# Impact of Cytomegalovirus (CMV) Reactivation after Umbilical Cord Blood Transplantation

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## Abstract

This study investigated the impact of pre-transplant CMV serostatus and post-transplant CMV reactivation and disease on umbilical cord blood transplant (UCBT) outcomes. Between 1994 and 2007, 332 patients with hematologic malignancies underwent UCBT and 54% were CMV seropositive. Pre-transplant recipient CMV serostatus had no impact on acute or chronic GVHD, relapse, disease free survival (DFS), or overall survival (OS). There was a trend toward greater day 100 treatment-related mortality (TRM) in CMV seropositive recipients (p=0.07). CMV reactivation occurred in 51% (92/180) of patients with no difference in myeloablative (MA) versus reduced-intensity conditioning (RIC) recipients (p=0.33). Similarly, reactivation was not influenced by the number of UCB units transplanted, the degree of HLA disparity, the $CD34^+$ or $CD3^+$ cell dose, or donor killer immunoglobulin-like receptor (KIR) gene haplotype. Rapid lymphocyte recovery was associated with CMV reactivation (p=0.02). CMV reactivation was not associated with acute (p=0.97) or chronic GVHD (p=0.65), nor did it impact TRM (p=0.88), relapse (p=0.62) or survival (p=0.78). CMV disease occurred in 13.8% of the CMV-seropositive patients, resulting in higher TRM (p=0.01) and lower OS (p=0.02). Thus, although recipient CMV serostatus and CMV reactivation have little demonstrable impact on UCB transplant outcomes, the development of CMV disease remains a risk, associated with inferior outcomes.

## Table of Contents

## Table of Contents (continued)

Page

## List of Tables

Page

## List of Figures

Page

## Introduction

Umbilical cord blood (UCB) is increasingly being used as an alternative donor source for hematopoietic cell transplantation (HCT). Compared to bone marrow (BM), UCB is relatively simple and safe to collect, rapidly available, and has a decreased likelihood of transmitting viral infections. UCB is associated with a lower risk of severe graft versus host disease (GVHD), despite significant HLA disparity between donor and recipient.[1] Since UCB T-cells are immunologically naïve, they do not offer passive immunity to the transplant recipient. Given these differences, there have been concerns for prolonged reconstitution of antigen specific immunity and increased risk for viral infections after UCB transplantation.[2, 3] In fact, some studies show fewer CMV-specific $CD4^+$ and $CD8^+$ T cells and a higher incidence of viral infections following UCB transplant.[4-7]

Cytomegalovirus (CMV) is thought to contribute significantly to HCT morbidity and mortality.[3, 8-10] Risk factors for CMV reactivation include prior CMV infection in the donor or recipient, GVHD, steroid therapy, T-cell depletion, and age.[10-16] Unlike PB or BM transplantation where CMV reactivation can arise from either the donor or recipient, after UCB transplantation CMV is almost exclusively due to reactivation of endogenous virus in the host. This is because CMV infection in newborns is rare and infected UCB units are not generally banked or used clinically.[17] To date, the incidence and risk factors for CMV reactivation have not been extensively described post-UCB transplantation. Here we report the incidence of CMV reactivation and its

impact on UCB transplant associated outcomes. We also explore risk factors for CMV reactivation in CMV seropositive recipients following UCB transplantation.

## Methods

*Transplant procedures*

Myeloablative (MA) conditioning was used in 227 consecutive patients with malignant hematological diseases and consisted of cyclophosphamide (CY 60 mg/kg x 2) and total body irradiation (TBI 13.2 Gy, 165 cGy twice daily x 4 days). From 1994-2000 this regimen included equine anti-thymocyte globulin (ATGAM, Pharmacia) 15 mg/kg every 12 hours on days -3 to -1 pre-transplant and methylprednisolone (MP) (1 mg/kg every 12 hours from days 5 to 19) (n=31). After 2000, ATG and methylprednisolone were replaced with fludarabine (FLU, 25 mg/m$^2$/d) on day -8 through -6 and mycophenolate mofetil (MMF, 1 g every 12 hrs from day -3 to day +30).[18] All patients also received cyclosporine A starting at day -3 and continuing for approximately 180 days. Following myeloablative conditioning either one (n=116) or two (n=111) UCB units were infused. Reduced intensity conditioning (RIC) consisted of CY (50 mg/kg), FLU (200 mg/m$^2$) and total body irradiation (TBI 2 Gy). RIC was followed by double UCBT in all patients (n=105).[19] Patients undergoing RIC single UCBT (n=17), RIC double UCBT with ATG in the preparative regimen (n=4), and transplantation for CLL (n=6) were excluded from this analysis due to small numbers of patients in those categories.

