed to locate a suitable donor, and by the possibility of performing transplants in the presence of 1or 2 HLA disparities in the donor/recipient pairs.[43,44] Both these factors can be relevant for treating children with JMML, whose disease, often running an aggressive clinical course, might not allow an extended period of time for finding a suitable unrelated bone marrow donor.

Two thirds of our patients had a normal karyotype, monosomy 7 being the most frequent cytogenetic anomaly. In contrast with a previous report documenting a negative impact of abnormal karyotype on the probability of OS after HSCT,[15] we found that neither monosomy 7 nor other cytogenetic abnormalities confer a worse prognosis. This finding supports a recently published study from the United Kingdom co-operative group on childhood MDS, which reported that, in children with JMML, monosomy 7 was associated with an outcome comparable to or even better than that of patients with normal karytotype.[45]

Splenectomy before HSCT, as well as spleen size at time of the allograft, did not appear to have an impact on post-transplant outcome. One could argue that patients given splenectomy before transplantation were those with the largest spleen and, thus, with the greatest tumor burden, this possibly being associated with a higher risk of treatment failure. However, the fact that spleen size

at time of transplantation influenced neither the risk of relapse nor the probability of survival in patients who did not undergo splenectomy does not support this hypothesis. The results of this study, as well those of previously published reports,[11,14,46] are not in favour of an indiscriminate use of splenectomy before transplantation, the potential advantages having to be weighed against the risks related to the procedure or to post-splenectomy infections. The indication of performing splenectomy has to be carefully evaluated for each single child, the presence of massive splenomegaly with evidence of hypersplenism and/or refractoriness to platelet transfusions being an argument for considering this procedure in order to promote engraftment, to hasten hematological recovery and to lower the risk of hemorrhagic complications.

Clinical remissions and long-term survival after AML-type combination therapy have been reported in small series of children with JMML.[8,47,48] Other investigators, however, pointed out that intensive chemotherapy is notably unsuccessful, especially in patients with aggressive disease.[6,9,49] Neither EFS was improved, nor relapse incidence was reduced in our patients who had received intensive chemotherapy before the allograft. Thus, in view of these results, intensive chemotherapy prior to allogeneic HSCT cannot be recommended.

The worse outcome of female patients, also confirmed in multivariate analysis, is a finding never reported in previously published studies on children with JMML, given HSCT.[10-15] There is no immediate explanation for this finding, although it is noteworthy that among females there was a relatively higher percentage of patients with both NF1 and a low platelet count at time of diagnosis.

In conclusion, this study indicate that HSCT, after a preparative regimen consisting of busulfan, cyclophosphamide and melphalan, may cure approximately 50% of patients with JMML and that nowadays results achievable using UD are comparable to those obtained employing an HLA-compatible related donor. Identification of factors influencing relapse rate and EFS can be of help in counseling patients. Disease recurrence remains the major cause of treatmente failure and novel strategies to lower the risk of relapse are warranted. In this regard, a reduction in both intensity and

duration of GVHD prophylaxis might favor the emergence of a GVL effect displayed by donor lymphocytes, thus contributing to better leukemia control.

Appendix: The following transplant teams enrolled patients in the present study:

Transplant Center	**Country**	**Principal investigator**
Berlin	Germany	Wolfram Ebell
Bologna	Italy	Andrea Pession
Cagliari	Italy	Franca Argiolu
Copenhagen	Denmark	Carsten Heilmann
Dublin	Ireland	Angus O'Marcaigh
Düsseldorf	Germany	Dagmar Dilloo
Erlangen	Germany	Wolfgang Holter
Essen	Germany	Bernhard Kremens
Frankfurt	Germany	Thomas Klingebiel
Freiburg	Germany	Charlotte Niemeyer
Geneva	Switzerland	Pierre Wacker
Genova	Italy	Giorgio Dini
Giessen	Germany	Alfred Reiter
Greifswald	Germany	James Beck
Hamburg	Germany	Hartmut Kabisch
Hannover	Germany	Karl Sykora
Jena	Germany	Felix Zintl
Kiel	Germany	Alexander Claviez
Leiden	The Netherlands	Elisabeth Korthof
Lund	Sweden	Albert Bekassy
Monza	Italy	Cornelio Uderzo
München	Germany	Monika Führer
Padova	Italy	Chiara Messina
Paraná	Brazil	Carmen S. Bonfim
Pavia	Italy	Franco Locatelli
Pisa	Italy	Claudio Favre
Prague	Czech Republic	Jan Starý
Tübingen	Germany	Peter Bader
Uppsala	Sweden	Johan Arvidson
Utrecht	The Netherlands	Tom Révész
Wien	Austria	Christina Peters

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Figure legends

Figure 1. Cumulative incidence of grade II-IV and grade III-IV acute GVHD in the overall cohort of patients studied.

Figure 2. Kaplan-Meier estimate of overall survival (Surv) and event-free survival (EFS) and cumulative incidence of relapse (RI) and transplant-related mortality (TRM) in the overall population.

Figure 3. Kaplan-Meier estimate of event-free survival (EFS) and transplant-related mortality (TRM) according to the type of donor employed.

Figure 4. Cumulative incidence of relapse (RI) and Kaplan-Meier estimate of event-free survival (EFS) according to age at diagnosis.

Figure 5. Kaplan-Meier estimate of event-free survival (EFS) according to patient's gender.

Figure 6. Kaplan-Meier estimate of event-free survival (EFS) in patients splenectomized before transplantation and according to spleen size at time of allograft.

Figure 7. Kaplan-Meier estimate of event-free survival (EFS) according to patient's karyotype.

Table I. Patient characteristics

Number of patients enrolled	100	
Patient gender (M / F)	67 / 33	
Patient age at diagnosis (years)	1.4	(0.1 – 14)
Patient age at HSCT (years)	2.5	(0.3 – 15)
Interval between diagnosis and HSCT (months)	6	(0.3 – 49)
WBC at diagnosis* (x 10^9/L)	34	(3 – 240)
Monocytes at diagnosis** (x 10^9/L)	5.5	(1 – 50)
Platelet count at diagnosis*** (x 10^9/L)	65	(9 – 530)
Percentage of HbF at diagnosis°	9	(0 – 80)
Karyotype:		
Normal karyotype	66	
Monosomy 7	20	
Trisomy 8	9	
Other abnormalities	4	
Missing / unknown	1	
Patients with clinical evidence of NF-1°°	14	
WBC at HSCT°°° (x 10^9/L)	9.6	(0.7 – 320)
Percentage of marrow blasts at HSCT°°°°	6	(0 – 85)
Splenectomy before HSCT	24	
Spleen size at HSCT^ (cm)	5	(0 – 16)
Patient HCMV serology		
Negative	57	
Positive	39	
Unknown	4	

* 3 unknown; ** 6 unknown; *** 3 unknown; ° 23 unknown; °° 8 unknown; °°° 8 unknown; °°°° 13 unknown; ^ 6 unknown.
Data are expressed as median and range or as percentage, as appropriate.

