Distribution Agreement

In presenting this thesis or dissertation as a partial fulfillment of the requirements for an advanced degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis or dissertation in whole or in part in all forms of media, now or hereafter known, including display on the world wide web. I understand that I may select some access restrictions as part of the online submission of this thesis or dissertation. I retain all ownership rights to the copyright of the thesis or dissertation. I also retain the right to use in future works (such as articles or books) all or part of this thesis or dissertation.

Signature:

_____ _____

Himalee S. Sabnis, MD, MS Date

Identification of Prognostic Features Associated with Survival in Juvenile Myelomonocytic Leukemia

By

Himalee S. Sabnis, M.D, M.S.

Master of Science

Clinical Research

_____

Todd Cooper, D.O.

Advisor

_____

Mitchell Klein, Ph.D.

Committee Member

_____

Muna Qayed, M.D., M.Sc.

Committee Member

Accepted:

_____

Lisa A. Tedesco, Ph.D.

Dean of the James T. Laney School of Graduate Studies

_____

Date

Identification of Prognostic Features Associated with Survival in Juvenile Myelomonocytic Leukemia (JMML)

By

Himalee S. Sabnis, M.D., M.S.
M.D., Seth Gordhandas Sunderdas Medical College, Mumbai, India 2002
M.S., State University of New York, Albany NY 2005

Advisor: Todd Cooper, D.O.

An abstract of
A thesis submitted to the Faculty of the
James T. Laney School of Graduate Studies of Emory University
in partial fulfillment of the requirements for the degree of
Master of Science
in Clinical Research
2015

ABSTRACT

Identification of Prognostic Features Associated with Survival in Juvenile Myelomonocytic Leukemia (JMML)

By Himalee S. Sabnis, M.D., M.S.

Juvenile Myelomonocytic Leukemia is a rare myeloproliferative disorder of childhood with a five year overall survival ranging between 52-64%. Allogeneic stem cell transplantation is regarded as the only curative treatment modality but up to 50% of all transplant recipients will relapse within the first year after transplantation. We studied 114 JMML patients enrolled on the North American JMML Project (NAJP) registry to determine whether allogeneic hematopoietic stem cell transplantation (HSCT) affected survival and to determine clinical and laboratory parameters that influence overall survival (OS). The two-year overall survival of all patients enrolled was 47% ± 0.05 with a median survival time of 449 days. We found that HSCT significantly improved the two-year survival of JMML patients (38% ± 0.07 versus 52% ± 0.06, p-value 0.024). Age at diagnosis, gender, lung disease, Neurofibromatosis type 1 (NF1), transplantation status, white blood count, platelet count, fetal hemoglobin levels and monosomy 7 were assessed as univariate predictors for OS in a Cox Proportional Hazards Model. Age at diagnosis >24 months (HR 1.98, 95% CI 1.04 – 3.76, p-value 0.03), presence of lung disease (HR 4.37, 95% CI 1.77 – 10.8, p-value 0.001), and platelet count <40K/µl (HR 0.46, 95% CI 0.24 – 0.86, p-value 0.01) were found to be adverse risk factors for overall survival in the multivariate model. Sub-analysis of the transplanted patients was done using the above mentioned covariates in addition to specific covariates applicable only to transplanted patients. Transplantation covariates that were studied included age at transplant, wait time between study enrollment to transplant and type of donor source (related versus unrelated). Analysis showed that presence of NF1 (HR 0.17, 95% CI 0.04 – 0.76, p-value 0.02), high white blood count >50 K/µl at diagnosis (HR 0.31, 95% CI 0.12 – 0.80, p-value 0.01) and wait time >120 days (HR 0.34, 95% CI 0.15 – 0.74, p-value 0.006) were favorable predictors for overall survival in patients that received a hematopoietic stem cell transplant. Future studies should examine the role of therapy for relapsed or recurrent disease in JMML as well as the effects of transplant regimens and transplant complications on overall survival.

Identification of Prognostic Features Associated with Survival in Juvenile Myelomonocytic Leukemia (JMML)

By

Himalee S. Sabnis, M.D., M.S.
M.D., Seth Gordhandas Sunderdas Medical College, Mumbai, India 2002
M.S., State University of New York, Albany NY 2005

Advisor: Todd Cooper, D.O.

A thesis submitted to the Faculty of the
James T. Laney School of Graduate Studies of Emory University
in partial fulfillment of the requirements for the degree of
Master of Science
in Clinical Research
2015

ACKNOWLEDGEMENTS

For their willingness and cooperation to be included in a national database for this rare disease, I must first thank all **parents and patients** with Juvenile Myelomonocytic Leukemia (JMML) and the creators of the North American JMML Project (NAJP), **Dr. Robert Castleberry, M.D.,** and **Dr. Peter Emanuel, M.D.** Without their dedication and support for maintaining this database over the years, this study would not have been possible.

For his constant guidance and unwavering support during my graduate school program and for his oversight in analyzing results and preparing this manuscript, I am especially grateful to my mentor **Dr. Todd Cooper, D. O.** He has devoted time and effort to my career development as a young investigator and has been my role model both personally and professionally. I can never thank him enough for his help and unfaltering confidence in my abilities.

For their exceptional help in data analysis, interpretation of results and preparation of this manuscript, I must thank the members of the Emory MSCR Program and Thesis Committee – **Dr. Mitchel Klein, Ph.D., Dr. Amita Manatunga, Ph.D., Dr. John E. McGowan, Jr., M.D** and **Dr. Muna Qayed, M.D, M.Sc** as well as my colleague **Dr. Joanna Newton, M.D, M.Sc.** They were always willing to make time in their busy schedules to meet with me and provide invaluable feedback about my project.

For his critical thinking, timely appraisal and unending support, I must thank **Dr. Sunil Raikar, M.D**. As my colleague in pediatric oncology, he is my biggest critic and as my fiancé, he is my biggest fan. I am immensely grateful for his review of my data analysis and interpretation.

Finally, I must thank the **Atlanta Clinical and Translational Science Institute – Research, Education, Training and Career Development (ACTSI-RETCD) KL2** Program for supporting my graduate school tuition and providing me with invaluable protected time to complete my degree.

Table of Contents

INTRODUCTION

Juvenile Myelomonocytic Leukemia (JMML) is a rare myeloproliferative disorder of childhood which accounts for 2-3% of all childhood malignancies. The annual incidence in the United States is estimated at 1.3 per million children 0-14 years of age [1]. The disease mainly affects young children (<4 years old) and tends to have a male predominance [2]. In the past the disease had been known by other names such as Juvenile Chronic Myeloid Leukemia (JCML), Chronic Myelomonocytic Leukemia of Infancy, and Infantile Monosomy 7 Syndrome. In 2008, the new World Health Organization (WHO) classification of hematopoietic neoplasms classified it under myelodysplastic/myeloproliferative diseases [3].

The diagnosis of JMML can be challenging since patients often present with symptoms that can mimic other infectious or immunologic disorders as well as hematologic malignancies. These symptoms may include fever, liver and spleen enlargement and an elevated white blood cell count and warrants prompt evaluation by disease experts. Laboratory characteristics that are especially seen in JMML include marked monocytosis, rapid proliferation of JMML cells in response to cytokine GM-CSF and elevated levels of fetal hemoglobin (HbF). In the last two decades, identification of molecular mutations in genes such as PTPN11 (protein tyrosine phosphatase, non-receptor type 11), NRAS (neuroblastoma rat sarcoma viral oncogene homolog), K-Ras (Kirsten rat sarcoma viral oncogene homolog), NF1 (Neurofibromatosis Type 1) and CBL (Casitas B-lineage Lymphoma proto-oncogene) has established a molecular basis for the disease in 85% of patients with JMML [4-8]. Using the existing clinical and laboratory parameters used to define the disease, the International JMML Working Group revised the diagnostic criteria in 2008 to account for these genetic mutations [1].

