Title: **Impact of route and adequacy of nutritional intake on outcomes of allogeneic hematopoietic cell transplantation for hematologic malignancies**

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Short running head: Nutrition and allogeneic stem cell transplantation

Abbreviations

ANS, artificial nutrition support

BM, bone marrow

BMI, body mass index

CI, confidence interval

CMV, cytomegalovirus

EBMT, European Blood and Marrow Transplantation

EN, enteral nutrition

ETF, enteral tube feed

GRFS, graft versus host disease-free and relapse-free survival

GvHD, graft-versus-host disease

HCT-CI, hematopoietic cell transplant co-morbidity index

HR, hazard ratio

NRM, non-relapse mortality

OR, odds ratio

PBSC, peripheral blood stem cells

PN, parenteral nutrition

Abstract

Background: Allogeneic hematopoietic cell transplantation (HCT) is often associated with poor oral intake due to painful mucositis and gastrointestinal sequalae that occur following a preparative regimen of intensive chemotherapy and/or total body radiation. Although attractive to assume that optimal nutrition improves HCT outcomes, there are limited data to support this. It is also unclear whether artificial nutrition support should be provided as enteral tube feeding or parenteral nutrition (PN).

Methods: We analysed day-100 non-relapse mortality (NRM), incidence of acute graft-versus-host disease (GvHD), acute gastrointestinal GvHD, 5-year survival and GvHD-free/relapse-free survival (GRFS) according to both route and adequacy of nutritional intake prior to neutrophil engraftment, together with other known prognostic factors, in a retrospective cohort of 484 patients who underwent allogeneic HCT for hematologic malignancy between 2000 and 2014.

Results: Multivariate analyses showed increased NRM with inadequate nutrition (hazard ratio (HR) 4.1; 95% confidence interval (CI) 2.2–7.2) and adequate PN (HR 2.9; 95% CI 1.6–5.4) compared to adequate enteral nutrition (EN) both $P<.001$. There were increased incidences of gastrointestinal GvHD of any stage and all GvHD ≥ grade 2 in patients who received PN (odds ratio (OR) 2.0; 95% CI 1.2–3.3; $P=.006$, and OR 1.8; 95% CI 1.1–3.0; $P=.018$, respectively), compared to adequate EN. Patients who received adequate PN and inadequate nutrition also had reduced probabilities of survival and GRFS at 5 years.

Conclusion: Adequate EN during the early transplantation course is associated with reduced NRM, improved survival and GRFS at 5 years. Furthermore, adequate EN

is associated with lower incidence of overall and gut acute GvHD than PN, perhaps because of its ability to maintain mucosal integrity, modulate the immune response to intensive chemo/radiotherapy and support the gastrointestinal tract environment, including gut microflora.

Key words*:* Allogeneic stem cell transplant, survival, graft-versus-host-disease, enteral nutrition, parenteral nutrition, non-relapse mortality, hematologic malignancy, artificial nutrition support.

Introduction

The side effects of allogeneic (donor) hematopoietic cell transplantation (HCT) frequently impair the ability of patients to consume an adequate diet. Patients receive intensive conditioning that may include high dose chemotherapy with or without total body irradiation, that can result in significant mucositis and other gastrointestinal sequelae. Oral intake declines rapidly in the first eight days after HCT and many patients consume less than 60% of their estimated energy requirements during this time (1). As a result nutritional status declines from transplant admission to discharge and this does not fully recover when assessed soon after discharge (2).

Although it might seem obvious that optimal nutrition is likely to improve outcomes of HCT, the data to support this are extremely limited. The best way in which to support the nutritional intake of HCT recipients is also unclear. Some patients are able to maintain an adequate nutrient intake by consuming a diet higher in energy and

protein. However, for some, particularly those receiving myeloablative conditioning, artificial nutrition support (ANS) will be required. Historically, parenteral nutrition (PN) has been widely used in transplant recipients experiencing significant gastrointestinal toxicities. It is well established that oral intake and enteral tube feeding (ETF) are more physiological and associated with less metabolic and infectious risks than PN. Moreover there may be particular benefits of EN for the HCT recipient, via maintenance of gut mucosal integrity and in supporting the gastrointestinal tract environment, including gut microflora, that can be impaired during HCT (3;4) Alterations in gut microflora have recently been implicated in the development of graft versus host disease (GvHD) (5-7), which is associated with significant morbidity and mortality following donor HCT.

In this study we analysed day-100 non-relapse mortality (NRM), incidence of acute graft-versus-host disease (GvHD) of any site, acute GvHD of the gastrointestinal tract, 5-year survival and GvHD-free/relapse-free survival (GRFS) after HCT according to both the route and adequacy of nutritional intake using a cohort of consecutive patients who underwent allogeneic HCT from a peripheral blood or bone marrow donor in a single institution.

Subjects and methods

Study cohort

All patients aged 17 or above who underwent their first HCT for hematologic malignancy at Hammersmith Hospital, using a sibling or unrelated donor between

January 2000 and December 2014 were eligible. Umbilical cord blood transplants and HLA haploidentical transplants were excluded.

Ethics

All patients were treated on institutional review board–approved protocols or standard treatment protocols and gave consent in accordance with the Declaration of Helsinki of 1975 as revised in 1983.

Nutritional support

At our centre, all allogeneic HCT patients are routinely reviewed early in their transplant admission by a specialist dietitian as a standard of their transplant care. All patients receiving myeloablative regimens are advised to have an enteral feeding tube inserted routinely after establishing good control of the emetogenic effects of the conditioning regimen and prior to development of mucositis. Any patient experiencing symptoms that impact their oral intake are referred by nursing and medical staff for more regular assessment by the specialist dietitian. Nutritional status is assessed from daily measurements of weight and body mass index (BMI) relative to pre-treatment weights. Adjusted dry weights are estimated (8) when signs of fluid accumulation are evident clinically or if there are unlikely short term weight gains. Energy and protein requirements are estimated using predictive formulae based on age, gender, physical activity and metabolic factors (9-11).

In all patients the criteria for initiation of ANS are: (a) patients' actual or anticipated oral intake below 1/3 of estimated requirements for 5 days or below 2/3 of estimated requirements for 10 days, (b) if 10% weight loss of pre-transplant weight, or (c) where significant weight loss with BMI less than 18 kg/ m2 occurred. Our preferred

method of ANS is ETF, but when ETF is not feasible or not tolerated by a patient, PN is recommended. PN is also recommended where there are overt signs of malabsorption of enteral nutrition e.g. intractable diarrhoea or vomiting. When indicated, ANS is introduced at a low rate and increase to tolerance over the first few days hence ANS of less than 4 days is considered unlikely to have been effective.

