**BIOST 537**

**Group 7**

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**Introduction**

Acute leukemia is a rapidly developing white blood cell cancer. Types of leukemia include both acute and chronic forms of lymphocytic or myeloid leukemia (ALL and AML, respectively), and AML is the primary form of adult leukemia [(1)](https://www.zotero.org/google-docs/?TQnz8Z). Henceforth “leukemia” will be used to refer to both AML and ALL. Survival rates for this cancer are low, and decrease with increasing age [(2)](https://www.zotero.org/google-docs/?RBrvOV). Chemotherapy followed by bone marrow transplant, a form of stem cell transplant, is one of the only available treatment options, and relapse of leukemia after treatment is common [(3)](https://www.zotero.org/google-docs/?1OkLRr).

Acute graft-versus-host disease (aGVHD) is a relatively common immune response to stem cell transplants, including bone marrow transplants [(4)](https://www.zotero.org/google-docs/?m2lIDj), and has been shown to offer some potential protection against relapse of leukemia [(1)](https://www.zotero.org/google-docs/?zmVXWn). However, aGVHD brings its own set of health complications, such as rashes, diarrhea, jaundice, and risk of other infections [(4)](https://www.zotero.org/google-docs/?1cOdwA), and is a primary cause of death following stem cell transplant [(5,6)](https://www.zotero.org/google-docs/?6noalO). The current standard of treatment for aGVHD is the administration of immunophilin and methotrexate, while the primary approach for prevention is immunosuppression [(6)](https://www.zotero.org/google-docs/?0lo5Da). The extent of potential benefits and risks of aGVHD and associated treatments in AML populations is still unknown, but given the potential association with relapse, these questions could have important implications for AML patient survival.

This analysis uses data collected by a multi-site study at four hospitals in the United States and Australia from March 1984 through June 1989 to evaluate the associations between bone marrow transplants, aGVHD, and relapse or death due to AML. Our analysis uses survival data techniques to examine disease-free survival in the study patients and the potential roles of FAB classification, baseline variables, and aGVHD in survival in this population.

**Methods**

*Data overview*

This analysis used data collected by a multi-site study at four hospitals in the United States and Australia from March 1984 through June 1989. The study included 137 leukemia patients enrolled at hospital visits and followed until time of death or completion of the study. Information was collected at the time of bone marrow transplantation, including patient age, sex (male or female), CMV status, as well as donor age, sex, and CMV status, hospital where recruited, disease subtype according to FAB grade (coded as 1 for grade 4 or 5 and AML and 0 otherwise), disease type (acute lymphoblastic, acute myelocytic, and high risk), as well as prophylactic use of methotrexate coded as a binary yes/no variable.

The researchers also created variables for time until death or end of study, time until relapse, time until aGVHD (coded 0 if the patient never experienced it and 1 otherwise), time until recovery of normal platelet levels, and binary variables for death at last follow up time, relapse, disease-free survival (1 for dead or relapse vs 0 for alive and disease-free), aGVHD at time of aGVHD, and recovery of normal platelet levels.

*Survival post bone marrow transplant*

The study did not involve delayed entry, given that the initiating event (bone marrow transplantation) happens at the beginning of the study. However, right-censoring was a concern because some participants had not been observed with the terminating event (relapse or death) before loss-to-follow-up or the end of the study. To account for right-censoring, we used a nonparametric Kaplan-Meier curve *(Figure 1)* to estimate the disease-free survival time for patients enrolled in this study. *Table 1* displays the summary statistics for patient baseline characteristics by different FAB classifications. Two-sample t-tests were used to determine if any baseline measurements differ significantly by FAB classification at the level of alpha=0.10.

As we did not expect hazard ratios to vary substantially over time, pairwise Cox Proportional Hazard (PH) models were used to evaluate the association between baseline characteristics and disease-free survival among all patients. P-values were obtained through a Wald test. Potential confounders were taken into account as follows: Since waiting time until transplant differs by hospital, and the medical equipment and healthcare service of a hospital can impact patients’ disease-free survival, we adjusted for hospital when investigating the association between waiting time and disease-free survival. Past studies have shown that predictive markers for CMV vary by sex, and that CMV largely affects young children. Females have much higher IgG and IgM compared to age-matched males, where IgG and IgM are excellent predictive markers for CMV infection [(10,](https://www.zotero.org/google-docs/?r4DcVP) [11)](https://www.zotero.org/google-docs/?kTzxGT). Hence, we adjusted for patients’ age and gender when investigating the association between CMV and disease-free survival. Given that age, gender and CMV status have shown to be associated with patients’ morbidity and mortality [(12)](https://www.zotero.org/google-docs/?QZntKK), we adjusted for them when investigating the association between disease-related predictors (disease group, FAB and prophylactic use of methotrexate) and disease-free survival.

