## Title

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## Journal

Biology of Blood and Marrow Transplantation, 23(2)

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## Publication Date

2017-02-01

## DOI

10.1016/j.bbmt.2016.11.001

Peer reviewed

HHS Public Access

Author manuscript

*Biol Blood Marrow Transplant*. Author manuscript; available in PMC 2018 April 30.

Published in final edited form as:

*Biol Blood Marrow Transplant*. 2017 February ; 23(2): 285–292. doi:10.1016/j.bbmt.2016.11.001.

# Clofarabine Plus Busulfan Is an Effective Conditioning Regimen for Allogeneic Hematopoietic Stem Cell Transplantation in Patients with Acute Lymphoblastic Leukemia: Long-term Study Results

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## Abstract

We investigated the long-term safety and disease-control data obtained with intravenous busulfan (Bu) combined with clofarabine (Clo) in patients with acute lymphoblastic leukemia (ALL) undergoing allogeneic hematopoietic stem cell transplantation (SCT). 107 patients with median age 38 years (range 19–64 years) received a matched sibling (n=52), or matched unrelated donor transplant (n=55) for ALL in first complete remission (n=62), second complete remission (n=28), or more advanced disease (n=17). Nearly half of the patients had high-risk cytogenetic profiles as defined by the presence of t(9;22) (n=34), t(4;11) (n=4), or complex cytogenetics (n=7). Clo 40 mg/m$^2$ was given once daily, each dose followed by pharmacokinetically-dosed Bu infused over three hours daily for 4 days, followed by hematopoietic cell infusion after two rest days. The Bu dose was based upon the drug clearance determined by a test Bu dose, 32 mg/m$^2$, given 48 hours prior to the high dose regimen. The target daily area under the curve (AUC) was 5,500 microMol-min for patients less than 60 years of age and 4000 microMol-min for patients older than 59 years of age. With a median follow-up of 3.3 years among surviving patients (1–5.8 years), the 2-year progression-free survival (PFS) rates for patients transplanted in CR1, CR2, or more advanced disease were 62%, 34%, and 35%, respectively. The regimen was well tolerated with non-relapse mortality (NRM) rates of 10% and 31% and at 100 days and 2 years, respectively. The incidence of grades II–IV and III–IV acute graft versus host disease (GVHD) were 35% and 10%, respectively; 18% patients developed extensive chronic GVHD. The 2-year overall survival (OS) rates for patients transplanted in CR1, CR2, or more advanced disease were 70%, 57%, and 35%, respectively. Among 11 patients older than 59 years treated with reduced dose Bu in CR1 (n=7) or

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CR2 (n=4), 4 remain alive and disease-free with a median follow up of 2.6 years (2–4.7 years). Only the presence of MRD at time of transplant was associated with significantly worse PFS and OS on multivariate analysis. The Clo-Bu combination provides effective disease control while maintaining a favorable safety profile. Overall survival and NRM rates compare favorably with traditional myeloablative TBI-based conditioning regimens.

**Keywords**

Acute lymphoblastic leukemia; Allogeneic hematopoietic stem cell transplantation; Transplant conditioning regimens

## INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (SCT) is an effective, potentially curative treatment option for adults with acute lymphoblastic leukemia (ALL), but may be associated with significant morbidity. Non-relapse mortality (NRM) rates between 20% and 45% have been reported for patients receiving a standard, total body irradiation (TBI)-based, myeloablative preparative regimen(1–3). In efforts to improve NRM, reduced intensity conditioning (RIC) regimens have been investigated, with improvements in acute NRM, but resulting in increased risk of relapse, especially for patients beyond first complete remission(4–6). In attempts to limit the toxicities associated with TBI-based, myeloablative regimens, we replaced radiation with a chemotherapy-only, double alkylator regimen consisting of intravenous (i.v.), pharmacokinetically (PK)-dosed busulfan (Bu), and melphalan (Mel)(7). We showed comparable disease control to radiation-based regimens, while decreasing acute regimen-related toxicities, but long-term NRM, primarily related to graft versus host disease (GVHD), remained substantial (55% at 2 years for patients older than 40 years(7).

We, and others, have shown good disease control and decreased toxicity when a second alykylator (Melphalan or cyclophosphamide) was replaced by the nucleoside analogue (NA) fludarabine (Flu) in the transplant conditioning regimen in children and adults with leukemia(8–15). We further hypothesized that replacing the NA Flu with the second generation NA clofarabine (Clo), which has single agent activity in refractory relapsed ALL(16, 17), would provide particularly good disease control in patients with ALL. The i.v. Clo-Bu combination was used for patients undergoing allogeneic SCT for ALL, and the early published results in 51 patients were encouraging, showing a low 100-day TRM of 6%, and a projected 1-year disease-free survival rate for patients transplanted in CR1 of 64% (18).

