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(Article begins on next page)

# **A Phase I/II Study of Stem Cell Transplantation Using a Single Cord Blood Unit Expanded Ex vivo with Nicotinamide**

## **Running Title: Nicotinamide-Expanded Single UCB Transplantation**

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## **Abstract**

### **Purpose**

Increasing the number of hematopoietic stem and progenitor cells within an umbilical cord blood (UCB) graft shortens the time to hematopoietic recovery following UCB transplantation. In this study, we assessed the safety and efficacy of an UCB graft that was expanded *ex vivo* in the presence of nicotinamide and transplanted following myeloablative conditioning as a stand-alone hematopoietic stem cell graft.

### **Patients**

Thirty-six patients with hematologic malignancies were transplanted at 11 sites.

### **Results**

The cumulative incidence of neutrophil engraftment at day 42 was 94%. Two patients experienced secondary graft failure attributable to viral infections. Hematopoietic recovery was compared to that observed in recipients of standard UCB transplantation as reported to the CIBMTR (n=146). The median time to neutrophil recovery was 11.5 days (95%CI:9-14 days) for recipients of nicotinamide-expanded UCB and 21 days (95%CI:20-23 days) for the comparator ( $p<0.001$ ). The median time to platelet recovery was 34 days (95%CI:32-42 days) and 46 days (95%CI:42-50 days) for the expanded and the comparator cohorts, respectively ( $p<0.001$ ). The cumulative incidence of grade II-IV acute GVHD at day 100 was 44%, and grade III/IV acute GVHD at day 100 was 11%. The cumulative incidence at 1-year of all chronic GVHD was 41%, and moderate/severe chronic GVHD was 10%. The 1-year cumulative incidences of non-relapse mortality and relapse were 24% and 27%, respectively. The 1-year probabilities of overall and disease-free survival were 51% and 49%, respectively.

### **Conclusion**

UCB expanded ex-vivo with nicotinamide shortens median neutrophil recovery by 9.5 days (95%CI: 7-12 days) and platelet recovery by a median 12 days (95%CI:3-17 days). This trial establishes feasibility, safety and efficacy of an ex-vivo expanded UCB unit as a stand-alone graft.

## **Introduction**

Despite remarkable improvement in outcomes of adult recipients of umbilical cord blood (UCB) transplantation, slow hematopoietic recovery continues to be the major limitation of this approach. Stemming from this delay in hematopoietic recovery are other disadvantages of UCB transplantation such as increased risk for infection, prolonged hospitalization and increased resource utilization. Early phase, single center studies have demonstrated that ex vivo expansion of UCB stem cells prior to transplantation has the potential to address this critical shortcoming. By expanding both hematopoietic stem and progenitor cells, the time to neutrophil recovery following myeloablative conditioning can be even more rapid than that following a mobilized peripheral blood stem cell graft.<sup>1-4</sup>

NiCord is an ex vivo expanded cell product derived from the CD133+ fraction of banked UCB that utilizes nicotinamide as the active agent that inhibits differentiation and enhances the functionality of cultured hematopoietic stem and progenitor cells. When nicotinamide is added to stimulatory hematopoietic cytokines, UCB-derived hematopoietic progenitor cell cultures demonstrate an increased frequency of phenotypically primitive CD34<sup>+</sup>CD38<sup>-</sup> cells and a substantial increase in bone marrow homing and engraftment potential of ex vivo expanded CD34<sup>+</sup> cells.<sup>5</sup> The ability of nicotinamide to expand both committed and long-term repopulating hematopoietic stem cells was confirmed in a first in human pilot study of NiCord. In this study, a second unmanipulated UCB unit was co-infused with the NiCord expanded unit to maintain patient safety. With long term follow-up, stable NiCord-derived hematopoiesis has now been observed for over 7 years. Based on these results, we conducted a multicenter, phase I/II study of NiCord transplanted as a single, expanded UCB graft following myeloablative conditioning.

## **METHODS**

### **Patient Eligibility**

Eligible patients were 12-65 years old with high-risk hematologic malignancies and no readily available matched sibling or matched unrelated adult donor.

The CIBMTR provided historical data on 1037 patients receiving UCB transplantation between 2010 and 2013. A cohort of patients was selected with characteristics as similar as possible to the phase I/II patients; selections for disease status, age, cell count, HLA-matching, and performance score criteria, resulted in a CIBMTR sample size of 146.

Accrual to the study occurred between 2014 and 2017. Of the 58 patients who enrolled in the trial, 10 became ineligible during the pre-transplant work-up and 5 withdrew due to logistical issues surrounding graft production. Forty-three patients were allocated to treatment on study. Seven of the 43 patients were not evaluable due to NiCord production complications. These patients underwent UCB transplantation with either an unmanipulated cord blood graft, or a combination of NiCord plus an unmanipulated cord blood graft.

The study was approved by the Institutional Review Boards of all participating institutions and the national regulatory authorities. All patients provided written informed consent. The study was performed in accordance with the International Conference on Harmonization Guidelines and Good Clinical Practice. Study registration; ClinicalTrials.gov NCT01816230.

