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CD56^{bright} natural killer regulatory cells in filgrastim primed donor blood or marrow products regulate chronic graft-*versus*-host disease: the Canadian Blood and Marrow Transplant Group randomized 0601 study results

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ABSTRACT

Randomized trials have conclusively shown higher rates of chronic graft-*versus*-host disease with filgrastim-stimulated apheresis peripheral blood as a donor source than unstimulated bone marrow. The Canadian Blood and Marrow Transplant Group conducted a phase 3 study of adults who received either filgrastim-stimulated apheresis peripheral blood or filgrastim-stimulated bone marrow from human leukocyte antigen-identical sibling donors. Because all donors received the identical filgrastim dosing schedule, this study allowed for a controlled evaluation of the impact of stem cell source on development of chronic graft-*versus*-host disease. One hundred and twenty-one evaluable filgrastim-stimulated apheresis peripheral blood and filgrastim-stimulated bone marrow patient donor products were immunologically characterized by flow cytometry and tested for their association with acute and chronic graft-*versus*-host disease within 2 years of transplantation. The immune populations evaluated included, regulatory T cells, central memory and effector T cells, interferon γ positive producing T cells, invariate natural killer T cells, regulatory natural killer cells, dendritic cell populations, macrophages, and activated B cells and memory B cells. When both filgrastim-stimulated apheresis peripheral blood and filgrastim-stimulated bone marrow were grouped together, a higher chronic graft-*versus*-host disease frequency was associated with lower proportions of CD56^{bright} natural killer regulatory cells and interferon γ-producing T helper cells in the donor product. Lower CD56^{bright} natural killer regulatory cells displayed differential impacts on the development of extensive chronic graft-*versus*-host disease between filgrastim-stimulated apheresis

peripheral blood and filgrastim-stimulated bone marrow. In summary, while controlling for the potential impact of filgrastim on marrow, our studies demonstrated that $CD56^{bright}$ natural killer regulatory cells had a much stronger impact on filgrastim-stimulated apheresis peripheral blood than on filgrastim-stimulated bone marrow. This supports the conclusion that a lower proportion of $CD56^{bright}$ natural killer regulatory cells results in the high rate of chronic graft-*versus*-host disease seen in filgrastim-stimulated apheresis peripheral blood. *clinicaltrials.gov Identifier: 00438958.*

Introduction

Filgrastim granulocyte-colony stimulating factor (G-CSF)-stimulated apheresis peripheral blood (G-PB) as a donor source is clinically well established due to rapid engraftment, ease of collection, and similar survival to marrow as a donor source. G-PB is limited by a significantly higher rate of chronic graft-*versus*-host disease (cGvHD),[1-8] purported to be due to the infusion of increased donor product T cell numbers.[9] Other studies have suggested that the $CD34^+$ cell donor load,[10,11] activated $HLA\text{-}DR^+$ T cells,[12] and possibly the total nucleated cell dose[13] impact on the development of cGvHD after G-PB transplantation, however, all such studies are limited by the lack of comparison to a control marrow transplanted population. Cell populations are associated with acute GvHD (aGvHD) after G-PB which includes dendritic cells[14] and Treg cells[15] but do not have any association with cGvHD. Other donor cell populations have found no association for either aGvHD or cGvHD.[16] To date no study has definitely established which immune cell populations are most responsible for the higher rate of cGvHD associated with the G-PB donor source compared to marrow. Until the specific, unique components in G-PB *versus* marrow as a source are identified, it remains difficult to develop graft manipulation strategies to modulate cGvHD.

The Canadian Blood and Marrow Transplant Group (CBMTG) undertook a definitive phase 3 trial comparing G-bone marrow (BM) with G-PB in sibling allografts for adults with hematologic malignancies. In that study, the CBMTG showed that cGvHD was lower with G-BM (HR=0.66; 95% CI 0.46 – 0.95; P=0.007).[17] This study presented an unprecedented opportunity to evaluate the impact of graft source on the development of cGvHD with both donor sources receiving G-CSF treatment using an identical regimen. The population was relatively homogenous as only human leukocyte antigen (HLA)-identical sibling donor (8/8 or 7/8 HLA-match) was used for predominantly myeloid malignancies with the only variable being the method of collection (i.e., marrow harvest *versus* apheresis). We hypothesized that one of the immune cell populations previously identified by correlative cGvHD biology and biomarkers studies would correlate with the induction of cGvHD by G-PB donor product. To test this hypothesis, we evaluated both G-BM and G-PB donor grafts combined for donor product immune cells for any specific cell types correlation with the development of cGvHD. Once identified, we evaluated the relative impact of each immune cell population on cGvHD for the relative impact of the two donor sources, G-PB *versus* G-BM, on the development of cGvHD. The immune populations evaluated included: regulatory T cells, central memory and effector T cells, interferon $(IFN)\gamma^+$ producing T cells, regulatory natural killer (NK) cells, invariant natural killer T (iNKT) cells, plasmacytoid and myeloid dendritic cells, macrophages, activated B cells, and memory B cells.

Methods

Clinical Study Design

Samples for the current study were obtained as part of a larger clinical study (CBMTG 0601), a randomized phase 3, parallel group trial conducted by the CBMTG at 13 centers in Canada, Saudi Arabia, Australia, New Zealand, and the USA. The institutional research ethics board at each center approved the trial and recipients and donors both gave informed consent before randomization. Recipients were between 16 and 65 years of age and with a hematologic malignancy. Donors were 7/8 or 8/8 HLA-matched siblings medically fit to receive G-CSF and undergo a marrow harvest or apheresis. This study has been described previously.[17]

Patient and Donor Characteristics

CBMTG 0601 comprised 223 donor-recipient pairs randomized between April 2007 and January 2012 with 223 evaluable pairs. Of the entire 223 evaluable patients from the clinical trial, 121 had evaluable samples for the current correlative studies. The primary analysis was performed on patients who had survived up to 2 years after BM transplantation (BMT) (> 95% of patients developed overall cGvHD by 2 years), with the omission of patients due to death and leukemia relapses that occurred before the onset of cGvHD (Table 1; n = 89). We found no significant difference between the 121 evaluated and the 102 not included in the analysis for cGvHD (65% *vs.* 59%), death (34% *vs.* 44%), relapses (29% *vs.* 24%), or time to cGvHD (day 180±112 *vs.* day 185±124), respectively. We defined overall cGvHD as including both limited and extensive cGvHD, and will from now on refer to overall cGvHD as cGvHD, unless specified as extensive cGvHD. Confirmatory analysis was performed on all evaluable patients including those with a death or relapse before 2 years.

Both subgroups had similar patient characteristics to the entire population in the study (Table 1). A comprehensive immune evaluation of T cells, B cells, NK cells, iNKT cells, macrophages, and dendritic cell populations was performed on the donor product for a number of immune populations (*Online Supplementary Table S1*) and tested for association with aGvHD and cGvHD within 2 years of transplantation. Additional evaluations examined the association of identified immune cell populations and the development of aGvHD and cGvHD for the graft source (G-PB or G-BM) and other clinical factors, including transplant related mortality (TRM), relapse, previous aGvHD before the onset of cGvHD, donor-recipient sex differences, acute myeloid leukemia (AML) *versus* no AML, total body irradiation (TBI) *versus* no TBI, recipient age and donor age.

Sample processing for biological studies using immunophenotyping and functional assays

Samples from allografts were couriered overnight at room temperature to a central laboratory located at BC Children's Hospital

Research Institute in Vancouver, Canada; peripheral blood mononuclear cells (PBMCs) were isolated and frozen on arrival. Batched samples were thawed using 1 x 10^6 viable cells per assay. Immunophenotyping and functional assays evaluated T cell, B cell, dendritic cell, monocyte, and NK cell populations (*Online Supplementary Table S1*). Data were acquired using LSR II flow cytometer (BD Biosciences) and analyzed by FlowJo v10 (TreeStar, Ashland, OR, USA). Details of the cell immunophenotyping strategy can be found in Table 1. Graft composition was evaluated as the percentage of cell population per donor lymphocytes. On smaller cell count samples, there was a prioritization for assays with immunophenotyping to be carried out first, followed by functional stimulations assays for cytokine production if sufficient samples were available.