Table II. Transplant procedure.

Donor:		
HLA-identical family donor	48	
Matched unrelated donor	52	
Donor gender (M / F)*	50 / 46	
Donor age (years)**	19	(1 – 54)
Donor HCMV serology:		
Negative	55	
Positive	38	
Unknown	7	
Stem cell source:		
Bone marrow	79	
Peripheral blood	14	
Cord blood	7	
Median mononuclear cell dose infused		
Bone marrow (x 10^8/Kg)	6.1	(0.6 – 18.9)
Peripheral blood (CD34+ x 10^6/Kg)	20	(10 – 30)
Cord blood (x 10^7/Kg)	5	(4 – 14)
GVHD prophylaxis:	*Matched family donor*	*Matched unrelated donor*
None	1	0
Cs-A	32	0
MTX	2	0
Cs-A + MTX	7	3
Cs-A + ALG or MoAb	3	3
Cs-A + MTX + ALG or MoAb	1	41
Cs-A ± PDN ± MTX ± MoAb	2	5

*4 unknown; ** unknown;
Data are expressed as median and range or as percentage, as appropriate.
Cs-A: Cyclosporin-A; MTX: short-term methotrexate; ALG: Anti-lymphocyte globulin; MoAb: monoclonal antibodies

Table III. Univariate analysis of 5-year event-free survival probability (EFS), cumulative incidence of relapse (RI) and cumulative incidence of transplant-related mortality (TRM).

		EFS		RI		TRM	
	N. of patients	Probability	(95% CI)	Cumulative incidence	(95% CI)	Cumulative incidence	(95% CI)
Overall probability or incidence	100	52%	(42 – 62)	35%	(27 – 46)	13	(8 – 22)
Patient gender							
Male	67	61%	(49 – 73)	30%	(20 – 44)	9%	(4 – 19)
Female	33	33%	(17 – 49)	45%	(31 – 66)	21%	(11 – 41)
P value		**0.0021**		**0.012**		0.065	
Donor gender							
Male	50	53%	(38 – 67)	41%	(30 – 59)	6%	(2 – 18)
Female	46	53%	(39 – 68)	25%	(15 – 42)	22%	(13 – 38)
Missing	4						
P value		N.S.		N.S.		**0.035**	
Age at diagnosis							
< 1 years	38	65%	(46 – 78)	17%	(8 – 35)	21%	(12 – 40)
1 – 2 years	18	67%	(45 – 88)	22%	(9 – 53)	11%	(3 – 41)
2 – 3 years	9	47%	(10 – 84)	53%	(27 – 100)	0%	- -
3 – 4 years	18	50%	(27 – 73)	44%	(27 – 75)	6%	(1 – 37)
≥ 4 years	17	16%	(0 – 34)	73%	(53 – 98)	12%	(3 – 43)
P value		**0.013**		**0.0003**		N.S.	
< 2 years	56	64%	(51 – 76)	18%	(10 – 32)	18%	(10 – 32)
2 - 4 years	27	50%	(31 – 70)	46%	(30 – 70)	4%	(1 – 25)
≥ 4 years	17	16%	(0 – 34)	73%	(53 – 98)	12%	(3 – 43)
P value		**0.0020**		**0.0001**		N.S.	
Interval diagnosis – HSCT							
<3 months	24	56%	(35 – 77)	31%	(17 – 59)	13%	(4 – 36)
3 – 6 months	29	54%	(35 – 73)	39%	(24 – 62)	7%	(2 – 28)
6 – 9 months	25	47%	(27 – 67)	37%	(22 – 62)	16%	(7 – 40)
≥ 9 months	22	50%	(29 – 71)	32%	(17 – 59)	18%	(7 – 44)
P value		N.S.		N.S.		N.S.	
Age at HSCT							
< 1 year	17	64%	(41 – 87)	12%	(3 – 45)	24%	(10 – 55)
1 – 2 years	27	66%	(48 – 84)	19%	(9 – 42)	15%	(6 – 37)
2 – 3 years	13	62%	(35 – 88)	23%	(9 – 62)	15%	(4 – 55)
3 – 4 years	18	55%	(32 – 78)	45%	(27 – 75)	0%	- -
≥ 4 years	25	23%	(6 – 40)	65%	(48 – 87)	12%	(4 – 35)
P value		**0.014**		**0.0008**		N.S:	
< 2 years	44	65%	(51 – 79)	16%	(8 – 32)	18%	(10 – 34)
2 - 4 years	31	57%	(39 – 75)	37%	(23 – 59)	6%	(2 – 25)
≥ 4 years	25	23%	(6 – 40)	65%	(48 – 87)	12%	(4 – 35)
P value		**0.0022**		**0.0008**		N.S.	

Continued . . .