### **Graft Selection**

To be eligible for participation in the study, patients were required to have an available cord blood unit matched at 4-6/6 HLA class I (HLA-A & HLA-B, low resolution) and class II (HLADRB1, high resolution) loci. The UCB unit was required to have pre-cryopreserved dose of  $\geq 8.0 \times 10^6$  CD34+ total cells as well as a pre-cryopreserved total nucleated cell dose (TNC) of

$\geq 1.8 \times 10^9$  delivering  $\geq 1.8 \times 10^7$  TNC/kg. The UCB unit must have been volume reduced and red blood cell depleted prior to cryopreservation.

Patients were required to have available an additional partially HLA-matched cord blood unit as a backup in case the expanded product did not pass the required quality control tests. The backup cord blood unit was required to be HLA-matched at 4-6/6 HLA class I (HLA-A & HLA-B, low resolution) and class II (HLA-DRB1, high resolution) loci with the patient, and have a pre-cryopreserved, total nucleated cell dose of at least  $2.5 \times 10^7$  TNC/kg.

### **NiCord (Gamida Cell) Production**

The NiCord-designated cryopreserved unit was delivered from the cord blood bank to a cGMP-compliant cell processing facility (Lonza, Maryland USA or Gamida Cell, Jerusalem Israel).

NiCord was manufactured as previously described.<sup>3</sup> Briefly, the unit underwent immunomagnetic bead selection for CD133 positive cells. The CD133 negative, T-cell-containing flow-through fraction was retained and re-cryopreserved. The CD133 positive fraction was cultured for  $21 \pm 2$  days. CD34<sup>+</sup> and CD3<sup>+</sup> cell content of the graft, as reported in this manuscript, was quantified prior to cryopreservation of the product.

### **Conditioning Regimens and Graft versus Host Disease Prophylaxis**

Three alternative myeloablative conditioning regimens were permitted for study participants. All dosing was based on 25% adjusted ideal body weight unless otherwise noted. Regimen A consisted of total body irradiation 1350 centigray delivered in 8 or 9 fractions on days -9 to -6 or -5, and either cyclophosphamide, 60 mg/kg given per institutional practice on days -4 and -3 or thiotepa 5 mg/kg administered over 4 hour IV infusion on days -11 and -10.<sup>6-8</sup> The third agent in

regimen A was fludarabine 40mg/m<sup>2</sup> given over 1 hour on days -5 to -2 when paired with thiotepa or 25 mg/m<sup>2</sup> on days -8 to -6 when paired with cyclophosphamide.

Regimen B consisted of thiotepa 5 mg/kg administered over 4 hour IV infusion on days -7 and -6, busulfan 3.2 mg/kg administered over 3 hour IV infusion on days -5 through -3 and fludarabine 50 mg/m<sup>2</sup> administered over 60 minute IV infusion on days -5 through -3.

Regimen C consisted of clofarabine 30mg/m<sup>2</sup> administered over 1 hour IV infusion on days -5 through -2, fludarabine 10mg/m<sup>2</sup> administered over 60 minute IV infusion on days -5 through -2 and busulfan using weight based dosing as per Bartelink et al. on days -5 through -2.<sup>9</sup>

GVHD prophylaxis was provided by a calcineurin inhibitor (tacrolimus or cyclosporine) and mycophenolate mofetil starting four days prior to transplantation. Mycophenolate mofetil was continued for a minimum of 60 days and the calcineurin inhibitor for minimum of six months following transplantation.

### **Supportive Care**

Granulocyte-colony stimulating factor (5µg/kg recipient body weight) was given daily starting on day +1 following transplantation until the absolute neutrophil count exceeded 1000 cells/µl. Anti-viral and anti-fungal prophylaxis was administered at the discretion of the transplant center. Anti-bacterial prophylaxis for the first 100 days following transplantation was required by protocol. The agent used was left to the discretion of the transplant center.

### **Laboratory and Clinical Assessments**

Donor chimerism was performed by the local transplant center on whole blood, CD15+ myeloid, and CD3+ T cells using quantitative analysis of informative microsatellite DNA sequences. Quantitative assessment of CD3, CD4, CD8, NK and B-cell recovery was performed on a subset of patients by the local transplant center (or designated referral laboratory) 2 months, 3 months, 6



months and 1 year following transplantation. The time to neutrophil engraftment was defined as the first of three consecutive measurements on different days with an absolute neutrophil count of  $0.5 \times 10^9/L$  or higher, and the time to platelet engraftment as the first of three consecutive measurements with a platelet count of  $20 \times 10^9/L$  or higher without platelet transfusion in the preceding 7 days.

### **Statistical Considerations**

Analysis was limited to the 36 patients transplanted with NiCord as a stand-alone graft. Database closure was on 16NOV2017. The trial follows patients for 365 days post-transplantation; longer follow-up is available from an observational follow-up study.

The primary endpoint was the cumulative incidence of neutrophil engraftment at 42 days with  $\leq 10\%$  host cells and the incidence of secondary graft failure. To facilitate comparison with CIBMTR data, engraftment without chimerism was evaluated here.

