Identifying Prognostic Factors in Acute Leukemia Patients: Survival Analysis of Bone Marrow Transplant Outcomes

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A B S T R A C T

Purpose: Bone marrow transplantation is a critical treatment for acute leukemia, a blood cancer, but patient prognosis varies based on multiple factors. This study aims to analyze survival outcomes following transplantation and identify baseline characteristics and post-transplant events associated with disease-free survival and relapse risk.
Methods: Using data from a multicenter study of 137 patients, we applied survival analysis techniques, including Kaplan-Meier estimation and Cox proportional hazards models, to examine time-invariant and time-varying associations between patient/donor characteristics, stage of initial disease, acute graft-versus-host disease (aGVHD) and platelet recovery with death and cancer relapse.
Results: The median disease-free survival time was 481, 95% CI: [363, 748] days. Leukemia disease classification, recruitment center and development of aGVHD had significant effect on the disease-free survival. We did not find significant impact of aGVHD or platelet recovery on relapse-free survival, when treating death as a competing event.
Discussion: Our findings provide insights into key prognostic factors affecting survival after transplantation. We assess the potential protective effect of aGVHD, the role of CMV status, and the impact of methotrexate prophylaxis. These results contribute to refining risk stratification and informing clinical decision-making for leukemia patients undergoing transplantation.

Leukemia is a cancer of the body's blood forming tissue, including the bone marrow and the lymphatic system. In acute leukemia, immature blood cells acquire mutations and cannot carry out their normal function, multiplying rapidly and crowding out healthy blood cells in the bone marrow. Fewer healthy white blood cells, red blood cells, and platelets cause the signs and symptoms of leukemia. Scientists attribute its development to a combination of genetic and environmental factors.

Bone marrow transplants are a standard treatment for acute leukemia. Prognosis for recovery may depend on risk factors known at the time of transplantation, such as patient and/or donor age and sex, stage of initial disease, and time from diagnosis to transplantation. The ultimate prognosis may change as the patient's post-transplantation experience unfolds, with the occurrence of events at random times during the recovery process, including the development of acute graft-versus-host disease (aGVHD) and the return of platelet counts to a normal level. Transplantation can be considered a failure when a patient's leukemia returns (relapse) or they die while in remission.

Study design and data source

A multicenter study was conducted to evaluate whether patient and donor characteristics as well as unfolding clinical events are predictive of death in patients receiving allogeneic marrow transplantation. Enrolled patients were prepared with a radiation-free conditioning regimen consisting of a combination of oral Busulfan and intravenous cyclophosphamide. Enrollment took place at four different hospitals in the United States (Philadelphia, PA and Columbus, OH) and Australia (Sydney and Melbourne).

Study population

Patients were enrolled between March 1, 1984, and June 30, 1989, and were followed until death or end of the study. All patients underwent radiation-free conditioning with a regimen of oral busulfan and intravenous cyclophosphamide before their allogeneic marrow transplantation. Unlike radiation-based conditioning, which destroys cancerous marrow but is associated with significant long-term toxicities, particularly in pediatric and young adult patients, chemotherapy-only regimens reduce the risk of secondary malignancies, endocrine dysfunction, and organ damage, while still effectively preparing the body for transplant and recovery.

Classification of disease. Acute leukemia is classified using the French American British (FAB) system and broader disease groupings. The available FAB classification is a binary variable distinguishing acute myelocytic leukemia (AML) grade 4/5 and otherwise. Additionally, a simplified disease grouping categorizes cases into acute lymphoblastic leukemia (ALL), low-risk acute myelocytic leukemia (AML), and high-risk AML. Identifying AML is particularly important because, unlike many other cancers, AML does not form solid tumors, which are typically used to assess cancer stage. Instead, AML classification and subtype play a crucial role in guiding treatment decisions.