Table 3, continued

Donor age							
< 10 years	31	60%	(42 – 78)	34%	(20 – 56)	6%	(2 – 25)
10 - 20 years	9	33%	(3 – 64)	33%	(13 – 84)	33%	(13 – 84)
≥ 20 years	39	47%	(31 – 63)	37%	(25 – 57)	16%	(8 – 33)
Missing	21						
P value		N.S.		N.S.		N.S.	
Leukocytes at diagnosis (x 10^9/L)							
< 20	25	51%	(31 – 71)	45%	(29 – 70)	4%	(1 – 27)
20 – 40	35	49%	(32 – 67)	30%	(18 – 51)	20%	(10 – 39)
40 – 60	17	40%	(17- 64)	36%	(19 – 69)	24%	(10 – 55)
60 – 80	11	64%	(35 – 92)	36%	(17 – 79)	0%	- -
≥ 80	9	63%	(30 – 97)	37%	(15 – 91)	0%	- -
Missing	3						
P value		N.S.		N.S.		0.09	
Monocytes at diagnosis (x 10^9/L)							
1 – 2	13	54%	(27 – 80)	31%	(14 – 70)	15%	(4 – 55)
2 – 3	15	51%	(25 – 78)	42%	(23 – 78)	7%	(1 – 44)
3 – 5	16	56%	(32 – 81)	31%	(15 – 65)	13%	(3 – 46)
5 – 10	25	38%	(19 – 58)	37%	(22 – 62)	25%	(12 – 50)
≥ 10	25	62%	(43 – 82)	34%	(19 – 59)	4%	(1 – 27)
Missing	6						
P value		N.S.		N.S.		N.S.	
Platelets at diagnosis (x 10^9/L)							
< 50	38	44%	(28 – 60)	42%	(29 – 62)	13%	(6 – 30)
50 – 100	27	48%	(28 – 69)	37%	(22 – 63)	15%	(6 – 37)
≥ 100	32	62%	(45 – 79)	29%	(17 – 50)	9%	(3 – 28)
Missing	3						
P value		N.S.		N.S.		N.S.	
Karyotype							
Normal	66	49%	(36 – 61)	37%	(27 – 52)	14%	(8 – 25)
Monosomy 7	20	68%	(46 – 89)	22%	(9 – 54)	10%	(3 – 37)
Other	13	46%	(19 – 73)	46%	(26 – 83)	8%	(1 – 51)
Missing	1						
P value		N.S.		N.S.		N.S.	
HbF percentage							
< 5%	26	61%	(42 – 80)	20%	(9 – 43)	20%	(9 – 43)
5 – 10%	14	43%	(17 – 69)	57%	(36 – 90)	0%	- -
10 – 40%	23	51%	(29 – 72)	32%	(17 – 59)	17%	(7 – 42)
≥ 40%	14	19%	(0 – 41)	81%	(62 – 100)	0%	- -
Missing	23						
P value		N.S.		**0.0083**		N.S.	
< 40%	63	53%	(41 – 66)	33%	(23 – 47)	14	(8 – 26)
≥ 40%	14	19%	(0 – 41)	81%	(62 – 100)	0	- -
Missing	23						
P value		0.07		**0.004**		N.S.	

Continued . . .

Table 3, continued

Clinical evidence of NF1							
No	78	55%	(43 – 66)	34%	(24 – 47)	12%	(6 – 22)
Yes	14	36%	(11 – 61)	50%	(30 – 84)	14%	(4 – 52)
Missing	8						
P value		N.S.		N.S.		N.S.	
Leukocytes at HSCT (x 10^9/L)							
< 10	46	53%	(38 – 68)	32%	(20 – 49)	15%	(8 – 30)
10 – 20	20	47%	(25 – 70)	37%	(20 – 67)	16%	(6 – 45)
20 – 40	17	45%	(20 – 69)	43%	(25 – 77)	12%	(3 – 43)
≥ 40	9	44%	(12 – 77)	56%	(31 – 100)	0%	- -
Missing	8						
P value		N.S.		N.S.			
Bone marrow blast percentage at HSCT							
< 5%	30	63%	(46 – 81)	20%	(10 – 41)	17%	(7 – 37)
5 – 20%	47	52%	(37 – 67)	35%	(24 – 52)	13%	(6 -27)
≥ 20%	10	0%		90%	(73 – 100)	10%	(2 – 64)
Missing	13						
P value		0.10		**0.017**		N.S.	
Spleen size at HSCT							
< 5 cm	34	61%	(44 – 78)	24%	(13 – 44)	15%	(7 – 33)
≥ 5 cm	36	44%	(26 – 62)	45%	(30 – 67)	11%	(4 – 28)
Splenectomized	24	48%	(28 – 69)	39%	(23 – 65)	13%	(4 – 36)
Missing	6						
P value		N.S.		N.S.		N.S.	
Pre-HSCT treatment							
None or Low-dose	84	52%	(41 – 63)	35%	(26 – 47)	13%	(8 – 23)
AML-Like	16	50%	(26 – 75)	38%	(20 – 71)	13%	(3 – 46)
P value		N.S.		N.S.		N.S.	
Donor							
Matched family donor	48	55%	(41 – 70)	35%	(23 – 52)	10%	(5 – 24)
Unrelated donor	52	49%	(35 – 63)	36%	(24 – 52)	16%	(8 – 30)
P value		N.S.		N.S.		N.S.	
Stem cell source							
Bone marrow	79	51%	(40 – 62)	35%	(26 – 47)	14%	(8 – 24)
Peripheral blood	14	55%	(28 – 82)	36%	(18 – 72)	9%	(1 – 59)
Cord blood	7	54%	(14 – 93)	32%	(10 – 100)	14%	(2 – 88)
P value		N.S.		N.S.		N.S.	
Stem cell dose (BM only)							
< $5x10^8$/Kg	22	58%	(37 – 79)	33%	(18 – 61)	9%	(2 – 34)
>= $5x10^8$/Kg	46	54%	(39 – 68)	31%	(20 – 48)	15%	(8 – 30)
Missing	11						
P value		N.S.		N.S.		N.S.	

Continued . . .