Treatment of JMML may require initial high dose chemotherapy for patients presenting with high disease burden and respiratory distress at diagnosis. However chemotherapy alone is not curative. The disease may follow an aggressive course and if left untreated, is rapidly fatal within 12 months. The overall survival is estimated to be between 52-64% with allogeneic hematopoietic stem cell transplantation (HSCT) providing the only cure [2, 3]. Relapse post-transplantation tends to occur early (4-5 months) and in approximately half of all patients transplanted [9]. These patients may be cured with a donor lymphocyte infusion or a second transplant however disease recurrence post transplantation still continues to be the major cause of mortality in JMML [10]. There has been no randomized trial comparing stem cell transplantation to other therapeutic modalities primarily because of the rare nature of the disease and due to the lack of other therapeutic options. However, there are a subset of patients whose clinical course is less aggressive and do not require a stem cell transplant for cure. Patients with Noonan's Syndrome show a predilection to develop JMML however the myeloproliferation spontaneously regresses in most patients [11]. Recent data suggests that patients with mutations in CBL gene may also follow a more indolent course and could potentially not need a stem cell transplant to achieve cure [12]. Most studies to date have focused on JMML patients that have been treated with an allogeneic stem cell transplant [2, 13, 14] therefore, randomized studies examining the effects of HSCT on overall survival in children with JMML have not been conducted. Given that there is subset of patients that does not require transplant to survive, it leads us to question if transplantation actually improves overall survival? Given that stem cell transplantation is currently the only potentially curative therapy in JMML, we hypothesized that allogeneic stem cell transplantation is associated with overall improved survival in these patients.

Though multiple studies have examined prognostic factors in patients with JMML, there is currently no universally accepted risk stratification for relapse or recurrence. Risk

stratification can identify patients who are at high risk of dying at presentation (these might benefit from early interventions such as pre-transplant chemotherapy) or those more likely to relapse after stem cell transplantation (these might benefit from early post-transplant interventions). Several studies have looked at clinical and laboratory criteria that might help define this high risk subset but no uniform consensus currently exists[15]. Host factors such as age, gender, disease factors such as platelet count and hemoglobin F levels at diagnosis and treatment related factors such as splenectomy, pre-transplant chemotherapy, type of donor among others have been evaluated within larger cohorts. Our cohort comprises of patients that were enrolled on the North American JMML (NAJP) registry and currently contains 114 evaluable JMML patients making it the one of the largest cohort of JMML patients that has been studied. Our cohort has 44 patients that did not undergo stem cell transplantation due to various reasons enabling us to compare the differences in clinical presentation between these patients and the patients who were transplanted. Given that age, platelet counts and levels of fetal hemoglobin had been cited in literature as possible prognostic factors [15], we hypothesized those patients that were older than 2 years of age or with platelet counts less than 40,000/µl or with fetal hemoglobin >40% at the time of presentation were more likely to have lower overall survival. These cutoffs were based on observations made in the literature.

BACKGROUND

The North American JMML Registry was established in 1985 at University of Alabama, Birmingham under the supervision of Dr. Robert Castleberry and Dr. Peter Emanuel. It enrolled JMML patients between the years 1985-2007 from North America. Dr. Todd Cooper became the Principle Investigator of NAJP in 2005. All patients were enrolled after central review of their clinical and laboratory data and samples were processed in Dr. Emanuel's laboratory for evaluation of hypersensitivity to GM-CSF. Data that was captured on the registry included demographic details, date of presentation, transplantation status and donor source, date of last follow up/death in addition to clinical features at presentation such as fever, spleen and liver enlargement, lung disease and NF1 status. Laboratory data included blood counts and bone marrow results at the time of presentation in addition to levels of fetal hemoglobin. Molecular diagnostic testing included monosomy 7 and bcr;abl chromosomal translocation status; however the newer molecular testing for PTPN11, RAS and CBL mutations was not available at the time of enrollment and was thus not included in the database.

Just as the diagnosis of JMML can be challenging, its treatment can be equally if not more complicated. The only curative modality for JMML at present continues to be allogeneic stem cell transplantation [12, 16]. It is true that allogeneic stem cell transplantation provides a possible chance of a cure but the relapse rate post-transplantation is fairly high (50%) so the decision for transplantation requires careful weighing of risks versus benefits for each patient [7]. Other therapeutic options that have been used in the last several decades to achieve disease control not cure have included low dose chemotherapy with agents such as etoposide/cytarabine, high dose chemotherapy with fludarabine and cytarabine, retinoids, alpha-interferon, splenectomy and newer targeted agents such as farnesyl transferase inhibitors (FTI's) [17-19]. The

role of pre-transplant therapy has been debated but no study to-date has determined its impact on overall survival post-transplant. However, most patients that present with symptoms will receive some form of treatment which usually includes low or high dose chemotherapy to achieve better disease control and to alleviate symptoms. It has been demonstrated that not all patients that fail to receive a stem cell transplant will succumb to their disease. It is known that patients with Noonan's syndrome present with JMML that can spontaneously regress but patients without this syndrome have also shown regression of their disease. Newer molecular diagnostics have identified mutations in NRAS ($NRAS^{G12S}$) and KRAS ($KRAS^{G12V}$) that follow a milder course towards regression and patients with CBL gene mutation also demonstrate an indolent course in some cases [20, 21]. Current standards of therapy and small patient numbers will not allow for a prospective, randomized clinical trial comparing OS in JMML patients that received BMT and those that did not. The NAJP registry did contain a number of patients that did not receive a stem cell transplant. Typical reasons for not receiving a transplant included patients who were too sick to receive transplant, lack of suitable donor options or spontaneous regression of the disease prior to transplantation. Therefore, we decided to perform a retrospective analysis of the NAJP database and compare the effect of stem cell transplant on overall survival.

Using the clinical and laboratory data available in JMML patients to date, four large studies have tried to determine whether any factors can be used to determine risk of poor outcome for JMML patients. In 2003, one of the earliest studies done by Passmore et al. in the United Kingdom, found that among 67 patients with JMML, the FPC score (elevated hemoglobin F >10%, high platelet count >40K/µl and cytogenetics) and age > 2 years were adverse prognostic factors for 5 year overall survival [22]. They also used the adult MDS (myelodysplastic syndrome) scoring system IPSS (International Prognostic

Scoring System) and applied it to the pediatric patients to determine its utility for risk stratification and found that IPSS was not helpful for prognostic classification in patients with JMML. Subsequently, data from a larger cohort of JMML patients (n=100) from the EWOG-MDS and EBMT Groups that all underwent hematopoietic stem cell transplantation was also analyzed for risk assessment [2]. This cohort primarily included patients from Germany, Italy, Netherlands, Sweden, Austria and the Czech Republic. Disease progression and relapse post-transplantation was the most frequent cause of death as predicted. In their univariate analysis, older age (>4 years), female gender, high percentage of fetal hemoglobin (>40%) and high blast percentage in the marrow at the time of transplantation (>20%) was indicative of leukemia relapse. However in the multivariate analysis, they found that only age at transplant >4 years (RR 2.96, 95% CL 1.26 – 6.92, p-value 0.012) to be the significant adverse risk factor for incidence of relapse [2]. During this period, discovery of the mutations in RAS, NF1, PTPN11 established a molecular basis of the disease in a large proportion of patients. Yoshida et al studied 71 patients in Japanese cohort that were diagnosed with JMML, 48 of which received a stem cell transplant [23]. This was one of the first studies incorporating gene mutation status into risk stratification. Univariate analysis showed that PTPN11 mutations, age >24 months and presence of cytogenetic abnormality were adverse prognostic factors for overall survival but were not associated with an inferior survival after transplantation in a multivariate model. The largest study in JMML patients thus far was recently published by Locatelli et al, utilizing data from four national registries, EUROCORD, EWOG-MDS, EBMT and CIBMTR. They studied 110 JMML patients who received umbilical cord blood transplantation and found that age >1.4 years (HR 2.3, 95% CL 1.2 – 4.3, p-value 0.009) and presence of monosomy 7 (HR 2.6, 95% CL 1.4 – 5, p-value 0.003) were adverse risk features for overall survival [14]. Mutational status was only known in 24 patients in this study and was not used for clinical correlation.