Health demographics and initial intervention. Patient and donor demographics include age at transplantation and sex. Wait time from diagnosis to transplantation, measured in days, may reflect disease severity and overall patient health, as older or critically ill patients may be weaker withstanding intensive chemotherapy or allogenic hematopoietic stem cell transplantation (allo-HSCT). Cytomegalovirus (CMV) immune status for both patient and donor were recorded at baseline. CMV is a common virus that remains dormant in the body after initial infection but can reactivate if the immune system is compromised. Research suggests CMV infection may alter immune function and negatively impact long-term health outcomes.[1] However, the significance of the donor's CMV status remains debated. Some studies propose that CMV reactivation post-transplant may lower the risk of leukemia relapses by stimulating protective T-cells that contribute to a graft-versus-leukemia (GVL) effect.[2] To prevent aGVHD and its associated complications, some patients received prophylactic methotrexate. GVHD, while a potentially serious complication, has also been linked to anti-leukemic effects, reducing relapse risk through an immune-mediated response.[3]

Subsequent clinical events. During recovery, key clinical milestones include the onset of aGVHD and the return of normal platelet counts. Available in the data is the times, in days, between transplantation to these events, if they occur. Additionally, the time from transplantation to leukemia relapse, if applicable, is recorded. The ultimate terminating outcome indicated in the data is mortality.

Methods

Statistical analysis

To estimate the disease-free survival time for patients enrolled in this study we produced a Kaplan-Meier survival curve and reported the median survival time as well as survival probability at 100 days, 1-year and 3-years after enrollment. Disease-free survival was defined as survival without leukemia relapses. Confidence interval (95%) for the survival function was constructed using the complementary log-log transformation method.

We used descriptive statistics to summarize the baseline measurements in the total sample and across different disease groups or different FAB classifications, with counts and percentages reported for discrete variables and median, interquartile range (IQR), and range reported for continuous variables. To test if any of these baseline variables are associated with differences in disease-free survival, we fit a Cox proportional hazards (PH) model including all baseline covariates to examine the hazard ratios for each variable, while accounting for all the other baseline variables. In this model, we were not able to adjust for prophylactic use of methotrexate due to some recruitment centers not having people who reported prophylactic use of methotrexate. Therefore, we also ran a log rank test to examine prophylactic use of methotrexate separately.

To evaluate the protective effect of aGHVD on survival from relapse and mortality, we fit two Cox proportional hazards models, one for disease-free survival and another for relapse-free survival. In both models, our target is the hazard ratio associated with the development of aGHVD, which is modeled as a time-varying covariate. In the relapse-free survival model, death is a competing event with relapse, because cancer relapse cannot occur after death, and modeling death as a censoring event may misrepresent relapse-free survival.

Next, we produced a subset of the data to include people who develop aGHVD. To test if any of the baseline variables are associated with differences in disease-free survival for this subset of patients who develop aGHVD, we fit another Cox PH model including all baseline covariates to examine the hazard ratios for each variable, while accounting for all the other baseline variables. Similar to when we tested for baseline differences with the whole sample, we were not able to adjust for prophylactic use of methotrexate due to some recruitment centers not having people who

reported prophylactic use of methotrexate. Therefore, we also ran a log rank test to examine prophylactic use of methotrexate separately.

To investigate the association of prophylactic use of methotrexate with risk of developing aGVHD, we fit a cox PH model using development of aGVHD as the terminating event and prophylactic use of methotrexate as the predictor. Since the relationship maybe confounded by perceived risk of developing aGVHD, we fit another model adjusting for factors used clinically to predict risk for aGVHD, including the patient's and donor's age, CMV status in patient and donor, waiting time for transplantation and whether the transplant was made from female donor to male patients.[4] We also adjusted for the recruitment site, because of its association with methotrexate use in the study.

To investigate the effects of platelet recovery, we fit two Cox PH models, one for disease-free survival and another for relapse-free survival. In both models, our target is the hazard ratio associated with platelet recovery, which is modeled as a time-varying covariate. In the second PH model, death is a competing event with relapse, because cancer relapse cannot occur after death, and modeling death as a censoring event may misrepresent relapse-free survival. Since we use an alpha level of 0.1 to test for statistical significance, we also report out 90% confidence intervals.

Results

Overall characteristics and disease-free survival

Table 1 describes the baseline characteristics for patients across different disease groups and Table 2 describes the baseline characteristics for patients across different FAB classifications and for the overall sample. The median age was 28 years. Across the different subgroups, patients had a higher median age as the disease group increased from the ALL (acute lymphoblastic leukemia) group to AML low risk (acute myelocytic leukemia) to AML high risk group, but had a similar median age across the two FAB classifications. Most of the participants were male (58.4%), with considerably less females in the acute lymphoblastic leukemia group and for those who did not have FAB grade 4 or 5 and AML. In the total sample, 49.6% of patients had a positive CMV status at baseline and 50.4% of patients had a negative CMV status at baseline. Though, less patients had a positive CMV status at baseline in the acute lymphoblastic leukemia group (39.5%), but more patients had a positive CMV status in the AML high risk group (60.0%). Wait time ranged from 24 to 2616 days and varied across the different disease groups and FAB classifications. Overall, most patients (55.5%) were recruited from The Ohio State University. Most patients (70.8%) reported no prophylactic use of methotrexate (70.8%), and this remained true across the different disease groups, but a higher proportion of patients with FAB grade 4 or 5 and AML reported prophylactic use of methotrexate (82.2%).