Table 3, continued

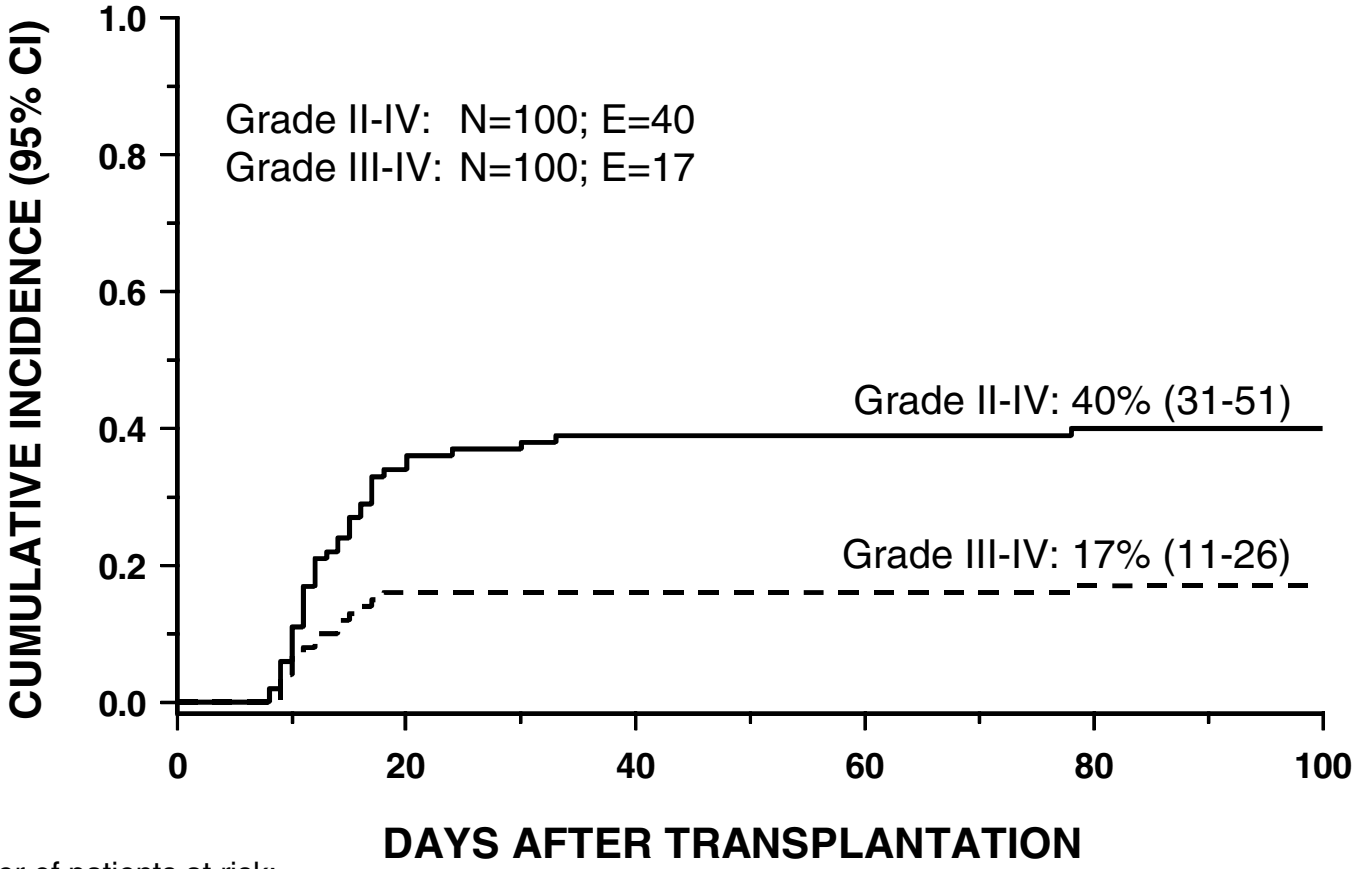
HCMV serology							
Neg/Neg	41	49%	(33 – 65)	39%	(26 – 58)	13%	(6 – 29)
Other	53	54%	(40 – 67)	33%	(22 – 49)	13%	(7 – 26)
Missing	6						
P value		N.S.		N.S.		N.S.	
Busulfan PK study							
Yes	25	60%	(40-79)	32%	(18-57)	8%	(2-30)
No	75	49%	(37-61)	36%	(27-59)	15%	(9-269
P value		N.S.		N.S.		N.S.	
GVHD prophylaxis							
All transplants:							
Monotherapy	35	59%	(42 – 75)	33%	(20 – 54)	9%	(3 – 25)
Combination treatment	14	36%	(11 – 61)	36%	(18 – 72)	29%	(12 – 65)
Serotherapy	51	52%	(37 – 66)	36%	(25 – 53)	12%	(6 – 25)
P value		N.S.		N.S.		N.S.	
Sibling donor transplants:							
Monotherapy	35	59%	(42 – 75)	33%	(20 – 54)	9%	(3 – 25)
Combination treatment	8	38%	(4 – 71)	38%	(11 – 92)	25%	(8 – 83)
Serotherapy	5	60%	(17 – 100)	40%	(14 – 100)	0%	- -
P value		N.S.		N.S.			
Unrelated donor transplants:							
Monotherapy	0	- -		- -		- -	
Combination treatment	6	33%	(0 – 71)	33%	(11 – 100)	33%	(11 – 100)
Serotherapy	46	51%	(36 – 66)	36%	(24 – 53)	13%	(6 – 28)
P value		N.S.		N.S.		0.08	
Acute GVHD occurrence							
Grade 0-I	60	48%	(35 – 61)	37%	(26 – 52)	15%	(8 – 28)
Grade II-IV	40	57%	(42 – 73)	33%	(21 – 51)	10%	(4 – 25)
P value		N.S.		N.S.		0.083	
Chronic GVHD							
Absent	73	59%	(48 – 71)	35%	(26 – 48)	6%	(2 – 15)
Present	13	67%	(41 – 94)	25%	(9 – 67)	8%	(1 – 51)
Not evaluable	14						
P value		N.S.		N.S.		N.S.	

*GVHD prophylaxis was defined as follows: **Monotherapy**: a single drug used (Cyclosporin-A or Methotrexate; the single patient who did not receive any GVHD prophylaxis was included in this group). **Combination treatment**: more than one drug used (Cyclosporin-A + Methotrexate or steroids), without the addition of anti-lymphocyte globulin or monoclonal antibodies. **Serotherapy**: any drug combination plus the addition of anti-lymphocyte globulin or monoclonal antibodies (Campath-1G).

Table IV. Multivariate analysis of variables influencing the probability of event-free survival (EFS), relapse incidence (RI) and transplant-related mortality (TRM). All variables with a P value ≤ 0.1 in univariate analysis were considered as covariates and included in the Cox proportional hazard regression model.

	Relative risk	(95% CI)	P
EFS			
Patient age at HSCT:			
≥ 4 years vs < 4 years	2.24	(1.07 – 4.69)	0.032
Patient gender:			
Female vs. Male	2.22	(1.09 – 4.50)	0.028
HbF %:			
≥ 40% vs. < 40%	1.20	(0.52 – 2.72)	N.S.
Bone marrow blast % at HSCT:			
5 – 19% vs. < 5%	1.70	(0.76 – 3.79)	N.S.
≥ 20% vs. < 5%	1.82	(0.64 – 5.15)	N.S.
RI			
Patient age at HSCT:			
≥ 4 years vs < 4 years	2.96	(1.26 – 6.92)	0.012
Patient gender:			
Female vs. Male	1.80	(0.77 – 4.20)	N.S.
HbF %:			
≥ 40% vs. < 40%	1.90	(0.79 – 4.54)	N.S.
Bone marrow blast % at HSCT:			
5 – 19% vs. < 5%	2.08	(0.78 – 5.55)	N.S.
≥ 20% vs. < 5%	2.06	(0.60 – 7.06)	N.S.
TRM			
Patient gender:			
Female vs. Male	2.18	(0.66 – 7.22)	N.S.
Donor gender:			
Female vs. Male	2.87	(0.76 – 10.87)	N.S.
WBC at diagnosis (x 10^9/L):			
≥ 30 vs. < 30	3.16	(0.68 – 14.69)	N.S.
GVHD Prophylaxis:			
Cs-A + MTX vs. monotherapy	3.95	(0.77 – 20.35)	N.S.
Cs-A + MTX + ALG vs. monotherapy	1.43	(0.35 – 5.92)	N.S.