Studies performed to date in JMML have several limitations. Most of these larger studies primarily followed patients that had received a stem cell transplant and did not have a large number of non-transplanted patients to be able to study outcomes separately within both groups. They also did not account for what are considered to be important variables such as the presence of lung disease or respiratory symptoms at diagnosis, the time interval from JMML diagnosis to time of transplantation or effects of other co-existing conditions such as neurofibromatosis. The patient population also tended to be more homogenous limited mainly to European and Japanese cohorts. Our study is the single, largest cohort of JMML patients that has been reported in the literature. It comprises of 70 patients who underwent stem cell transplantation and 44 patients who did not get transplanted. In addition to evaluating the role of previously considered risk factors such as host factors (age and gender) and disease-specific factors (platelet count, monosomy 7 and fetal hemoglobin), we also have the ability to study the effects of previously unstudied factors such as lung disease and NF1 on overall survival. The time from diagnosis to transplant has recently been speculated to be an important predictor in overall survival of JMML patients on the recently concluded JMML Children's Oncology Group (COG) trial AAML 0122 (Todd Cooper, personal communication, manuscript in press). We will examine this predictor in our cohort to ascertain its impact on patient survival.

The two aims of our study are to compare the overall survival of JMML patients who underwent transplantation versus those that did not and to identify clinical and laboratory parameters that are associated with a worse prognosis in JMML.

METHODS

Hypothesis

In JMML patients, allogeneic hematopoietic stem cell transplantation is associated with increased survival. Patients who are older than 2 years at the time of diagnosis, have platelet counts < 40K/µl or fetal hemoglobin levels >40% at diagnosis have a worse overall survival.

Study Design

This is a retrospective cohort analysis of JMML patients enrolled on the North American JMML (NAJP) registry between 1985 – 2007.

Patients

Patients in North America (Mainly United States of America and Canada) were enrolled on the NAJP registry. Patients were considered to have JMML if they met the following eligibility criteria:

1. Category 1 (all the following must be fulfilled)
    a. Absence of t(9;22) BCR/ABL fusion gene
    b. Absolute Monocyte Count >1000/ µl
    c. <20% blasts in bone marrow
2. Category 2 (at least two of the following)
    a. Circulating Myeloid precursors
    b. White blood cell count >10,000/ µl
    c. Increased Fetal hemoglobin
    d. GM-CSF Hypersensitivity

All parents signed informed and written consent prior to enrollment in the registry. Patients that received a hematopoietic stem cell transplant from matched related or matched unrelated donor sources were designated to the transplant group.

Measurements

Outcomes: The primary outcome measure was overall survival which was defined as the time between study enrollment and either death or last follow up. The cause of death was noted to be progressive or relapsed disease in majority of the patients. In patients that underwent a transplant; we did not have sufficient information to distinguish transplant related mortality from disease related events.

Predictor: Transplantation status, as defined above

Covariates: In the multivariate analysis, we controlled for demographic factors namely age and gender, disease related factors such as NF1 status, presence of lung disease and laboratory parameters at presentation including white blood cell (WBC) count, platelet count, fetal hemoglobin levels (HbF) and presence of monosomy 7. The time interval between enrollment on the registry to transplantation was also included as a covariate for transplanted patients.

Analytic Plan

Descriptive Statistics: Frequency tables were constructed to describe the characteristics of the whole cohort. Variables examined for the entire cohort included demographic variables, duration of follow up, clinical and laboratory characteristics.

Analysis of Baseline Characteristics: Patients were stratified into either transplant ('Allogeneic HSCT') or no transplant ('No HSCT') group and differences in their baseline clinical, demographic characteristics and primary outcome (categorical variables) were

compared using Pearson's Chi-square test. Differences in laboratory parameters (continuous variables) between the two groups were compared using two sample t-test.

Survival Analysis: The Kaplan-Meier Method was used to calculate the overall survival (OS) estimates for all patients stratified based on transplantation status. Two year estimates (days 730) was reported for both groups using time of enrollment on the study for time-to-event analysis. Log-log curves were used to determine that proportional hazards assumption was met. The Log rank test was used to compare the survival estimates between the two groups. The median time to transplantation was also calculated. This median time was used as a covariate in survival analysis since patients who were unable to survive this time interval would not have had an equal chance of receiving a transplant due to early mortality and may be a source of bias. One year survival was calculated for the two strata (Allogeneic HSCT versus No HSCT) accounting for this early mortality.

Hazard Ratios for OS (overall survival): The hazard ratios (HR) for overall survival with the 95% confidence interval limits were calculated using Cox Proportional Hazards model. Important known covariates namely age, gender, white blood count, platelet count, fetal hemoglobin, monosomy 7 status along with transplantation as a predictor were included in the model.

Interactions: Interactions between neurofibromatosis and transplantation as well as lung disease and transplantation was accounted for. The interaction between NF1 and transplantation and lung disease and transplantation was found to be significant and the term was included in the final model.

Assessment of Potential Bias: Selection bias exists for the HSCT variable, since providers were more likely to send patients who were sicker at the time of diagnosis to transplant versus not, since transplantation is the only curative option; more patients overall were referred for transplantation versus not and it is likely that some patients

were not able to survive the time needed for transplantation to be an option (generally considered 6 weeks – several months). To control for these factors we compared the baseline characteristics of both transplanted and non-transplanted groups.

RESULTS

Patients

A total of 140 patients were included on the NAJP registry but only 114 patients met criteria for inclusion in this study as they had their outcomes documented in the registry (**Table 1**). The 114 patients were followed for a median time period of 411.5 days (range 6-5677 days) and 44 patients (39.5%) were alive at the time of last follow up. Two thirds of all patients were males (n=76) as expected given the male predominance of the disease. The median age of all patients was 15.5 months at the time of diagnosis (range 1-72 months). Of the 114 patients, 70 patients (61.4%) underwent hematopoietic stem cell transplantation.

Clinical and laboratory characteristics of the entire cohort were analyzed (**Table 2 and 3**). All patients presented with splenomegaly (median spleen size - 5 cm) which according to the newer diagnostic criteria is a hallmark of the disease [1]. Hepatomegaly (median liver enlargement – 4 cm) was noted in 90.3% of all patients.

Lymphadenopathy was the next most common clinical finding at presentation, seen in 52.3% of all patients followed by skin disease (42.9%) and lung disease (19.8%). Skin disease was typically noted to be in the form of maculo-papular rash and lung disease was defined by the presence of x-ray findings and respiratory symptoms present at the time of diagnosis. Among co-existing conditions, 14.4% patients had the clinical diagnosis of Neurofibromatosis type 1 at presentation. As expected, laboratory parameters demonstrated elevated WBC counts, monocytosis, low percentage of peripheral blasts and elevated levels of fetal hemoglobin. JMML-specific laboratory findings noted in the cohort included GM-CSF hypersensitivity in two-thirds (75.4%) and presence of monosomy 7 in 14.5% of all patients.

All patients were further divided into two groups based on their transplantation status into 'Allogeneic HSCT' or 'No HSCT' groups. The baseline clinical and laboratory characteristics of the two groups were compared and no significant difference in any variables was noted except for the presence of neurofibromatosis type 1 in both groups (**Table 4 and 5**). The Allogeneic HSCT group had a larger percentage of patients with NF1 as compared to the No HSCT group (20% versus 5%, p-value 0.03). The median wait time from the time of enrollment on the study to transplant for all transplanted patients was 138.5 days (range 34-1178, SD 208.5).