The Kaplan-Meier curve for disease-free survival is shown in Figure 2. The median disease-free survival time is 481 days, 95% CI: [363, 748]. The probability of disease-free survival at 100 days, 1 year and 3 years is 0.825 [0.750, 0.879], 0.583 [0.496, 0.660] and 0.395 [0.312, 0.476], respectively.

For the total sample using a Cox PH model with an alpha level of 0.1, and accounting for all the other baseline covariates (besides prophylactic use of methotrexate), we find that there is a statistically significant difference in disease-free survival for recruitment centers, disease groups, and FAB classification at baseline. Specifically, comparing Alfred to The Ohio State University and adjusting for the other baseline covariates in the model, the hazard of relapse is estimated to be 1.85 times larger (HR=1.85; 90% CI=1.03, 3.30; p=0.08). Comparing Hahnemann to The Ohio State University and adjusting for the other baseline covariates in the model, the hazard of relapse is estimated to be approximately 66.3% smaller (HR=0.34; 90% CI= 0.17, 0.69; p=0.01). Comparing the AML low risk group to the ALL group and adjusting for the other baseline covariates in the model, the hazard of relapse is estimated to be 57.4% smaller (HR=0.43; 90% CI= 0.23, 0.78; p=0.02). Comparing one FAB subtype (grade 4 or 5 and AML) to the other and adjusting for the other baseline covariates in the model, the hazard of relapse is estimated to be 2.43 times larger (HR=2.43; 90% CI=1.53, 3.88; p=0.002). When separately comparing those who reported prophylactic use of methotrexate and those who did not report prophylactic use of methotrexate for the full sample, we do see a statistically significant difference at the alpha level 0.1 (X^2=2.8; p=0.09).

Protective effects of aGVHD

For disease-free survival, the hazard ratio associated with the occurrence of aGVHD is 1.72 (90% CI: 1.11-2.65), adjusting for covariates. In other words, comparing two patients of the same covariates, one who did develop aGHVD during the study period and another who did not, the instantaneous risk of death or relapse for the first patient is 1.72 greater than times the second, throughout the study period. For relapse-free survival, the hazard ratio associated with the occurrence of aGVHD is 0.83 (90% CI: 0.41-1.68), controlling for covariates related to patient/donor health and state of initial cancer. We have sufficient evidence that aGVHD is positively associated with disease-free survival. The latter confidence interval for the hazard ratio ranges above and below 1, therefore we do not have sufficient

evidence that aGVHD is associated with better or worse relapse-free survival. Figure 2 displays the disease-free survival and relapse-free survival hazard ratios.

Factors associated with disease-free survival among patients who develop aGVHD

Using a Cox proportional hazards model with an alpha level of 0.1, we find that there is a statistically significant difference in disease-free survival among the patients who develop aGVHD, when accounting for all the other baseline covariates (besides prophylactic use of methotrexate), for sex, recruitment centers, and disease groups at baseline. Comparing males to females, adjusting for the other baseline covariates in the model, the hazard of relapse is estimated to be approximately 81.8% smaller (HR=0.18; 90% CI= 0.04, 0.94; p=0.09). Comparing St. Vincent to The Ohio State University, adjusting for the other baseline covariates in the model, the hazard of relapse is estimated to be 42.8 times larger (HR=42.8; 90% CI=2.11, 870.0; p=0.04). Comparing Hahnemann to The Ohio State University, adjusting for the other baseline covariates in the model, the hazard of relapse is estimated to be 99.6% smaller (HR=0.004, 90% CI=0.0002, 0.07; p=0.001). Comparing the AML low risk group to the ALL group, adjusting for the other baseline covariates in the model, the hazard of relapse is estimated to be 24.8 times larger (HR=24.8; 90% CI=3.05, 202.0; p=0.01). Comparing AML high risk group to ALL group, adjusting for the other baseline covariates in the model, the hazard of relapse is estimated to be 150.0 times larger (HR=150.0; 95% CI=7.25, 3103.0; p=0.007). When separately comparing those who reported prophylactic use of methotrexate and those who did not report prophylactic use of methotrexate among the patients who develop aGVHD, we do not see statistically significant differences (X^2=0.9; p=0.3).