We have completed the trial and accrued 107 patients. This report presents the long-term follow-up results of this trial.

## PATIENTS AND METHODS

### Patient eligibility and study treatment

This was a prospective, phase II single arm study investigating the combination of Bu and Clo in patients with ALL. Enrollment began in October 2009 and completed in July 2015, and we are reporting the outcomes for adult patients treated consecutively during this time period. Patient eligibility and study methods were detailed in our previous publication (18). Briefly, patients were between 18 and 65 years of age, with an available human leukocyte antigen (HLA) matched related donor or 8/8 unrelated donor undergoing first allogeneic SCT. Additional eligibility criteria included a Zubrod performance status of 0 or 1, adequate organ function, and absence of active infection. Patients with active CNS disease were excluded.

The transplant conditioning regimen consisted of Clo 40 mg/m$^2$ infused over one hour followed by pharmacokinetically-dosed Bu infused over 3 hours once daily for 4 days followed by hematopoietic cell infusion after 2 rest days. The therapeutic dose was determined by the drug clearance determined from a pharmacokinetic test dose of IV Bu, 32 mg/m$^2$ infused over 45 minutes two days before the first therapeutic Bu dose. The therapeutic i.v. Bu dose targeted an average daily AUC of 5,500 microMol-min for patients less than 60 years of age or 4,000 microMol-min for patients older than 59 years. Collection of blood and methods for PK analyses were performed as previously reported (7, 19).

Phenytoin 600 mg orally was used during and one day after completion of i.v. Bu therapy, starting the evening before the first dose (20). Graft versus host disease (GVHD) prophylaxis consisted of a combination of tacrolimus and mini-dose methotrexate. Patients who received unrelated donor products additionally received rabbit anti-thymocyte globulin for a total 4 mg/kg infused over three days beginning three days prior to SCT. Institutional transplant guidelines for antimicrobial, antifungal, and antiviral prophylaxis were followed as previously reported(21). Patients with a prior history of CNS involvement received craniospinal XRT immediately prior to transplant conditioning or post transplant intrathecal pre-emptive therapy, as feasible; patients without history of CNS involvement of leukemia did not receive any CNS therapy beyond completing their recommended primary treatment(22). Finally, patients whose leukemia was positive for the Philadelphia chromosome (Ph) were started on maintenance therapy with tyrosine kinase inhibitor (TKI) upon normalization of blood counts following SCT to continue for up to 5 years.

### Definitions and clinical outcome variables

The disease stage at transplantation was defined using established criteria based on bone marrow morphology. Criteria for complete response included normal cytogenetics, the absence of circulating blasts, less than 5% marrow blasts, and normalization of complete blood counts (CBC). Response was documented as best response occurring by day 30 following SCT. Standard morphologic criteria were used to diagnose recurrent disease. Molecular response measured by quantitative polymerase chain reaction (PCR) analysis for BCR-ABL rearrangement was obtained when possible. Multiparameter flow cytometry, with a sensitivity of 0.01%, was used to further assay for minimal residual disease (MRD). MRD

and PCR analyses were not used to assign disease stage or document relapse. Hematologic recovery was defined as the first day the patient had an absolute neutrophil count of $0.5 \times 10^9$/L or higher for 3 consecutive days. Platelet recovery was defined as occurring on the first of 7 consecutive days with a platelet count of $20 \times 10^9$/L or higher without transfusion support. Failure to engraft by day +30 was considered primary engraftment failure. Hematopoietic chimerism was evaluated in peripheral blood (with myeloid and T- lineage sorting) by restriction fragment length polymorphisms using PCR methods to determine donor engraftment. Mixed chimerism was defined as the presence of any detectable (≥1%) recipient DNA in addition to donor-derived DNA in myeloid or T-lineage cells.

Overall survival (OS) was estimated from the time of SCT until death from any cause, and patients still alive at last follow-up were administratively censored. Progression-free survival (PFS) was estimated from SCT until the date of relapse or death from any cause. Patients alive and disease-free at last follow-up were censored. Non-relapse mortality (NRM) was defined as death from any cause other than disease progression or relapse. Acute and chronic GVHD were graded based on standard criteria (23–24).