Survival Analysis

Using Kaplan-Meier method, the 2 year - overall survival of all patients on the registry was 47% ± 0.05 with a median survival time of 449 days (**Figure 1**). The stratified Kaplan-Meier analysis was used to determine the overall survival for both transplanted and non-transplanted patients. The 2 year OS for non-transplanted patients was significantly lower than those that underwent stem cell transplantation (38% ± 0.07 versus 52% ± 0.06, p-value 0.024) (**Figure 2**). From the survival curves, it was apparent that early mortality (<200 days) played an important role in the worse outcome of the No HSCT group. The earliest transplantation in our cohort occurred at 34 days with a median time to transplant of 138.5 days. It is well established that patients with JMML often present with fulminant disease resulting in high mortality within the first few weeks of presentation. There were 18/44 patients in the No HSCT group that died prior to 138 days. In order to overcome this selection bias, we chose to compare the survival of the remaining 26 patients with patients who had received a transplant. For this analysis in our Allogeneic HSCT group, we used the difference between the date of transplant to the time of last follow up as our time to event to account for the variability in the time needed for each patient to get to transplant. Almost all deaths in both groups occurred

within one year of follow up so for this analysis we estimated the one year survival. The one year - OS of the No HSCT group was higher than the Allogeneic HSCT group (65% ± 0.09 versus 50% ± 0.07) but the difference was not statistically significant (p-value 0.17) (**Figure 3**).

Predictors for Overall Survival

Univariate Analysis: Age at diagnosis, gender, lung disease, Neurofibromatosis, transplantation status, white blood count, platelet count, fetal hemoglobin levels and monosomy 7 were assessed as univariate predictors for OS in Cox Proportional Hazards Model (**Table 6**).The following predictors were found to significantly affect overall survival: age at diagnosis >24 months (HR 2.15, 95% CI 1.29 – 3.6, p-value 0.003), presence of lung disease (HR 2.25, 95% CI 1.26 – 4.01, p-value 0.006), coexisting neurofibromatosis (HR 0.33, 95%CI 0.12 – 0.91, p-value 0.03), transplantation status (HR 0.56, 95% CI 0.33 – 0.94, p-value 0.03), platelet count (HR 0.39, 95% CI 0.23 – 0.65, p-value 0.0003) and fetal hemoglobin levels (HR 1.88, 95% CI 1.08 – 3.05, p-value 0.02). These variables were included in multivariate analysis for OS.

Interactions: In the previous analysis for distribution of patients between Allogeneic HSCT and No HSCT group, it was found that patients with NF1 were disproportionately higher in the Allogeneic HSCT group. To examine if this difference impacted the effect of transplantation on overall survival, an interaction term for NF1 and transplantation status was included in multivariate model. Patients with respiratory disease at presentation may do poorly due to lung injury prior to transplant and to account for this, we also included an interaction term between lung disease and transplant status (**Table 7A and B**). Both these interaction terms were found to be significant and were included in the final multivariate model.

Multivariate Model: Age at diagnosis >24 months (HR 1.98, 95% CI 1.04 – 3.76, p-value 0.03), presence of lung disease (HR 4.37, 95% CI 1.77 – 10.8, p-value 0.001), and platelet count <40K/µl (HR 0.46, 95% CI 0.24 – 0.86, p-value 0.01) continued to be adverse risk factors for overall survival in the multivariate model (**Table 8**). In patients with NF1, after controlling for other covariates, transplantation was associated with significantly improved overall survival (HR 0.03, 95% CI 0.002– 0.62, p-value 0.02). This was not true in patients without NF1 (HR 0.59, 95% CI 0.28– 1.21, p-value 0.15). The interaction term for lung disease and transplantation was not significant in this final model and thus hazard ratios were not examined within patients who had lung disease versus not.

Sub-analysis of only transplanted patients

We wanted to further study the cohort of patients who had undergone a hematopoietic stem cell transplant in order to determine if there were any clinical or laboratory predictors of poor prognosis. The graft source included both matched related and unrelated sources which did not have an impact on overall survival (**Figure 4**).

Univariate Analysis: In addition to the clinical and laboratory features considered for the analysis of the entire cohort, specific covariates applicable to transplanted patients namely age at transplant, wait time between study enrollment to transplant and type of donor source (related versus unrelated) were also analyzed using Cox Proportional Hazards model (**Table 9**). Presence of NF1 (HR 0.18, 95% CI 0.04 – 0.77, p-value 0.02), high white blood count >50 K/µl at diagnosis (HR 0.43, 95% CI 0.18 – 0.99, p-value 0.04), high platelet count >40 K/µl at diagnosis (HR 0.47, 95% CI 0.23 – 0.95, p-value 0.03), hemoglobin F level < 40% (HR 2.31, 95% CI 1.12 – 4.74, p-value 0.02) and time to transplant >120 days (HR 0.44, 95% CI 0.22 – 0.9, p-value 0.02) were found to be favorable prognostic features for survival.

Multivariate Analysis: Presence of NF1 (HR 0.17, 95% CI 0.04 – 0.76, p-value 0.02), high white blood count >50 K/µl at diagnosis (HR 0.31, 95% CI 0.12 – 0.80, p-value 0.01) and wait time >120 days (HR 0.34, 95% CI 0.15 – 0.74, p-value 0.006) continued to be significant predictors for overall survival in the multivariate model.

DISCUSSION

Studies to date in JMML have studied patients that primarily underwent hematopoietic stem cell transplantation without specific emphasis on JMML patients that survived this disease without transplantation. While a randomized controlled trial would be the ideal way to study the significance of transplantation in patients with JMML, given the rare incidence of this disease, such an endeavor is practically improbable. Analysis of the demographic variables in our NAJP cohort showed similar age, gender predominance and overall survival comparable to similar JMML studies in the literature. Disease recurrence/relapse was the primary cause of mortality in our patient population independent of the therapy received. With 114 patients, it is the largest JMML cohort that has been studied thus far in North America and included 44 patients that did not undergo HSCT providing us with the unique opportunity to analyze both transplanted and non-transplanted patients simultaneously.

In our study, we did not find any significant differences in the clinical and laboratory characteristics between the Allogeneic HSCT and No HSCT groups except for the distribution of patients with Neurofibromatosis Type 1 who were present in a higher proportion within patients who had received a transplant. Though reasons for this disparity are unclear it is possible that patients that were diagnosed with NF1 were closely followed at the outset for development of myeloproliferative conditions given the association between the two diseases. This may have resulted in preferential referral of these patients for HSCT unlike patients who did not have NF1 and in whom diagnosis of JMML may have been delayed. Analysis of overall survival defined as time interval between diagnosis and study enrollment to time of last follow up showed that patients that received an allogeneic transplant had significantly better overall survival as compared to the non-transplanted patients. This analysis however, would not be able to

account for those patients that presented with aggressive disease at the onset and were unable to survive long enough to be eligible for transplantation. The average time for a patient to get to hematopoietic stem cell transplantation can range from as little as 6 weeks to several months from diagnosis. We re-analyzed our cohort using the median time to get to transplantation in our patients (138 days) as time zero for patients within our non-transplanted strata and the date of transplant as time zero for our transplanted strata. This accounted for the selection bias that that might have occurred with early deaths within the non-transplanted group and this survival analysis did not show a difference in the overall survival between patients transplanted versus not. This alludes to the fact that though transplantation is the only modality for cure, focus should be placed on pre-transplant therapies that will enable more patients to get to transplantation, if overall survival in JMML is to be improved. We recognize that our study has certain limitations given its retrospective nature. Important variables that might influence outcome that were unavailable in the database included lack of genetic mutation data in JMML patients, details of the kind of alternative therapy if any, that was given to the patients in the No HSCT arm or if the patients who underwent transplantation and relapsed, received a second HSCT.