Cumulative Incidence of Acute GVHD



Number of patients at risk:

100	66	54	54	52	49	Grade II-IV
100	84	77	76	74	71	Grade III-IV

Figure 1

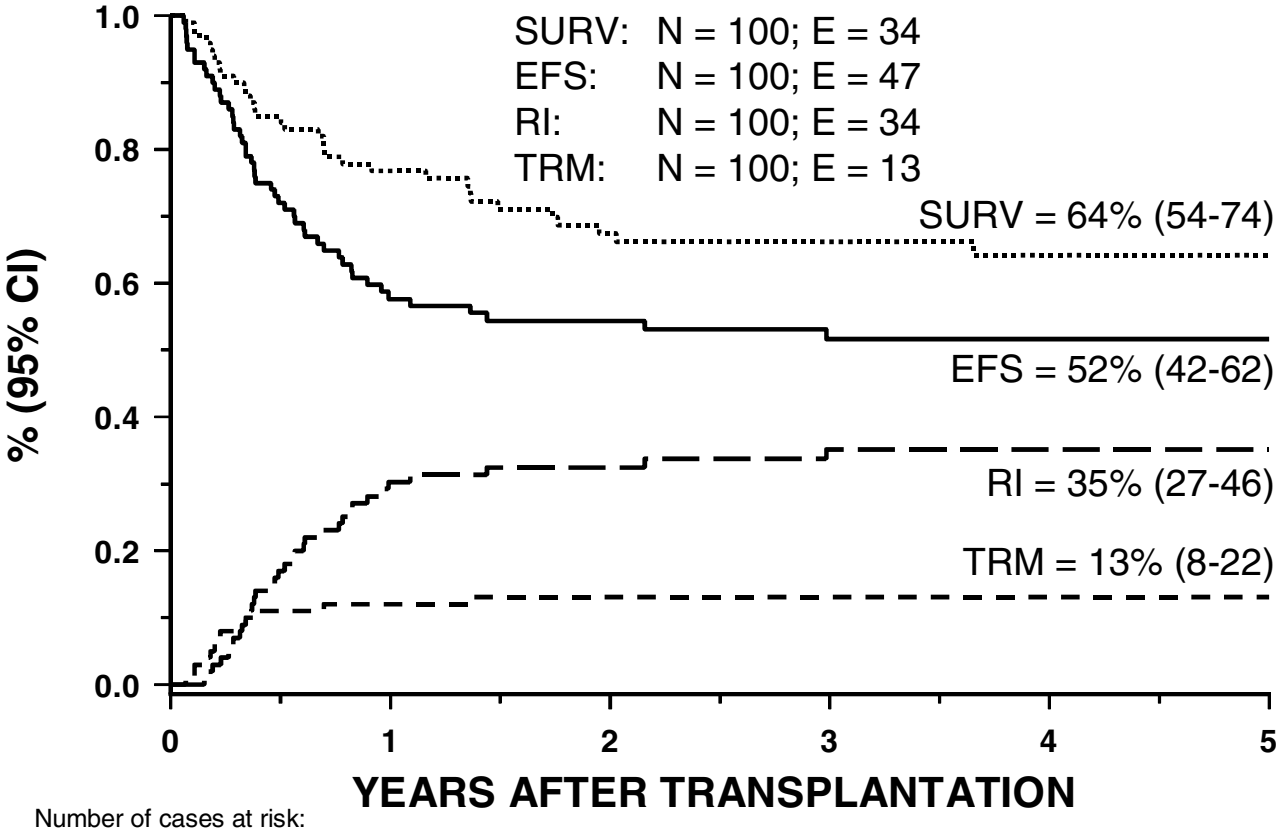

Overall Survival, Event-Free Survival,
Relapse Incidence and Transplant-Related Mortality
SURV: N = 100; E = 34
EFS: N = 100; E = 47
RI: N = 100; E = 34
TRM: N = 100; E = 13
SURV = 64% (54-74)
EFS = 52% (42-62)
RI = 35% (27-46)
TRM = 13% (8-22)
% (95% CI)
1.0
0.8
0.6
0.4
0.2
0.0
0
1
2
3
4
5
YEARS AFTER TRANSPLANTATION
Number of cases at risk:
100 72 54 43 29 17 SURV
100 55 45 35 23 13 EFS, RI, TRM

Figure 2.