In the current study, nutritional support between the date of hospital admission for HCT and the date of neutrophil engraftment (recovery) was reviewed and recorded for each patient. All patients with established ANS were classified as requiring either ETF, PN or both ETF and PN during some, or all of the time to engraftment. Patients that did not receive either modality, or received it for less than 4 days, were designated as having oral intake.

During data collection it became apparent that, firstly there were low numbers of patients that successfully received enteral tube feeding, therefore oral intake and tube feeding patients were grouped together to form an enteral nutrition group. Secondly a number of patients defaulted to the “oral intake” group due to a lack of access or tolerance to ANS, rather than due to their ability to eat adequately. For the same reasons, some ANS episodes started late or terminated early. In order to isolate the effect of poor nutritional intake within each modality, overall nutritional intakes were categorised as either broadly adequate or clearly inadequate. For patients on oral intake alone, this was considered adequate unless there was a documented unmet need for ANS, according to our above stated criteria, for 4 or more days. ANS episodes were considered adequate if they started as planned and ended due to a successful transition to oral intake or an alternative method of support.

Using a combination of the route and adequacy of nutritional intakes, subjects were categorised into three nutrition groups: 1. **Adequate enteral nutrition** – patients who maintained an adequate nutritional intake either orally or those that also required 4 or more days of ETF. 2. **Adequate parenteral nutrition** – patients that achieved adequate nutritional intakes during the period that included 4 or more days of PN. 3. **Inadequate nutrition** - those with inadequate oral intake and a documented unmet need for ANS.

Statistics

Follow-up data were available on all patients. The main endpoints of the study were 5 year survival, GvHD-free/relapse-free survival (GRFS), NRM, defined as death without previous relapse/progression at 100 days after the date of hematopoietic cell infusion; incidence of acute GvHD at any site (grade II or above) and acute GvHD of the gut of any grade. Acute GvHD was graded according to standard criteria and events in GRFS included grade 3-4 acute GvHD, systemic therapy-requiring chronic GvHD, relapse, or death (12). All patients were considered assessable for acute GvHD after day +1 from the hematopoietic cell infusion, however, patients who did not survive to day 100 and did not have acute GvHD were excluded from the acute GvHD analyses. Neutrophil engraftment was defined as absolute neutrophil count not lower than 1000/microL for 3 consecutive days. Route and adequacy of nutritional intake groups were compared using the Chi-squared or Mann-Whitney test as appropriate. The Kaplan-Meier method was used to produce survival and GFRS curves, with groups compared using with the log-rank test. Variables with P-values <0.20 were entered into stepwise Cox-regression analyses to find the best models. Cumulative incidence curves for non-relapse mortality were constructed in

the competing risks framework considering relapse as the competing event. Differences between cumulative incidence curves were tested using the Gray method, and factors with P < .20 in univariate analysis, were entered into a multivariate regression analysis using the Fine and Gray model with a forward stepping procedure. Event data for grade 2–4 acute GvHD and gut acute GvHD were described as simple proportions, with groups compared using the Chi-squared test and logistic regression analysis with a forward stepping procedure being utilised to find independent prognostic factors. Statistical analyses were performed with IBM SPSS Statistics 24.0 and R version 3.2.2 (the CRAN project; www.cran.r-project.org). Our pre-declared endpoints for this study were GvHD incidence and severity and early (transplant-related) mortality and survival. The decision to include 5 year survival data was made post hoc. This is an accepted measure of cure within the HCT setting and was included to allow comparability with interventions in other studies. It must be noted that since not pre-specified, the 5-year analyses should be considered exploratory. All statistical tests were two sided, and P < .05 was used to indicate statistical significance.

This study has not been registered as clinical trial. Participants were not prospectively allocated to an intervention hence criteria for registration was not met.

Results

Patient characteristics

We identified 512 patients who met the inclusion criteria. We excluded 26 patients for whom there was no detailed information on nutritional support and a further 2 patients who died within 3 days of transplantation in whom nutritional intake

adequacy could not be evaluated. The remaining 484 patients were included in the analyses. A total of 245 (51%) patients had adequate enteral nutrition (EN) either orally (N = 198) or with use of ETF (N = 47). Patients in whom ETF could not be established for the required time, received PN (N = 148, 31%) in order to provide adequate nutrition. The remaining 91 (19%) patients had inadequate nutrition due to either curtailed ANS (N = 33) or a failure to start ANS because of a lack of feeding access via any route (N = 58). **Figure 1** indicates the route of intake for the study patients and their classification into nutritional group and **Table 1** shows the exact length of feeding episodes between the dates of transplant and engraftment, categorised according to route and adequacy of nutritional intake.

Figure 1. Flow chart to determine the route and adequacy of nutritional intake between hematopoietic cell transplant and neutrophil engraftment.

Subjects were categorised into three nutrition groups: 1. **Adequate enteral nutrition (EN)** – patients who maintained an adequate nutritional intake either orally or those that also required 4 or more days of enteral tube feeding (ETF). 2. **Adequate parenteral nutrition (PN)** – patients that achieved adequate nutritional intakes during the period that included 4 or more days of PN. 3. **Inadequate nutrition** - those with a documented unmet need for artificial nutrition support (ANS) for 4 or more days before engraftment.