*Impact of aGVHD on relapse or death*

To evaluate the association between baseline characteristics and disease-free survival among patients who developed aGVHD, we fit the same pairwise Cox PH models as described in the section above, while restricting the study population to those who were observed with aGVHD. A Cox PH model adjusting for patients’ age, CMV status, donor and recipient sex match and prophylactic use of methotrexate was used to assess if the occurrence of aGVHD after transplantation is associated with decreased risk of relapse. We also used a Cox PH model to assess the association between prophylactic use of methotrexate and the risk of developing aGVHD. The association was assessed with and without adjusting for age, FAB and disease group, given that age, AML and ALL have been shown to affect methotrexate use as well as the risk of developing aGVHD [(15)](https://www.zotero.org/google-docs/?SbCjtk). Kaplan Meier curves were used to estimate the survival function of time from transplant until onset of aGVHD separately for patients either administered methotrexate or not.

Both a cox PH model and a generalized gamma accelerated failure time (AFT) model were applied to evaluate whether the occurrence of aGVHD after transplantation is associated with improved disease-free survival. The generalized gamma distribution was chosen due to its high flexibility and the potential to better fit the data. We adjusted for patients’ age, CMV status, donor and recipient sex match and prophylactic use of methotrexate because they were shown to be linked with both the exposure and the outcome. Older age, CMV and sex mismatch between donor and recipient were considered as risk factors for aGVHD, morbidity, and mortality [(13)](https://www.zotero.org/google-docs/?ngq0Oq). Prophylactic use of methotrexate has been shown to be associated with decreased risk of aGVHD and longer disease-free survival [(14)](https://www.zotero.org/google-docs/?0OS2Zj).

*Platelet level and disease-free survival*

A Cox PH model and a generalized Gamma AFT model were used to investigate whether recovery of normal platelet level is associated with improved disease-free survival, adjusting for patients’ age, gender, disease group and prophylactic use of methotrexate. Platelet count at 100 days post-transplant has been shown to be an important predictor of survival in leukemia patients undergoing bone marrow transplants [(9)](https://www.zotero.org/google-docs/?AahCG1). There is evidence suggesting that older age, male, ALL, AML and methotrexate use are linked with lower platelet count or reactivity [(16–18)](https://www.zotero.org/google-docs/?ESGCDm). A separate Cox PH model was used to investigate whether recovery of normal platelet level is associated with a decreased risk of relapse, while taking into account the same set of confounders.

**Results**

*Patient population*

This study consisted of 92 individuals with disease subtype FAB grade 4 or 5 and AML and 45 individuals with an “other” disease subtype (Table 1). Between disease subtypes, the average age of patents were approximately equal, as was the sex distribution. The proportion of CMV positive individuals was also not significantly different between the two FAB groups. This also held true for the donor’s age and sex. The proportion of donors who were CMV positive was statistically higher in patients with FAB grade 4 or 5 and AML (p=0.094). On average, patients with FAB grade 4 or 5 and AML had shorter wait times than the other group, but the difference was not statistically significant. The proportion of individuals who use methotrexate was significantly lower in patients with FAB grade 4 or 5 and AML (p=0.063). All hospitals were represented in both FAB groups, and the distribution of hospital recruitment did not significantly differ between them.