Several large studies have tried to determine host-, disease- and treatment- specific factors that may enable risk stratification of JMML patients. This would help the clinician identify the high risk patients early, allowing for close follow-up of these patients as well as potential early referral to centers with experience in dealing with JMML. We evaluated both previously studied (e.g. age, gender) and lesser studied but clinically accepted (e.g. lung disease) risk factors in our analysis and found that patients who were older (>24 months at diagnosis), had lung disease and elevated platelet counts had a significantly worse prognosis in our multivariate analysis. Transplantation

significantly influenced overall survival in patients who also had co-existing neurofibromatosis though this association should be interpreted with caution given the small number of NF1 patients in the No HSCT group (2/44). Our 2 year cutoff for age is similar to that demonstrated in some cohorts though a cutoff of 1.4years was demonstrated in the most recent EUROCORD/CIBMTR study [14, 22, 24, 25]. Older patients may have poorer outcomes given that they may have fewer donor options available at the time of presentation. Lung disease has long been considered one of the most life-threatening manifestations of the disease at the time of presentation and it is not surprising that in our analysis this was a found to be a strong adverse prognostic factor. Our study is the first study however, to specifically quantify this association. Elevated fetal hemoglobin and male gender which have been debated in the literature to be adverse risk factors were not significant in our final analysis.

Though matched related sibling donors are considered the ideal source for stem cells umbilical cord blood as the donor source is an especially important for this disease given that most patients present <3 years of age. Within the group of patients that underwent transplantation, we found no difference in overall outcomes for patients that had received a matched related donor graft versus those that had received matched unrelated grafts (umbilical cord blood, bone marrow or peripheral blood stem cells). Interestingly, patients who had elevated white blood counts, neurofibromatosis and who had a delay in their time to transplant great than 120 days did significantly better in transplant. It is likely that patients who had elevated white blood counts at diagnosis were more likely to receive cytoreductive chemotherapy prior to transplant thereby reducing disease burden at the time of transplant and resulting in improving outcomes. It is known that the clinical severity of JMML can vary widely between patients. Most patients will present with moderate to severe disease symptoms due to elevated white blood cell counts. The

patients who had a longer wait time to get to transplant may have had a less aggressive presentation.

Overall, our ability to compare JMML patients that underwent hematopoietic stem cell transplantation versus those who did not in this large, heterogenous sample of patients within North America is a major strength of the study. Central review of clinical and laboratory information ensured the uniform quality of data which was used in our analysis and we were able to quantify associations between co-morbid conditions such as Neurofibromatosis type 1 as well as disease severity (e.g. lung disease) and JMML. Future studies should examine the role of therapy for relapsed or recurrent disease in JMML as well as the effects of transplant regimens and transplant complications on overall survival.

CONCLUSION

Hematopoietic stem cell transplantation does have a significant impact on overall survival in JMML patients however early mortality continues to be an important concern. Older age (>24 months), low platelet counts (<40K/µl) and presence of lung disease are adverse prognostic factors for overall survival in all patients. In patients undergoing transplantation, absence of NF1, low white blood cell count (<50K/µl) and shorter wait times to transplant (<120 days) are associated with poorer prognosis.

REFERENCES

1. Chan, R.J., et al., *Juvenile myelomonocytic leukemia: a report from the 2nd International JMML Symposium*. Leuk Res, 2009. **33**(3): p. 355-62.
2. Locatelli, F., et al., *Hematopoietic stem cell transplantation (HSCT) in children with juvenile myelomonocytic leukemia (JMML): results of the EWOG-MDS/EBMT trial*. Blood, 2005. **105**(1): p. 410-9.
3. Vardiman, J.W., *The World Health Organization (WHO) classification of tumors of the hematopoietic and lymphoid tissues: an overview with emphasis on the myeloid neoplasms*. Chem Biol Interact, 2010. **184**(1-2): p. 16-20.
4. Flotho, C., et al., *RAS mutations and clonality analysis in children with juvenile myelomonocytic leukemia (JMML)*. Leukemia, 1999. **13**(1): p. 32-7.
5. Wutz, D., et al., *Gene expression of the hematopoietic cell phosphatase in juvenile myelomonocytic leukemia*. Leuk Lymphoma, 1999. **35**(5-6): p. 491-9.
6. Side, L.E., et al., *Mutations of the NF1 gene in children with juvenile myelomonocytic leukemia without clinical evidence of neurofibromatosis, type 1*. Blood, 1998. **92**(1): p. 267-72.
7. Niemeyer, C.M., et al., *Germline CBL mutations cause developmental abnormalities and predispose to juvenile myelomonocytic leukemia*. Nat Genet, 2010. **42**(9): p. 794-800.
8. Kratz, C.P., et al., *The mutational spectrum of PTPN11 in juvenile myelomonocytic leukemia and Noonan syndrome/myeloproliferative disease*. Blood, 2005. **106**(6): p. 2183-5.
9. Locatelli, F. and B. Lucarelli, *Treatment of disease recurrence after allogeneic hematopoietic stem cell transplantation in children with juvenile myelomonocytic leukemia: a great challenge still to be won*. Pediatr Blood Cancer, 2013. **60**(1): p. 1-2.

10. Neudorf, S., et al., *Chemotherapy and donor leukocyte infusions for relapsed juvenile myelomonocytic leukemia (JMML).* Bone Marrow Transplant, 2004. **33**(10): p. 1069.

11. Hasle, H., *Malignant diseases in Noonan syndrome and related disorders.* Horm Res, 2009. **72 Suppl 2**: p. 8-14.

12. Locatelli, F. and C.M. Niemeyer, *How I treat juvenile myelomonocytic leukemia.* Blood, 2015. **125**(7): p. 1083-90.

13. Yabe, M., et al., *Transplantation for juvenile myelomonocytic leukemia: a retrospective study of 30 children treated with a regimen of busulfan, fludarabine, and melphalan.* Int J Hematol, 2015. **101**(2): p. 184-90.

14. Locatelli, F., et al., *Analysis of risk factors influencing outcomes after cord blood transplantation in children with juvenile myelomonocytic leukemia: a EUROCORD, EBMT, EWOG-MDS, CIBMTR study*. Blood, 2013. **122**(12): p. 2135-41.

15. Dvorak, C.C. and M.L. Loh, *Juvenile myelomonocytic leukemia: molecular pathogenesis informs current approaches to therapy and hematopoietic cell transplantation.* Front Pediatr, 2014. **2**: p. 25.

16. Chang, T.Y., C.C. Dvorak, and M.L. Loh, *Bedside to bench in juvenile myelomonocytic leukemia: insights into leukemogenesis from a rare pediatric leukemia.* Blood, 2014. **124**(16): p. 2487-97.

17. Castleberry, R.P., et al., *A pilot study of isotretinoin in the treatment of juvenile chronic myelogenous leukemia.* N Engl J Med, 1994. **331**(25): p. 1680-4.

18. Emanuel, P.D., et al., *Inhibition of juvenile myelomonocytic leukemia cell growth in vitro by farnesyltransferase inhibitors.* Blood, 2000. **95**(2): p. 639-45.

19. Emanuel, P.D., K.M. Shannon, and R.P. Castleberry, *Juvenile myelomonocytic leukemia: molecular understanding and prospects for therapy*. Mol Med Today, 1996. **2**(11): p. 468-75.

20. Matsuda, K., et al., *Spontaneous improvement of hematologic abnormalities in patients having juvenile myelomonocytic leukemia with specific RAS mutations.* Blood, 2007. **109**(12): p. 5477-80.

21. Imamura, M., et al., *Juvenile myelomonocytic leukemia with less aggressive clinical course and KRAS mutation.* Pediatr Blood Cancer, 2008. **51**(4): p. 569.

22. Passmore, S.J., et al., *Paediatric myelodysplastic syndromes and juvenile myelomonocytic leukaemia in the UK: a population-based study of incidence and survival.* Br J Haematol, 2003. **121**(5): p. 758-67.