### Statistical methods

The trial completed accrual in July 2015, and this is the final report of the 107 adult patients treated with matched related or unrelated donors on this study. Patients undergoing transplant with syngeneic donors were excluded from this analysis. The primary outcomes for this single-arm trial were safety and overall survival. Bayesian early stopping rules based on the observed rates of these 2 outcomes, as compared to historical data, were implemented (25). The methods of Gooley, Fine, and Gray were used to compare the cumulative incidence of NRM vs. the competing risk of relapse, separately by age group (<40 vs ≥ 40) and by disease stage. Overall survival and PFS were analyzed using the Kaplan-Meier estimator (26), and univariate and multivariate Cox proportional hazards models. The factors age (≥40 vs. <40 years), cytogenetic risk (high vs. intermediate), immune phenotype (T- vs. B-lineage), donor relation (MUD vs. sib), and presence of MRD in patients with morphologic remission (CR1, CR2, or CR3) were investigated in univariate and multivariate analyses for OS and PFS. Survival curves were generated and the log-rank test was used to compare groups. Hazard ratios and 95% confidence intervals were estimated. With respect to cytogenetic risk group, only the subset of patients in high and intermediate groups were compared. Descriptive statistics were used to summarize patient demographics. The cumulative incidence of GVHD and relapse were calculated with death as a competing risk.

## RESULTS

### Patient and treatment characteristics

Patient demographics and baseline disease characteristics are listed in Table 1. One hundred and seven patients with median age of 37 years (range 19–64 years), with 11 patients older than 59 years, were evaluated on this study. The median time from diagnosis to transplant was 9.2 months (range 2.3–118.2 months). The majority of patients had high risk features at diagnosis with 32% (n=34) having an elevated WBC count and 43% (n=45) having high-risk cytogenetics, defined as presence of the t(9;22), t(4;11), or complex karyotype, defined as ≥5

cytogenetic abnormalities. Additionally, 8 patients presented with CNS involvement and 14 patients presented with lymph node involvement at time of diagnosis. At time of transplant, 58% (n=62) were in CR1, 26% (n=28) were in CR2, and 16% of patients had more advanced disease (CR3, n=2; incomplete recovery of counts, n=9; blasts >5%, n=6). Among the patients in CR, 30% had MRD present (n=28). Among 34 patients with the t(9;22) translocation, 15 patients (44%) were started on TKI maintenance therapy at a median of 2.2 months (range 1.3–14.4) following SCT. The majority of patients received dasatinib (n=10) at a median dose of 100 mg; four patients received imatinib and one patient received ponatinib. All of the Ph+ patients received TKI therapy with their chemotherapy prior to being referred for transplant.

## Graft content and engraftment

Stem cell graft characteristics and hematopoietic recovery data are summarized in Table 2. Approximately half of the patients received a matched related donor transplant (49%) and half matched unrelated donor SCT (51%). The source of stem cells was peripheral blood for the majority of patients. The median days to neutrophil and platelet recovery were 11 (range 10–25 days) and 14 (range 8–109 days), respectively. Two patients died before day 30 following transplant due to infection and ensuing end-organ failure, leaving 105 patients evaluable for chimerism assessment. Full donor chimerism by day 30 was achieved in 70% of patients; 92% of patients eventually achieved full donor chimerism defined as 100% donor T-cells and myeloid cells. Eight patients remained mixed chimera in their T-cell component, ranging from <50% to 99% donor T cells, and 4 of these patients eventually relapsed. One of these patients remained severely cytopenic and required a stem cell boost. Of note, among the 11 patients 60 years and older, who received reduced dose Bu, 10 attained full donor chimerism at median of 44 days; 1 patient remained mixed chimera in the T cell component at 84% and had disease progression at 2 months post SCT.

## Response, relapse, and progression-free survival

Among 105 patients evaluable for response (2 patients not evaluable due to early death), 13 patients cleared their disease, 91 patients maintained their remission, and one patient remained with active disease. Thirty-eight patients progressed at a median of 5.6 months (range 1.9–50.2) following SCT, with a 2-year progression or death probability of 38% for patients transplanted in CR1, 66% for patients transplanted in CR2, and 65% for patients transplanted with more advanced disease. Three patients had isolated CNS relapse, with two of these patients having a prior history of CNS involvement. One patient relapsed in the CNS nearly two years after SCT, was re-induced into remission with XRT and intrathecal therapy followed by consolidation with a second transplant and remains in remission. One patient relapsed in the CNS 2.7 months post SCT, was re-induced into remission with XRT and intrathecal therapy, and died 4 years later due to GVHD complications. The last patient relapsed in the CNS nearly one year post SCT, was re-induced into remission with intrathecal chemotherapy, consolidated with systemic chemotherapy, and died 3 years later from infection.