*Graft-versus-host disease prophylaxis, diagnosis, and treatment*

Graft-versus-host diseases (GVHD) prophylaxis consisted of cyclosporine/mycophenolate mofetil (CsA/MMF, n=257) or cyclosporine/methylprednisone/anti-thymocyte globulin (CsA/MP/ATG, n=75).[20] Diagnosis of acute and chronic GVHD was based on standard clinical criteria and biopsy when available. Staging was based upon published criteria [21] and treatment of acute GVHD (aGVHD) clinical stage II or greater was with methlyprednisolone ($\geq$48 mg/m$^2$ intravenously or oral equivalent) daily for a minimum of 2 weeks prior to a taper over 8 weeks.

*CMV screening and prophylaxis*

Prior to conditioning, all patients were assessed for CMV exposure by serology using enzyme immunoassay. Patients with a CMV IgG antibody level greater than 10.0 EU/mL were considered positive. After transplant all patients underwent weekly screening for CMV reactivation by either pp65 antigenemia (prior to 2006) or PCR (after 2006) until day +100 post-UCBT. CMV seropositive recipients received high dose acyclovir prophylaxis (500 mg/m2 [10-12 mg/kg] IV every 8 hours or 800 mg [18 mg/kg pediatric] PO 5 times daily) until day 100. CMV seronegative recipients who were herpes simplex virus (HSV) seropositive received low dose acyclovir prophylaxis (250 mg/m2 [5 mg/kg] IV every 12 hours or 400 mg [9 mg/kg pediatric] PO twice daily) until day +60. CMV reactivation was defined as either CMV antigenemia ($\geq$2 pp65 positive

cells/50,000), DNAemia (≥500 copies by quantitative polymerase chain reaction [PCR]), or culture of CMV from blood, body fluid or tissue. For the purposes of this analysis, the maximum interval allowed for a single reactivation episode was 30 days.[22] After clearance of CMV for more than 30 days, a subsequent episode of CMV reactivation was considered an additional event. CMV reactivation was treated with ganciclovir (induction [5 mg/kg IV twice daily] for 2 weeks, followed by maintenance [5 mg/kg IV daily] for an additional 6 weeks). Foscarnet (induction [60 mg/kg IV every 8 hours], followed by maintenance [90 mg/kg IV daily]) was used in place of ganciclovir in patients prior to absolute neutrophil count (ANC) recovery. All blood products were CMV seronegative or leukoreduced by filtration. CMV disease was defined as detection of virus in end organ tissues including lung (bronchoalveolar lavage sample [BAL]) and GI tract (histopathologic changes consistent with CMV).

*Data collection*

Data regarding patient characteristics and outcomes were prospectively collected by the Biostatistical Support Group at the University of Minnesota in the HCT database. CMV serostatus and reactivation data were assessed for completeness and accuracy by retrospective review of patient records. Data was analyzed retrospectively. Protocols were approved by the University of Minnesota Institutional Review Board. All patients and/or their legal guardians provided IRB-approved signed informed consent in accordance with the Declaration of Helsinki.

*Killer Ig Receptor (KIR) Gene Content Analysis*

The presence or absence of 16 KIR genes was determined using a high-throughput SNP-based Sequenom MassARRAY system (Sequenom, San Diego, CA) and the matrix-assisted laser desorption/ionization–time-of-flight mass spectrometry (MALDI-TOF MS) platform for the large scale KIR genotyping of DNA samples as previously described.[23] Samples with at least 1 KIR B haplotype-defining locus (*KIR2DL5, 2DS1, 2DS2, 2DS3, 2DS5,* or *3DS1*) were assigned the genotype B/X and samples lacking all KIR B loci were assigned the genotype A/A.[23]