*Survival post bone marrow transplant*

Disease-free survival probability declined rapidly from day 0 to day 750 (approximately 2.8 years) after bone marrow transplantation (Figure 1). The declining rate slowed substantially after day 750. Noticeably, the disease-free survival probability stayed constant from day 1100 to day 2200. The median time from transplant until death for the whole population was 481 (95% CI: 363 - 748) days. We found that disease type 2 (z = -2.24, p = 0.025) and FAB type (z = -2.68, p = 0.0073) were both associated with disease-free survival at the alpha = 0.10 significance level (Table 2). Patients with disease group 2 had 48.9% (95% CI: 8.1%, 71.6%) lower hazard of death or relapse than those in disease group 1, adjusting for age, gender and CMV status. Patients with FAB grade 4 or 5 and AML had 84.0% (95% CI: 17.9%, 187.1%) higher hazard of death or relapse compared to other patients, adjusting for age, gender, and CMV status.

*Impact of aGVHD on relapse or death*

We did not find a significant association between prophylactic use of methotrexate and risk of developing aGVHD (Figure 2). Based on a Cox PH model, the estimated hazard of developing aGVHD among patients with prophylactic use of methotrexate is 0.74 (95% CI: 0.30, 1.84) times the hazard of developing aGVHD among patients without prophylactic use of methotrexate (p=0.52). After adjusting for age, FAB and disease group, the estimated hazard of developing aGVHD among patients with prophylactic use of methotrexate is 0.53 (95% CI: 0.21, 1.37) times the hazard of developing aGVHD among patients without prophylactic use of methotrexate (p=0.19). We still failed to reject the null hypothesis that the hazard of developing aGVHD is equal between patients with and without prophylactic use of methotrexate.

We realized that among patients who develop aGVHD, donor age (z = -1.32, p = 0.063) and disease type 3 (z=1.95 , p=0.051) were associated with disease-free survival (Table 3). Comparing two subgroups of patients whose donor age is one year apart, the estimated hazard of death or relapse is 1.07 (95% CI: 1.00, 1.15) times higher in the subgroup with older donor age. Comparing two subgroups of patients that agree on age, gender and CMV status, one group with disease type 1 and the other group with disease type 3, the estimated hazard of death or relapse is 5.74 (95% CI: 0.99, 33.19) times higher in the subgroup with disease type 3. Based on a Cox PH model, we did not find a significant association between aGVHD and hazard of relapse (p = 0.33), adjusting for patient age, CMV status, and prophylactic use of methotrexate. Similarly, we did not find a significant association between aGVHD and disease-free survival (p = 0.65). An AFT model informed us that the estimated mean (median) disease-free survival time in the aGVHD+ group is 1.01 (95% CI: 0.95, 2.94) times the disease-free survival time is in the aGVHD- group (p=0.99), accounting for patient age, CMV status, and prophylactic use of methotrexate.

*Platelet level and disease-free survival*

When adjusting for age, sex, disease group, and methotrexate treatment, we discovered that recovery of normal platelet level was associated with a 78.0% (95% CI: (59.9%, 87.9%), z = -4.96, p = 7.2 x 10-7) reduced hazard of death or relapse, but was not significantly associated with a decreased risk of relapse (p=0.88). Age, sex, disease group or prophylactic use of methotrexate were not found to be significantly associated with the hazard of relapse or death. Additionally, based on a generalized Gamma AFT model, we found that the estimated mean (median) time until relapse or death was 20.9 (95% CI: (8.3, 52.7), p = 1.2 x 10-10) times longer for those with recovered platelet level than those without. We also noticed that patients in disease group 2 had significantly longer mean (median) disease-free survival time as well as lower hazard of death/relapse compared to those in disease group one, adjusting for age, sex, prophylactic use of methotrexate, and recovery of normal platelet level.

**Discussion**

Synopsis

This analysis examined differences in relapse or death due to AML after bone marrow transplant, as well as the implications of aGVHD and platelet level on these outcomes. To do so, we leveraged Kaplan-Meier survival curves, Cox proportional hazards models, and accelerated failure time models. We found that the time from transplant to approximately three years post-transplant has the largest decrease in survival probability, and that the average time until relapse or deaths was 481 days. Additionally, we found that the hazard of relapse varied by disease type and FAB type. We did not find any significant relationship between methotrexate and aGVHD. There is also no evidence suggesting an association between aGVHD and death or relapse. Therefore, we do not consider aGVHD as an important prognostic event. Recovery of platelet levels had large implications for disease-free survival, with those with recovered platelet levels having a substantially lower hazard of death or relapse and a longer disease-free survival time. However, recovery of platelet levels was not found to be associated with the risk of relapse alone.