Prophylactic use of methotrexate and development of aGVHD

The Kaplan-Meier estimates of the probability of not developing aGVHD for patients who were administered prophylactic methotrexate compared to those who were not are shown in Figure 3. There is no significant difference in HR of developing aGVHD among patients who were administered prophylactic methotrexate compared to those who were not in the unadjusted model (HR = 0.742, 90% CI: [0.345, 1.6], p =0.52) and in the model adjusted for covariates (HR = 0.543, 90% CI: [0.234, 1.26], p = 0.23). However, adjusting for potential confounders increases the precision of the HR estimate and shifts it towards a protective effect.

Significance of platelet recovery

For disease-free survival, the ratio associated with platelet recovery is 0.42 (90% CI: 0.25-0.69), adjusting for covariates. For relapse-free survival, the ratio associated with platelet recovery is 1.11 (90% CI: 0.48-2.58), adjusting for covariates. In other words, comparing two patients of the same covariates, one whose platelet count returned to a normal level during the study period and another who did not, the instantaneous risk of relapse for the first patient is 1.11 times the second, throughout the study period. We have sufficient evidence that platelet recovery is positively associated with disease-free survival. The latter confidence interval for the hazard ratio ranges above and below 1, therefore we do not have sufficient evidence that platelet is associated with better or worse relapse-free survival. Figure 4 displays the disease-free survival and relapse-free survival hazard ratios.

Conclusion

Implications and Limitations

We do observe some baseline differences across the different groups reported, which is to be expected since some of these baseline variables could be associated with different disease progression. Our results suggest that aGVHD is positively associated with disease-free survival, though not relapse-free survival. We found that prophylactic use of methotrexate was not associated with the probability of developing aGVHD. In addition, we found that platelet recovery is associated with disease-free survival, though not relapse-free survival. Overall, we found preliminary evidence of some factors that can be used for patient prognosis in this area.

Our results should be considered with some limitations. The use of Cox PH has limitations, including the assumption that hazard ratios are proportional over time. Therefore, if the differences we observe with the Cox PH models are not proportional over time, then our estimates might be biased. Moreover, the correlations among some of the predictors (e.g., disease group and FAB classification) may lead to imprecise estimates of their coefficients. Sensitivity analysis using univariate Cox PH models showed similar results (results not shown). If there was a time gap between transplantation and enrollment, patients who died before enrollment could take place were not included in the study, presenting a problem of left truncation. In addition, this study used a small sample size and future research could benefit from larger-scale research studies.

Tables and Figures

Table 1: Baseline Characteristics Across the Different Disease Groups

	ALL (Acute lymphoblastic leukemia) n=38	**AML low risk (Acute myelocytic leukemia) n=54**	**AML high risk n=45**
Patient Age (years)			
Median (IQR)	22.5 (9.75)	29.5 (11)	32 (15)
Range	15 to 42	13 to 50	7 to 52
Patient Sex, n (%)			
Male	26 (68.4)	30 (55.6)	24 (53.3)
Female	12 (31.6)	24 (44.4)	21 (46.7)
Patient CMV Status, n (%)			
CMV positive	15 (39.5)	26 (48.1)	27 (60)
CMV negative	23 (60.5)	28 (51.8)	18 (40)
Donor Age (years)			
Median (IQR)	26 (12.75)	29.5 (14.5)	29 (16)
Range	5 to 48	12 to 54	2 to 56
Donor Sex, n (%)			
Male	26 (68.4)	34 (63.0)	28 (62.2)
Female	12 (31.6)	20 (37.0)	17 (37.8)
Donor CMV Status, n (%)			
CMV positive	17 (44.7)	22 (40.7)	19 (42.2)
CMV negative	21 (55.3)	32 (59.3)	26 (57.8)
Wait Time (days)			
Median (IQR)	199.5 (333)	120 (90)	210 (135)
Range	74 to 2616	30 to 450	24 to 900
Recruitment Center, n (%)			
Ohio State University (Columbus, OH)	21 (55.3)	27 (50.0)	28 (62.2)
Alfred (Melbourne, Australia)	8 (21.1)	5 (9.3)	4 (8.9)
Vincent (Sydney, Australia)	9 (23.7)	7 (13.0)	7 (15.6)
Hahnemann (Philadelphia, PA)	0 (0)	15 (27.8)	6 (13.3)
Prophylactic use of methotrexate, n (%)			
Yes	17 (44.7)	12 (22.2)	11 (24.4)
No	21 (55.3)	42 (77.8)	34 (75.6)