Competing risks for engraftment were death, progression/relapse, and second transplant; for GVHD were death, primary and secondary graft failure, and progression/relapse; for non-relapse mortality was progression/relapse; and for progression/relapse was death.

Due to differences in the age distribution between the phase I/II study and the retrospective cohort, unadjusted and age-adjusted cumulative incidence curves were calculated; age-adjusted curves for the CIBMTR cohort were weighted by the proportion of patients in the phase I/II trial in age-strata  $\leq 18$ , 19-39 and  $\geq 40$  years. Comparison graphs are provided showing the weighted cumulative incidence. If the last observed time in the NiCord group did not extend to the comparison time point, the last observed cumulative incidence is carried forward to the cohort comparison time point. Calculations of standard errors for the unadjusted and adjusted cumulative incidence confidence intervals were based on the Aalen and delta method, respectively. Differences between times to engraftment were tested using a Van Elteren test

stratified on age groups for the days to the event. For these tests, patients not engrafting were assigned a time to event larger than any patient with an event. Median time to an event is calculated among those with an event, with 95% confidence intervals based on confidence interval calculations for rank statistics. Confidence intervals (95%) for the difference between median times to engraftment were estimated using the non-parametric bootstrap.

SAS 9.4, Stata 15, R Studio, and R3.3.1 or higher were used for these analyses.

## **RESULTS**

### **Patient and Stem Cell Transplant Characteristics**

Characteristics of the 36 patients transplanted with NiCord as a stand-alone graft are described in Table 1. Eleven centers in the United States, Europe and Asia (Singapore) enrolled patients on the study.

### **Graft Characteristics**

Characteristics of the NiCord graft prior to and following expansion are shown in Figure 1. The median total CD34+ cell content of the cord blood unit as reported by the cord blood bank prior cryopreservation and NiCord expansion was  $0.13 \times 10^8$  (range  $0.8-0.25 \times 10^8$ ). Following NiCord expansion, the CD34 content of the graft increased by 33-fold to a median  $4.5 \times 10^8$  (range  $1.6-13.1 \times 10^8$ ) CD34+ cells. This resulted in a median CD34+ cell dose of  $6.3 \times 10^6/\text{kg}$  (range  $1.4-14.9 \times 10^6/\text{kg}$ ). The CD3+ T-cells in the NiCord graft were contained solely in the unexpanded, CD133 negative fraction.

CD3+ T-cell content of the negative fraction was a median of  $2.0 \times 10^8$  (range  $0.7-14.0 \times 10^8$ ) resulting in a median CD3+ content of  $2.4 \times 10^6/\text{kg}$  (range  $0.7-24.0 \times 10^6/\text{kg}$ ).

### **Hematopoietic Recovery**

The age-adjusted cumulative incidence of neutrophil engraftment at 42 days following transplantation was 94% for NiCord recipients and 85% for the CIBTMR comparator cohort (Figure 2A). By 21 days following transplantation, 89% of NiCord recipients had achieved neutrophil engraftment. Neutrophil engraftment was faster for NiCord recipients ( $p < 0.001$ ). Among patients who engrafted, the median time to neutrophil recovery was 11.5 days (95% CI: 9-14 days) for NiCord recipients and 21 days (95% CI: 20-23 days) for the CIBMTR comparator cohort. The age-adjusted cumulative incidence of platelet engraftment at 100 days following transplantation was 81% for NiCord recipients, and 63% for CIBMTR comparator cohort (Figure 2B). Platelet engraftment was faster among NiCord recipients ( $p < 0.001$ ). For patients achieving platelet recovery, the median time to platelet recovery was 34 days (95% CI: 32-42 days) and 46 days (95% CI: 42-50 days) for NiCord and CIBMTR comparator cohort, respectively.

Whole blood chimerism was available for 26 patients at 100 days following transplantation. Twenty-five patients (97%) had  $\geq 95\%$  donor, and one had 57% donor whole blood chimerism. Lineage-specific myeloid and T-cell chimerism was available in a subset of patients ( $n = 22$ ) at day 100. Twenty patients had  $> 90\%$  donor chimerism in both fractions. Two patients had mixed chimerism at day 100; one was 57% in the myeloid fraction and 3% in the T-cell fraction and the other was 100% in the myeloid fraction and 10% in the T-cell fraction.

One patient experienced primary graft failure. Two patients experienced secondary graft failure; one occurring at day 19 concurrent with high titer human herpes virus 6 viremia and the second occurring at 262 concurrent with a lethal adenovirus infection.

### **Graft versus Host Disease**

The cumulative incidence of grade II-IV and grade III-IV acute GVHD at day 100 was 44% (95% CI, 28%-60%) and 11% (95% CI, 3%-24%; Figure 3AB), respectively. The cumulative

incidence of chronic GVHD at one year was 41% (95% CI, 24%-57%). The cumulative incidence of moderate to severe chronic GVHD was 10% (95% CI, 2%-24%; Figure 3CB).