*Statistical analysis*

Patient and transplant characteristics across transplant type were compared using the Chi-square test or Fisher's exact test. Continuous factors were compared using the general Wilcoxon test. The primary endpoint was the cumulative incidence of CMV reactivation. Other endpoints included overall survival (OS), disease relapse, transplant related mortality (TRM), acute GVHD (aGVHD), and chronic GVHD (cGVHD). The cumulative incidence of CMV reactivation, disease relapse, acute GVHD, and chronic GVHD were calculated by treating deaths from other causes as competing risks.[24] The cumulative incidence of TRM was calculated by treating relapse as a competing risk. The statistical endpoint of survival was estimated by the Kaplan-Meier method.[25] Statistical comparison of the time-to-event curves between groups was completed by the

Log-Rank test. The time dependent effect of CMV reactivation on OS, disease relapse, TRM, aGVHD, and cGVHD was assessed using Cox regression analysis.

A Cox proportional hazards model was used to model the independent effect of potential predictors of CMV reactivation including: age (0-10 vs. 11-17 vs. 18-35 vs. 36+), weight, cell dose (quartiles of TNC, $CD34^+$, and $CD3^+$), disease risk (standard vs. high), conditioning regimen and number of UCB units, HLA disparity (4/6 vs. 5/6 vs. 6/6), GVHD prophylaxis, time-dependent GVHD, and gender.[26]

## Results

*Patient characteristics*

Between 1994 and 2007, 332 patients underwent HCT for hematologic malignancies using UCB at the University of Minnesota. Prior to transplant, 54% of patients (n=180) were CMV seropositive and 46% (n=152) were CMV seronegative. Patient characteristics for these patients are summarized in Table 1. CMV seronegative patients were more likely to have high risk disease and GVHD prophylaxis consisting of CsA/MP/ATG. The two groups were similar with respect to age, number of cord blood units used, conditioning regimen, HLA disparity, gender, and diagnosis.

*Predictors of CMV reactivation in CMV seropositive recipients*

The incidence of CMV reactivation was 51% (92/180) among CMV seropositive transplant recipients. Among CMV seronegative recipients, the incidence of CMV infection was 1.3% (2/152) and these recipients were not analyzed further. The median

time to CMV reactivation was 40 days (range 9-95 days). As shown in table 2, there was a similar time to reactivation regardless of conditioning regimen and number of units transplanted, HLA disparity of the engrafting unit, recipient age, gender, or disease risk.

There was no difference in CMV reactivation for recipients of a myeloablative vs. RIC transplant (p=0.33) (Figure 1). CMV reactivation (38% vs. 52%, p=0.93) and disease (0% vs. 15%, p=0.60) were similar regardless of whether the SCT occurred from 1994-1999 or 2000-2007. CMV reactivation was similar regardless of GVHD prophylaxis regimen (p=0.8) (Figure 2). Likewise in multivariate analysis, neither acute [RR=1.0 (95% CI 0.5-2.0), p=0.97] nor chronic GVHD [RR=1.2 (95% CI 0.6-2.1), p=0.65] impacted CMV reactivation.

The infused T-cell content did not influence CMV reactivation (p=0.82). In contrast, lymphocyte recovery was associated with CMV reactivation. Patients with an absolute lymphocyte count (ALC) >0.2 $x10^8$/L at day 28 post-transplant (n=100) were more likely to have CMV reactivation compared to those with ALC <0.2 $x10^8$/L (n=60) [61% (95% CI 50-72%) vs. 37% (95% CI 25-49%) p=0.02]. CMV reactivation did not impact secondary graft failure. Among patients with CMV reactivation, 2.2% (2/92) had secondary graft failure compared to 3.4% (3/88) of patients which did not experience CMV reactivation (p=0.62).

Donor KIR haplotype (A/A vs. B/X) was determined in a subset of samples where DNA was available for KIR typing (n=65). Twenty patients engrafted with KIR A/A haplotype donor and 55% (11/20) had CMV reactivation, whereas 58% (26/45) of those

engrafted with a KIR B/X donor showed reactivation. Thus, donor KIR haplotype appeared to not impact recipient CMV reactivation (p=0.83).