The 2-year PFS probability for the entire group was 51%. As expected disease stage had an impact on PFS, with 2-year PFS probability of 62%, 34% and 35% for patients transplanted

in CR1, CR2, and with more advanced disease, respectively (Figure 1A). Only the presence of MRD at time of transplant was significantly associated with lower PFS, with a hazard ratio of 2.15, p=0.02. (Figure 2A).

### Overall survival

With a median follow-up of 3.3 years among surviving patients (range 1–5.8 years), the 2-year OS rates for patients transplanted in CR1, CR2, or more advanced disease were 70%, 57%, and 35%, respectively (Figure 1B). The same factors investigated for PFS were evaluated for their impact on OS, and again, only the presence of MRD at time of transplant was highly associated with worse survival in mulitvariate analysis with a hazard ratio of 2.54, p=0.01 (Figure 1B).

### Toxicity, NRM, and GVHD

Regimen-related toxicities assessed by the NCI CTCAE version 3 are detailed in Table 3. The most commonly observed toxicities involved the GI tract, and were mild grades 1 or 2 nausea (100%) and/or diarrhea (48%), and grade II mucositis (65%). Reversible elevation in liver enzymes was commonly noted (85%), and reversible elevation in creatinine was noted in 9% of patients. Six patients developed sinusoidal obstructive syndrome or veno-occlusive disease (VOD), with median time to onset 25.5 days post SCT (range 7–37 days). The majority of these patients had a prolonged period of treatment prior to transplant, median time to transplant 17.5 months (range 3.7–118.2 months), with one fatality due to irreversible VOD. Non-relapse mortality rates at 100 days and 2 years were 6% and 18%, respectively. There was no regimen-related death within the first 100 days of transplant in the 11 patients 60-years and older. However, there was a trend for significantly higher NRM in patients greater than 40 years-old (p=0.07, Figure 3) on univariate analysis. There were 44 deaths: infection (n=7), GVHD (n=10), VOD (n=1), metastatic colon cancer at 10.4 months post SCT (n=1), and relapse (n=25).

The cumulative incidence of grades II–IV and III–IV acute GVHD were 35% and 10%, respectively; there was no statistically significant difference between patients receiving grafts from matched related- and unrelated donors. The cumulative incidence of chronic GVHD was 29%, with 18% experiencing extensive GVHD. Again, no difference was noted between allotypes (Table 4).

## DISCUSSION

The availability of an effective, TBI-free pre-transplant conditioning regimen for ALL patients is necessary to avoid the long-term toxicities documented with the use of radiation, in particular those including impaired growth and cognitive function (27, 28), the increased incidence without plateau in secondary malignancies(29, 30), and the increased incidence of a cardiometabolic trait leading to diabetes and to accelerated atherosclerotic cardiovascular disease (31, 32). The combination of busulfan and cyclophosphamide (Bu-Cy) has historically been an alternative to TBI. We previously explored Bu-Mel as an alternative to BuCy2 (Bu 16 mg/kg and Cy 120 mg/kg) in lymphoid malignancies. However, the double alkylator regimen had similarly high NRM and GVHD rates compared with standard TBI-

based regimens (7). The replacement of Cy with the NA Flu has shown good efficacy with reduced toxicity. We demonstrated excellent results with the Flu-Bu combination in patients undergoing transplant for AML(8), and our results were corroborated in prospective, multicenter, randomized studies of Flu-Bu vs. Bu- Cy(33)(13) in AML, in which the patients treated with Flu-Bu had a significantly lower rate of NRM rate compared with Bu-Cy2, with similar overall and disease-free survival in the two groups. The Flu-Bu combination has also been tested in ALL. Santarone and colleagues demonstrated encouraging results in patients with ALL treated with PK- guided i.v. Flu-Bu with an 2-year OS rate of 63% for patients transplanted in first complete remission (CR1)(12); importantly long-term NRM was only 18% at 2 years(12). In an updated report on their series, with 65 patients transplanted with Flu-Bu in CR1 for ALL, the 2-year OS was 65%, and NRM remained low at 14%(14). Based on the single agent activity of clofarabine in refractory or relapsed ALL, we hypothesized that a combination of clofarabine and i.v. busulfan would be particularly effective in in this setting. The in vitro data obtained in our cell line models of ALL supported this, provided that attention was paid to optimizing the sequence and timing of the two agents(34–36) Indeed our early reported results were encouraging(18). Now, with a median follow-up time of 3.3 years among survivors, the 2-year OS and NRM rates for patients transplanted in CR 1 are 68% and 18%, respectively. These results compare favorably with reports of adult patients treated with myelo-ablative, TBI-based regimens in CR1, with reported survival rates of approximately 45%–55% and NRM rates of 20–35% (2, 37,38).