Study endpoints

The primary biologic endpoint of the analysis was an association of cGvHD with a number of subpopulations of T cell, B cell, NK cell, iNKT cell, macrophages, and dendritic cell populations. aGvHD and cGvHD were characterized according to Przepiorka *et al.*[18] and Sullivan *et al.*,[19] respectively. Any GvHD was defined as chronic GvHD and/or acute GvHD (grade 1 – IV aGvHD). Chronic GvHD was defined as an initial diagnosis of cGvHD within 2 years of transplantation.

Statistical analysis

The effect of candidate immune cell populations on the development of cGvHD (within a 2 year period from transplantation) was tested using a univariate logistic regression model. Patients

Table 1. Baseline characteristics: overall *vs.* studied populations.

	Overall Population in clinical trial (N = 223)		Confirmatory population including early deaths, relapse[1] (N = 121)		Primary studied population excluding early deaths, relapses[1] (N = 89)	
Variable	G-BM (%) N=113	G-PB (%) N=110	G-BM (%) N=52	G-PB (%) N=69	G-BM (%) N=39	G-PB (%) N=50
Disease Stage						
Early	71 (63)	77 (70)	34 (65)	49 (71)	26 (67)	33 (66)
Late	42 (37)	33 (30)	18 (35)	20 (29)	13 (33)	17 (34)
Disease						
CML	7 (6)	6 (5)	5 (10)	5 (7)	5 (13)	4 (8)
AML	53 (47)	54 (49)	26 (50)	35 (58)	18 (46)	27 (54)
MYELO	15 (13)	9 (8)	5 (10)	7 (10)	4 (11)	6 (12)
Other	38 (34)	41 (37)	16 (30)	22 (32)	12 (31)	13 (26)
Conditioning Regimen						
BU+CY	65 (58)	57 (51)	35 (67)	36 (52)	29 (74)	29 (58)
CY+TBI	2 (2)	2 (2)	0	0	0	0
Other:			0	0	0	0
Fludarabine + Melphalan	40 (35)	41 (37)	16 (30)	24 (35)	9 (23)	13 (26)
VP-16 + TBI	5 (4)	7 (6)	1 (3)	6 (9)	1 (3)	5 (10)
Fludarabine+Busulfan	1 (1)	3 (3)	0	2 (3)	0	2 (4)
Donor Sex						
Male	66 (58)	66 (60)	30 (58)	44 (64)	22 (57)	32 (64)
Female	47 (42)	44 (44)	22 (42)	25 (36)	17 (43)	18 (36)
Donor median age (range)	43 (18 – 66)	41 (18 – 64)	45.5 (18-62)	44 (18-66)	49 (20-62)	46 (19-64)
Donor CMV Status						
Positive	69 (61)	57 (52)	26 (50)	26 (38)	19 (49)	20 (40)
Negative	44 (39)	53 (48)	26 (50)	43 (62)	20 (51)	30 (60)
Recipient Sex						
Male	69 (61)	57 (52)	34 (65)	39 (57)	25 (64)	29 (58)
Female	44 (39)	53 (48)	18 (35)	30 (43)	14 (36)	21 (42)
Recipient median age (range)	43 (16 – 63)	46 (16 – 64)	48 (20-63)	46 (16-64)	49 (22-63)	47 (17-59)
Recipient CMV Status						
Positive	62 (55)	59 (54)	23 (44)	32 (46)	17 (44)	22 (44)
Negative	51 (45)	51 (46)	29 (56)	37 (54)	22 (66)	28 (56)

[1]Early deaths and relapses were defined as occurring before 2 years post BMT or before the onset of cGvHD. BU: busulfan; CY: cyclophosphamide; VP=16 = etoposide; CMV: cytomegalovirus; MYELO: myelodysplastic disease; CML: Chronic Myeloid Leukemia; AML; Acute Myeloid Leukemia; G-BM: G bone marrow; G-PB: G peripheral blood; TBI: total body irradiation.

who relapsed or those who died before occurrence of cGvHD were excluded from the logistic regression analysis, since it could not be established whether or not they would have developed cGvHD. Of the initial 121 patients, 89 met the inclusion criteria. As these were exploratory analyses no statistical adjustments were made for multiple comparisons, thus a *P*-value threshold of 0.01 was used in the primary analysis and 0.05 in all other secondary analyses. All analyses were performed using MATLAB. For the two cell populations that were found to be significant, due to smaller cell numbers in some donor graft samples, 7 of the 89 patients did not have immunophenotyping for $CD56^{bright}$ NK_{reg} cells and 11 of the 89 patients did not have functional stimulation and immunophenotyping for the $CD4^+$ T cell $IFN\gamma^+$ (Figure 1).

To confirm that excluding patients who displayed relapse or died before cGvHD did not introduce biases, we also employed a univariate Cox proportional hazards model[20] with those patients included, and used the time to cGvHD onset as the response. The time to cGvHD for said patients was considered as censored under the Cox model.

As to visualization, the identified cell populations were plotted with values split by GvHD status. Multivariate analysis on all significant cell populations identified in the univariate analysis was also performed to test for the unique effect of each cell population. This analysis was applied only on patients that had all these cell populations, resulting in 75 and 94 patients being evaluated for the logistic regression model and Cox model, respectively (Figure 1). Furthermore, logistic regression was applied to examine the effect of aGvHD, sex, TBI, recipient age, donor age, AML, death, relapse as well as donor source on the identified cell populations. Moreover, with patients split by cGvHD status, optimal cut points for the identified cell populations were determined by plotting their receiver operating characteristic (ROC) curve and by finding the point on the ROC curve that is closest to the point of perfect sensitivity and specificity. Lastly, we examined the interaction effect between donor source and the identified significant markers on cGvHD status using logistic regression and the Cox model.

Role of the funding source

The National Cancer Institute of the National Institutes of Health (NIH) funded the study herein following peer review, but had no direct influence on the study design, the collection, analysis, and interpretation of data, in the writing of the report or in the decision to submit the paper for publication. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Evaluation of donor graft composition for immune populations associated with the development of cGvHD

Immunophenotypic and functional evaluations were performed for a large number of $CD4^+$ and $CD8^+$ T cell, NK cell, B cell, macrophages, plasmacytoid and myeloid dendritic cell, iNKT cell, and regulatory T cell populations as outlined in *Online Supplementary Table S1* and correlated with the presence of cGvHD. We also evaluated the activation status of $CD4^+$and $CD8^+$ T cells by CD25 and human leukocyte antigen – antigen D related (HLA-DR) expression and found no difference. Initial analyses evaluated candidate immune cell populations for correlation with cGvHD followed by analysis for extensive cGvHD. The two donor sources, G-PB and G-BM, were grouped together for these analyses. We found no significant associations (at $P<0.01$) with cGvHD except for two populations, $CD56^{bright}$ NK_{reg} cells (Table 2; $P=0.003$) and $IFN\gamma^+$ $CD4^+$ T cells ($P=0.002$).

A confirmatory analysis was further performed that included patients who either died or developed a leukemia relapse before the onset of cGvHD and before 2 years following transplantation. These included 107 patients for CD56bright NK_{reg} cells and 100 patients for $IFN\gamma^+$ $CD4^+$ T cells as a marker (Figure 1). We confirmed the results of

Table 2. Association between donor cell population numbers with development of cGvHD (excludes all patients with a death or relapse before 2 years or overall cGvHD).