Additional variables not associated with CMV reactivation were gender (p=0.57), disease risk (standard vs. high, p=0.48), conditioning regimen (p=0.33), and cell dose/kg, defined as total nucleated cells/kg (p=0.75) or $CD34^+$/kg (p=0.12). There was a trend toward increased age impacting CMV reactivation in multivariate analysis, with patients 18 years and older having a relative risk of CMV reactivation of 1.5 (95% CI 1.0-2.5, p=0.06).

*Impact of recipient CMV seropositivity on UCBT outcomes*

Recipient CMV positive serostatus is frequently associated with inferior transplant outcomes.[9, 27-30] The impact of pre-transplant CMV serostatus was determined on transplant related outcomes. While CMV seropositive recipients showed a trend toward greater day 100 TRM (p=0.07), CMV serostatus was not associated with aGVHD (p=0.8), cGVHD (p=0.24), OS (p=0.55) or disease relapse (p=0.78) (Table 3).

*Impact of CMV reactivation on transplant associated outcomes for CMV seropositive recipients*

We next analyzed whether CMV reactivation impacted transplant related outcomes. CMV reactivation was not associated with TRM [RR=1.0 (95% CI 0.5-1.8), p=0.88], leukemia relapse [RR=1.2 (95% CI 0.6-2.1), p=0.62], or OS [RR=0.9 (95% CI 0.6-1.5), p=0.78]. In addition, CMV reactivation was not associated with the

development of aGVHD [RR=1.0 (95% CI 0.5-2.0), p=0.97] or cGVHD [RR=1.2 (95% CI 0.6-2.1), p=0.65] (Table 4).

CMV seropositive recipients were also assessed based on the number of CMV reactivation events post-transplant. Eighty patients had one reactivation and 12 patients had 2 reactivations. The number of CMV reactivations (1 vs. 2) was not predictive of progression free survival (p=0.60), TRM (p=0.31), or OS (p=0.31).

Of the CMV positive recipients, 13.9% (25/180) developed CMV disease involving the following sites: respiratory (n=16), GI (n=6) or multi-organ (n=3). Among patients with CMV disease, TRM was 48% (95% CI 27%-69%), aGVHD occurred in 17% (95% CI 0%-38%), cGVHD in 10% (95% CI 0%-22%) and OS was 43% (95% CI 23%-63%). Comparing patients with CMV disease to those who reactivated but did not develop disease, there was increased TRM [RR=3.9 (95% CI 1.4-11.2), p=0.01] and decreased overall survival [RR=2.4 (95% CI 1.1-5.2), p=0.02]. The relative risk of relapse [RR=1.3 (95% CI 0.4-3.9), p=0.62] and cGVHD [RR=0.6 (95% CI 0.1-2.6), p=0.51] were not significantly different between patients with CMV reactivation who did and did not develop CMV disease.

**Discussion**

We analyzed the impact of recipient CMV serostatus and the consequences of CMV reactivation on transplant associated outcomes using 332 patients with hematological malignancies undergoing myeloablative or RIC followed by transplantation with either one or two UCB units. When compared to CMV seronegative

patients, CMV seropositive recipients had similar outcomes. CMV reactivation did not alter TRM, GVHD, relapse or survival, however patients that developed CMV disease had higher TRM and lower OS, but relapse and cGVHD were unaffected.

Previous reports have found that CMV reactivation in the post-UCB setting varies between 21% and 100%.[11, 31] CMV reactivation post-bone marrow (BM) and peripheral blood (PB) transplant have ranged from 12.8% to 22% in study groups containing both CMV seronegative and seropositive donors and recipients and 52% to 69% in CMV seropositive donors and recipients.[11, 32-34] The rate of CMV reactivation in this study was 51% and there was no difference for patients undergoing RIC or myeloablative conditioning. We and others have shown that the rate of CMV reactivation after UCB transplantation is not different when compared to more traditional hematopoietic cell sources such as BM or PB.[11, 32-34]

To date, only five studies have focused on CMV reactivation following UCB transplantation.[3, 11, 31, 35, 36] The largest of these studies included 140 Japanese adults, all of whom received reduced intensity conditioning.[3] Similar to our findings, CMV reactivation (antigenemia) occurred in 55% of patients at a median of day +35. These investigators observed that a low $CD34^+$ cell dose was a risk factor for CMV reactivation. In contrast, we found no impact of either total mononuclear, $CD34^+$ or $CD3^+$ cell dose on CMV reactivation. Tomonari evaluated 101 Japanese adults, all of whom received myeloablative conditioning.[36] Sixty-five percent of these patients had CMV reactivation. There was no significant difference between CMV seronegative and

CMV seropositive patients with respect to TRM, aGVHD and cGVHD, leukemia relapse and OS, similar to our findings.