The administration of PK-guided busulfan allows for accurate dose delivery within a tighter range in systemic drug exposure (7), and contributed to the low toxicity profile of the regimen. Transient transaminitis was common, as was also reported by Magenau and colleagues(39). Six patients developed VOD, with one patient having fatal VOD, and this was also comparable to the Flu-Bu experience reported by others (8, 11, 14). All of these patients had extensive therapy prior to HCT with median time from diagnosis to HCT of 17.5 months. Interestingly, none of the 13 refractory patients who were treated with inotuzumab ozogamicin developed VOD. Inotuzumab is a CD22 monoclonal antibody conjugated to the toxin calecheamicin that is very effective in relapsed ALL(40) but associated with hepatic toxicity, and has been noted to be associated with increased VOD rates across our various transplant regimens(41). The 100-day NRM rate of 6% was comparable to what was reported for Flu-Bu(14), and markedly lower than the 19% reported by Doney and colleagues using TBI-based regimens(42).

The cumulative incidence of acute GVHD, grades 2–4 35% and grades 3–4 10% (Table 4), is comparable to reports from radiation-based(37) and non-radiation based regimens(12), and is substantially lower than that observed in our prior combination of Bu with melphalan(43). Reducing the incidence of GVHD, especially in older patients, may significantly benefit the risk of late NRM, since 7 (14%) of our patients died of GVHD-related complications. Notably, the 2-year NRM rate ranged from 12% to 27% based on age greater than or less than 40 years (Figure 2), and again NRM compares favorably to data reported using myelo-ablative TBI-based regimens with 2-year NRM 20–35% based on age (35 years)(2). Importantly, the generally low NRM rate for all patients translates into an

equal survival rate for younger vs. older patients (Fig 3). This observation is critical to studies that don't show benefit to transplant versus chemotherapy due to higher NRM(2, 44).

We(8, 20, 45), and others(10–12) have shown the optimal daily Bu AUC dose to be within the window of 3800 microMol-min (when used with low-dose TBI) to 6080 microMol-min when combined with NA. Indeed, in the study by Kunter et al, patients with daily Bu AUC targeting 5300 microMol-min had a better OS compared to those targeting >6000 microMol-min (p=.04)(14). Thus, we targeted a (median) daily AUC of 5500 microMol-min for our younger patients for maximum disease control, and 4000 microMol-min for our patients greater than 59 years in efforts to minimize toxicity. We have shown reliable engraftment with this lower dose Bu in combination with fludarabine in patients with myeloproliferative disorders receiving allogeneic SCT(46). The rate of 100% engraftment at day 30 was similar between the low and high Bu AUC dose. Five of the 11 older patients remain alive and disease-free, with 3 patients dying from disease progression and one from GVHD; one patient had pre-existing cardiac disease and died from cardiac failure and one died from pre-existing metastatic colon cancer.

Despite a high CR rate following SCT, relapse remains the major cause of treatment failure. This is strikingly evident when patients are evaluated based on the presence of MRD at time of SCT; patients with positive MRD have significantly lower PFS and OS (Figure 2). Thus, the regimen needs to be modified to improve disease control. We have published data in AML that the addition of a second NA results in added synergy and reduced relapse(47). Furthermore, we have published data that the addition of biologic modifiers, such as histone deacetylase inhibitors or DNA methyltransferase inhibitors, add synergy to cytotoxic chemotherapy combinations in lymphoma and leukemia cells lines(36) (48). Importantly, biologic modifiers have non-overlapping toxicities with cytotoxic agents, and thus would not be expected to increase regimen-related toxicity. Therefore, our current trial for advanced ALL patients is a combination of Bu-Clo-Flu + the histone deacetylase inhibitor vorinostat, and early results are encouraging.

In conclusion, the Clo-Bu preparative regimen produces good disease outcomes in patients with high-risk disease and/or older age, and provides patients with an alternative to TBI-based regimens. Importantly, the regimen's excellent toxicity profile provides a platform to study novel additional approaches to improve disease control which remains the main reason for treatment failure.