### **Non-relapse mortality, Relapse, Survival**

The median follow-up of surviving patients is 14 months (range 5-36 months). The 1-year cumulative incidence of non-relapse mortality was 24% (95% CI, 11%-40%; Figure 4A). The 1-year cumulative incidence of relapse was 27% (95% CI, 13%-43%; Figure 4B). The 1-year probability of overall survival was 51% (95% CI, 33%-67%), and disease-free survival was 49% (95% CI, 31%-65%; Figure 5).

### **Transplant Course and Toxicity**

Primary hospital discharge occurred at a median of 20 days (range 0-61 days) following transplantation. Recipients of the NiCord graft spent a median of 73 days alive and out of hospital during the first 100 days following UCB transplantation. NiCord infusion was well tolerated, with hypertension reported as the most common adverse event attributable to NiCord infusion. One grade 3 hypertension and one grade 2 hypersensitivity reaction were attributed to NiCord infusion. Of the 16 subjects who died, 8 (50%) were attributable to relapsed disease, 5 (31%) to infection, 2 (13%) to GVHD and 1 (6%) to organ failure.

### **Immune reconstitution**

Lymphoid immune recovery was monitored in a subset of 27 patients following transplantation of NiCord. Figure 6 demonstrates the CD3, CD4, CD8, CD19 and NK cell recovery during the first 12 months following transplantation.

## **DISCUSSION**

NiCord is an ex-vivo expanded UCB graft designed specifically to address the limitations arising from low hematopoietic stem and progenitor cell dose and resultant delayed engraftment times seen in adult UCB transplantation recipients. We show that transplantation of NiCord is safe, is effective in reducing the time to hematopoietic recovery, and does not require co-infusion of a second unmanipulated UCB unit.

The use of dual UCB grafts has vastly expanded the accessibility of UCB transplantation to adult patients who lack an adequately sized single UCB graft.<sup>7,8</sup> However, the problem of delayed hematopoietic recovery was not addressed by this technique. Dual UCB transplantation also provided the ideal platform for clinical development of modified cord blood grafts. The initial strategies of UCB expansion were designed as a temporary bridge to what was expected to be prolonged, durable hematopoiesis by a second unmanipulated cord blood unit, co-infused with the expanded unit. Delaney and colleagues were the first to demonstrate that transplantation of UCB stem cells, expanded for 16 days in the presence of Delta 1 Notch ligand, resulted in a median 10-day reduction in time to neutrophil recovery compared to conventional dual UCB transplantation.<sup>1</sup>

NiCord was designed to be a stand-alone graft and differed from the preceding expanded UCB products in that the T-cell fraction from the unit was retained and re-cryopreserved prior to culture. This important difference allowed NiCord the potential to become the dominant unit following co-infusion with an unmanipulated cord blood unit.<sup>3</sup> Wagner and colleagues subsequently reported results of a phase I/II dual UCB transplant study where one unit was expanded in the presence of StemRegenin-1.<sup>4</sup> They too co-infused T-cells recovered prior to culture, allowing for long-term persistence of the expanded graft.

This study the first to show that an expanded UCB unit can be infused as a stand-alone graft and is capable of providing robust, durable hematopoiesis. One patient (3%) experienced primary graft failure, a rate well below the graft failure rate following stem cell transplantation

from bone marrow grafts.<sup>10</sup> However, a larger sample size is required to confirm the potential for NiCord to reduce the risk of primary graft failure. Two patients experienced secondary graft failure. While stem cell exhaustion cannot be completely ruled out, high titer adenovirus and human herpes virus 6 infections are the most plausible explanation for these events.

The median time to neutrophil recovery is 20 days following myeloablative HLA-identical allogeneic bone marrow transplantation, and 15 days following HLA-identical mobilized peripheral blood stem cell (PBSC) transplantation.<sup>10</sup> This 5-day reduction in time to neutrophil recovery for PBSC recipients translated into a significant reduction of bacterial infections during the first 100 days following transplantation.<sup>11</sup> The median time to neutrophil recovery following myeloablative haploidentical peripheral blood stem cell transplantation using post-transplant cyclophosphamide as GVHD prophylaxis is 16-19 days.<sup>12,13</sup> Transplantation of NiCord as a single, ex vivo expanded UCB graft results in an estimated median time to neutrophil recovery of 11.5 days (95% CI: 9-14 days). In a separate study, Anand and colleagues compared the infection risk in standard myeloablative UCB transplant recipients to those transplanted with NiCord at Duke University. NiCord recipients engrafted a median 13.5 days quicker than standard UCB transplant recipients which translated into a clear reduction in the incidence bacterial infections (RR, 0.39; P=0.003).<sup>14</sup>

Rapid hematopoietic recovery also contributes to fewer days spent in the hospital. Using a large cohort of patients from the CIBMTR, Ballen and colleagues showed that compared to adult bone marrow or PBSC transplantation, patients receiving standard myeloablative UCB transplantation spent the fewest days alive and out of the hospital during the first 100 days following transplantation (Bone marrow-69 days, PBSC- 75 days, dual UCB- 55 days). NiCord recipients in this study spent a median of 73 days alive and out of hospital during the first 100 days, a number comparable to that of PBSC transplantation.