Explanations and Comparisons

Previous work has shown that bone marrow transplant is associated with improvements in disease-free survival [(19)](https://www.zotero.org/google-docs/?8xnXP4). Additionally, FAB grade 5 is associated with very low rates of disease-free survival, which is in line with our finding that the hazard of disease was higher in those with grades 4 or 5 [(20)](https://www.zotero.org/google-docs/?6vsWF9). Additionally we found that low risk AML had the lowest risk of relapse or disease, compared to all acute lymphoblastic leukemia and high risk AML. Some studies report the survival rates of those with ALL are higher than those with subtypes of AML, in contrast with our results [(21)](https://www.zotero.org/google-docs/?njjf0s). Of note is that our analysis examined disease-free survival, indicating that AML may have lower rates of remission and a lower survival rate post-remission than ALL.

We also did not find any significant association between infection with cytomegalovirus (CMV) and disease-free survival, although CMV has previously been associated with change in risk of relapse or death after bone marrow transplant. [(7,8)](https://www.zotero.org/google-docs/?TFPzJB). We do not anticipate a lack of statistical power to underlie our null result, as CMV status is evenly distributed in our study population - 69 out of 137 patients had CMV. Instead, we attribute the non-significant finding to the lack of certainty on the biological importance of CMV on disease-free survival.

Our sample size of those who developed aGVHD was quite small (26 patients), and the insufficient statistical power may contribute to our non-significant findings for the association between aGVHD and disease-free survival/relapse. The null result is consistent with a past study showing that acute and later chronic GVHD are major hazards that are not related to relapse (6). Excessive immunosuppression to limit GVHD can magnify the risks of opportunistic infection (e.g., reactivation of Epstein–Barr virus infection and lymphoproliferative disease) and recurrence of leukemia. Acute or chronic GVHD may augment graft-versus-leukemia protection against relapse of leukemia, but more severe GVHD does not enhance antitumor effects (6). However, there is also past research indicating that aGVHD is linked with improved survival [(22)](https://www.zotero.org/google-docs/?Khy9PI). Hence, the relationship between aGVHD and survival/relapse is still controversial and needs to be further explored. Additionally, we were unable to show any relationship between methotrexate and risk of aGVHD, which has also been established. Our sample size of those with aGVHD who used methotrexate was only six patients.

We did find that recovery of platelet levels was associated with improvements in disease-free survival, in line with previous studies [(9)](https://www.zotero.org/google-docs/?UzbAtu). There were only 17 patients whose platelet levels did not recover, and of those 17, 16 relapsed within the first year and a half since bone marrow transplant. This, in conjunction with the sharp decrease in survival rates during the first three years points to this time period as crucial for overall patient survival and recovery.

Limitations

This work is limited by the small sample sizes in certain patient stratifications, which increases the uncertainty of study results and restricts the power of our statistical analysis. Especially when the analysis is limited to individuals who develop aGVHD, a sample size of 26 results in very large standard deviations for the estimated coefficients. Besides, we assume proportional hazards in our Cox proportional hazard analyses, which may have varying degrees of justification. When this assumption fails, Cox PH model may have little power to detect difference in hazard between subgroups. While we attempted to adjust for all potential confounders, disease-free survival after leukemia has been shown to have other confounding factors not included in this analysis. One such factor is race, as survival rates have been shown to be disparate across racial and ethnic groups. Another factor is socioeconomic status, as people with higher socioeconomic status tend to live longer and healthier, given their access to various resources. When these factors happen to be linked with our exposure, the true association of interest can be masked or modified. Additionally, there may be some selection bias as this analysis only included people who were eligible for this study and have been matched for a bone marrow transplant in the four given hospitals. The study population may differ from the ideal target population, which includes everyone who needed and received a bone marrow transplant.

Next steps and implications

Future studies should attempt to increase the sample size of patients who developed aGVHD post bone marrow transplant. Additionally, this analysis extends over the course of roughly 7 years, when the median time until death or relapse is 183 days. This indicates that similar studies should allocate their resources towards recruitment and interventions rather than aspects associated with extended follow-up. We also found the first three years after bone marrow transplantation to be most pivotal in overall disease-free recovery. Future interventions and studies may benefit from examining this period in detail, and potentially developing therapeutics that increase survival during this period.