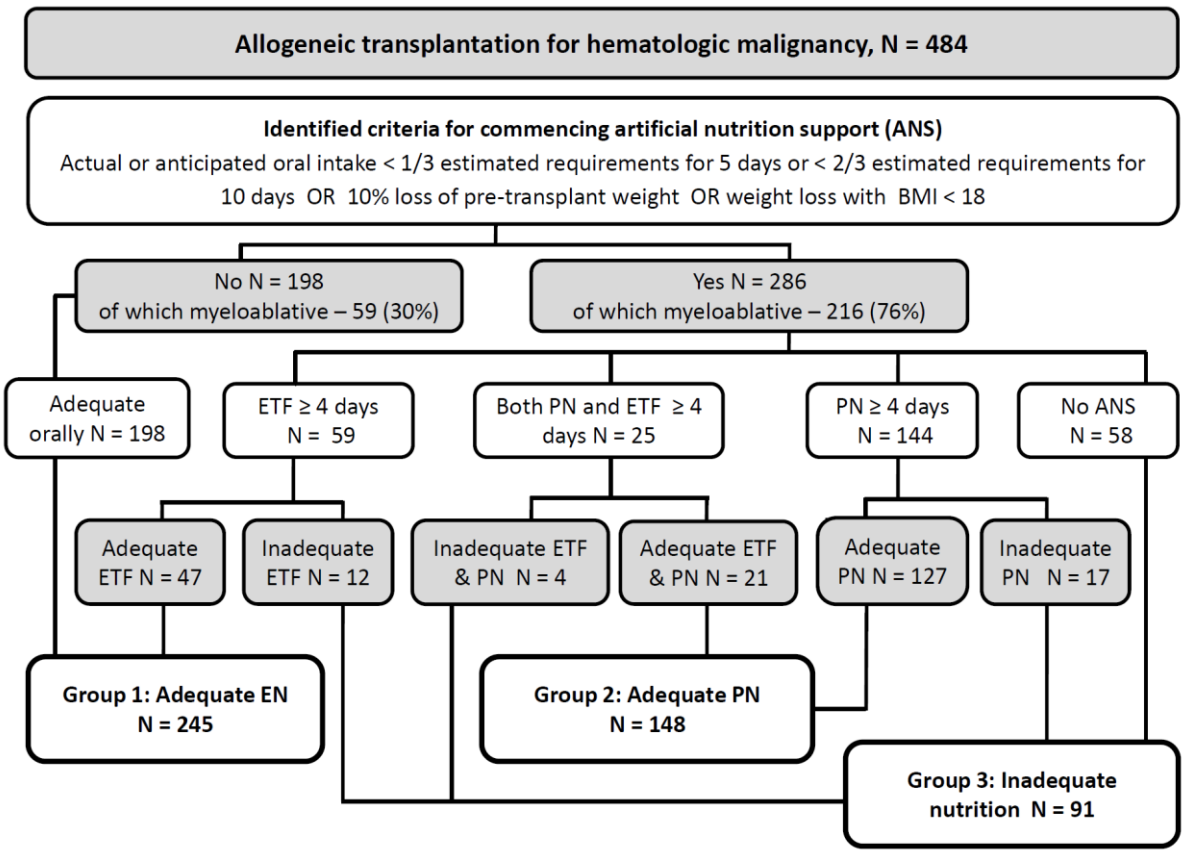


Table 1. Length of feeding episodes between transplant and neutrophil engraftment categorised according to route and adequacy of nutritional intake

	N	ETF, median days (range)	PN, median days (range)	Days to engraftment, median (range)
Adequate EN:				
Oral intake alone	198	0 (0 – 3)	0 (0 – 3)	19 (7 – 42)
With established ETF	47	12 (4 – 61)	0 (0 – 3)	20 (10 – 35)
OVERALL adequate EN	245	0 (0 – 61)	0 (0 – 3)	19 (7 - 42)
Adequate PN:				
With established PN	127	0 (0 – 3)	16 (4 – 68)	21 (11 – 38)
With established PN and ETF	21	7 (2 – 18)	15 (4 – 22)	23 (15 – 47)
OVERALL adequate PN	148	0 (0 – 18)	16 (4 – 68)	22 (11 – 47)
Inadequate nutrition:				
Oral intake alone	58	0 (0 – 3)	0 (0 – 3)	21 (11 – 34)
ETF given	12	7 (4 - 49)	0 (0 – 3)	19 (12 – 30)
PN given	17	0 (0 – 3)	8 (4 - 25)	22 (13 – 32)
ETF and PN given	4	16 (15 – 20)	12 (5 – 37)	32 (30 – 36)
OVERALL inadequate nutrition	91	0 (0 – 49)	0 (0 - 37)	21 (11 – 36)

EN, enteral nutrition; ETF, enteral tube feeding; PN, parenteral nutrition.

Overall, 285 (59%) of patients received myeloablative conditioning and 199 (41%) reduced intensity conditioning (RIC), as defined by the European Blood and Marrow Transplantation (EBMT) criteria (13). The characteristics of the study population, donors, and transplants according to nutritional group are summarised in **Table 2**.

196 **Table 2. Patient and transplant characteristics according to category of**

197 **nutritional route and adequacy**

Variable	All (%)	Adequate EN (%)	Adequate PN (%)	Inadequate nutrition (%)	*P*
All	484	245 (51)	148 (31)	91 (19)	-
Age group (years)					
Younger than 20	10 (2)	3 (1)	5 (3)	2 (2)	.001
20 to 40	212 (44)	87 (36)	84 (57)	41 (45)	
41 to 60	227 (47)	134 (55)	53 (36)	40 (44)	
Older than 60	35 (7)	21 (9)	6 (4)	8 (9)	
Gender					
Male	305 (63)	149 (61)	101 (68)	55 (60)	.29
Female	179 (37)	96 (39)	47 (32)	36 (40)	
Diagnosis					
Acute leukaemia	158 (33)	73 (30)	57 (39)	28 (31)	< .001
CML	186 (38)	81 (33)	70 (47)	35 (38)	
Lymphoma & CLL	83 (17)	56 (23)	12 (8)	15 (16)	
MDS & MPN	37 (8)	27 (11)	4 (3)	6 (7)	
Myeloma	20 (4)	8 (3)	5 (3)	7 (8)	
EBMT disease risk					
Early	229 (47)	110 (45)	75 (51)	44 (48)	.24
Intermediate	139 (29)	70 (29)	47 (32)	22 (24)	
Late	116 (24)	65 (27)	26 (18)	25 (28)	
BMI (kg/m^2)					
Underweight (less than 20)	36 (7)	15 (6)	14 (10)	7 (8)	.042
Healthy (20 – 24.9)	181 (37)	90 (37)	63 (43)	28 (31)	
Overweight (25 – 30)	216 (45)	121 (49)	50 (34)	45 (49)	
Obese (over 30)	51 (11)	19 (8)	21 (14)	11 (12)	