Table 2: Baseline Characteristics Across the French American British (FAB) Classifications

	FAB grade 4 or 5 and AML n=45	**Otherwise n=92**	**Total N=137**
Patient Age (years)			
Median (IQR)	28 (14)	27 (13.25)	28 (14)
Range	7 to 50	13 to 52	7 to 52
Patient Sex, n (%)			
Male	24 (53.3)	56 (60.9)	80 (58.4)
Female	21 (46.7)	36 (39.1)	57 (41.6)
Patient CMV Status, n (%)			
CMV positive	24 (53.3)	44 (47.8)	68 (49.6)
CMV negative	21 (46.7)	48 (52.2)	69 (50.4)
Donor Age (years)			
Median (IQR)	28 (15)	28.5 (14)	28 (14)
Range	2 to 48	5 to 56	2 to 56
Donor Sex, n (%)			
Male	30 (66.7)	58 (63.0)	88 (64.2)
Female	15 (33.3)	34 (37.0)	49 (35.8)
Donor CMV Status, n (%)			
CMV positive	14 (31.1)	44 (47.8)	58 (42.3)
CMV negative	31 (68.9)	48 (52.2)	79 (57.7)
Wait Time (days)			
Median (IQR)	150 (105)	180 (130)	178 (130)
Range	60 to 780	24 to 2616	24 to 2616
Recruitment Center, n (%)			
Ohio State University (Columbus, OH)	28 (62.2)	48 (52.2)	76 (55.5)
Alfred (Melbourne, Australia)	3 (6.7)	14 (15.2)	17 (12.4)
Vincent (Sydney, Australia)	5 (11.1)	18 (19.6)	23 (16.8)
Hahnemann (Philadelphia, PA)	9 (20.0)	12 (13.0)	21 (15.3)
Prophylactic use of methotrexate, n (%)			
Yes	37 (82.2)	32 (34.8)	40 (29.2)
No	8 (17.8)	60 (65.2)	97 (70.8)

Figure 1. Kaplan-Meier curve of disease-free survival.

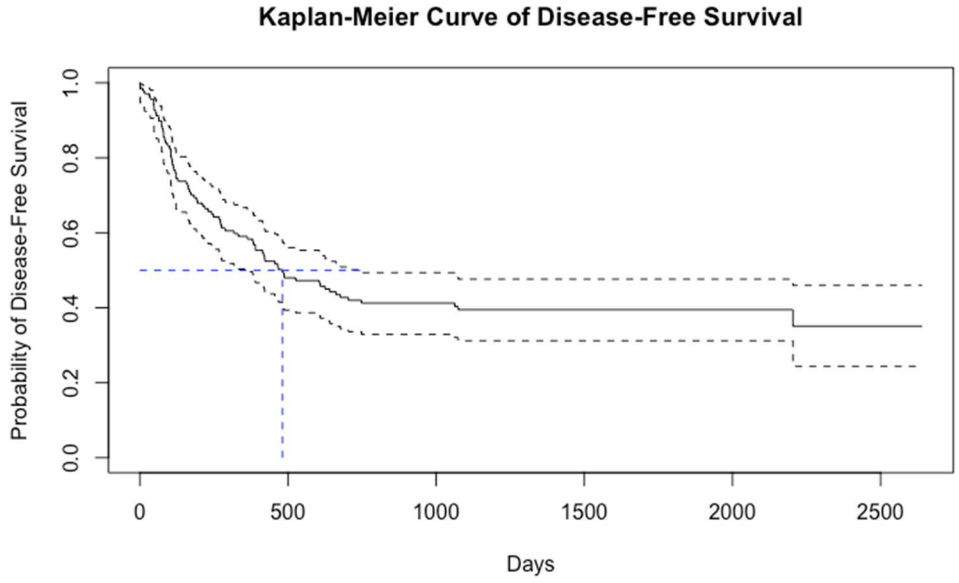


Figure 2. Forest plot of hazard ratios. Estimates were generated from two Cox PH models, each considering separate terminating events (relapse or death, or only relapse).