Variable	Percentage cells per total lymphocytes – Independent variable[1] *P* (Wald test)	Odds Ratio (95% CI)
$CD56^{bright}$ NK_{reg} cells		
Overall cGvHD (N = 821)	0.003	0.13 (0.03- 0.49)
Extensive cGvHD (N = 78)	0.005	0.19 (0.06-0.61)
$CD4^+$ T cells $IFN\gamma$		
Overall cGvHD (N = 78)	0.002	0.77 (0.66- 0.91)
Extensive cGvHD (N = 75)	0.002	0.83 (0.74-0.94)
	Percentage cells per total lymphocytes – Multivariate analysis[2]	
$CD56^{bright}$ NK_{reg} cells		
Overall cGvHD	0.02	0.16 (0.03- 0.74)
Extensive cGvHD	0.01	0.19 (0.05-0.69)
$CD4^+$ T cells $IFN\gamma$		
Overall cGvHD	0.007	0.77 (0.64- 0.93)
Extensive cGvHD	0.01	0.84 (0.73-0.96)

[1]The multivariate analysis based on logistic regression model was performed only on patients who had both values resulting in a decrease to N=75. [2]The multivariate analysis based on the Cox proportional hazards model was performed only on patients who had both values resulting in a decrease to N=94. cGvHD: chronic graft-*versus*-host disease; IFN: interferon; NK: natural killer.

the logistic regression analysis for both CD56^{bright} NK$_{reg}$ cells with cGvHD (*Online Supplementary Table S2*; OR=0.54, *P*=0.02) and IFNγ^{+} CD4^{+} T cells with cGvHD (*Online Supplementary Table S2*, OR=0.93, *P*=0.001). Further analysis focused on these two populations (CD56^{bright} NK$_{reg}$ cells and IFNγ^{+} CD4^{+} T cells) as outlined below.

Association of donor CD56^{bright} NK$_{reg}$ cell composition with the development of cGvHD

Significant associations between a lower percentage of donor CD56^{bright} NK$_{reg}$ cells per total lymphocytes and development of any GvHD (aGvHD and/or cGvHD; *P*=0.003) as well as cGvHD only (Figure 2A and Table 2; OR=0.13; *P*=0.003) were found. Further analysis, limited to extensive cGvHD alone, was similar with logistic regression (Figure 2B, OR=0.19; *P*=0.005). A significant association was also found with aGvHD status (*P*=0.02). We confirmed that the CD56^{bright} NK$_{reg}$ cell population was the classic regulatory NK (NK$_{reg}$) population by further evaluation for expression of CD335 (NKp46), CD336 (NKp44), and CD337 (NKp30) on all samples from the study population of G-BM and G-PB NK$_{reg}$ cells (Figure 2C). The expression of CD335 (NKp46) was found to be significantly higher in CD56^{bright} NK$_{reg}$ cells with comparable expression of CD337 in both subpopulations consistent with the NK$_{reg}$ phenotype.[21,22] From now on, we will refer to this population (CD3^{-}, CD56^{bright}/CD335^{bright}/perforin^{-}/granzyme B^{-}/CD16$^{+/-}$) as CD56^{bright} NK$_{reg}$ cells. We evaluated whether cytomegalovirus (CMV) seropositivity of the donor impacted on the presence of CD56^{bright} NK$_{reg}$ cells present, and found no significant difference in CD56 bright NK$_{reg}$ cells for CMV seropositive (0.59±0.50% CD56^{bright} NK$_{reg}$ cells per total lymphocytes) *versus* CMV

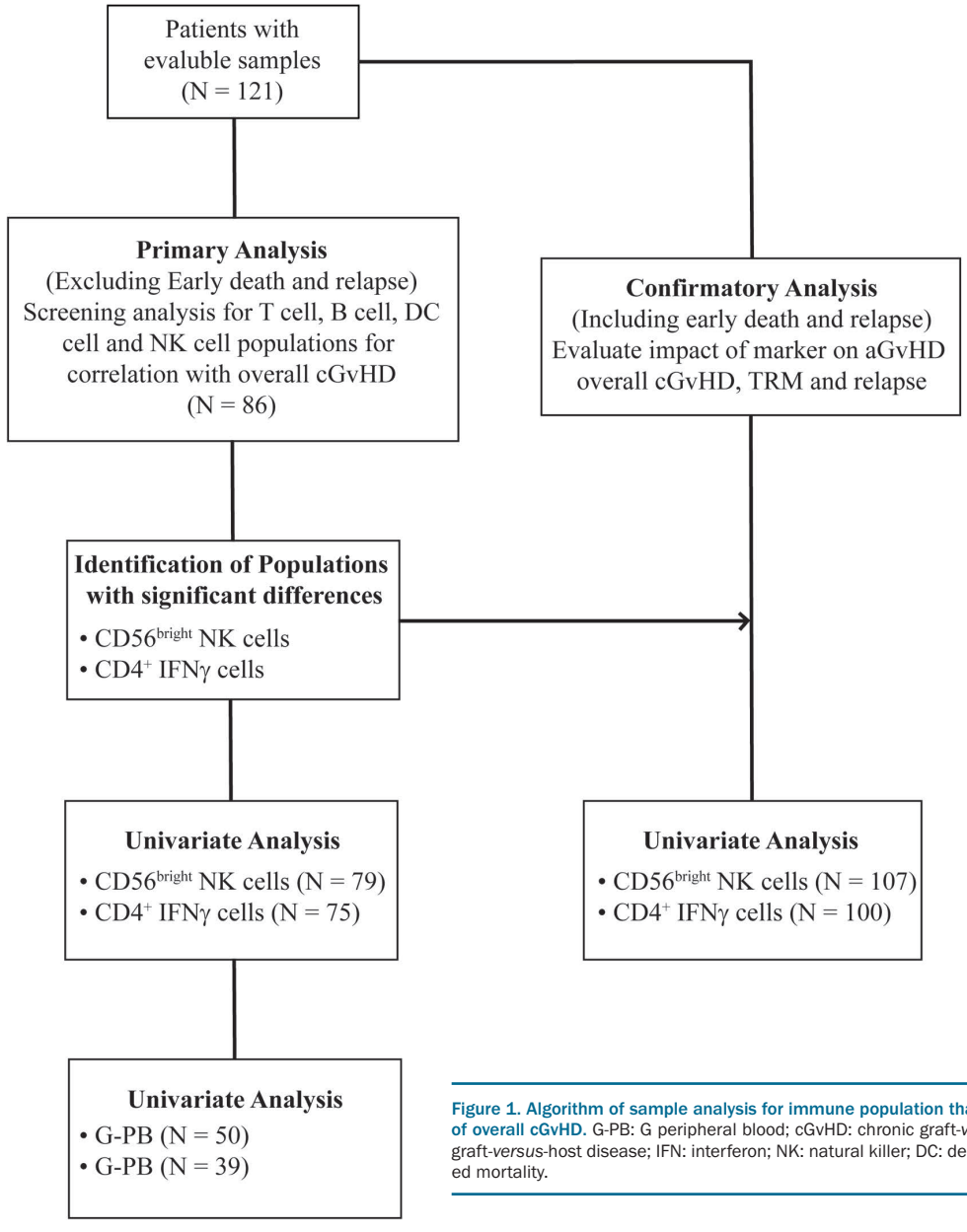

Figure 1. Algorithm of sample analysis for immune population that correlate with the development of overall cGvHD. G-PB: G peripheral blood; cGvHD: chronic graft-*versus*-host disease; aGvHD: acute graft-*versus*-host disease; IFN: interferon; NK: natural killer; DC: dendritic cells; TRM: transplant related mortality.

seronegative (0.55±0.42%, *P*=0.65) donors. A ROC curve to predict the development of cGvHD by 2 years was calculated for the CD56^{bright} NK$_{reg}$ population, and we found an area under the curve (AUC) of 0.85 (Figure 2D).

Association of donor IFNγ producing CD4$^+$ T cells with the development of cGvHD

Our group, and others, have previously shown that lower numbers of IFNγ producing cells were associated with cGvHD.[23] Cytokine production was measured after mitogen stimulation *in vitro* (PMA/Ionomycin). We identified a significant association between lower numbers of IFNγ-producing CD4$^+$ T cell population (CD4$^+$/CD3$^+$/IFNγ$^+$/IL-4$^-$/IL-17$^-$) and development of any GvHD (either aGvHD and/or cGvHD; *P*=0.002) and cGvHD alone (Figure 3A and Table 2, OR=0.77, *P*=0.002). Further analysis, limited to extensive cGvHD alone, was similar for logistic regression (Figure 3B and Table 2, OR=0.83, *P*=0.001). A ROC analysis revealed an AUC of 0.88 (Figure 3C).