23. Yoshida, N., et al., *Correlation of clinical features with the mutational status of GM-CSF signaling pathway-related genes in juvenile myelomonocytic leukemia.* Pediatr Res, 2009. **65**(3): p. 334-40.

24. Bresolin, S., et al., *Gene expression-based classification as an independent predictor of clinical outcome in juvenile myelomonocytic leukemia.* J Clin Oncol, 2010. **28**(11): p. 1919-27.

25. Yoshida, N., et al., *[Prognosis of 75 patients with juvenile myelomonocytic leukemia: prospective study by MDS committee in the Japanese Society of Pediatric Hematology].* Rinsho Ketsueki, 2011. **52**(12): p. 1853-8.

TABLES AND FIGURES

TABLE 1: Characteristics of patients on North American JMML Project (NAJP) registry

Demographic Variable	Number	Percentage
Total Patients	**141**	
Outcome data present	**114**	**44 (39.5 %) Alive**
Follow Up Time	6-5677 days	
Median	411.5 days	
Gender		
Males	76	66.7 %
Females	38	33.3 %
Age	1-72 months	
Median	15.5 months	
Transplantation Status		
Allogeneic Transplant	70	61.4 %
No Transplantation	44	38.6 %

TABLE 2: Clinical characteristics of patients on North American JMML Project (NAJP) registry

Clinical Variable	Positive (No. of patients)*	Percentage
Splenomegaly (Median Size – 5 cm)	114 (114)	100
Hepatomegaly (Median Size – 4 cm)	102 (113)	90.3
Lymphadenopathy	57 (109)	52.3
Skin Disease	48 (112)	42.9
Lung Disease	22 (111)	19.8
GM-CSF Hypersensitivity	86 (114)	75.4
Neurofibromatosis	16 (111)	14.4
Monosomy 7	16 (110)	14.5

*Data missing on some patients

TABLE 3: Laboratory characteristics of patients on North American JMML Project (NAJP) registry

Laboratory Results	Minimum – Maximum	Median
WBC count/µl*	11 – 123 K	35 K
Hemoglobin(g/dl)	4 – 17	9
Hematocrit	11 – 50	28
MCV (µl)*	62 – 107	79
Platelet Count/ µl	4 – 702 K	64.5 K
Absolute Monocyte Count/ µl	1005 – 40480	5110
Peripheral Blasts %	0 – 18	1
Bone Marrow Blasts %	0 – 20	5
Fetal Hemoglobin (HbF) %	0 – 94	20

(*Abbreviations: WBC – White Blood Cell, MCV – Mean Corpuscular Volume)

TABLE 4: Comparison of clinical characteristics present at diagnosis between patients who received hematopoietic stem cell transplant versus no transplant

Characteristics	Allogeneic HSCT N=70	No HSCT N=44	p-value*
Age	21 months (SD 16)	21.7 months (SD 20)	0.85
Gender			0.79
Male	46 (66%)	30 (68%)	
Female	24 (34%)	14 (32%)	
Lymphadenopathy			0.24
Present	38 (57%)	19 (45%)	
Absent	29 (44%)	23 (55%)	
Skin Disease			0.57
Present	31 (45%)	17 (40%)	
Absent	38 (55%)	26 (60%)	
Lung Disease			0.09
Present	10 (15%)	12 (28%)	
Absent	58 (85%)	31 (72%)	
Neurofibromatosis Type 1			***0.03***
Present	14 (20%)	2 (5%)	
Absent	56 (80%)	39 (95%)	
Hepatomegaly			0.4
Present	61 (88%)	41 (93%)	
Absent	8 (12%)	3 (7%)	
Outcome			0.35
Alive	40 (47%)	29 (65%)	
Dead	30 (43%)	15 (35%)	

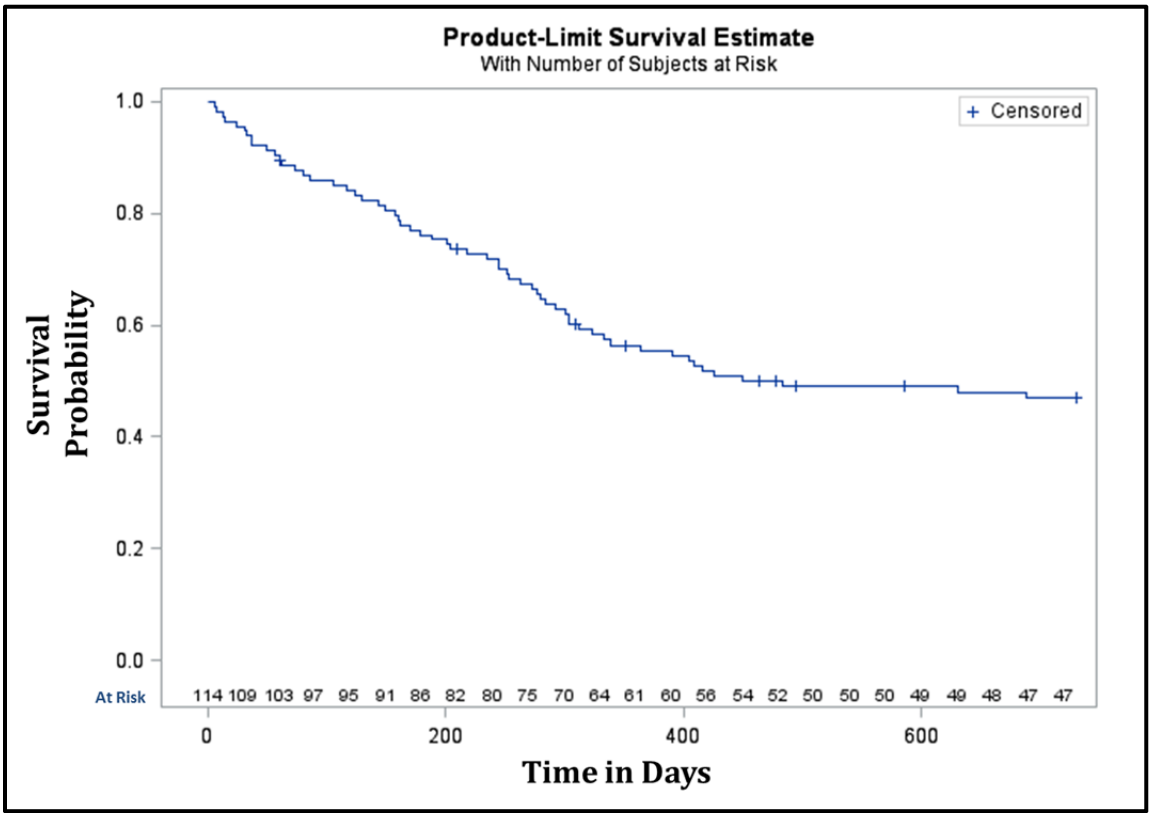
*p-value determined by Pearson's chi-square test, <0.05 (two-sided) considered significant

TABLE 5: Comparison of laboratory characteristics present at diagnosis between patients who received hematopoietic stem cell transplant versus no transplant

Characteristics	Allogeneic HSCT N=70 Mean (SD)	No HSCT N=44 Mean (SD)	p-value*
WBC count/µl	43.3 (25)	39.9 (22.2)	0.45
Hemoglobin(g/dl)	9.2 (1.6)	9.3 (2.6)	0.97
Hematocrit	28.3 (4.9)	28.1 (7.2)	0.81
MCV (µl)	79.4 (8.5)	81.2 (7.4)	0.29
Platelet Count/ µl	83.5 (99)	96.3 (102)	0.51
Absolute Monocyte Count/ µl	7083.3 (6179.7)	6884.2 (7084.7)	0.87
Peripheral Blasts %	2.5 (3.4)	2.4 (3.7)	0.85
Bone Marrow Blasts %	6.2 (4.9)	5.3 (4.1)	0.32
Fetal Hemoglobin (HbF) %	25.8 (21.4)	23.7 (24)	0.67
GM-CSF Hypersensitivity			0.59#
Present	54 (77%)	32 (73%)	
Absent	16 (23%)	12 (27%)	