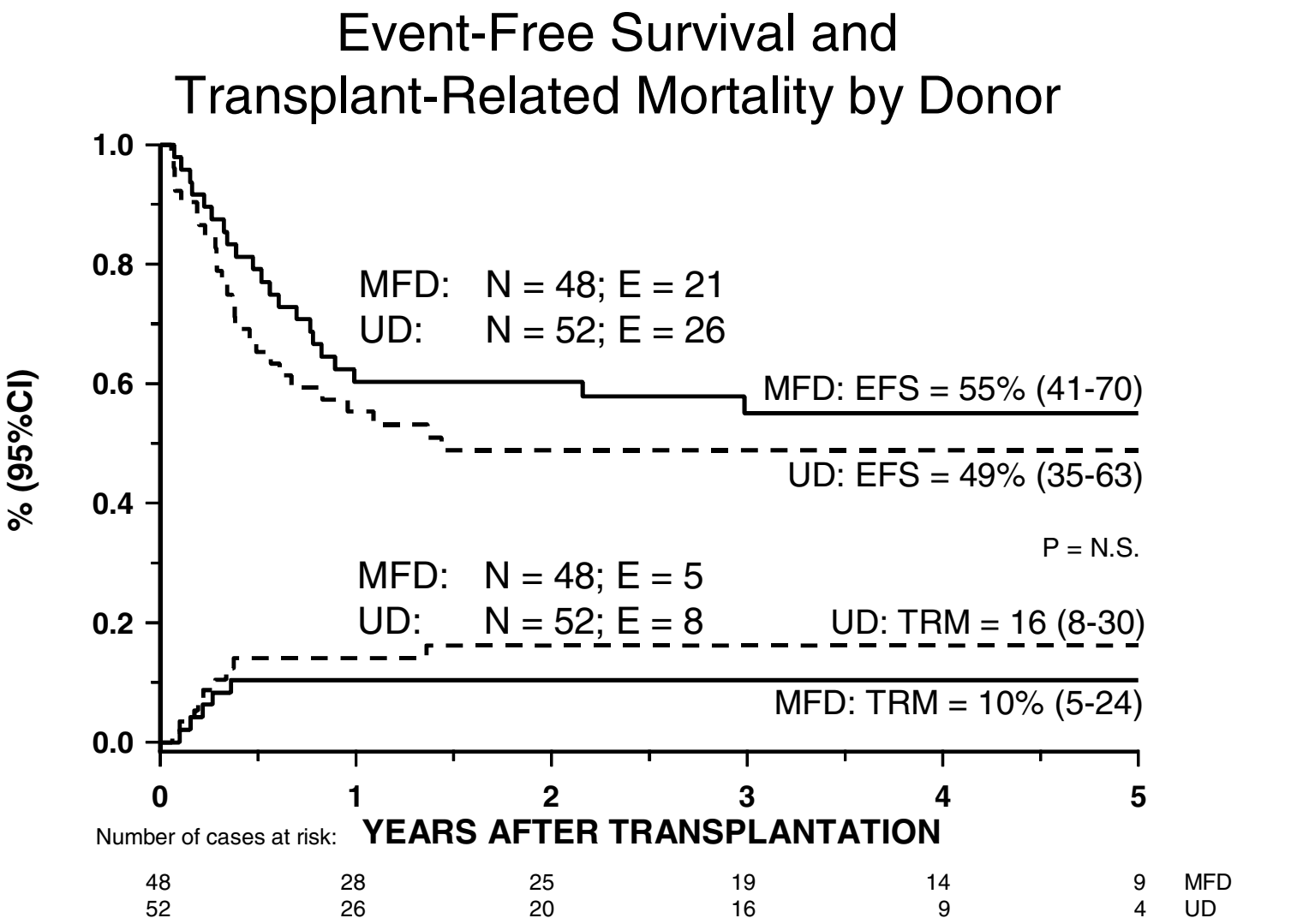
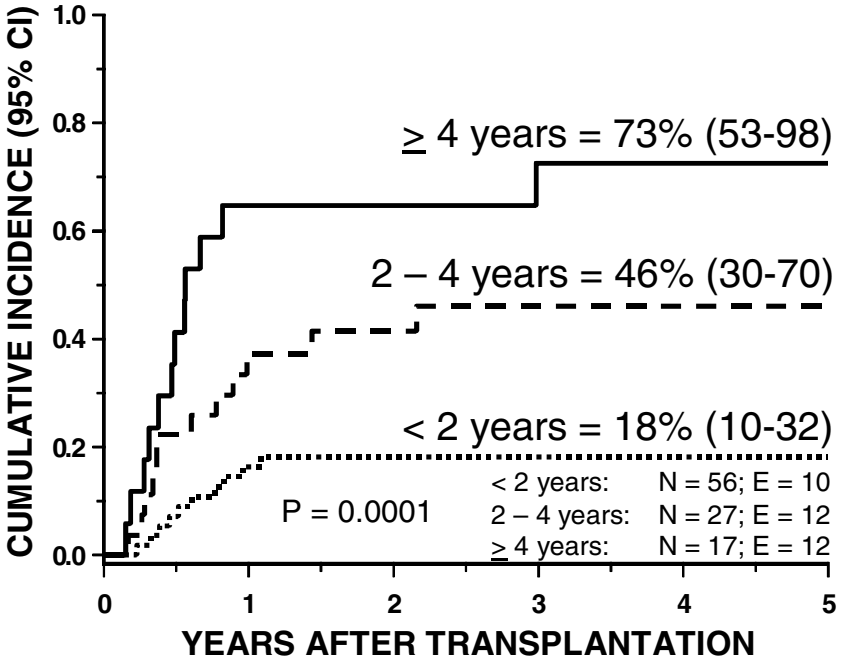
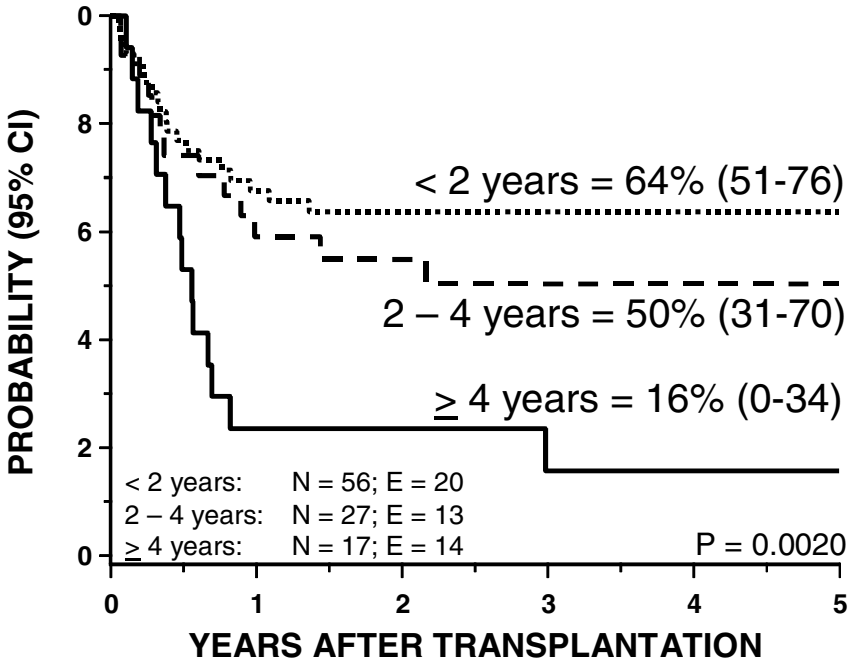

Event-Free Survival and
Transplant-Related Mortality by Donor
% (95%CI)
1.0
0.8
0.6
0.4
0.2
0.0
MFD: N = 48; E = 21
UD: N = 52; E = 26
MFD: EFS = 55% (41-70)
UD: EFS = 49% (35-63)
P = N.S.
MFD: N = 48; E = 5
UD: N = 52; E = 8
UD: TRM = 16 (8-30)
MFD: TRM = 10% (5-24)
0
1
2
3
4
5
YEARS AFTER TRANSPLANTATION
Number of cases at risk:
48 28 25 19 14 9 MFD
52 26 20 16 9 4 UD

Figure 3.