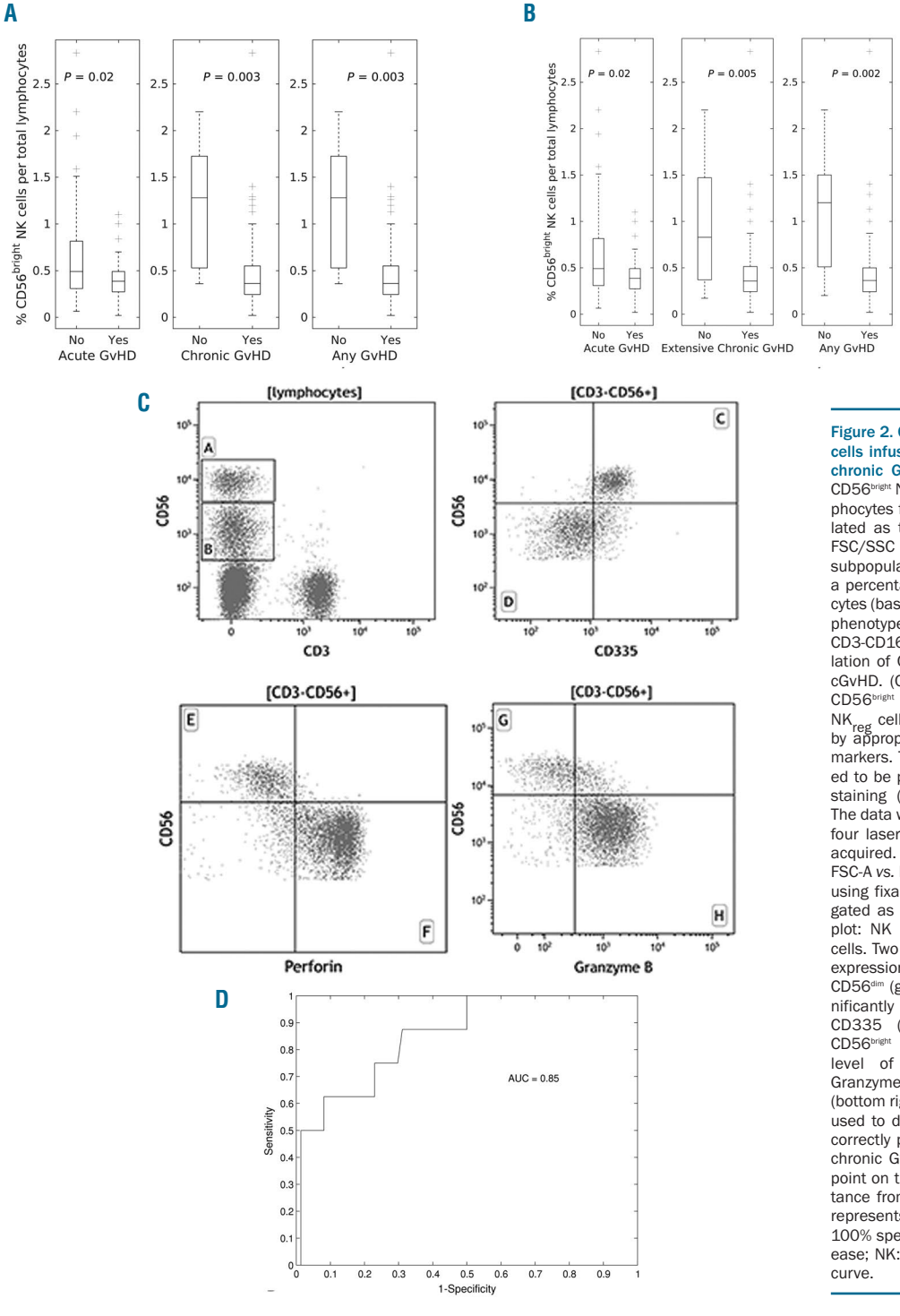

Figure 2. Correlation of donor CD56^{bright} NK$_{reg}$ cells infusion characteristics with acute and chronic GvHD. (A) Box and whisker plot of CD56^{bright} NK$_{reg}$ cells percentage per total lymphocytes for overall cGvHD, which was calculated as the total nucleated cells gated on FSC/SSC plots, then the percentage of each subpopulation of interest was determined as a percentage of the total number of lymphocytes (based on forward and side scatter). The phenotype of CD56^{bright} NK cells was CD56^{bright} CD3-CD16-perforin-granzyme B. (B) The correlation of CD56^{bright} NK$_{reg}$ cells with extensive cGvHD. (C) Representative dot plot showing CD56^{bright} and CD56^{dim} subpopulations of NK$_{reg}$ cells. Mononuclear cells were stained by appropriate conjugated mAbs for surface markers. The cells were then fixed and treated to be permeable, followed by intracellular staining (*Online Supplementary Table S1*). The data was acquired by LSRII equipped with four lasers. A minimum of 1x10^6 cells were acquired. Single cells were gated based on FSC-A *vs.* FSC-H and dead cells were excluded using fixable viability dye. Lymphocytes were gated as FSC lo and SSC lo. Upper left dot plot: NK cells were defined as CD3$^-$CD56$^+$ cells. Two populations are revealed based on expression of CD56, CD56^{bright} (gate A) and CD56^{dim} (gate B). CD56^{bright}cells express a significantly higher level of activating receptor CD335 (NKp46) compared to CD56^{dim}. CD56^{bright} cells express a significantly lower level of molecules involved in killing, Granzyme B (bottom left dot plot) and Perforin (bottom right dot plot). (D) A ROC analysis was used to determine an 'optimal' cut point for correctly predicting the occurrence of overall chronic GvHD. 'Optimal' was defined as the point on the ROC curve with the shortest distance from the point (0, 1). The point (0, 1) represents the ideal, 100% sensitivity and 100% specificity. GvHD: graft-*versus*-host disease; NK: natural killer; AUC: area under the curve.