Donor match					
Matched sibling	248 (51)	136 (56)	56 (38)	56 (62)	.002
Matched unrelated	182 (38)	86 (35)	71 (48)	25 (28)	
Mismatched unrelated	54 (11)	23 (9)	21 (14)	10 (11)	
Conditioning					
Myeloablative	285 (59)	98 (40)	131 (89)	56 (62)	< .001
Reduced intensity	199 (41)	147 (60)	17 (12)	35 (39)	
Previous autograft					
No	437 (90)	219 (89)	139 (94)	79 (87)	.16
Yes	47 (10)	26 (11)	9 (6)	12 (13)	
Patient / Donor Sex					
Other combination	384 (80)	188 (77)	124 (84)	72 (79)	.28
Male / Female	99 (20)	56 (23)	24 (16)	19 (21)	
Missing data	*1 (<1)*				
Patient CMV serology					
Positive	202 (42)	100 (41)	71 (48)	31 (34)	.13
Negative	277 (57)	142 (58)	77 (52)	58 (64)	
Missing data	*5 (1)*				
Donor CMV serology					
Positive	238 (49)	109 (44)	82 (55)	47 (52)	.11
Negative	238 (49)	131 (53)	64 (43)	43 (47)	
Missing data	*8 (2)*				
Cells infused					
PBSC	326 (67)	184 (75)	88 (59)	54 (59)	.004
BM	155 (32)	59 (24)	59 (40)	37 (41)	
PB + BM	3 (1)	2 (1)	1 (1)	0 (0)	
CD34+ cells infused					
Less than 4.00 x 10^6	115 (24)	40 (16)	46 (31)	29 (32)	< .001
More than 3.99 x 10^6	313 (65)	173 (71)	87 (59)	53 (58)	
Missing data	*56 (12)*				

Era (years)					
2000 – 2004	199 (41)	82 (33)	70 (47)	47 (52)	.007
2005 – 2009	138 (29)	78 (32)	42 (28)	18 (20)	
2010 – 2014	147 (30)	85 (35)	36 (24)	26 (29)	
HCT-CI					
0-1	250 (51)	130 (57)	70 (50)	50 (58)	.17
2-3	148 (31)	75 (33)	55 (39)	18 (21)	
More than 3	56 (12)	22 (10)	16 (11)	18 (21)	
Missing data	*30 (6)*				

BM, bone marrow; CLL, chronic lymphocytic leukaemia; CML, chronic myeloid leukaemia; CMV, cytomegalovirus; EBMT, European Blood and Marrow Transplantation Society; EN, enteral nutrition; HCT-CI, HCT comorbidity index; MDS, myelodysplastic syndromes; MPN, myeloproliferative neoplasms; PB, peripheral blood; PBSC, peripheral blood stem cells; PN, parenteral nutrition.

Nutritional intake and non-relapse mortality

The probability of NRM for the whole cohort was 14.7% (95% confidence interval (CI): 12 – 18). The effects of nutritional group on NRM were initially studied together with other patient, disease and transplant factors in univariate analyses; significant results of which are summarised in **Supplementary Table 1**.

Subsequent multivariate analysis, showed significantly increased NRM in the adequate PN (HR 2.9; 95% confidence interval (CI) 1.6 – 5.4) and inadequate nutrition (HR 4.1; 95% CI 2.2 – 7.2) groups compared to those with adequate EN (all $P < .001$, **Table 3**, **Figure 2**(A)). HRs for NRM were also significantly associated with age, category 40-60 years (HR 1.9; 95% CI 1.1 – 3.1; $P = .026$) and > 60 years (HR 3.1; 95% CI 1.5 – 6.8; $P = .004$) compared to those < 40 years old, previous

autograft (HR 2.4; 95% CI 1.3 – 4.5; *P* = .007) and positive recipient cytomegalo virus (CMV) serology (HR 1.8; 95% CI 1.1 – 3.1; *P* = .028).

Nutritional intake and acute GvHD

There were 439 and 438 evaluable cases respectively for acute GvHD grade II or greater and gastrointestinal acute GvHD of any grade after exclusion of patients that died before day 100 without acute GvHD. Acute grade II or greater GvHD was observed in 179 (41%) patients and any gastrointestinal acute GvHD was documented in 153 (35%) patients. After univariate analyses (significant results summarised in Supplementary Table 1) the effects of nutritional intake were studied in multivariate analyses as summarised in Table 3: There were increased incidences of both acute GvHD ≥ grade 2 and gastrointestinal GvHD of any stage in patients who received PN (odds ratio (OR) 2.0; 95% CI 1.2 – 3.3; *P* = .006, and OR 1.8; 95% CI 1.1 – 3.0; *P* = .018, respectively), compared to adequate EN. Other significant covariates in the model for increased risk of both overall acute GvHD ≥ grade 2 and gut GvHD were the use of myeloablative conditioning versus RIC (OR 0.5; 95% CI 0.3 – 0.7; *P* = .001 and OR 0.4; 95% CI 0.3 – 0.7; *P* < .001, respectively) and female donor to male recipient versus other combinations (OR 1.7; 95% CI 1.0 – 2.7; *P* = .047 and OR 1.8; 95% CI 1.1 – 3.0; *P* = .025, respectively).

235 **Table 3. Factors significantly associated in multivariate analyses of NRM, acute GVHD, survival and GRFS**