UCB transplantation has a 30-year track record of providing a hematopoietic stem cell transplant option for patient without an available matched adult donor. Many adult recipients require two UCB units to ensure reliable engraftment. However, the addition of a second unit significantly increases the expense of the transplant, is associated with delayed platelet recovery and a higher incidence of chronic GVHD.<sup>18</sup> This study suggests that NiCord obviates the need for a second UCB graft. NiCord paves the way for use of smaller, better matched units for adult patients that otherwise could not be used due to excessive risk of graft failure. The study demonstrates the feasibility of an ex vivo expanded hematopoietic stem cell product manufactured in a centralized cell processing facility and distributed internationally to 3 continents. It is hypothesized that the ongoing prospective multi-center, phase III registration clinical trial comparing NiCord to standard myeloablative UCB transplantation will provide confirmation of the findings presented in this study.

## **Acknowledgments**

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## **Authorship**

Contribution: M.H., G.S. contributed to the design of the trial, enrolled patients on the trial, reviewed data, interpreted results and jointly drafted the manuscript. J.K. contributed to the design of the trial. D.C., D.V., F.F., J.B., S.N., M.J., J.W., J.K., L.K., N.M., P.S., R.H., W.H., P.M. enrolled patients on the trial and reviewed the manuscript. S.W., B.B., L.F. interpreted results and helped draft the manuscript. All study authors had full access to the data and had final responsibility for the decision to submit for publication.

Conflict of Interest: The authors declare no competing financial interests.

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## Figure Legends

**Figure 1, NiCord Graft Characteristics.** Median (range) Total nucleated cell content, median (range) CD34+ cell content and median (range) CD34+ cell dose are shown prior to and following ex-vivo expansion of the umbilical cord blood unit. Pre-expansion values represent cell content as reported by the cord blood bank prior to cryopreservation of the umbilical cord blood unit.

**Figure 2, Hematopoietic Recovery.** Cumulative incidence of neutrophil by day 42 (A) and platelet recovery by day 100 (B) among recipients of NiCord and a comparable retrospective cohort from the Center for International Blood and Marrow Transplant Research

**Figure 3, Acute and Chronic Graft versus Host Disease.** Cumulative incidence of grade II-IV (A) and grade III-IV (B) acute GVHD (C) mild/moderate/severe chronic GVHD and (D) moderate/severe chronic GVHD following transplantation with NiCord.

**Figure 4, Non-relapse Mortality and Relapse.** Cumulative incidence of non-relapse mortality (A) and relapse (B) following transplantation with NiCord

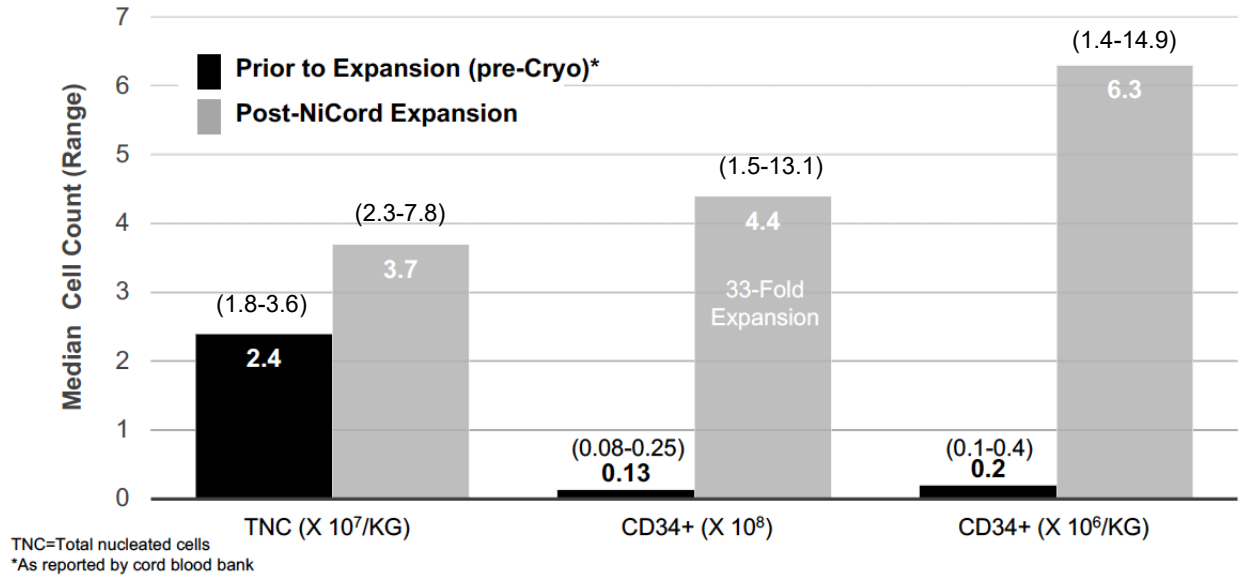
**Figure 5, Disease-free and Overall Survival.** Kaplan-Meier estimate of disease-free and overall survival following transplantation with NiCord

**Figure 6, Immune Reconstitution.** Quantitative recovery of CD3 (A), CD4 (B), CD8 (C), CD19 (D) and NK-cell (E) measured at day 70, day 100, 6-months and 1-year following transplantation with NiCord.