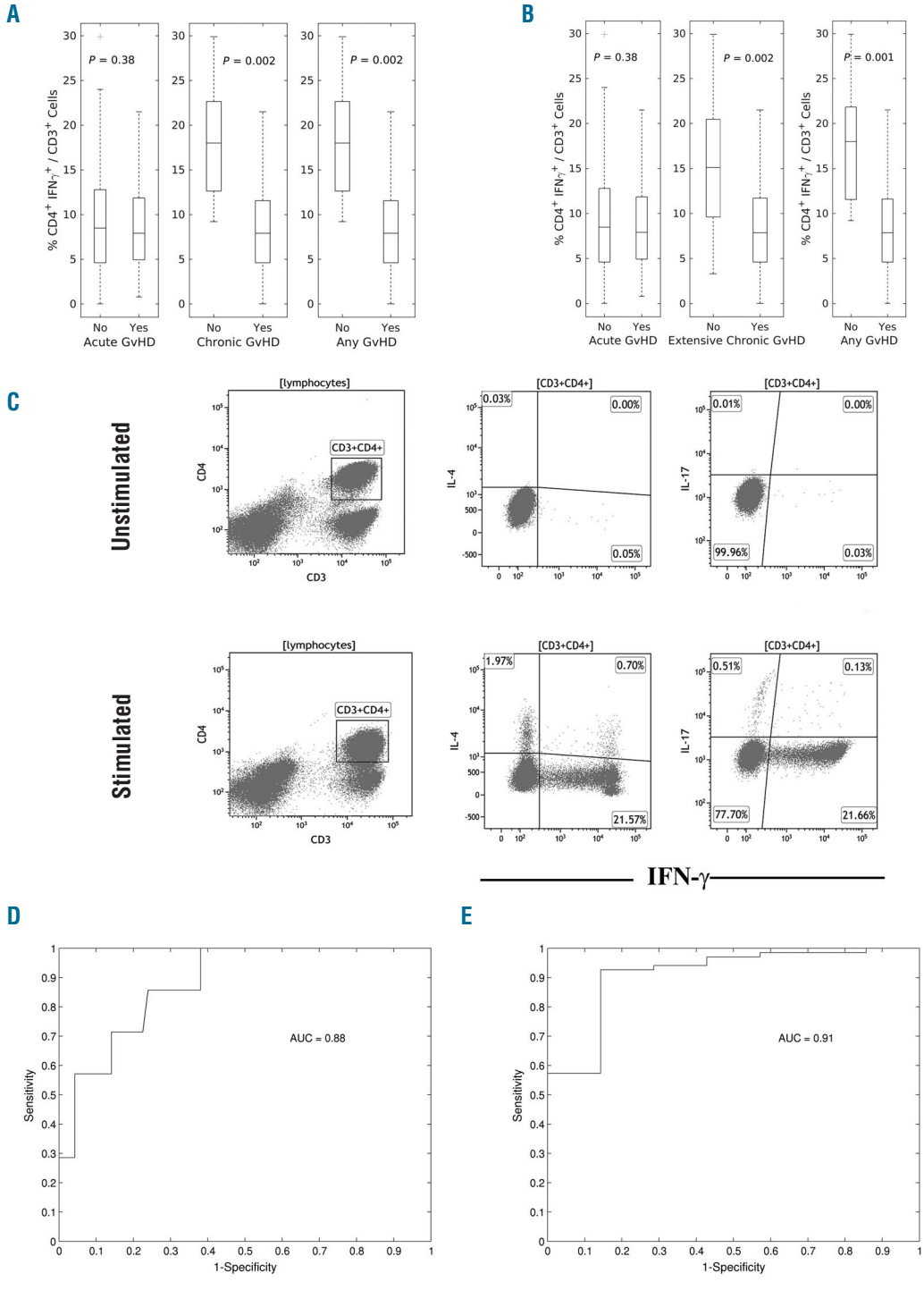

Figure 3. Correlation of donor IFNγ producing CD4⁺ T cells infusion characteristics with acute and chronic GvHD. (A) Box and whisker plot of the correlation of IFNγ producing CD4⁺ T cells percentage with overall cGvHD. The population was calculated as a subpopulation of CD3⁺ lymphocytes. PBMCs were stimulated with PMA (100ng/ml)/Ionomycin (1 μg/ml) in the presence of monensin for 6 hours. Unstimulated cells treated with monensin were used as control. At the end of incubation, the cells were harvested and surface markers were stained for CD3, CD4, and CD8, in addition to fixable viability to distinguish dead cells. Intracellular staining was performed to detect production of IL-17, IL-4 and IFNγ. The data was acquired using BD LSRII and analyzed by FlowJo v9. Hierarchical gating; 1: lymphocytes were selected based on FSC/SSC, 2: exclusion of dead cells, 3: selection of CD3⁺ T cells, and 4: determination of IFNγ producing CD4⁺ T-cells. The data is presented as % of CD4⁺IFNγ⁺ T cells per CD3⁺ lymphocytes. (B) Correlation of IFNγ producing CD4⁺ T cells with extensive cGvHD; (C) A representative dot plot of INFγ⁺ T helper cells. Mononuclear cells were seeded at a density of 1x10⁶ per milliliter of culture medium (RPMI-1640 supplemented with 10% heat-inactivated FBS and 2mM l-glutamine), and the cells incubated in CO2 incubator providing 95% oxygen and 5% CO2 at 37 degrees centigrade. The cells were stimulated for 6 hours with 100ng/ml PMA and 1 μg/ml Ionomycin in presence of golgi inhibitor monensin (BD Biosciences; following manufacturers' instructions). As control, cells were cultured without stimulator, but received monensin. The cells were then harvested and surface staining was performed to detect CD3⁺CD4⁺ cells. Then the cells were fixed and treated to be permeable. Intracellular staining was performed to detect IFNγ, IL-4 and IL-17. At least 1x10⁶ cells were acquired. Gating hierarchy; 1: single cells (FSC-A *vs.* FSC-H), 2: viable cells (fixable viability dye negative), and 3: lymphocytes 4-CD3⁺CD4⁺ cells. The percentage of Th1 (IFNγ⁺), Th17 (IL-17⁺) and Th2 (IL-4⁺) were determined after setting quadrant based on unstimulated cells (upper row). Dot plots in lower row show cytokine expression after stimulation. D) A ROC analysis was used to determine an 'optimal' cut point for correctly predicting the occurrence of chronic GvHD. The optimal cut point for IFNγ producing CD4⁺ T cells/CD3⁺ T cells was 13.9%. (E) Logistic regression was performed with the two identified markers as predictors and overall cGvHD status as response. Each sample was given an estimated probability of overall cGvHD based on the fitted model. The ROC was generated by applying different probability thresholds. GvHD: graft-*versus*-host disease; IFN: interferon; AUC: area under the curve.

A multivariable logistic regression analysis was performed on samples with both $CD56^{bright}NK_{reg}$ cells and $IFN\gamma^+$ $CD4^+$ T cells (N=94). The decrease from the original 121 to 94 patients was due to small cell numbers in donor samples and patients with relapse or death before cGvHD who were further removed (Table 2). We found that both $IFN\gamma^+$ $CD4^+$ T cells (OR=0.84; *P*=0.01) and $CD56^{bright}NK_{reg}$ cells (OR=0.19; *P*=0.01; Table 2) maintained their significance, suggesting that each of these cell populations has some unique attributes that significantly relate to cGvHD status. We also found that the combination of $CD56^{bright}$ NK_{reg} cells and $IFN\gamma^+$ $CD4^+$ T cells resulted in a higher ROC AUC of 0.91 (Figure 3D) than that of using each cell population alone.

Correlation of clinical factors and donor immune populations

Each of the two cell markers was evaluated for any impact that the following clinical factors may have on their interpretation: clinical donor and recipient age, sex mismatch between donor and recipient, AML *versus* no AML, TBI *versus* no TBI, and presence or absence of aGvHD. Because all donors were related, 7/8 or 8/8 HLA matches and received a myeloablative preparative regimen these variables were not evaluated. Only the $IFN\gamma^+$ $CD4^+$ T cell donor population correlated with a modest decrease in transplant related mortality (*Online Supplementary Table S3*).

Evaluation of the impact of donor immune populations on probability of G-BM and G-PB developing cGvHD

The CBMTG 0601 trial was a prospective randomized non-blinded study comparing donor G-CSF stimulated marrow *versus* G-CSF stimulated peripheral blood. Specific, well-defined clinical endpoints, including cGvHD, were documented up to 2 years post-transplant; this allowed us to directly compare the impact of each cell population in marrow (G-BM) *versus* peripheral blood (G-PB) allografts. Using an interaction test, we evaluated whether either of the two populations were different in terms of their impact on G-BM *versus* G-PB. While the $CD56^{bright}$ NK_{reg} cell population showed no significant impact on overall cGvHD in either donor source (Figure 4A; *P*=0.15), it did show a significant impact on the development of extensive cGvHD (Figure 4B; *P*=0.05) after G-PB transplantation compared to G-BM. By contrast, $IFN\gamma^+$ $CD4^+$ T cells appeared to have no significant impact