Relapse Incidence and EFS by Age at diagnosis
(years)





Number of cases at risk:

56	35	28	22	16	10	< 2 yrs
27	15	13	11	5	2	2 – 4 yrs
17	4	4	2	2	1	≥ 4 yrs

Number of cases at risk:

56	35	28	22	16	10	< 2 yrs
27	15	13	11	5	2	2 – 4 yrs
17	4	4	2	2	1	≥ 4 yrs

Figures 4A and 4B.

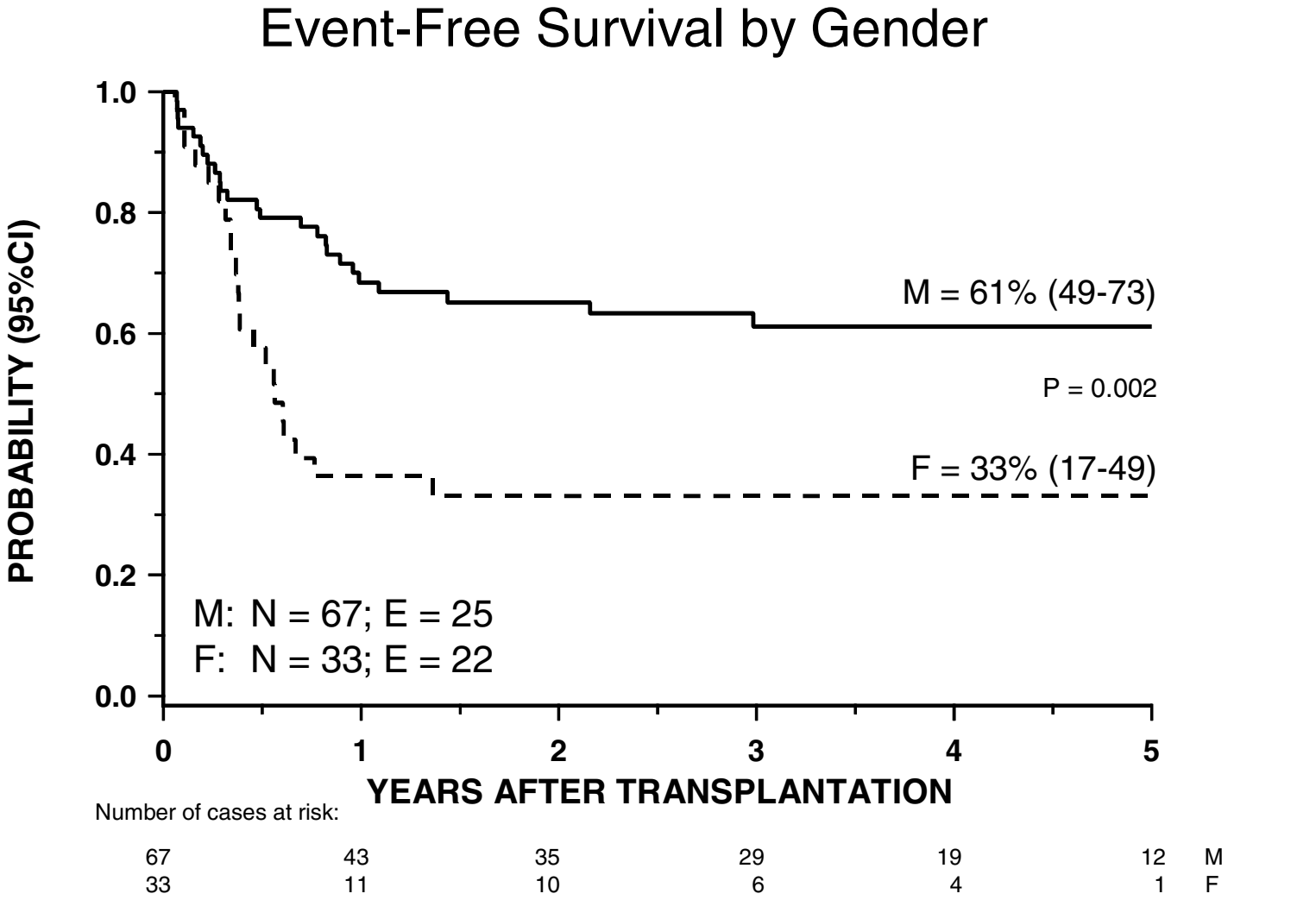

Event-Free Survival by Gender
PROBABILITY (95%CI)
1.0
0.8
0.6
0.4
0.2
0.0
M = 61% (49-73)
P = 0.002
F = 33% (17-49)
M: N = 67; E = 25
F: N = 33; E = 22
0
1
2
3
4
5
YEARS AFTER TRANSPLANTATION
Number of cases at risk:
67 43 35 29 19 12 M
33 11 10 6 4 1 F

Figure 5.