	NRM at 100d (N = 479)			Acute GvHD grade 2-4 (N = 438)			Gut acute GvHD any grade (N = 437)			Survival at 5yrs (N = 454)			GRFS at 5yrs (N = 454)		
	N	HR (95% CI)	*P*	N	OR (95% CI)	*P*	N	OR (95% CI)	*P*	N	HR (95%CI)	*P*	N	HR (95%CI)	*P*
Nutritional intake group															
Adequate EN	242	1.00		231	1.00		231	1.00		227	1.00		227	1.00	
Adequate PN	148	2.9 (1.6 – 5.4)	< .001	132	2.0 (1.2 - 3.3)	.006	131	1.8 (1.1 - 3.0)	.018	141	1.6 (1.2-2.1)	.003	141	1.6 (1.3-2.1)	< .001
Inadequate (all routes)	89	4.1 (2.2 – 7.2)	< .001	75	1.3 (0.7 - 2.2)	.38	75	1.3 (0.7 - 2.3)	.39	86	1.7 (1.2-2.3)	.003	86	1.6 (1.2-2.1)	.004
Recipient age (years)															
Younger than 40	212	1.00				NS			NS	204	1.00				NS
40-60	229	1.9 (1.1 - 3.1)	.026							212	1.5 (1.2-2.0)	.003			
At least 60	38	3.1 (1.5 - 6.8)	.004							38	2.3 (1.5-3.7)	< .001			
Previous autograft															
No	434	1.00				NS			NS	408	1.00				NS
Yes	45	2.4 (1.3 - 4.5)	.007							46	1.6 (1.1-2.3)	.019			
Recipient CMV															
Negative	202	1.00				NS			NS			NS			NS
Positive	277	1.8 (1.1 - 3.1)	.028												
Recipient / Donor Sex															NS
Other combination			NS	353	1.00		352	1.00				NS			
Male / Female				85	1.7 (1.0 - 2.7)	.047	85	1.8 (1.1 - 3.0)	.025						
Conditioning regimen															NS
Myeloablative			NS	262	1.00		262	1.00				NS			
Reduced intensity				176	0.5 (0.3 – 0.7)	.001	175	0.4 (0.3 - 0.7)	< .001						
EBMT Disease Risk															
Early			NS			NS			NS	215	1.00		215	1.00	
Intermediate										125	1.5 (1.1-2.1)	.009	125	1.7 (1.3-2.3)	.019
Late										114	19 (1.3-2.6)	< .001	114	2.0 (1.5-2.7)	< .001
HCT-CI															
0-1			NS			NS			NS	250	1.00		250	1.00	
2-3										148	1.4 (1.1-1.9)	.012	148	1.3 (1.0-1.7)	.024
More than 3										56	2.2 (1.5-3.1)	< .001	56	1.8 (1.3-2.5)	.001

CI, confidence interval; CMV, cytomegalovirus; EBMT, European Blood and Marrow Transplantation; EN, enteral nutrition; GRFS, graft versus host disease-free and relapse-free survival; GvHD, graft-versus-host disease; HCT-CI, hematopoietic cell transplant co-morbidity index; NRM, non-relapse mortality; NS, not statistically significant; PBSC, peripheral blood stem cells; PN, parenteral nutrition.

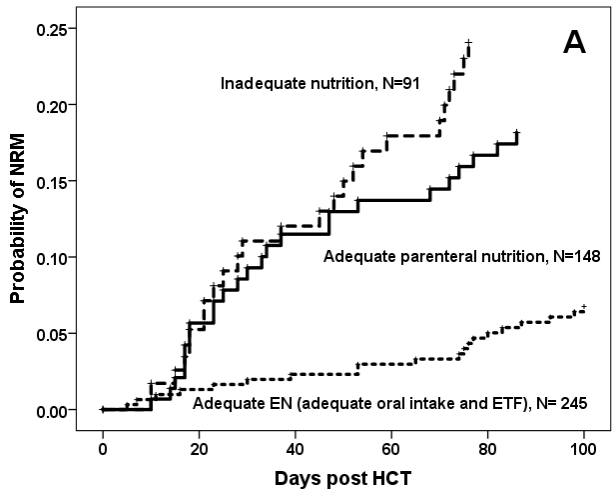
Association of nutritional intake with survival and GRFS at 5 years

The probability of survival at 5 years for the whole cohort was 49% (95% CI: 45 – 54). Statistically significant factors associated with survival in univariate analyses are summarised in Supplementary Table 1. Multivariate analysis showed an increased risk of death in the adequate PN (HR 1.6; 95% CI 1.2 – 2.1, *P* = .003) and inadequate nutrition (HR 1.7; 95% CI 1.2 – 2.3. *P* = .003) groups compared to those with adequate EN (Table 3, Figure 2(B)) even when adjusted for other disease, patient and transplant factors.

The probability of GRFS was 34% (95% CI: 30 – 38) and similarly to survival, multivariate analysis performed after univariate analysis (Supplementary Table 1) showed lower GRFS associated with the adequate PN (HR 1.6; 95% CI 1.3 – 2.1, *P* < .001) and inadequate nutrition (HR 1.6; 95% CI 1.2 – 2.1, *P* = .004) groups compared to adequate EN (Table 3, Figure 2(C)).

Figure 2. Adjusted probabilities according to nutritional take group (from multivariate analyses shown in Table 3) of:

(A) non-relapse mortality at 100 days after hematopoietic cell transplant (HCT); (B) 5-year survival after HCT; (C) 5-year graft versus host disease-free and relapse-free survival (GRFS) after HCT. Lines represent nutritional intake group; solid lines – inadequate nutrition, dotted lines – adequate parenteral nutrition, dashed lines – adequate enteral nutrition (EN) comprising oral intake and enteral tube feeding (ETF).



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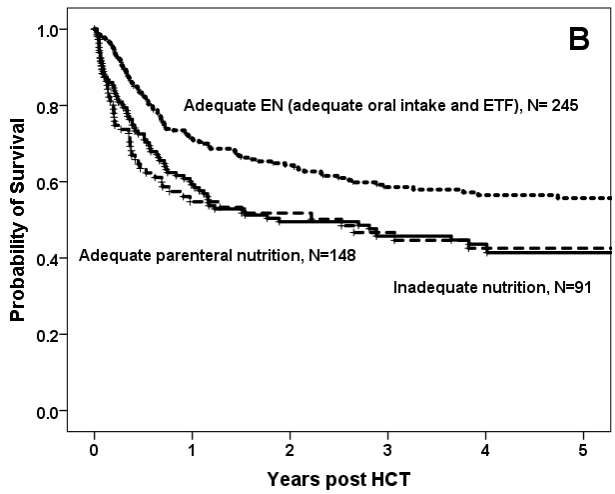

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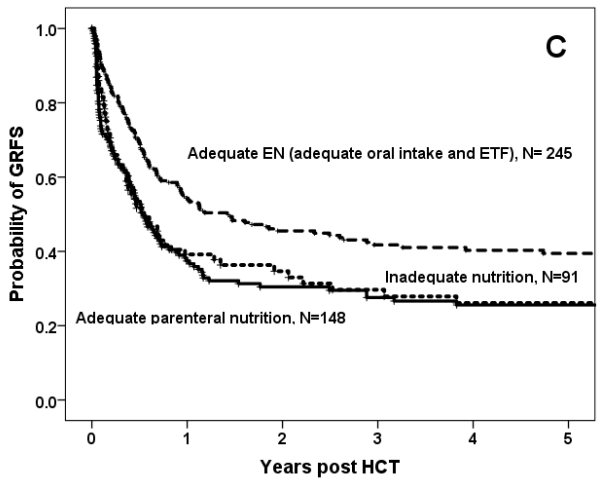