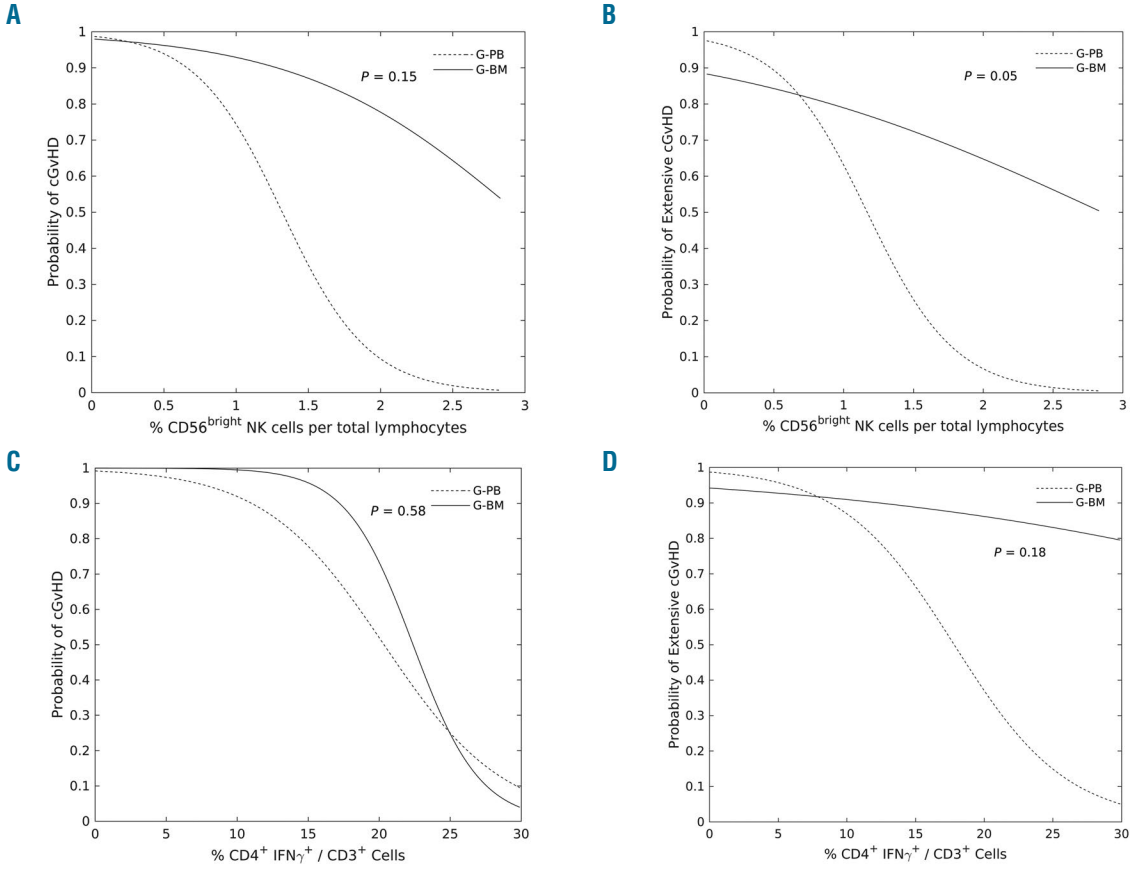

Figure 4. Impact of donor IFNγ producing $CD4^+$ T cells and $CD56^{bright}$ NK_{reg} cells infusion characteristics on cGvHD by donor source using G-PB or G-BM transplantation. (A) The estimated probability of overall cGvHD by treatment (donor source G-BM *versus* G-PB) as a function of the $CD56^{bright}$ cells per total lymphocytes. B) Estimated probability of extensive cGvHD as a function of $CD56^{bright}$ cells per total lymphocytes by donor source (G-PB or G-BM). C) Estimated probability of overall cGvHD as a function of donor IFNγ producing $CD4^+$ T cells by donor source (G-PB or G-BM); D) Estimated probability of extensive cGvHD as a function of donor IFNγ producing $CD4^+$ T cells by donor source (G-PB or G-BM). GvHD: graft-*versus*-host disease; IFN: interferon.

on either G-BM or G-PB and later development of cGvHD (Figure 4C; P=0.58) or extensive cGvHD (Figure 4D; P=0.18).

Discussion

G-CSF-mobilized peripheral blood apheresis donor product is used by a large number of BMT centers, despite the fact that it has a significantly higher rate of cGvHD compared to harvested bone marrow donor product. This major limitation could be minimized if the immune cellular component that influences the higher rate of cGvHD associated with G-PB were characterized. The CBMTG 0601 protocol comparing marrow *versus* apheresis peripheral blood donor product offered a unique opportunity to evaluate the impact of apheresis PB when both donor populations were treated with an identical dosing schedule of G-CSF and collected with identical timing. Moreover, the study population were all adults (> 16 years of age) with related donors (HLA 8/8 and 7/8 matching); using a minimization randomization ensured that the recipient populations were matched for important contributing factors such as preparative regimen and underlying disease.[17] A comprehensive evaluation of donor immune cell components that had previously been associated with the development of cGvHD allowed for an identification not only of populations associated with both donor sources, but, more importantly, of those associated with G-PB. We initially evaluated both G-BM and G-PB together and found two significant associations in two donor cell populations for both overall and extensive cGvHD. Both had inverse relationships with the development of overall cGvHD (IFNγ$^+$ CD4$^+$ T cells and CD56^{bright} NK$_{reg}$ cells) suggesting regulatory functions. We then looked at the populations in a broader context to ensure that the observations were consistent, and found: a) no correlation with other factors except aGvHD in NKreg cell and TRM in the T cell population, and b) the association was consistent when a secondary analysis included patients that died or relapsed before 2 years after BMT. We also found that the inclusion of both populations together increased the association with cGvHD. Having looked at the two populations in the overall population, we subsequently looked at the impact on cGvHD in the two donor sources of graft (G-BM and G-PB) separately. We found that the NK$_{reg}$ population had a proportionately greater impact on extensive cGvHD in G-PB compared to G-BM. This controlled evaluation supports the importance of CD56^{bright} NK$_{reg}$ cells as a suppressive immune population on cGvHD in related donor G-PB transplantation.

It is now well established that strategies that impact the graft cellular composition at the time of transplant can impact the development of cGvHD many months later. As an example, *in vivo* depletion of T cell and B cells with either anti-thymocyte globulin or alemtuzumab, *in vivo* depletion of activated T cell and B cell populations using post transplantation cyclophosphamide, and *ex vivo* depletion of T and B cell populations in haploidentical transplants can reduce the cumulative incidence of cGvHD.[24-30]

A number of donor cell populations have been associated with the onset of cGvHD. These include T cells, B cells and dendritic cells.[31-33] We have previously shown that activated B cells (CpG oligodeoxynucleotide (ODN) responsive, TLR9$^+$) are associated with increased cGvHD, whereas regulatory T cells and IFNγ producing T cells are associated with decreased cGvHD.[23,34] G-CSF administration may influence allograft cellular composition in marrow and peripheral blood products.[35] One study found that the number of donor naïve and memory T cell subsets correlated with infections and aGvHD, and were impacted by whether the graft source was unstimulated marrow or G-CSF-stimulated apheresis donor product.[21] The most comprehensive study was BMT CTN 0201,[36] which evaluated the impact of donor G-PB *versus* unstimulated marrow as the donor product. The BMT CTN 0201 study analysis differed from our study in that their primary analysis focused on: a) unrelated donor sources, b) unstimulated marrow as the control rather than G-BM as in our analysis, and c) overall survival rather than cGvHD. They found that plasmacytoid dendritic cells (pDCs) and naïve T cells were associated with improved overall survival but not with cGvHD. Similar to our study, they found that the T cell content of the G-PB was higher than that of BM grafts. In spite of these differences, they found no increased incidence of cGvHD associated with donor graft CD8$^+$ or CD4$^+$ T cell populations, including those expressing CD45RA, CCR7, and CD62L, CD127, and Ki-67, for regulatory cells or for NKT cells. In the BMT CTN 0201 study, it appears that neither of the two populations identified in the current study, CD56^{bright} NK$_{reg}$ cells or IFNγ producing CD4$^+$ T cells, were included in their evaluations.

Our study reports a strong association of CD56^{bright} NK$_{reg}$ cells with a lower rate of cGvHD in both G-BM and G-PB. CD56^{bright} NK cells were first described in 1992 as IL-2 responsive group with the high affinity IL-2 receptor.[37] CD56^{bright} NKp46 cells (NKregs) have been associated with lower GvHD in other small trials.[38] The CD56^{bright} NK$_{reg}$ population has abundant immunoregulatory cytokines, is located primarily in secondary lymphoid tissues, and has low cytotoxicity. The cytokine-secreting CD56^{bright} CD16^{dim} cells express high levels of inhibitory CD94/NKG2A complex, CD25, and CD117, recognize HLA-E but lack inhibitory major histocompatibility complex (MHC) class 1a allele specific KIRs.[39] Unfortunately, KIR data was not collected as part of these studies and could not be further evaluated in these analyses. Expression of CD117 and NKp46 are typical for some populations of innate lymphocytes, associated with a lack of acute GVHD,[40] which we observed in this study. Our group has previously shown an inverse relationship of CXCR3$^+$ CD56^{bright} NK cells with the onset of cGvHD in a large adult population,[41] further supporting the important role of this population in cGvHD. Moreover, the impact of G-CSF on the induction of CD34$^+$ progenitors for growth into an innate lymphoid effector population appears to be different in marrow *versus* PB.[42]