Our findings show that CMV seropositivity pre-transplant and CMV reactivation do not negatively impact transplant outcomes. This is in contrast to prior studies where UCB transplant recipients had inferior outcomes following CMV reactivation.[3, 8, 31, 37] Although in our study TRM was not significantly different between CMV seronegative and CMV seropositive recipients, CMV seropositive recipients did show a trend toward greater day 100 TRM ($p=0.07$). Curiously, CMV reactivation was not associated with higher TRM.

In this analysis, follow-up was limited to day +100 because patients are typically transitioned to their home institution at that time. As a result, data regarding CMV reactivation after this time point was not available. However, late CMV reactivation is increasingly being reported and a recent study found late CMV reactivation to be as high as 31% [38]. In addition, data for this study, including patient characteristics and outcomes, was collected prospectively, however the outcome analysis was done retrospectively. Perhaps a larger prospective study which captures late CMV reactivation events and progression to CMV disease could help determine whether the trend toward increased TRM among CMV seropositive recipients becomes significant with longer follow-up. However, a subset analysis of this study revealed very few CMV reactivation events beyond day +100 (not shown).

High dose acyclovir prophylaxis, as used in this study, has been shown to be equivalent to ganciclovir in preventing CMV antigenemia and disease while having a

lower side effect profile with respect to neutropenia and bacterial infections.[39] Prophylactic ganciclovir has toxicities such as myelosuppression and increased risk of infection.[33] Moreover, ganciclovir may cause delayed recovery of CMV-specific T-cell immunity resulting in increased late CMV disease.[40] Perhaps the use of high dose acyclovir prophylaxis, rather than ganciclovir, impacted the severity and consequence of CMV reactivation in our patients.

We found that CMV disease developed in 13.8% of CMV seropositive patients and among 27.1% of those who reactivated. These findings are similar to previous UCB transplant studies which have reported disease rates of 12% to 13% among seropositive UCB recipients and 23% to 29% for patients with CMV reactivation.[3, 11, 14] As would be expected, we found a lower OS and higher TRM among patients with CMV reactivation who experienced disease compared to those who did not develop CMV disease.

Previously investigators have found CMV to be associated with GHVD [3, 28, 29], and the immune suppression used for GVHD treatment may diminish graft vs. leukemia reactions. Interestingly, two recent studies showed a reduction in leukemia relapse rate in pediatric CMV seropositive donors and recipients, particularly in children where prophylaxis was omitted and only pre-emptive therapy was used.[37, 41] There was no association between CMV and GVHD or disease recurrence in our study however; we did not use the above approach.

Absolute lymphocyte count (ALC) at day +30 post-transplant is predictive of survival in patients with a number of malignant diseases including ALL, AML, myeloma,

Hodgkin's disease and Non-Hodgkin's lymphoma following autologous or allogeneic transplantation.[42-48] While studies have varied in their ALC cut-off and day of analysis, patients with low ALCs (<1.75-3.0 x$10^8$/L) early after transplant (day +21-30) showed inferior survival compared to patients with higher ALCs.[43-48] In a subsequent study, ALC <2.0 was found to be predictive of CMV infection in adult UCBT patients in univariate analysis.[33] In contrast, we found that an ALC <2.0 at day +28 was associated with a lower incidence of CMV reactivation (37% vs. 61%, p=0.02). However, the competing risk of death among patients with ALC <2.0 was much higher (27%) than that for patients with ALC ≥2.0 (4%).