* p-value determined by two sided t-test
#p-value determined by Pearson's chi-square test

FIGURE 1: Overall survival of all patients (n=114)

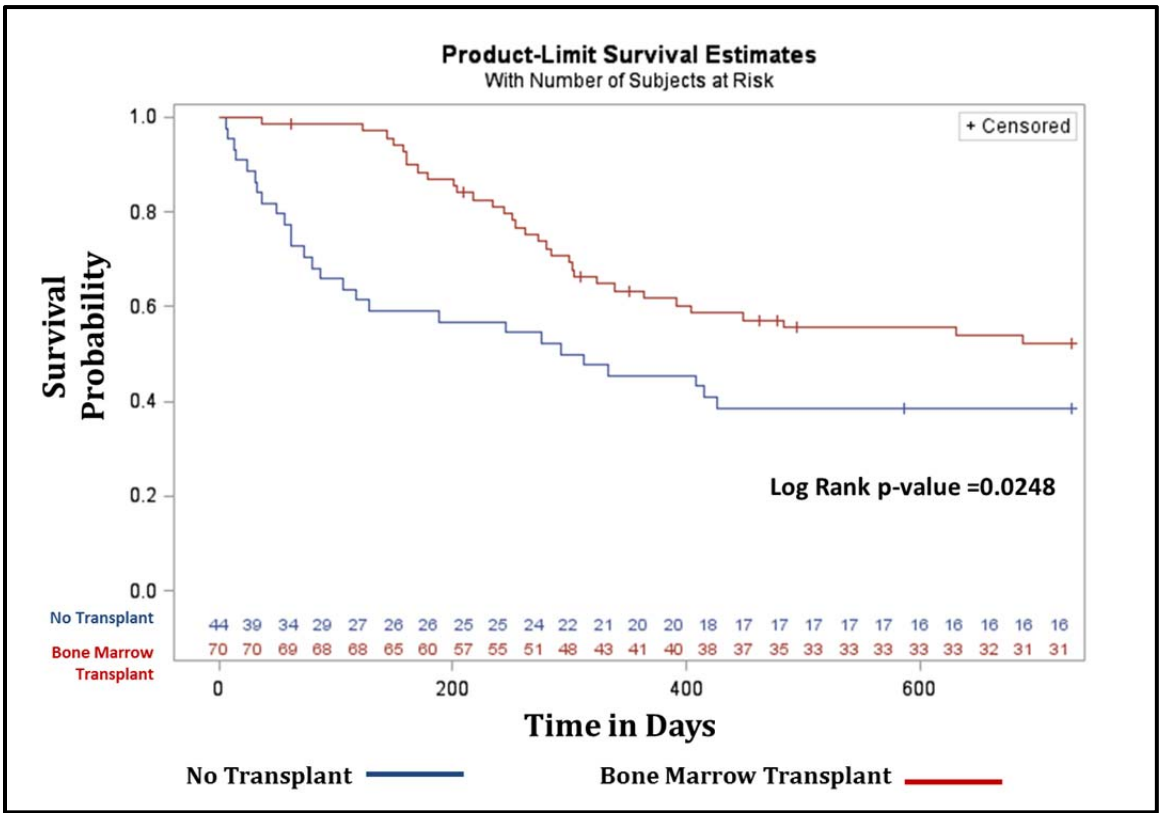


2 year OS = 47% ± 0.05

(95% CL: 37 % - 56%)

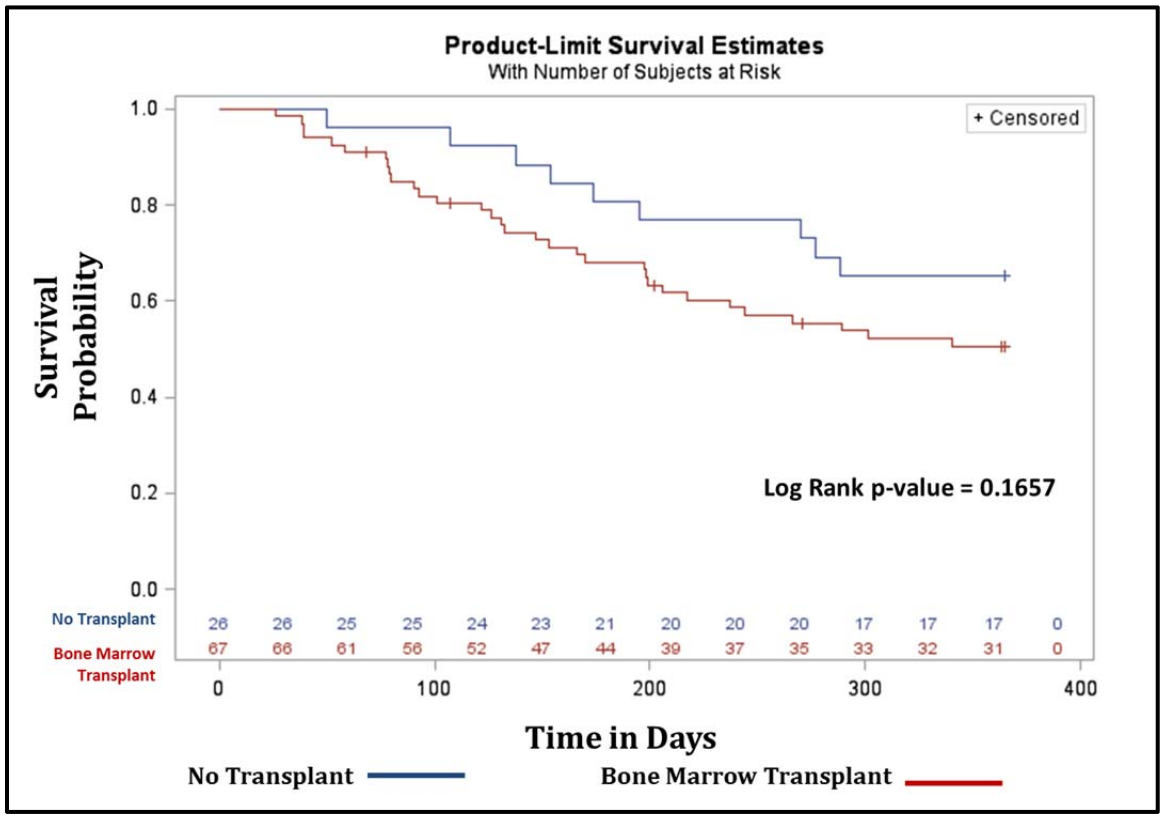
Median Survival time = 449 days

FIGURE 2: Two year overall survival of all patients depending on transplantation status



	Allogeneic HSCT	No HSCT
2 year Overall Survival 95% CL	52% ± 0.06 39 – 63 %	38% ± 0.07 24 – 52 %
Mean Survival (days)	495	252

FIGURE 3: One year overall survival of all patients on NAJP registry depending on transplantation status after accounting for early mortality (within 138 days of presentation)



	Allogeneic HSCT	No HSCT
1 year Overall Survival	50% ± 0.06	65% ± 0.09
95% CL	37 – 62 %	44 – 80 %
Mean Survival (days)	245	251

TABLE 6: Univariate analysis of clinical and laboratory parameters in overall survival (n=114)