## Acknowledgments

Dr. Borje Andersson is a consultant to Otsuka America Pharmaceuticals, Inc. (OAPI).

Funding source: This work supported in part by the Cancer Center Support Grant (NCI Grant P30 CA016672).

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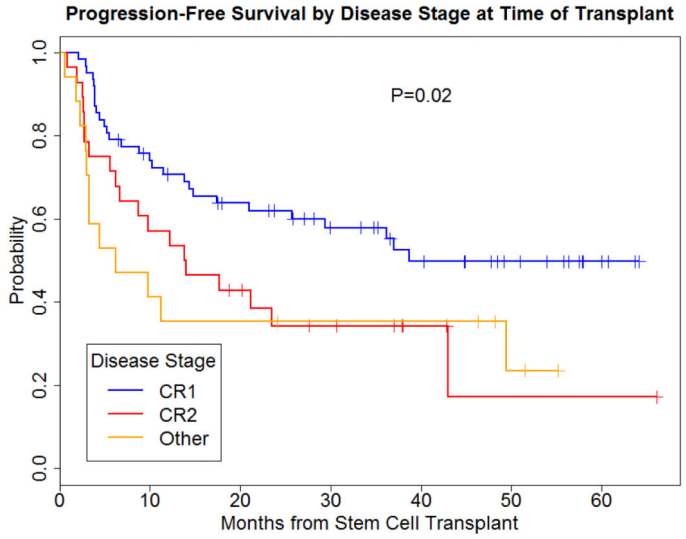
## Highlights

- Long-term outcomes using a radiation-free SCT conditioning regimen for ALL.
- Study results are comparable to radiation-containing regimens.
- Myeloablative clofarabine plus busulfan is an effective SCT regimen for adult ALL.

A.




B.

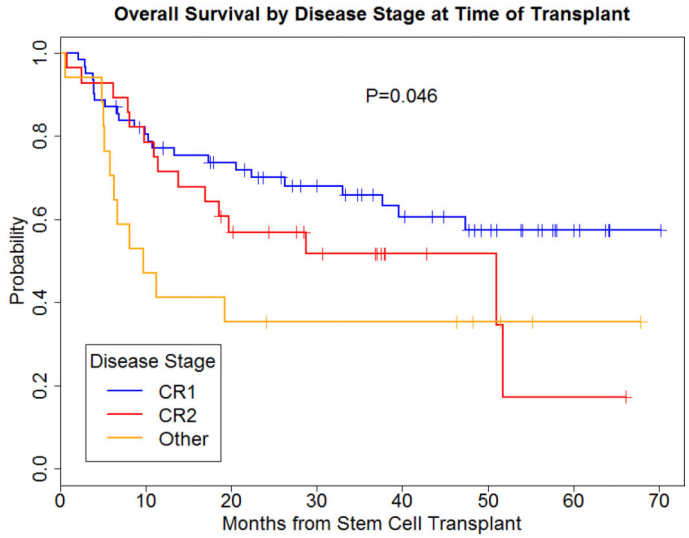
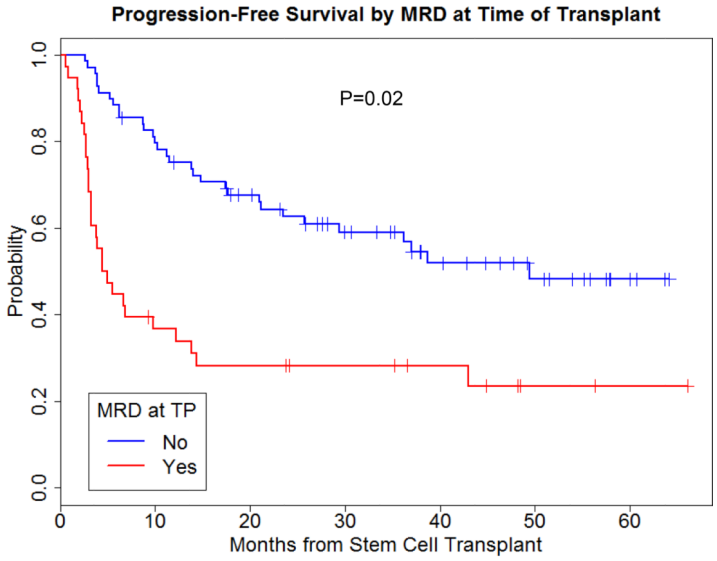



**Figure 1.**
Progression-free and overall survival by disease stage at time of transplant.

A.