KIRs are a polymorphic family of surface receptors expressed by NK cells and some T cells. Depending upon the individual receptor, KIR can either positively or negatively regulate lymphocyte activation and function. Individuals vary in the number of KIR genes contained within their genome and have been referred to as either haplotype A or B, depending upon the relative absence or presence of activating KIR, respectively.[49] Prior studies show that donor KIR haplotype may be associated with CMV reactivation. More specifically, recipients of HCT from a donor with a KIR B/X haplotype [50] or those that express KIR2DS2 [51] have a decreased incidence of CMV reactivation. In our study, donor KIR haplotype did not appear to influence recipient CMV reactivation (p=0.83), however KIR haplotype was only available for a subset of patients.

In summary, we could not identify a relationship between either CMV serostatus or reactivation following UCBT and transplantation outcomes. As well, the rate of CMV

reactivation in this study is similar to that reported for other HSC sources.[11, 32, 34]. Further studies are required to elucidate whether the trend toward greater day 100 TRM among CMV seropositive recipients following UCB transplant reaches significance, especially in the setting of late reactivation. Perhaps, current CMV prophylaxis and vigilant pre-emptive treatment strategies have reduced the historical significance of pretransplant CMV serostatus for most UCB transplant recipients, given the similar TRM, relapse, OS, aGVHD, and cGVHD. However, some patients still develop CMV disease, which continues to be associated with higher TRM and lower OS.

Table 1. Characteristics of all patients based on pre-transplant CMV exposure

| Variable | CMV positive recipient | CMV negative recipient | p |
|---|---|---|---|
| **Overall** | 180 (54%) | 152 (46%) | |
| **Conditioning Regimen and Number of UCB Units** | | | 0.07 |
| Myeloablative single (CY/FLU/TBI) | 25 (14%) | 16 (10%) | |
| Myeloablative single (CY/TBI/ATG) | 31 (17%) | 44 (29%) | |
| Myeloablative double (CY/FLU/TBI) | 66 (37%) | 45 (30%) | |
| RIC double (CY/FLU/TBI) | 58 (32%) | 47 (31%) | |
| **HLA disparity** | | | 0.74 |
| (engrafting unit) | | | |
| 4/6 | 79 (47%) | 65 (47%) | |
| 5/6 | 72 (43%) | 63 (46%) | |
| 6/6 | 16 (10%) | 10 (7%) | |
| **Age** | | | 0.07 |
| 0-10 | 29 (16%) | 38 (25%) | |
| 11-17 | 33 (18%) | 17 (11%) | |
| 18-35 | 48 (27%) | 33 (22%) | |
| 35+ | 70 (39%) | 64 (42%) | |
| **Gender** | | | 0.46 |
| Male | 103 (57%) | 93 (61%) | |
| Female | 77 (43%) | 59 (39%) | |
| **Diagnosis** | | | 0.67 |
| Acute leukemia | 125 (69%) | 104 (68%) | |
| Other leukemia/MDS | 16 (9%) | 18 (12%) | |
| Lymphoma | 34 (19%) | 28 (18%) | |
| Other malignancy | 6 (3%) | 2 (1%) | |
| **Disease Risk** | | | 0.04 |
| Standard | 70 (39%) | 43 (28%) | |
| High | 110 (61%) | 109 (72%) | |
| **GVHD Prophylaxis** | | | 0.01 |
| CSA/MMF | 149 (83%) | 108 (71%) | |
| CSA/methylprednisone/ATG | 31 (17%) | 44 (29%) | |

Standard risk disease = acute leukemia CR1, MDS, CML CP1

Table 2. Incidence of CMV reactivation (in pre-transplant CMV seropositive patients) through day 100