The other immune suppressive population that had an equal impact regardless of donor source, G-BM and G-PB, was the association of lower proportion IFNγ$^+$ CD4$^+$ T cells with cGvHD. We have previously observed that an increase in IFNγ was associated with a lower onset of late cGvHD in pediatric hematopoietic stem cell transplantation (HSCT) recipients, but had hypothesized that it would have been secreted by an NK$_{reg}$ population[21] as opposed to a CD4$^+$ T cell population. Murine models have shown that the role of IFNγ in GvHD appears to be variable depending on specific times post BMT, as early administration of recombinant IFNγ prevents CD4$^+$ T cell–mediated GVHD.[43] Support for this hypothesis

comes from the fact that donors who have microsatellite polymorphisms with decreased IFNγ production have higher rates of cGvHD.[44] In mouse models, high IFNγ production by NK T cells results in lower rates of cGvHD.[45] Interestingly, IFNγ is not necessary for the development of GvHD in many murine GvHD models,[46,2] and disease can progress despite a lack of IFNγ. The role of $IFN\gamma^+$ $CD4^+$ T cells in the induction of immune tolerance is not well understood. One mechanism may be that classic Th1 $IFN\gamma^+$ $CD4^+$ helper T cells induce immune tolerance *via* the activation of Th1 natural T_{reg} (nT_{reg}).[47] Another possibility is that IFNγ inhibits donor T-cell expansion by promoting apoptosis and suppressing proliferation, thereby eliminating alloreactive T cells in GvHD tissues by interacting with recipient non-hematopoietic cells and upregulating programmed cell death (PD)-1L expression.[48] A third possibility is that the $IFN\gamma^+$ $CD4^+$ T cell population represents a Th1 Treg population[49] that is primed to progress to an IL-10 producing Treg or Tr1 cell population. Lastly, IFNγ–licensed mesenchymal stem cells inhibit proliferation of activated T cells through both an indoleamine 2,3-dioxygenase (IDO) and, possibly, PD-1 dependent manner.[50] Whatever their role, this population requires further study in its potential to predict a later onset of cGvHD.

One question is whether we could define a threshold of either $CD56^{bright}NK_{reg}$ cells or $INF\gamma^+$ $CD4^+$ T cells which are required to be infused per Kg of the recipient. We found that the proportion in the donor product (cells per lymphocytes) and not the infused number of cells per Kg for both $CD56^{bright}$NK cells (P=0.64) and $IFN\gamma^+$ CD4+ T cells (P=0.94) was of the greatest importance, suggesting that for regulatory cells there is a proportional relationship with other cell populations. Thus, focusing on the proportion of the regulatory cell populations such as $CD56^{bright}$NK cells and $IFN\gamma^+$ $CD4^+$ T cells in relation to the total cells infused is more relevant as a strategy, rather than that of achieving a certain threshold dose.

In summary, while controlling for the potential impact of G-CSF on marrow, our studies demonstrated that $CD56^{bright}$ NK_{reg} cells had a much stronger impact on G-PB than on G-BM. This supports the conclusion that a lower proportion of $CD56^{bright}$ NK_{reg} cells results in the high rate of cGvHD seen in G-PB, thus validating the development of strategies to increase the proportion of $CD56^{bright}$ NK_{reg} cells after G-PB transplantation. Strategies could include alternative mobilization agents that selectively increase $CD56^{bright}$ NK_{reg} cells, expansion *ex vivo* followed by adoptive transfer, and *in vivo* $CD56^{bright}$ NK_{reg} cell expansion *via* the administration of low dose IL-2 after transplantation.

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References

1. Lee SJ, Logan B, Westervelt P, et. al. Patient-reported outcomes in 5-year survivors who received bone marrow vs. peripheral blood unrelated donor transplantation: long-term follow-up of a randomized clinical trial. JAMA Oncol. 2016;2(12):1583-1589.
2. Burns LJ, Logan BR, Chitphakdithai P, et. al. Recovery of unrelated donors of peripheral blood stem cells versus recovery of unrelated donors of bone marrow: a prespecified analysis from the Phase III Blood and Marrow Transplant Clinical Trials Network Protocol 0201. Biol Blood Marrow Transplant. 2016;22(6):1108-1116.
3. Anasetti C, Logan BR, Lee SJ, et. al. Blood and Marrow Transplant Clinical Trials Network. Peripheral-blood stem cells versus bone marrow from unrelated donors. N Engl J Med. 2012;367(16):1487-1496.
4. Couban S, Simpson DR, Barnett MJ, et. al. Canadian Bone Marrow Transplant Group A randomized multicenter comparison of bone marrow and peripheral blood in recipients of matched sibling allogeneic transplants for myeloid malignancies. Blood. 2002;100(5):1525-1531.
5. Chu R, Brazauskas R, Kan F, et. al. Comparison of outcomes after transplantation of G-CSF-stimulated bone marrow grafts versus bone marrow or peripheral blood grafts from HLA-matched sibling donors for patients with severe aplastic anemia. Biol Blood Marrow Transplant. 2011;17(7):1018-1024.
6. Nagafuji K, Matsuo K, Teshima T, et. al. Peripheral blood stem cell versus bone marrow transplantation from HLA-identical sibling donors in patients with leukemia: a propensity score-based comparison from the Japan Society for Hematopoietic Stem Cell Transplantation registry. Int J Hematol. 2010;91(5):855-864.
7. Eapen M, Logan BR, Confer DL, et. al. Peripheral blood grafts from unrelated donors are associated with increased acute and chronic graft-versus-host disease without improved survival. Biol Blood Marrow Transplant. 2007;13(12):1461-1468.
8. Schrezenmeier H, Passweg JR, Marsh JC, et al. Worse outcome and more chronic GVHD with peripheral blood progenitor cells than bone marrow in HLA-matched sibling donor transplants for young patients with severe acquired aplastic anemia. Blood. 2007;110(4):1397-1400.
9. Goldman J. Peripheral blood stem cells for allografting. Blood. 1995;85(6):1413-1415.
10. Dhédin N, Prébet T, De Latour RP, et. al. Extensive chronic GVHD is associated with donor blood CD34+ cell count after G-CSF mobilization in non-myeloablative allogeneic PBSC transplantation. Bone Marrow Transplant. 2012;47(12):1564-1568.
11. Mohty M, Bilger K, Jourdan E, et. al. Higher doses of CD34+ peripheral blood stem cells are associated with increased mortality from chronic graft-versus-host disease after allogeneic HLA-identical sibling transplantation. Leukemia. 2003;17(5):869-875.
12. Vasu S, Geyer S, Bingman A, et. al. Granulocyte colony-stimulating factor-mobilized allografts contain activated immune cell subsets associated with risk of acute and chronic graft-versus-host disease. Biol Blood Marrow Transplant. 2016;22(4):658-668.
13. Gallo S, Woolfrey AE, Burroughs LM, et. al. Marrow grafts from HLA-identical siblings for severe aplastic anemia: does limiting the number of transplanted marrow cells reduce the risk of chronic GvHD? Bone Marrow Transplant. 2016;51(12):1573-1578.
14. Arpinati M, Chirumbolo G, Urbini B, et. al. Acute graft-versus-host disease and steroid treatment impair CD11c+ and CD123+ dendritic cell reconstitution after allogeneic peripheral blood stem cell transplantation. Biol Blood Marrow Transplant. 2004;10(2):106-115.
15. Ding L, Zhu H, Yang Y, et. al. The absolute number of regulatory T cells in unmanipulated peripheral blood grafts predicts the occurrence of acute graft-versus-host disease post haplo-identical hematopoietic stem cell transplantation. Leuk Res. 2017;56:13-20.
16. Vela-Ojeda J, García-Ruiz Esparza MA, Reyes-Maldonado E, et. al. Clinical relevance of NK, NKT, and dendritic cell dose in patients receiving G-CSF-mobilized peripheral blood allogeneic stem cell transplantation. Ann Hematol. 2006;85(2):113-120.
17. Couban S, Aljurf M, Lachance S, et. al. Filgrastim-stimulated bone marrow compared with filgrastim-mobilized peripheral blood in myeloablative sibling allografting for patients with hematologic malignancies: a randomized Canadian Blood and