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Discussion

To our knowledge, this is the largest report, with the longest follow up to date, on the role of nutrition on outcomes of allogeneic transplantation for hematologic malignancy and the first to include assessment of nutritional adequacy. Although the 5-year analyses were post-hoc rather than pre-specified (and hence should be considered exploratory), data from this report potentially support two major conclusions. First, adequate EN during the early transplantation course was associated with reduced early mortality and improved survival and GRFS compared to adequate PN and inadequate nutrition. The probability of NRM for patients with adequate EN was 8.2% compared to 17.6% in those who received adequate PN and 27.5 % in patients with inadequate nutrition. Second, in line with previous studies (14-18), EN was associated with a reduced risk of acute GvHD compared to PN. Grade II to IV GvHD was observed in 32% and gut GvHD in 27% of patients who received adequate EN compared to 55% and 48% respectively of patients with adequate PN. These data provide further evidence for the clinical relevance of ANS as a potentially modifiable risk factor for both early and 5 year mortality.

Patients undergoing HCT struggle to consume an adequate diet at a time when requirements for nutrition are higher than usual and there is consensus that nutritional intakes should be optimised, including enteral and/or parenteral nutrition support where appropriate (19;20). These recommendations are supported by studies linking early mortality to nutritional status i.e. BMI recorded prior to HCT, although this may simply be a surrogate measure for disease severity (reviewed recently by Baumgartner *et al,* 2017) (21). More direct evidence in support of ANS has been missing. Assessments of weight and BMI are confounded by fluid

accumulation, which is common in the early post-transplantation period. This can, particularly in patients who received PN, potentially overstate their nutritional status.

A particular strength of our study is that we only used weight and BMI as parameters to identify the need for commencing ANS, whereas nutritional adequacy was assessed from the patient record. Any patient referred for oral or artificial nutritional support was under review by a specialist dietitian, hence inadequate intake by whatever route could be identified and be analysed separately from patients with good oral intake or effective ANS.

It is well established that EN may serve therapeutic roles beyond providing metabolic substrates, due to its trophic effects on the gut mucosa hence benefits in terms of bacterial translocation, systemic infection and its ability to modulate the stress response. In addition there is also evidence of economic gains from EN (22). However, PN is still widely used after allogeneic HCT, due to relatively poor tolerance of ETF and because venous access is already established in these patients. There are a few retrospective studies in HCT recipients that suggest superiority of EN over PN; for example, reductions in infections (23) and less early mortality and incidence of GvHD (16-18). More recent studies have retrospectively analysed outcomes of HCT cohorts where the patients were systematically offered ETF in preference to PN. In these studies EN is associated with reduced duration of febrile neutropenia, faster neutrophil engraftment, reduced risk of acute GVHD and better survival at 100 days compared to PN (14;15;24).

The relative advantage of EN could be explained by the known metabolic and other complications of PN. A pro-inflammatory effect of PN may also impact both NRM and GvHD (25;26). There are several plausible potential mechanisms for a beneficial

effect of EN on the maintenance of gut mucosal integrity and support of the GI tract environment, including cytokine production and host gut microflora. Gut associated lymphoid tissue plays an important role in the immune system. EN stimulates enterocyte turnover and supports the gut mucosal barrier and thus reduces translocation of bacteria and other inflammatory stimuli. Gut permeability changes as a result of changes to microbiota and strategies to modulate the gut microbiota after HCT are of increasing interest (27). Commensal bacteria are predominantly non-pathogenic and have roles in immune regulation and maintenance of host barrier defence against pathogens. Short-term changes to the diet or PN infusion result in rapid and significant changes to the host microbiome and intestinal barrier function (3;28).

Allogeneic HCT itself is accompanied by dramatic changes to the gut microbiota and there is increasing evidence that these changes to the microbiota may contribute to the development of post-transplant complications including GvHD (29). In keeping with the concept of gut nourishment we categorised patients with any PN episode of 4 or more days into the PN group regardless of any other periods of tube feeding or oral intake. This ensured those patients with 4 or more days of an inadequately nourished gut (despite having adequate nutrition overall), were captured together. This is in contrast to other retrospective cohort studies where patients receiving both EN and PN were categorised into an enteral nutrition group.

The obvious limitation of this study is its retrospective nature. We can only comment on associations without making causative links. Despite considering many known prognostic factors and performing multivariate analysis the nutritional support may only be a surrogate factor. For example, the inadequate nutrition group may represent more complex patients with severe gastrointestinal toxicity that prevented

enteral feeding, in combination with sepsis requiring removal of their central access jeopardising PN. Similar bias is possible when comparing EN and PN and will hopefully be resolved in an undergoing prospective randomised trial (30).

In conclusion, our data show that adequate nutrition during the period to engraftment after allogeneic HCT is associated with improved NRM, survival and GRFS. Adequate EN is associated with significantly better results for these outcomes than adequate PN. Furthermore, adequate EN, predominantly via oral intake, may be associated with lower incidence of overall and gut acute GvHD when compared to PN, perhaps because of its ability to maintain gut mucosal integrity and for support of the gastrointestinal tract environment, including gut microflora.

These data provide evidence for the clinical relevance of ANS as a potentially modifiable risk factor for outcomes of HCT. Although the retrospective and non-randomised nature of this study can only indicate association, the improved survival and reduced incidence of acute GvHD that we identify, warrant further research into the potential benefits of enteral nutrition support in these patients.

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Reference List

(1) Walrath M, Bacon C, Foley S, Fung HC. Gastrointestinal side effects and adequacy of enteral intake in hematopoietic stem cell transplant patients. Nutr Clin Pract 2014;30(2):305-10.

(2) de Defranchi RL, Bordalejo A, Canueto I, Villar A, Navarro E. Evolution of nutritional status in patients with autologous and allogeneic hematopoietic stem cell transplant. Support Care Cancer 2014;23(5):1341-7.

(3) Demehri FR, Barrett M, Teitelbaum DH. Changes to the Intestinal Microbiome With Parenteral Nutrition: Review of a Murine Model and Potential Clinical Implications. Nutr Clin Pract 2015;30(6):798-806.

(4) Cresci GA, Bawden E. Gut Microbiome: What We Do and Don't Know. Nutr Clin Pract 2015;30(6):734-46.