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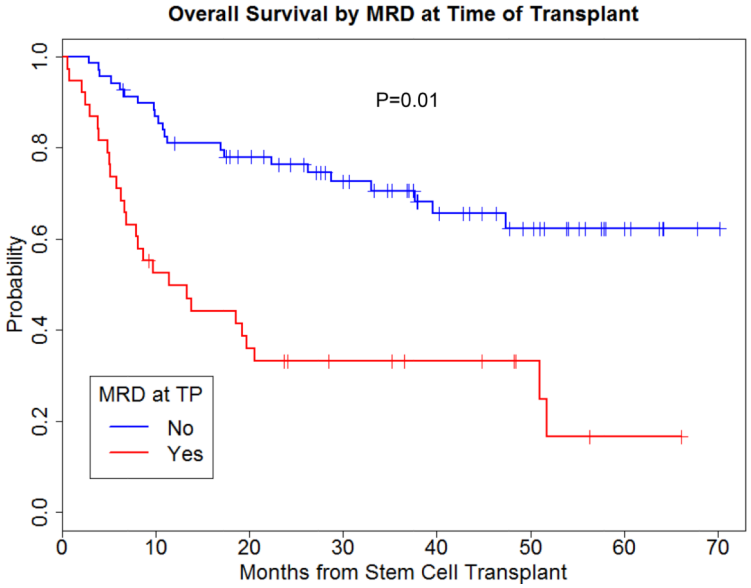