**Tables and Figures**

Table 1. Demographic and clinical characteristics of study participants by FAB

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Variable | Level | FAB grade 4 or 5 and AML | Other |  | p-value |
| Number of Patients | | 45 | 92 |  |  |
| Age (mean (SD)) | | 27.89 (9.81) | 28.60 (9.48) |  | 0.685 |
| Gender (%) | Female | 21 (46.7) | 36 (39.1) |  | 0.512 |
|  | Male | 24 (53.3) | 56 (60.9) | | |
| CMV Status (%) | CMV Positive | 21 (46.7) | 48 (52.2) |  | 0.672 |
|  | CMV Negative | 24 (53.3) | 44 (47.8) | | |
| Donor Age (mean (SD)) | | 26.96 (11.13) | 29.00 (9.67) |  | 0.271 |
| Donor Gender (%) | Female Donor | 15 (33.3) | 34 (37.0) |  | 0.821 |
|  | Male Donor | 30 (66.7) | 58 (63.0) | | |
| Donor CMV (%) | Donor CMV Negative | 31 (68.9) | 48 (52.2) |  | 0.094\* |
|  | Donor CMV Positive | 14 (31.1) | 44 (47.8) | | |
| Wait time in days (mean (SD)) | | 206.33 (163.85) | 308.72 (426.93) |  | 0.123 |
| Disease group (%) | ALL (Acute Lymphoblastic Leukemia) | 0 ( 0.0) | 38 (41.3) |  | <0.001\* |
|  | Low Risk AML (Acute Myelocytic Leukemia) | 18 (40.0) | 36 (39.1) | | |
|  | High Risk AML | 27 (60.0) | 18 (19.6) | | |
| Methotrexate Status (%) | No Methotrexate | 37 (82.2) | 60 (65.2) |  | 0.063\* |
|  | Yes Methotrexate | 8 (17.8) | 32 (34.8) | | |
| Recruiting Hospital (%) | The Ohio State University (Columbus, OH) | 28 (62.2) | 48 (52.2) |  | 0.206 |
|  | Alfred (Melbourne, Australia) | 3 ( 6.7) | 14 (15.2) | | |
|  | St. Vincent (Sydney, Australia) | 5 (11.1) | 18 (19.6) | | |
|  | Hahnemann (Philadelphia, PA) | 9 (20.0) | 12 (13.0) | | |
| Survival Status (%) | Alive | 11 (24.4) | 45 (48.9) |  | 0.011\* |
|  | Dead | 34 (75.6) | 47 (51.1) | | |
| Relapse Status (%) | No Relapse | 23 (51.1) | 72 (78.3) |  | 0.002\* |
|  | Relapse | 22 (48.9) | 20 (21.7) | | |
| aGVHD Status (%) | No aGVHD | 37 (82.2) | 74 (80.4) |  | 0.985 |
|  | aGVHD | 8 (17.8) | 18 (19.6) | | |
| Disease Free Survival Status (%) | Disease free survival | 10 (22.2) | 44 (47.8) |  | 0.007\* |
|  | Dead or Relapsed | 35 (77.8) | 48 (52.2) | | |
| Platelet Recovery Status (%) | No Platelet Recovery | 5 (11.1) | 12 (13.0) |  | 0.963 |
|  | Platelet Recovery | 40 (88.9) | 80 (87.0) | | |

Table 2. Estimated hazard ratio of death/relapse for patients with different baseline measurements

|  |  |  |  |
| --- | --- | --- | --- |
|  | Estimated Coefficient (Exponentiated) | 95% CI | p-value |
| Patient Age | 1.01 | (0.99, 1.04) | 0.34 |
| Patient Sex (Male) | 0.79 | (0.51, 1.23) | 0.30 |
| Patient CMV | 1.13 | (0.72, 1.78) | 0.58 |
| Donor Age | 1.01 | (0.99, 1.04) | 0.25 |
| Donor Sex (Male) | 0.99 | (0.63, 1.55) | 0.97 |
| Donor CMV | 1.00 | (0.64, 1.56) | 0.99 |
| Waiting Time | 0.9998 | (0.9992, 1.00) | 0.58 |
| Disease Group 2 | 0.51 | (0.28, 0.92) | 0.025\* |
| Disease Group 3 | 1.30 | (0.74, 2.32) | 0.36 |
| FAB | 1.84 | (1.18, 2.87) | 0.0073\* |
| Prophylactic Use of Methotrexate | 1.39 | (0.86, 2.26) | 0.18 |