| Variable | All | # events | Day 100 CMV reactivation (95% CI) | Median days to CMV reactivation (range) | p |
|---|---|---|---|---|---|
| **Overall** | 180 | 92 | 51% (43-59%) | 40 (9-95) | |
| **Type** | | | | | 0.33 |
| Full single (CY/FLU/TBI) | 25 | 9 | 36% (17-55%) | 40 (18-56) | |
| Full single (CY/TBI/ATG) | 31 | 14 | 45% (27-63%) | 33.5 (11-56) | |
| Full double (CY/FLU/TBI) | 66 | 35 | 53% (40-66%) | 39 (9-95) | |
| RIC double (CY/FLU/TBI) | 58 | 34 | 59% (45-73%) | 43.5 (12-83) | |
| **HLA disparity** (engrafting unit) | | | | | 0.22 |
| 4/6 | 79 | 45 | 57% (45-69%) | 39 (9-95) | |
| 5/6 | 72 | 33 | 46% (34-58%) | 38 (12-82) | |
| 6/6 | 16 | 9 | 56% (30-82%) | 45 (12-83) | |
| **Age** | | | | | 0.04 |
| 0-10 | 29 | 11 | 38% (20-56%) | 38 (12-55) | |
| 11-17 | 33 | 14 | 42% (25-59%) | 32 (9-95) | |
| 18-35 | 48 | 25 | 52% (36-68%) | 43 (18-84) | |
| 35+ | 70 | 42 | 60% (47-73%) | 43 (12-82) | |
| **Gender** | | | | | 0.57 |
| Male | 103 | 51 | 50% (40-60%) | 38 (9-84) | |
| Female | 77 | 41 | 53% (41-65%) | 43 (11-95) | |
| **Disease Risk** | | | | | 0.48 |
| Standard | 70 | 35 | 50% (38-62%) | 44 (9-95) | |
| High | 110 | 57 | 52% (42-64%) | 39 (11-83) | |
| **TNC (total x $10^7$/kg)** | | | | | 0.75 |
| 1st Quartile | 44 | 22 | 50% (34-66%) | 42.5 (12-70) | |
| 2nd Quartile | 46 | 24 | 52% (36-68%) | 43.5 (9-83) | |
| 3rd Quartile | 43 | 23 | 53% (37-69%) | 34 (19-95) | |
| 4th Quartile | 47 | 23 | 49% (34-64%) | 38 (12-77) | |
| **CD34 (total x $10^5$/kg)** | | | | | 0.12 |
| 1st Quartile | 40 | 17 | 43% (27-59%) | 44 (28-76) | |
| 2nd Quartile | 39 | 23 | 59% (42-76%) | 39 (11-82) | |
| 3rd Quartile | 40 | 19 | 48% (32-64%) | 46 (9-84) | |
| 4th Quartile | 42 | 25 | 60% (34-76%) | 34 (12-95) | |
| **CD3 (total x $10^5$/kg)** | | | | | 0.82 |
| 1st Quartile | 35 | 16 | 46% (30-62%) | 35.5 (11-56) | |
| 2nd Quartile | 41 | 24 | 59% (42-75%) | 39 (19-76) | |
| 3rd Quartile | 42 | 22 | 52% (46-68%) | 43 (9-83) | |
| 4th Quartile | 41 | 22 | 54% (37-71%) | 36.5 (12-95) | |

| | | | | | |
|---|---|---|---|---|---|
| **ALC (total x $10^8$/L)** | | | | | 0.02* |
| <2.0 | 60 | 22 | 37% (25-49%) | 45 (22-83) | |
| ≥2.0 | 100 | 61 | 61% (50-72%) | 38 (9-95) | |
| Missing | 20 | 9 | 15% (0-38%) | 40 (12-56) | |

*p-value only compares non-missing groups

Table 3. Univariate analysis of pre-transplant CMV serostatus and transplant associated outcomes

| **Variable**<br><br>CMV serostatus at transplant | **All** | **# events** | **1 year (range)** | **p** |
|---|---|---|---|---|
| **Overall survival** | | | | 0.55 |
| Negative | 152 | 53 | 65% (57-72%) | |
| Positive | 180 | 67 | 63 (55-69%) | |
| **Disease relapse** | | | | 0.78 |
| Negative | 152 | 43 | 28% (21-35%) | |
| Positive | 180 | 51 | 28% (21-35%) | |
| **Transplant related mortality** | | | | 0.07 |
| Negative | 152 | 24 | 16% (10-22%) | |
| Positive | 180 | 43 | 24% (18-30%) | |
| **Acute GVHD** (Grade II-IV) | | | **Day 100** | 0.80 |
| Negative | 152 | 77 | 51% (43-59%) | |
| Positive | 180 | 84 | 47% (39-55%) | |
| **Chronic GVHD** | | | | 0.24 |
| Negative | 152 | 30 | 19% (13-25%) | |
| Positive | 180 | 42 | 21% (15-27%) | |