Parameter	Hazard Ratio	Hazard Ratio 95% CI	p-value*
Age in months			***0.003***
< 24	Ref		
>= 24	2.16	1.29 – 3.60	
Gender			0.26
Male	Ref		
Female	1.36	0.80 – 2.31	
Lung Disease			***0.006***
Absent	Ref		
Present	2.25	1.26 – 4.01	
Neurofibromatosis			***0.03***
Absent	Ref		
Present	0.33	0.12 – 0.91	
Transplantation			***0.03***
No	Ref		
Yes	0.56	0.33 – 0.94	
White Blood Cell Count			0.69
< 50 K/µl	Ref		
>= 50 K/µl	1.12	0.65 – 1.93	
Platelet count			***0.0003***
<40 K/µl	Ref		
>=40 K/µl	0.39	0.23 – 0.65	
Hemoglobin F			***0.02***
< 40%	Ref		
>= 40%	1.88	1.08 – 3.05	
Monosomy 7			0.55
Absent	Ref		
Present	1.24	0.61 – 2.53	

* p-value < 0.05 (tow-sided) considered significant

TABLE 7A: Interaction between NF1 and transplantation status

Parameter	Hazard Ratio	Hazard Ratio 95% CI	p-value*
No NF1			0.29
No Transplant	Ref		
Transplant	0.74	0.43 – 1.28	
NF1			***0.007***
No Transplant	Ref		
Transplant	0.07	0.009 – 0.49	

* p-value < 0.05 (two-sided) considered significant

TABLE 7B: Interaction between lung disease and transplantation status

Parameter	Hazard Ratio	Hazard Ratio 95% CI	p-value*
Lung Disease Absent			0.39
No Transplant	Ref		
Transplant	0.76	0.41 – 1.42	
Lung Disease Present			***0.004***
No Transplant	Ref		
Transplant	0.21	0.07 – 0.59	

* p-value < 0.05 (two-sided) considered significant

TABLE 8: Multivariate analysis of clinical and laboratory parameters in overall survival

Parameter	Hazard Ratio	Hazard Ratio 95% CI	p-value*
Age in months			***0.03***
< 24	Ref		
>= 24	1.98	1.04 – 3.76	
Lung Disease			***0.001***
Absent	Ref		
Present	4.37	1.77 – 10.8	
No NF1			0.15
No Transplant	Ref		
Transplant	0.59	0.29 – 1.21	
NF1			***0.02***
No Transplant	Ref		
Transplant	0.04	0.002 – 0.62	
Platelet count			***0.01***
<40 K/µl	Ref		
>=40 K/µl	0.46	0.24 – 0.86	
Hemoglobin F			0.36
< 40%	Ref		
>= 40%	1.34	0.71 – 2.56	

* p-value < 0.05 (two-sided) considered significant

FIGURE 4: Two year overall survival of all patients that received hematopoietic stem cell transplantation depending on donor source

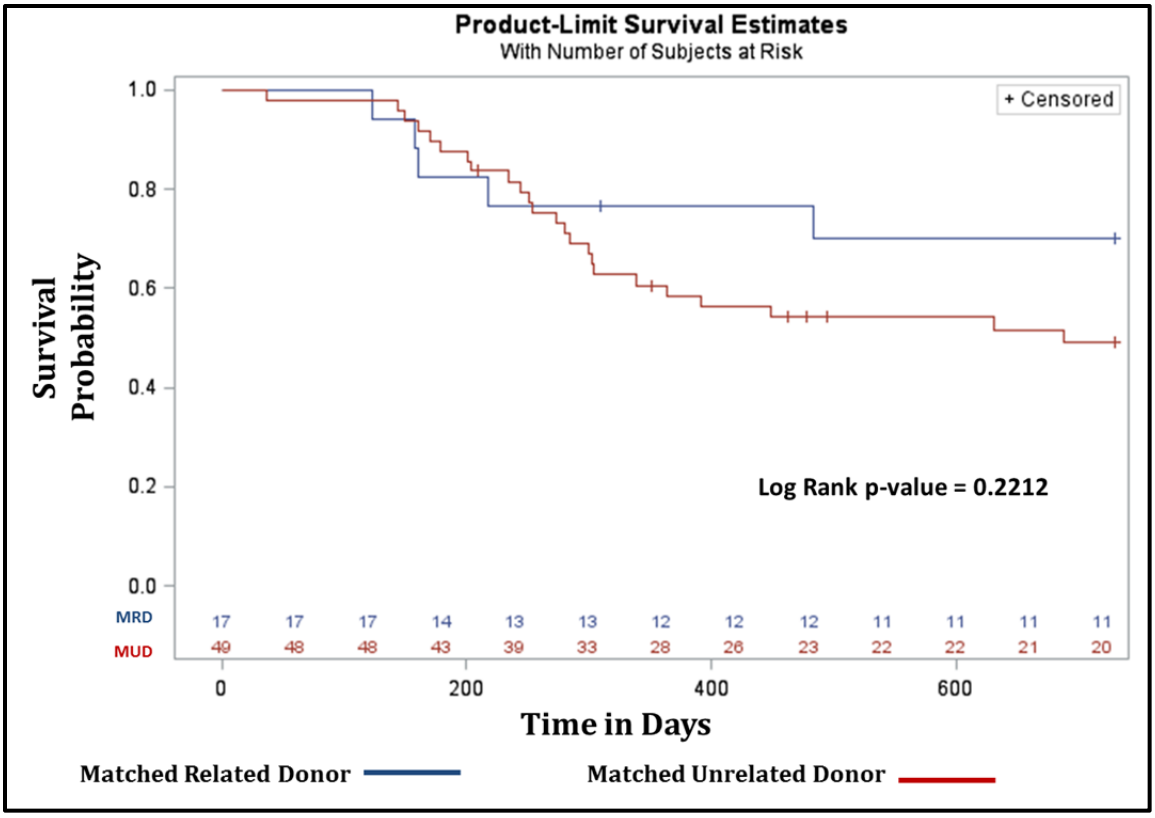


TABLE 9: Univariate analysis of clinical and laboratory parameters in overall survival within patients that underwent hematopoietic stem cell transplantation

Parameter	Hazard Ratio	Hazard Ratio 95% CI	p-value*
Age in months			0.26
< 24	Ref		
>= 24	1.49	0.74 – 2.97	
Gender			0.84
Male	Ref		
Female	0.92	0.45 – 1.92	
Lung Disease			0.80
Absent	Ref		
Present	1.13	0.44 – 2.96	
Neurofibromatosis			***0.02***
Absent	Ref		
Present	0.18	0.04 – 0.77	
White Blood Cell Count			***0.04***
< 50 K/µl	Ref		
>= 50 K/µl	0.43	0.18 – 0.99	
Platelet count			***0.03***
<40 K/µl	Ref		
>=40 K/µl	0.47	0.23 – 0.95	
Hemoglobin F			***0.02***
< 40%	Ref		
>= 40%	2.31	1.12 – 4.74	
Monosomy 7			0.55
Absent	Ref		
Present	0.70	0.21 – 2.30	
Time to Transplant from study enrollment			***0.02***
<120 days	Ref		
>120 days	0.44	0.22 – 0.9	

* p-value < 0.05 (two-sided) considered significant

TABLE 10: Multivariate analysis of clinical and laboratory parameters in overall survival of patients that underwent hematopoietic stem cell transplantation

Parameter	Hazard Ratio	Hazard Ratio 95% CI	p-value*
Neurofibromatosis			***0.02***
Absent	Ref		
Present	0.17	0.04 – 0.76	
White Blood Cell Count			***0.01***
< 50 K/µl	Ref		
>= 50 K/µl	0.31	0.12 – 0.80	
Platelet count			0.45
<40 K/µl	Ref		
>=40 K/µl	0.74	0.33 – 1.63	
Hemoglobin F			0.71
< 40%	Ref		
>= 40%	1.16	0.50 – 2.68	
Time to Transplant from study enrollment			***0.006***
<120 days	Ref		
>120 days	0.34	0.15 – 0.74	

* p-value < 0.05 (two-sided) considered significant