(5) Staffas A, Burgos da SM, van den Brink MR. The intestinal microbiota in allogeneic hematopoietic cell transplant and graft-versus-host disease. Blood 2017;129(8):927-33.

(6) Mathewson ND, Jenq R, Mathew AV, Koenigsknecht M, Hanash A, Toubai T, Oravecz-Wilson K, Wu SR, Sun Y, Rossi C, et al. Gut microbiome-derived metabolites modulate intestinal epithelial cell damage and mitigate graft-versus-host disease. Nat Immunol 2016;17(5):505-13.

(7) Teshima T, Reddy P, Zeiser R. Acute Graft-versus-Host Disease: Novel Biological Insights. Biol Blood Marrow Transplant 2016;22(1):11-6.

(8) Mendenhall CL. Protein-calorie malnutrition in alcoholic liver disease. In: Watson RR, Watzl B, editors. Nutrition and Alcohol.Florida: CRC Press; 1992. p. 363-84.

(9) Barak N, Wall-Alonso E, Sitrin MD. Evaluation of stress factors and body weight adjustments currently used to estimate energy expenditure in hospitalized patients. JPEN J Parenter Enteral Nutr 2002;26(4):231-8.

(10) Schofield WN. Predicting basal metabollic rate, new standards and review of previous work. Hum Nutr Clin Nutr 1985;39:5-41.

(11) Henry CJK. Basal metabollic rate studies in humans: measurement and development of new equations. Public Health Nutrition 2005;8(7a):1133-52.

(12) Holtan SG, DeFor TE, Lazaryan A, Bejanyan N, Arora M, Brunstein CG, Blazar BR, MacMillan ML, Weisdorf DJ. Composite end point of graft-versus-host disease-free, relapse-free survival after allogeneic hematopoietic cell transplantation. Blood 2015;125(8):1333-8.

(13) Aoudjhane M, Labopin M, Gorin NC, Shimoni A, Ruutu T, Kolb HJ, Frassoni F, Boiron JM, Yin JL, Finke J, et al. Comparative outcome of reduced intensity and myeloablative conditioning regimen in HLA identical sibling allogeneic haematopoietic stem cell transplantation for patients older than 50 years of age with acute myeloblastic leukaemia: a retrospective survey from the Acute Leukemia Working Party (ALWP) of the European group for Blood and Marrow Transplantation (EBMT). Leukemia 2005;19(12):2304-12.

(14) Seguy D, Duhamel A, Rejeb MB, Gomez E, Buhl ND, Bruno B, Cortot A, Yakoub-Agha I. Better outcome of patients undergoing enteral tube feeding after

myeloablative conditioning for allogeneic stem cell transplantation. Transplantation 2012;94(3):287-94.

(15) Seguy D, Berthon C, Micol JB, Darre S, Dalle JH, Neuville S, Bauters F, Jouet JP, Yakoub-Agha I. Enteral feeding and early outcomes of patients undergoing allogeneic stem cell transplantation following myeloablative conditioning. Transplantation 2006;82(6):835-9.

(16) Svahn BM, Remberger M, Heijbel M, Martell E, Wikstrom M, Eriksson B, Milovsavljevic R, Mattsson J, Ringden O. Case-control comparison of at-home and hospital care for allogeneic hematopoietic stem-cell transplantation: the role of oral nutrition. Transplantation 2008;85(7):1000-7.

(17) Svahn BM, Remberger M, Myrback KE, Holmberg K, Eriksson B, Hentschke P, Aschan J, Barkholt L, Ringden O. Home care during the pancytopenic phase after allogeneic hematopoietic stem cell transplantation is advantageous compared with hospital care. Blood 2002;100(13):4317-24.

(18) Mattsson J, Westin S, Edlund S, Remberger M. Poor oral nutrition after allogeneic stem cell transplantation correlates significantly with severe graft-versus-host disease. Bone Marrow Transplant 2006;38(9):629-33.

(19) August DA, Huhmann MB. A.S.P.E.N. clinical guidelines: nutrition support therapy during adult anticancer treatment and in hematopoietic cell transplantation. JPEN J Parenter Enteral Nutr 2009;33(5):472-500.

(20) Arends J, Bachmann P, Baracos V, Barthelemy N, Bertz H, Bozzetti F, Fearon K, Hutterer E, Isenring E, Kaasa S, et al. ESPEN guidelines on nutrition in cancer patients. Clin Nutr 2016;(16):10.

(21) Baumgartner A, Bargetzi A, Zueger N, Bargetzi M, Medinger M, Bounoure L, Gomes F, Stanga Z, Mueller B, Schuetz P. Revisiting nutritional support for allogeneic hematologic stem cell transplantation-a systematic review. Bone Marrow Transplant 2017;52(4):506-513

(22) Cangelosi MJ, Auerbach HR, Cohen JT. A clinical and economic evaluation of enteral nutrition. Curr Med Res Opin 2011;27(2):413-22.

(23) Sheean PM, Freels SA, Helton WS, Braunschweig CA. Adverse clinical consequences of hyperglycemia from total parenteral nutrition exposure during hematopoietic stem cell transplantation. Biol Blood Marrow Transplant 2006;12(6):656-64.

(24) Guieze R, Lemal R, Cabrespine A, Hermet E, Tournilhac O, Combal C, Bay JO, Bouteloup C. Enteral versus parenteral nutritional support in allogeneic haematopoietic stem-cell transplantation. Clin Nutr 2014;33(3):533-8.

(25) Demirer S, Aydintug S, Ustun C, Turkmen E, Tuzun A, Simsek S, Basaran O, Celebi H, Demirer T. Comparison of the efficacy of medium chain triglycerides with long chain triglycerides in total parenteral nutrition in patients with hematologic malignancies undergoing peripheral blood stem cell transplantation. Clin Nutr 2000;19(4):253-8.

(26) Muscaritoli M, Conversano L, Torelli GF, Arcese W, Capria S, Cangiano C, Falcone C, Rossi FF. Clinical and metabolic effects of different parenteral nutrition regimens in patients undergoing allogeneic bone marrow transplantation. Transplantation 1998;66(5):610-6.

(27) Andermann TM, Rezvani A, Bhatt AS. Microbiota Manipulation With Prebiotics and Probiotics in Patients Undergoing Stem Cell Transplantation. Curr Hematol Malig Rep 2016;11(1):19-28.