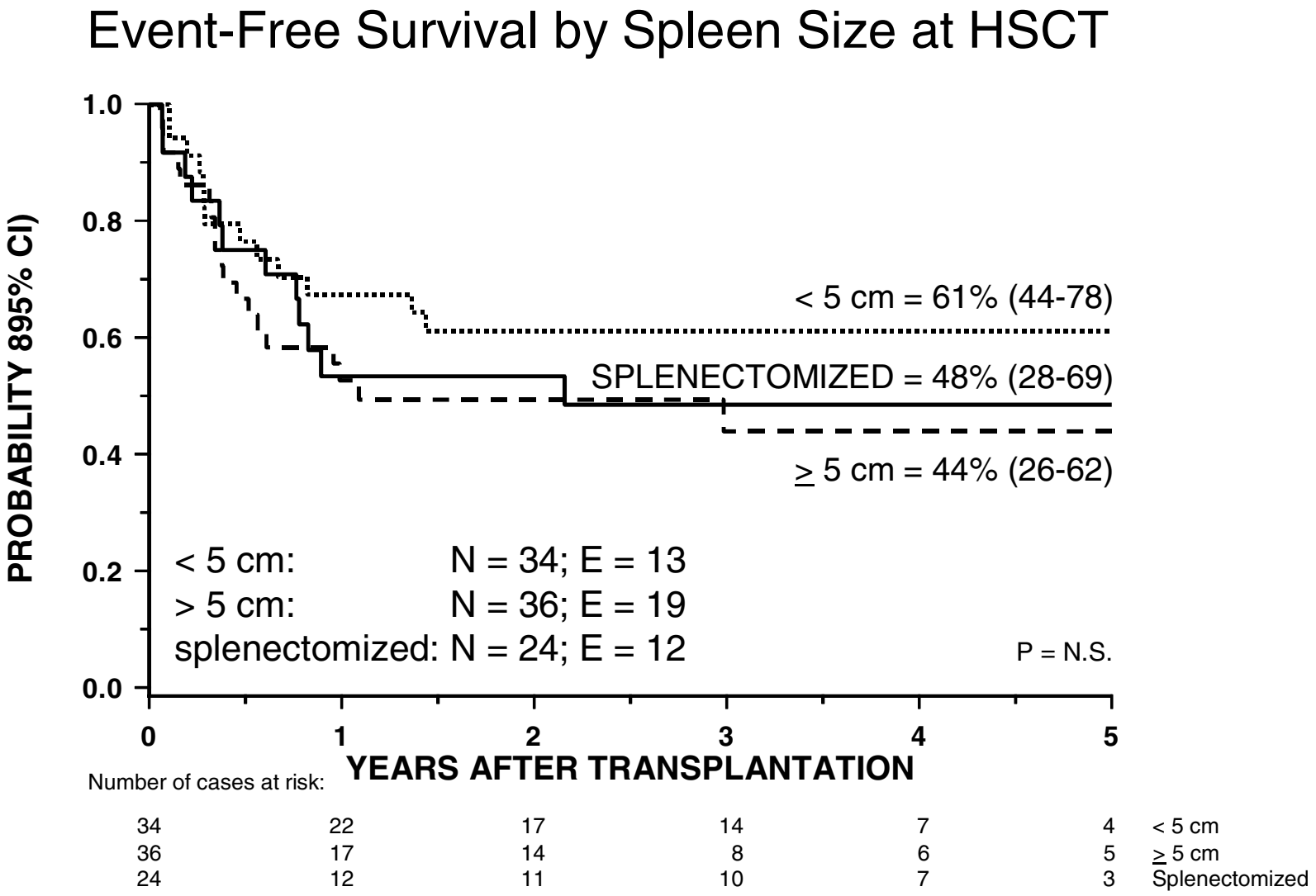

Event-Free Survival by Spleen Size at HSCT
PROBABILITY 895% CI)
1.0
0.8
0.6
0.4
0.2
0.0
< 5 cm = 61% (44-78)
SPLENECTOMIZED = 48% (28-69)
≥ 5 cm = 44% (26-62)
< 5 cm: N = 34; E = 13
> 5 cm: N = 36; E = 19
splenectomized: N = 24; E = 12
P = N.S.
0
1
2
3
4
5
YEARS AFTER TRANSPLANTATION
Number of cases at risk:
34 22 17 14 7 4 < 5 cm
36 17 14 8 6 5 ≥ 5 cm
24 12 11 10 7 3 Splenectomized

Figure 6.

Event-Free Survival by Cytogenetic Abnormalities

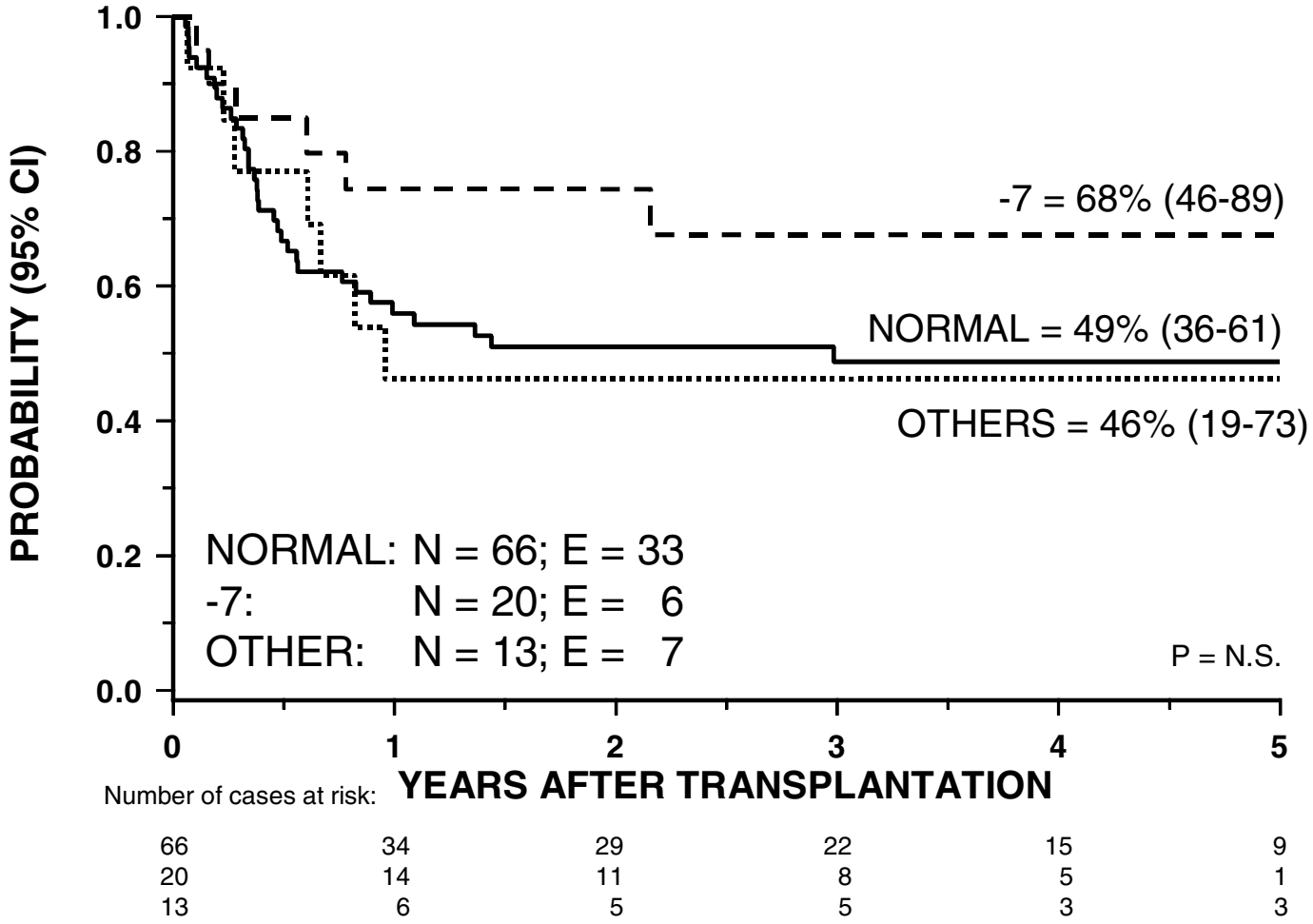


Figure 7.