Table 3. Estimated hazard ratio of death/relapse for aGVHD+ patients with different baseline measurements

|  |  |  |  |
| --- | --- | --- | --- |
|  | Estimated Coefficient (Exponentiated) | 95% CI | p-value |
| Patient Age | 1.02 | (0.98, 1.07) | 0.31 |
| Patient Sex (Male) | 1.32 | (0.48, 3.66) | 0.59 |
| Patient CMV | 0.59 | (0.20, 1.79) | 0.35 |
| Donor Age | 1.07 | (1.00, 1.15) | 0.063\* |
| Donor Sex (Male) | 0.84 | (0.31, 2.26) | 0.73 |
| Donor CMV | 1.46 | (0.47, 4.52) | 0.52 |
| Waiting Time | 1.00 | (0.9993, 1.0009) | 0.84 |
| Disease Group 2 | 1.78 | (0.40, 7.88) | 0.45 |
| Disease Group 3 | 5.74 | (0.99, 33.19) | 0.051\* |
| FAB | 1.84 | (0.64, 5.31) | 0.26 |
| Prophylactic Use of Methotrexate | 2.65 | (0.72, 9.73) | 0.14 |

Figure 1. Kaplan-Meier estimate of disease-free survival time among study participants

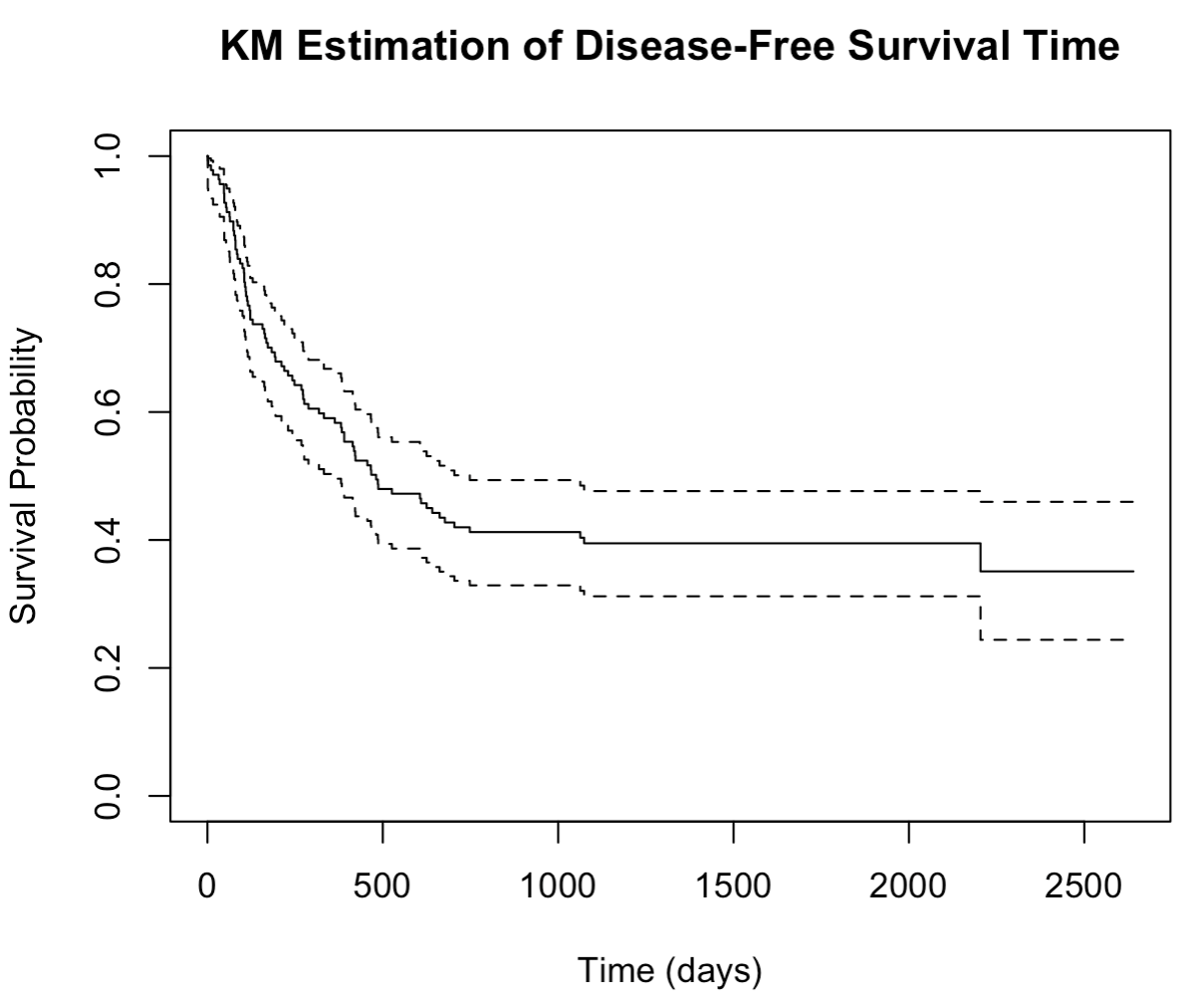
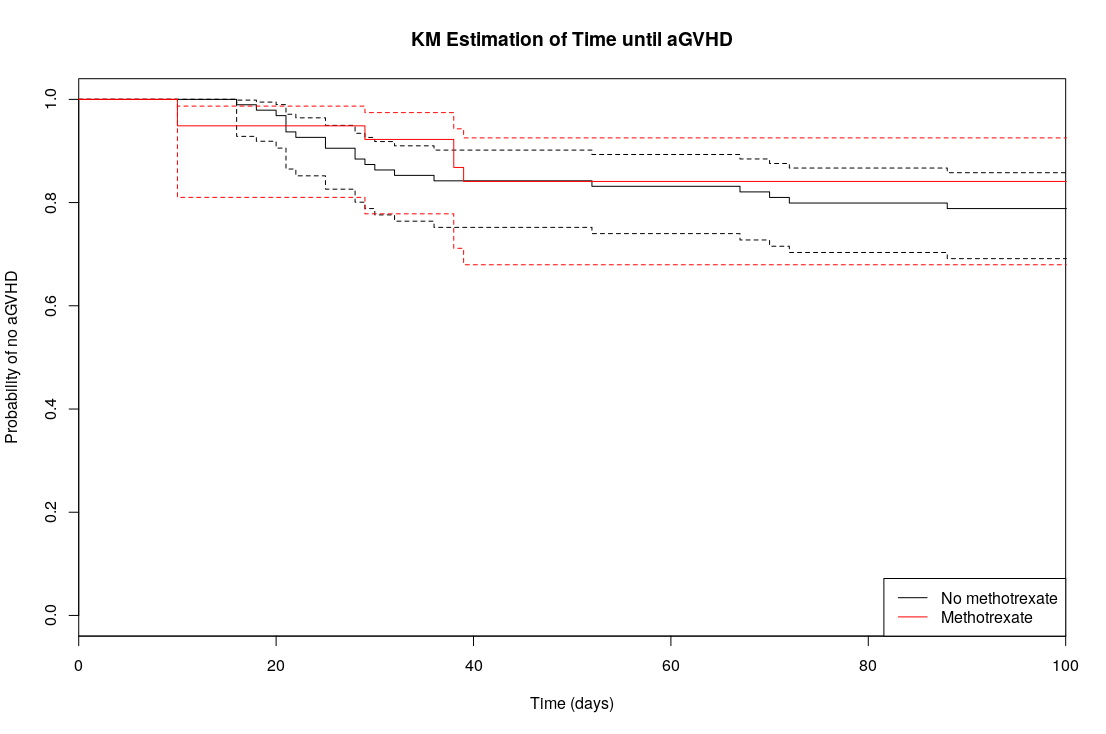


Figure 2. Kaplan-Meier estimate of time until aGVHD by prophylactic use of methotrexate



Code is available at <https://github.com/mwalte10/Class-projects/blob/main/biostats_final_group_project.R>.

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