Table 4. CMV reactivation and transplant related outcomes

| Variable | All | # events | 1 year (range) | Relative Risk (95% CI) | p |
|---|---|---|---|---|---|
| **No CMV reactivation** | | | | 1.0 | |
| **CMV reactivation** | | | | | |
| Overall survival | 92 | 32 | 64% (53-73%) | 0.9 (0.6-1.5) | 0.78 |
| Disease relapse | 89 | 27 | 31% (21-41%) | 1.2 (0.6-2.1) | 0.62 |
| Transplant related mortality | 89 | 20 | 30% (20-40%) | 1.0 (0.5-1.8) | 0.88 |
| Acute GVHD (Grade II-IV) | 56 | 12 | **Day 100** 22% (11-33%) | 1.0 (0.5-2.0) | 0.97 |
| Chronic GVHD | 89 | 20 | 22% (18-30%) | 1.2 (0.6-2.1) | 0.65 |

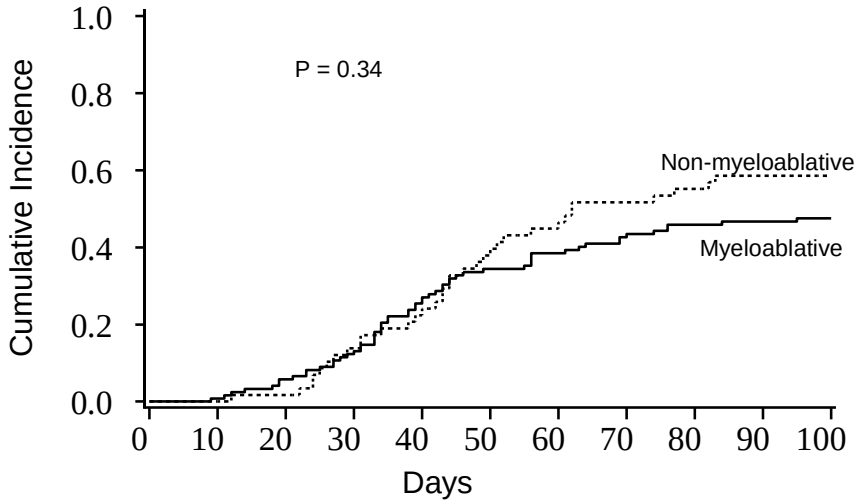


**Figure 1. Cumulative incidence of CMV reactivation by conditioning regimen.** There is no significant difference between the myeloablative and non-myeloablative conditioning.

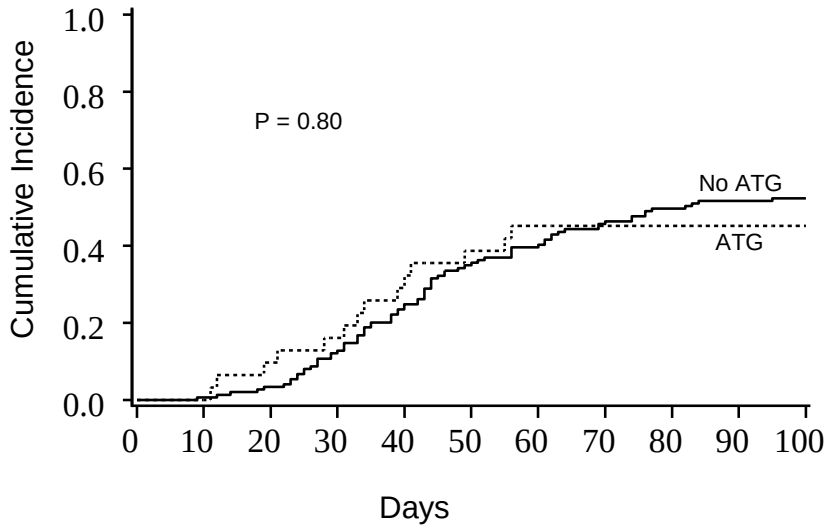


**Figure 2. Cumulative incidence of CMV reactivation by GVHD prophylaxis regimen.** There is no significant difference between the ATG containing regimen (CSA/methylprednisone/ATG) and the non-ATG containing regimen (CSA/MMF).

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