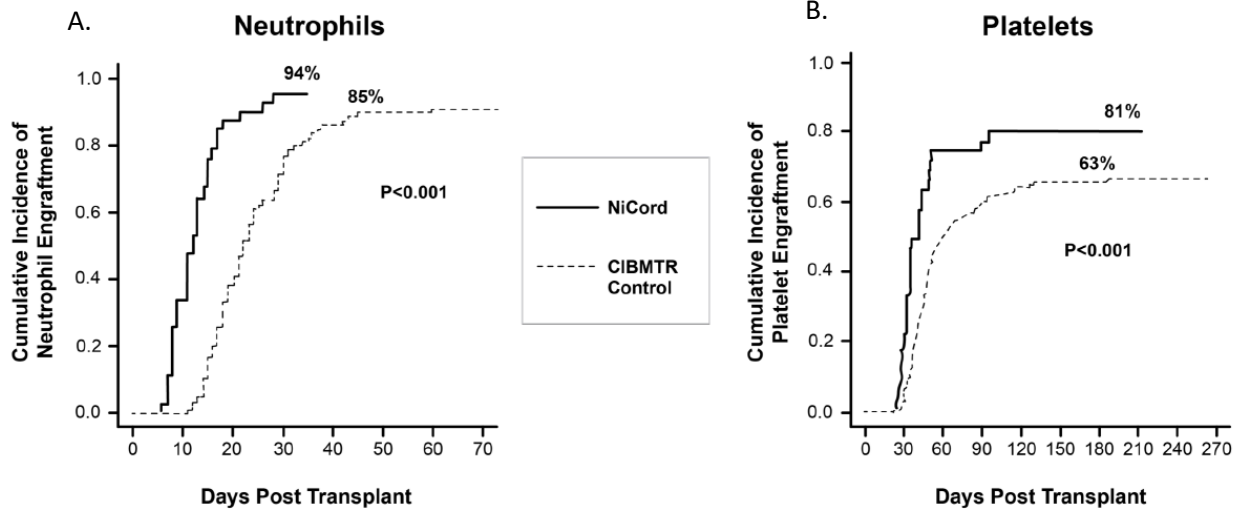
**Table 1. Patient Characteristics**

		N	%
Number of Patients		36	100.0
Gender	Male	20	55.6
	Female	16	44.4
Age (years)	Median: 44 (range 13-63)	.	.
Primary Diagnosis	ALL	9	25.0
	AML	17	47.2
	MDS	7	19.4
	Non-Hodgkin's Lymphoma	0	0.0
	Hodgkin's Disease	1	2.8
	CML	2	5.6
Adjusted Disease Risk Index <sup>19</sup>	Low	8	22.2
	Intermediate	15	41.7
	High	13	36.1
Performance Status	100	13	36.1
	90	16	44.4
	80	6	16.7
	70	1	2.8
Patient CMV Status	Positive	25	69.4
	Negative	10	27.8
	Indeterminate	1	2.8
HLA Match Score	4/6	26	72.2
	5/6	8	22.2
	6/6	2	5.6
Conditioning Regimen	Regimen A (TBI 1350, Fludarabine, Cyclophosphamide/Thiotepa)	13	36.1
	Regimen A.1 (TBI 1320, Fludarabine, Cyclophosphamide)	2	5.6
	Regimen B (Thiotepa, Busulfan, Fludarabine)	19	52.8
	Regimen C (Clofarabine, Fludarabine, Busulfan)	2	5.6
Weight at enrollment	Median 75kg (Range 42-127kg)	.	.