**Figure 2.**
Progression-free and overall survival by MRD at time of transplant.

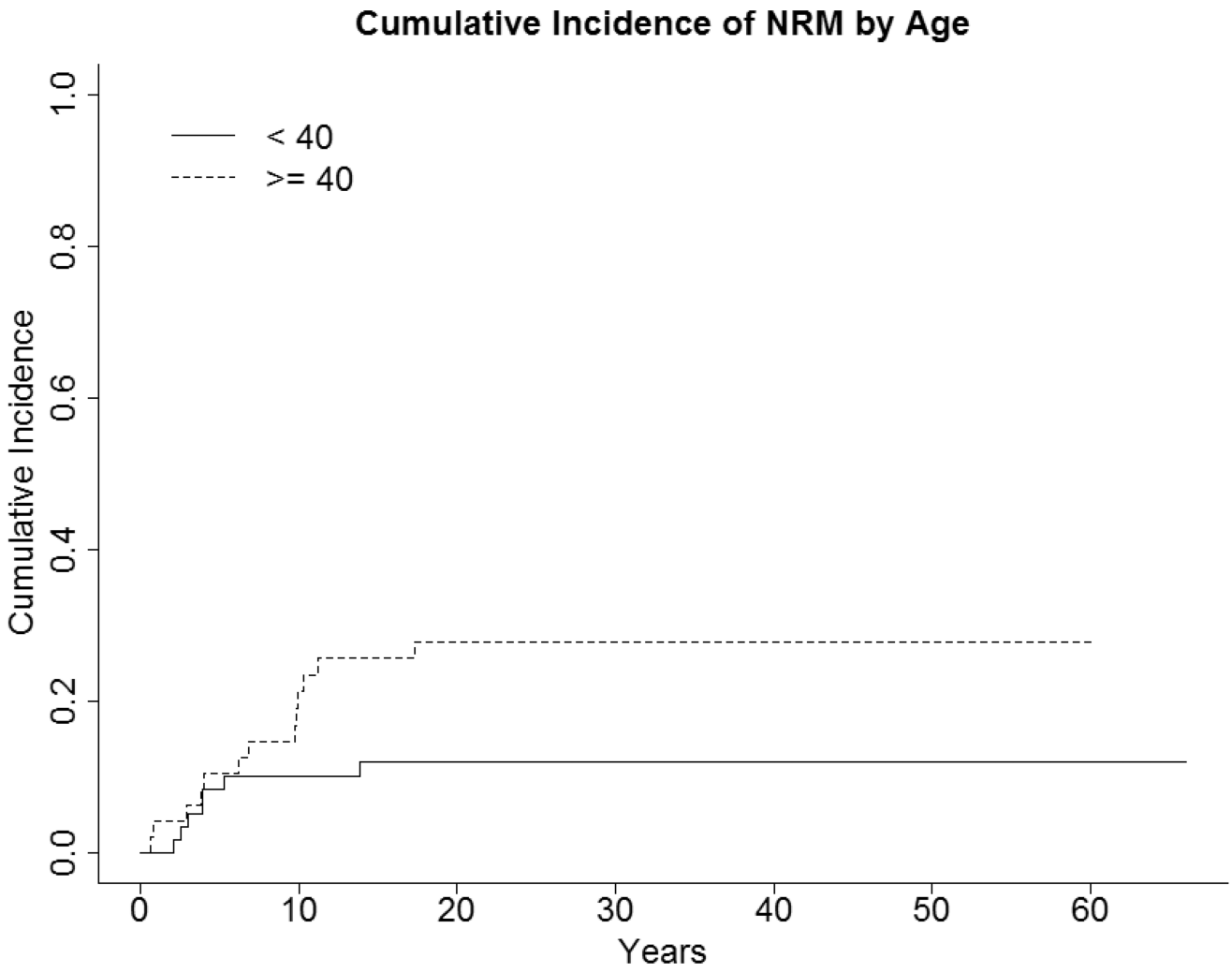


**Figure 3.**
Non-relapse mortality by age at time of transplant

**Table 1**

Patient characteristics at diagnosis, N=107

| Patient Characteristic | No. |
|---|---|
| Median age (range) | 37 (19–64) years |
| Greater than 59 years | 11 |
| Sex, male/female | 64/43 |
| Disease lineage (%) | |
| B-lineage | 91 (85) |
| T-lineage | 16 (15) |
| WBC count at diagnosis (%) | |
| <30,000/μL | 58 (54) |
| 30,000/μL–100,000/μL | 13 (12) |
| >100,000/μL | 21 (20) |
| Unknown | 15 (14) |
| Cytogenetics at diagnosis (%) | |
| Diploid | 27 (25) |
| Other | 27 (25) |
| 9;22 | 34 (32) |
| 4;11 | 4 (4) |
| Complex | 7 (7) |
| Unknown | 8 (7) |
| Time to achieve CR (%) | |
| Within 4 weeks | 43 (40) |
| >4 weeks | 59 (55) |
| Unknown | 5 (5) |
| Disease status at transplant (%) | |
| CR1 | 62 (58) |
| CR2 | 28 (26) |
| CR3 | 2 (2) |
| Incomplete recovery of counts | 9 (8) |
| >5% blasts | 6 (6) |
| CR, n=92 | |
| MRD present (%) | 28 (30) |
| MRD absent (%) | 64 (70) |
| Med chemo. regimens pre-SCT (range) | 1 (1–5) |
| Median months to SCT (range) | 9.2 (2.3–118.2) |

**Table 2**

Graft characteristics at transplant, and hematopoietic recovery

| Characteristic | No. |
|---|---|
| Donor type (%) | |
| Matched related | 52 (49) |
| Matched unrelated | 55 (51) |
| Stem cell source (%) | |
| Bone marrow | 34(32) |
| Peripheral blood | 73 (68) |
| Median days to ANC recovery (range)[1] | 11 (10–25) |
| Median days to platelet recovery (range)[1, 2] | 13 (8–109) |

[1] Count recovery defined in text of manuscript.

[2] Eight patients did not have platelet recovery.

**Table 3**

Regimen related toxicity in 107 patients

| Toxicity, n | Gr I | Gr II | Gr III | Gr IV | Gr V |
|---|---|---|---|---|---|
| Liver | | | | | |
| Bilirubin elevation | 7 | 22 | 9 | 0 | 0 |
| Transaminitis | 36 | 23 | 32 | 0 | 0 |
| VOD | 0 | 0 | 5 | 0 | 1 |
| Gastrointestinal tract | | | | | |
| Diarrhea | 38 | 13 | 5 | 0 | 0 |
| Nausea | 6 | 61 | 1 | 0 | 0 |
| Mucositis | 9 | 70 | 26 | 0 | 0 |
| Urinary tract/kidney | | | | | |
| Creatinine elevation | 5 | 5 | 1 | 0 | 0 |
| Skin | | | | | |
| Rash | 11 | 7 | 3 | 1 | 0 |
| Neurologic | | | | | |
| Headache | 4 | 2 | 0 | 0 | 0 |

**Table 4**

Cumulative incidence of acute and chronic GVHD

| | No. (%) |
|---|---|
| Acute GVHD | |
| Grades II–IV | 19/54 (35) |
| Grades III–IV | 5/50 (10) |
| Chronic | |
| Limited and/or extensive | 8/28 (29) |
| Extensive | 4/22 (18) |