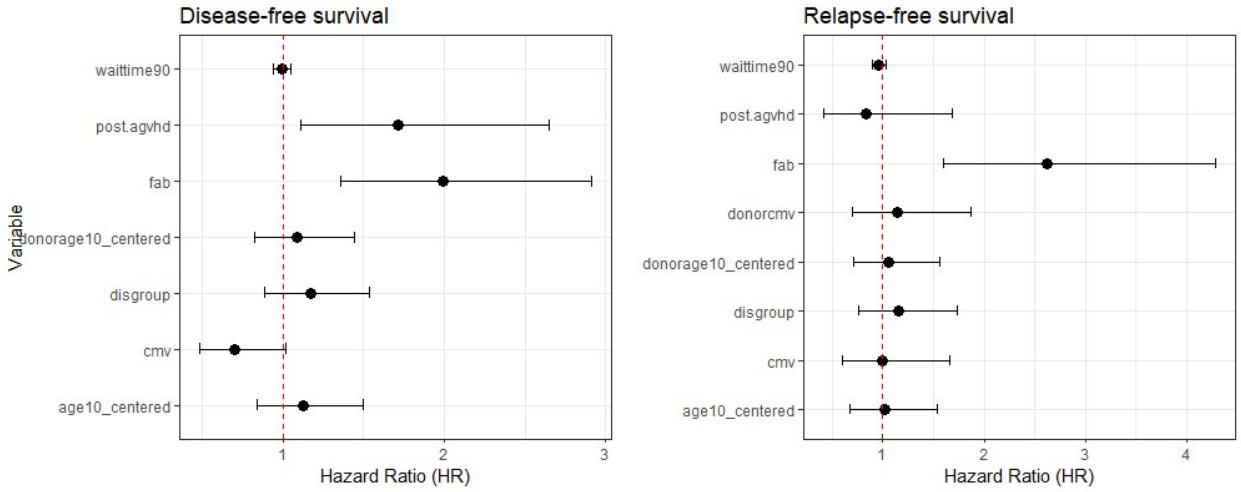
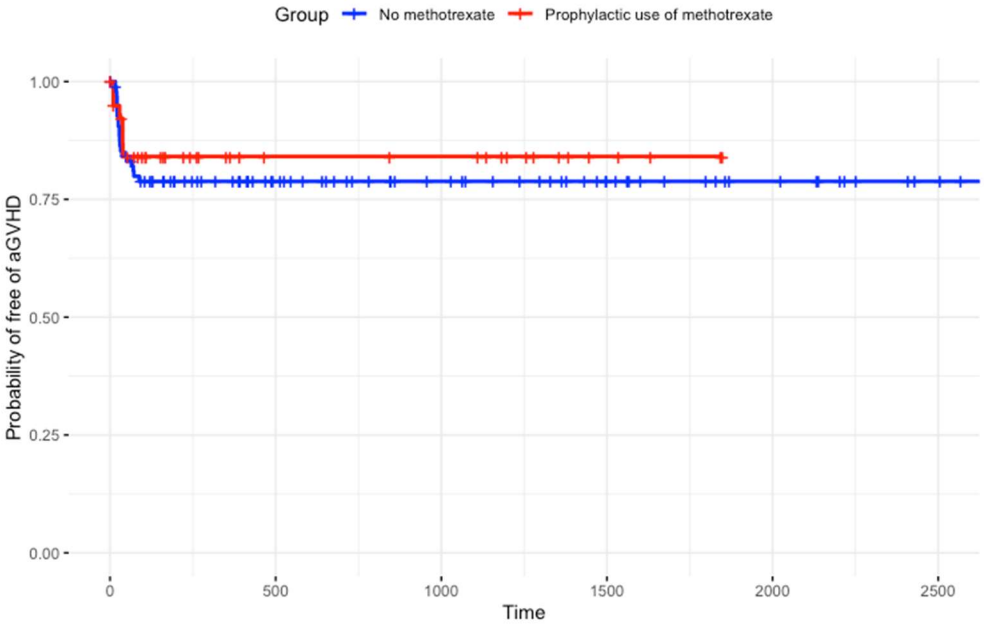


Figure 3. Survival from aGVHD development. Estimates were generated from a Kaplan-Meier model.



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