Marrow Transplant Group Study. Biol Blood Marrow Transplant. 2016; 22(8):1410-1415.
18. Przepiorka D, Weisdorf D, Martin P, et.al. Consensus conference on acute GVHD grading bone marrow transplant. 1995; 15:825-828.
19. Sullivan K. Acute and chronic graft versus host disease in man. Int J Cell Cloning 1986;4 Suppl 1:42-93.
20. Cox, D. R.; Oakes, D. 1984 Analysis of Survival Data. New York: Chapman & Hall. ISBN 041224490X.
21. Yakoub-Agha I1, Saule P, Depil S, et. al. Comparative analysis of naïve and memory CD4+ and CD8+ T-cell subsets in bone marrow and G-CSF-mobilized peripheral blood stem cell allografts: impact of donor characteristics. Exp Hematol. 2007; 35(6):861-871.
22. Shaw BE, Apperley JF, Russell NH, et al. Unrelated donor peripheral blood stem cell transplants incorporating pre-transplant in-vivo alemtuzumab are not associated with any increased risk of significant acute or chronic graft-versus-host disease. Br J Haematol. 2011;153(2):244-252.
23. Rozmus J , Schultz KR, Wynne K, et, al. Early and late overall chronic graft-versus-host disease (overall cGvHD) in children is characterized by different Th1/Th2 cytokine profiles: findings of The Children's Oncology Group Study (COG), ASCT0031. Biol Blood Marrow Transplant. 2011;17(12):1804-1813.
24. Bacigalupo A, Lamparelli T, Barisione G, et. al. Gruppo Italiano Trapianti Midollo Osseo (GITMO). Thymoglobulin prevents chronic graft-versus-host disease, chronic lung dysfunction, and late transplant-related mortality: long-term follow-up of a randomized trial in patients undergoing unrelated donor transplantation. Biol Blood Marrow Transplant. 2006;12(5):560-565.
25. Wolschke C, Zabelina T, Ayuk F, et. al. Effective prevention of GVHD using in vivo T-cell depletion with anti-lymphocyte globulin in HLA-identical or -mismatched sibling peripheral blood stem cell transplantation. Bone Marrow Transplant. 2014; 49(1):126-130.
26. Devillier R, Granata A, Fürst S, et. al. Low incidence of chronic GVHD after HLA-haploidentical peripheral blood stem cell transplantation with post-transplantation cyclophosphamide in older patients. Br J Haematol. 2017;176(1):132-135.
27. Kanakry CG, O'Donnell PV, Furlong T, et.al. Multi-institutional study of post-transplantation cyclophosphamide as single-agent graft-versus-host disease prophylaxis after allogeneic bone marrow transplantation using myeloablative busulfan and fludarabine conditioning. J Clin Oncol. 2014;32(31):3497-3505.
28. Carnevale-Schianca F, Caravelli D, Gallo S, et. al. Post-transplant cyclophosphamide and tacrolimus-mycophenolate mofetil combination prevents graft-versus-host disease in allogeneic peripheral blood hematopoietic cell transplantation from HLA-matched donors. Biol Blood Marrow Transplant. 2017;23(3):459-466.
29. Bashey A, Zhang X, Sizemore CA, et. al. T-cell-replete HLA-haploidentical hematopoietic transplantation for hematologic malignancies using post-transplantation cyclophosphamide results in outcomes equivalent to those of contemporaneous HLA-matched related and unrelated donor transplantation. J Clin Oncol. 2013; 31(10):1310-1316.
30. Li Pira G, Malaspina D, Girolami E, et. al. Selective Depletion of αβ T Cells and B Cells for Human Leukocyte Antigen-Haploidentical Hematopoietic Stem Cell Transplantation. A Three-Year Follow-Up of Procedure Efficiency. Biol Blood Marrow Transplant. 2016;22(11):2056-2064.
31. Arai S, Sahaf B, Narasimhan B, et. al. Prophylactic rituximab after allogeneic transplantation decreases B-cell alloimmunity with low chronic GVHD incidence. Blood. 2012;119(25):6145-6154.
32. Delia M, Pastore D, Mestice A, et al. Outcome of allogeneic peripheral blood stem cell transplantation by donor graft CD3+/Tregs ratio: a single-center experience. Biol Blood Marrow Transplant. 2013; 19(3):495-499.
33. Nachbaur D, Kircher B. Dendritic cells in allogeneic hematopoietic stem cell transplantation. Leuk Lymphoma. 2005; 46(10):1387-1396.
34. She K, Gilman AL, Aslanian S, et al. Altered Toll-like receptor 9 responses in circulating B cells at the onset of pediatric chronic GVHD. Biol Blood Marrow Transplant. 2007;13(4):386-397.
35. Shier LR, Schultz KR, Imren S, et al. Differential effects of granulocyte colony-stimulating factor on marrow- and blood-derived hematopoietic and immune cell populations in healthy human donors. Biol Blood Marrow Transplant. 2004;10(9):624-634.
36. Waller EK, Logan BR, Harris WA, et al. Improved survival after transplantation of more donor plasmacytoid dendritic or naïve T cells from unrelated-donor marrow grafts: results from BMTCTN 0201. J Clin Oncol. 2014;32(22):2365-2372.
37. Baume DM, Robertson MJ, Levine H, Differential responses to interleukin 2 define functionally distinct subsets of human natural killer cells. Eur J Immunol. 1992;22(1):1-6.
38. Larghero J, Rocha V, Porcher R, et al. Association of bone marrow natural killer cell dose with neutrophil recovery and chronic graft-versus-host disease after HLA identical sibling bone marrow transplants. Br J Haematol. 2007;138(1):101-109.
39. Karrich JJ, Cupedo T. Group 3 innate lymphoid cells in tissue damage and graft-versus-host disease pathogenesis. Curr Opin Hematol. 2016;23(4):410-415.
40. Munneke JM, Björklund AT, Mjösberg JM, et al. Activated innate lymphoid cells are associated with a reduced susceptibility to graft-versus-host disease. Blood. 2014; 124(5):812-821.
41. Kariminia A, Holtan SG, Ivison S, et al. Heterogeneity of chronic graft-versus-host disease biomarkers: the only consistent association is with CXCL10 and CXCR3+ NK cells. Blood. 2016;127(24):3082-3089.
42. Moretta F, Petronelli F, Lucarelli B, et al. The generation of human innate lymphoid cells is influenced by the source of hematopoietic stem cells and by the use of G-CSF. Eur J Immunol. 2016;46(5):1271-1278.
43. Bogunia-Kubik K, Mlynarczewska A, Wysoczanska B, Lange A. Recipient interferon-gamma 3/3 genotype contributes to the development of chronic graft-versus-host disease after allogeneic hematopoietic stem cell transplantation. Haematologica. 2005;90(3):425-426.
44. Baker J, Verneris MR, Ito M, Shizuru JA, Negrin RS. Expansion of cytolytic CD8(1) natural killer T cells with limited capacity for graft- versus-host disease induction due to interferon gamma production. Blood. 2001;97(10):2923-2931.
45. Lu Y, Waller EK. Dichotomous role of interferon-gamma in allogeneic bone marrow transplant. Biol Blood Marrow Transplant. 2009;15(11):1347-1353.
46. Fu J, Wang D, Yu Y, et al. T-bet is critical for the development of acute graft-versus-host disease through controlling T cell differentiation and function. J Immunol. 2015; 194(1):388-397.
47. Hall BM, Tran GT, Verma ND, et al. Do natural T regulatory cells become activated to antigen specific T regulatory cells in transplantation and in autoimmunity? Front Immunol. 2013;4:208.
48. Wang H, Yang YG. The complex and central role of interferon-γ in graft-versus-host disease and graft-versus-tumor activity. Immunol Rev. 2014;258(1):30-44.
49. Cope A, Le Friec G, Cardone J, Kemper C. The Th1 life cycle: molecular control of IFN-γ to IL-10 switching. Trends Immunol. 2011;32(6):278-286.
50. Chinnadurai R, Copland IB, Patel SR, Galipeau J. IDO-independent suppression of T-cell effector function by IFN-γ licensed human mesenchymal stromal cells. J Immunol. 2014;192(4):1491-1501.