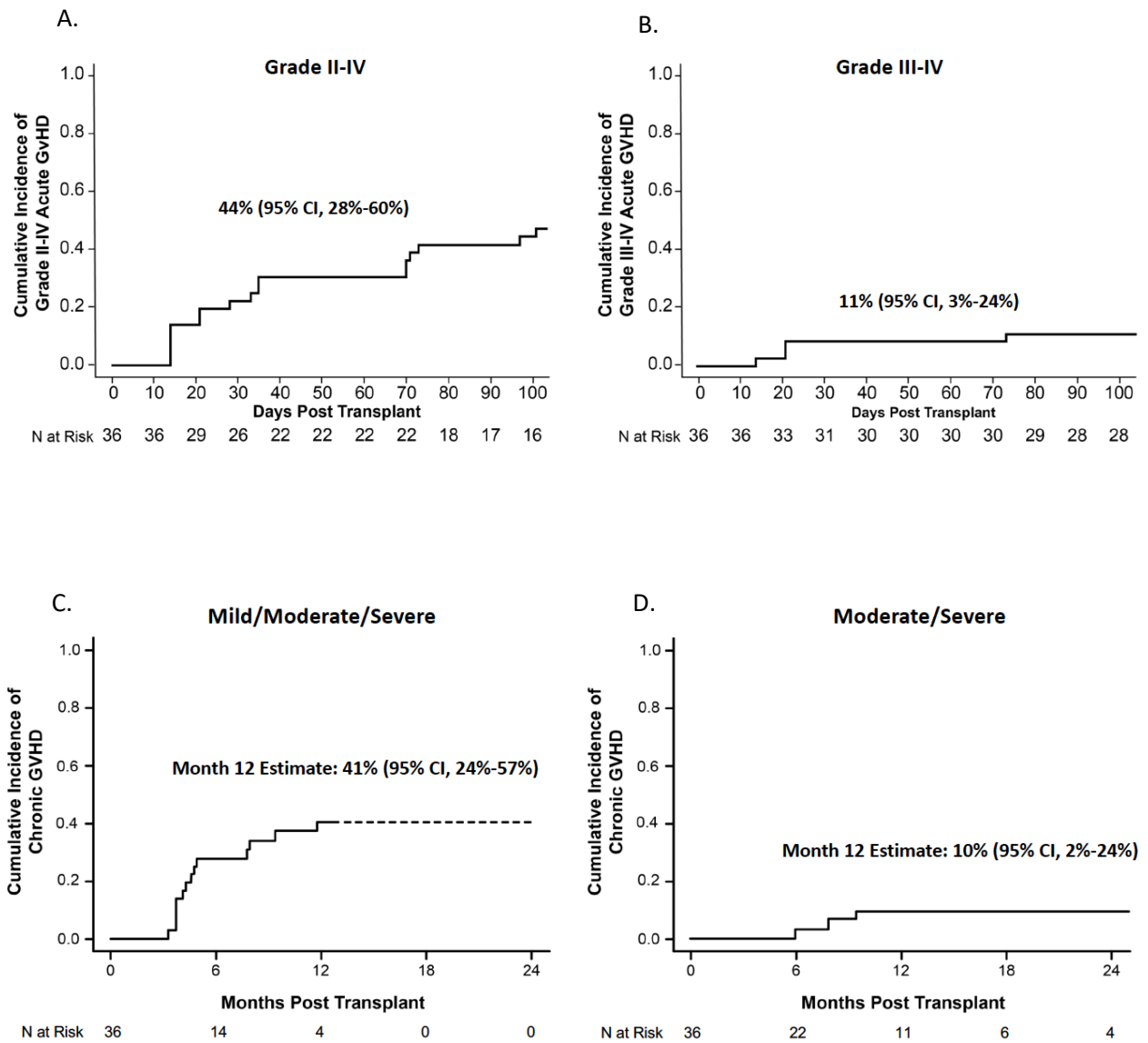
**Figure 1. NiCord Graft Characteristics**



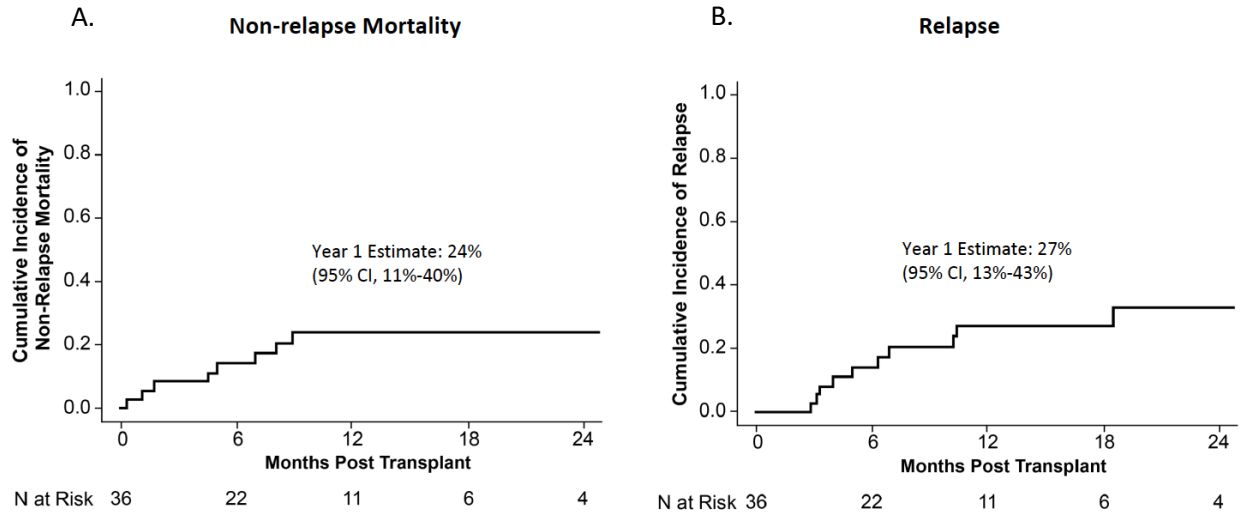
**Figure 2. Neutrophil and Platelet Engraftment**