(28) David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, Ling AV, Devlin AS, Varma Y, Fischbach MA, et al. Diet rapidly and reproducibly alters the human gut microbiome. Nature 2014;505(7484):559-63.

(29) Docampo MD, Auletta JJ, Jenq RR. Emerging Influence of the Intestinal Microbiota during Allogeneic Hematopoietic Cell Transplantation: Control the Gut and the Body Will Follow. Biol Blood Marrow Transplant 2015;21(8):1360-6.

(30) Lemal R, Cabrespine A, Pereira B, Combal C, Ravinet A, Hermet E, Bay JO, Bouteloup C. Could enteral nutrition improve the outcome of patients with haematological malignancies undergoing allogeneic haematopoietic stem cell transplantation? A study protocol for a randomized controlled trial (the NEPHA study). Trials 2015;16:136.

Supplementary Table 1. Univariate analyses of factors on NRM, acute GvHD, survival and GRFS.

Results shown where *P* < .2

	N	NRM at 100d % (95% CI) (N = 484)	*P*	Acute GvHD (%) (N = 439)		*P*	Gut acute GvHD (%) (N = 438)		*P*	Survival at 5 years % (95%CI) (N = 484)	*P*	GRFS at 5 years % (95%CI) (N = 484)	*P*
				grade 0-I	grade II-IV		No	Yes					
Overall		14.7 (12-18)	-	260 (59)	179 (41)	-	285 (65)	153 (35)	-	49 (45-54)	-	34 (30-38)	-
Nutritional intake group													
Adequate EN	245	8.2 (5-12)	< .001	157 (68)	75 (32)	< .001	169 (73)	63 (27)	< .001	54 (48-61)	<.001	39 (33-46)	.004
Adequate PN	148	17.6 (12-25)		59 (45)	73 (55)		68 (52)	63 (48)		46 (38-55)		30 (22-39)	
Inadequate (all routes)	91	27.5 (20-38)		44 (59)	31 (41)		48 (64)	27 (36)		42 (32-53)		27 (17-39)	
Recipient sex													
Male	305	_	> .2	175 (62)	109 (38)	.17	194 (69)	89 (31)	.039	_	> .2	_	> .2
Female	179			85 (55)	70 (45)		91 (59)	64 (41)					
Recipient age (years)													
Younger than 40	214	10.3 (7-15)	.017	_		> 0.2	_		> .2	60 (53-67)	< .001	40 (34-48)	.019
40-59	232	16.8 (13-22)								43 (37-50)		30 (24-37)	
At least 60	38	26.3 (15-45)								15 (3-66)		195 (10-31)	
Recipient CMV													
Negative	202	9.4 (6-14)	.004	_		> 0.2	_		> .2	53 (46-60)	.03	_	> .2
Positive	277	18.8 (15-24)								46 (40-53)			
Data missing	*5*												
Recipient / Donor Sex													
Other combination	384	_	> .2	217 (62)	136 (38)	.067	238 (68)	114 (32)	.032	_	> .2	_	> .2
Male / Female	99			43 (51)	42 (49)		47 (55)	38 (45)					
Data missing	*1*												
Stem cell source													
BM	154	_	> .2	73 (51)	71 (49)	.01	83 (58)	61 (42)	.02	57 (50-65)	.009	_	> .2
PBSC	326			185 (64)	106 (36)		200 (69)	90 (31)		44 (39-50)			
Data missing	*4*												
CD34 positive cell dose													
Less than 4.0 x 10^6	115	_	> .2	53 (23)	55 (34)	.017	58 (23)	50 (36)	.007	_	> .2	_	> .2
More than 3.99 x 10^6	313			176 (77)	106 (66)		192 (77)	89 (64)					
Data missing	*56*												

	N	NRM at 100d % (95% CI) (N = 484)	*P*	Acute GvHD (%) (N = 439)		*P*	Gut acute GvHD (%) (N = 438)		*P*	Survival at 5 years % (95%CI) (N = 484)	*P*	GRFS at 5 years % (95%CI) (N = 484)	*P*
				grade 0-I	grade II-IV		No	Yes					
Previous autograft													
No	437	13.5 (11-17)	.017	231 (58)	171 (42)	.013	29 (81)	7 (19)	.042	52 (28-57)	< .001	36 (32-41)	.002
Yes	47	25.5 (16-41)		29 (78)	8 (22)		256 (64)	146 (36)		19 (10-36)		13. (6-29)	
Era (years)													
2000 – 2004	199	_	> .2	_		> .2	113 (60)	74 (40)	.20	55 (48-63)	.023	_	> .2
2005 – 2009	138						85 (70)	37 (30)		45 (38-55)			
2010 – 2014	147						87 (67)	42 (33)		42 (32-53)			
EBMT disease risk													
Early	229	10.5 (7-15)	.004	130 (61)	84 (39)	.12	143 (67)	71 (33)	.05	61 (55-68)	< .001	44 (38-51)	
Intermediate	139	14.4 (10-22)		65 (52)	60 (48)		71 (57)	54 (43)		44 (36-53)		28. (21-37)	<.001
Late	116	23.3 (17-32)		65 (65)	35 (35)		71 (72)	28 (28)		31 (23-41)		19 (12-28)	
Conditioning regimen													
Myeloablative	285	_	> .2	131 (50)	131 (50)	< .001	146 (56)	116 (44)	< .001	54 (49-61)	.015	_	> .2
Reduced intensity	199			129 (73)	48 (27)		139 (79)	37 (21)		40 (34-49)			
HCT-CI													
0-1	250	10.8 (8-15)	.017	_		> .2	_		> .2	58 (52-64)	< .001	41 (35-48)	< .001
2-3	148	16.9 (12-24)								39 (31-48)		27 (20-36)	
More than 3	56	28.6 (19-43)								24 (15-40)		14 (7-30)	
Data missing	*30*												

BM, bone marrow; CI, confidence interval; CMV, cytomegalovirus; EBMT, European Blood and Marrow Transplantation; EN, enteral nutrition; GRFS, graft versus host disease-free and relapse-free survival; GvHD, graft-versus-host disease; HCT-CI, hematopoietic cell transplant co-morbidity index; NRM, non-relapse mortality; NS, not statistically significant; PBSC, peripheral blood stem cells; PN, parenteral nutrition.

END