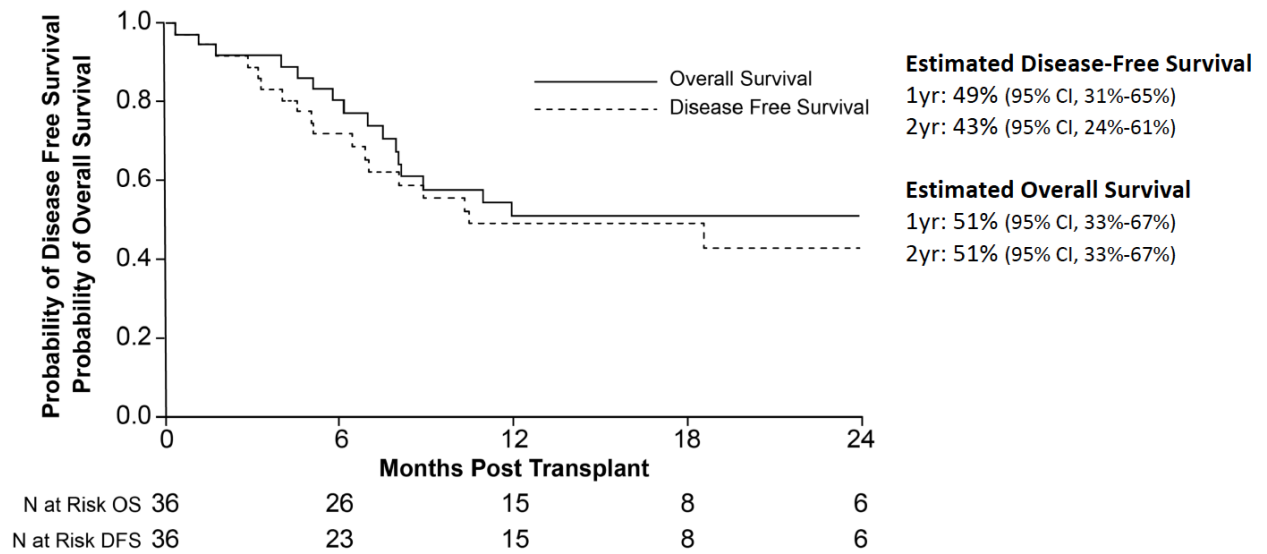
**Figure 3. Acute and Chronic Graft versus Host Disease**



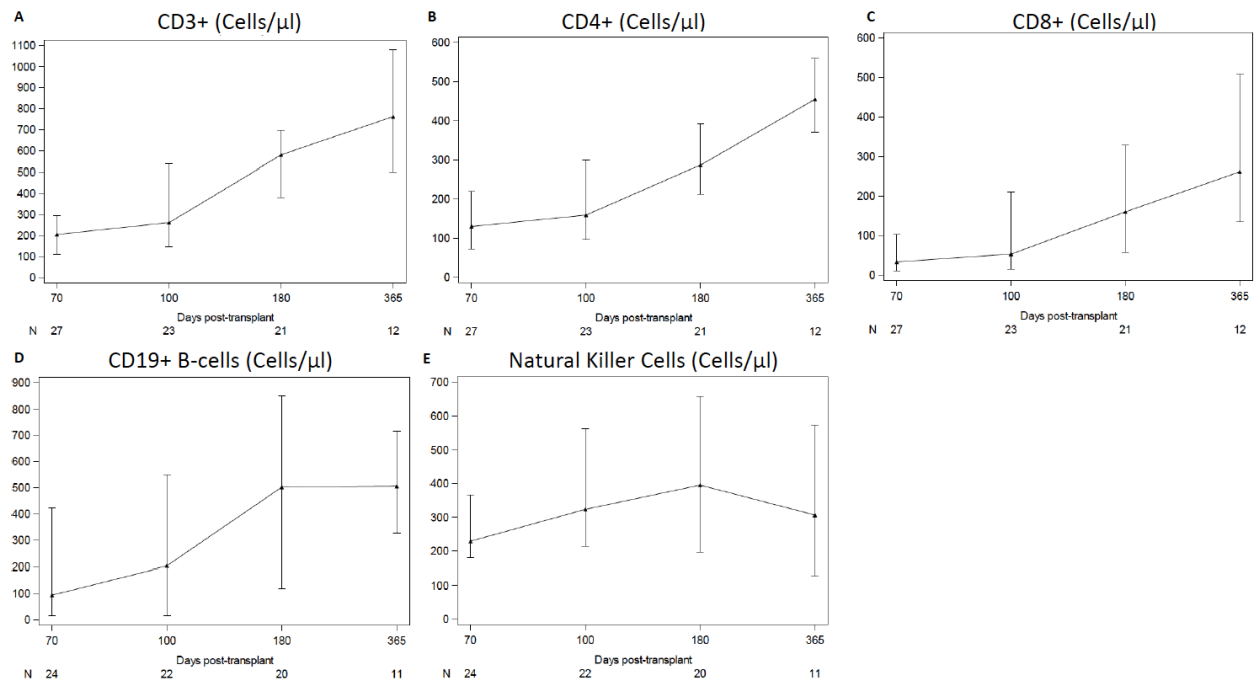
**Figure 4. Non-relapse Mortality and Relapse**



**Figure 5. Disease-free and Overall Survival**



**Figure 6. Quantitative Lymphocyte Recovery following NiCord Transplantation**





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