Neutrophil extracellular traps, endothelial injury and use of abatacept for graft-*versus*-host disease prophylaxis

The early post-transplant period is crucial for patients undergoing allogeneic hematopoietic stem cell transplantation (HSCT), as the foundation for later complications resulting from early endothelial injury is laid in this time. Interventions during this period can play a defining role in outcomes of HSCT. Abatacept is a recombinant T-cell co-stimulation blockade agent that has been shown to be effective in reducing acute graft-*versus*-host disease (GvHD).[1-3] Abatacept improves not only inflammatory disease but also cardiovascular and pulmonary outcomes in patients with rheumatological disorders, perhaps suggesting activities broader than T-cell inhibition.[4-6] Neutrophil extracellular traps (NET) consist of decondensed chromatin surrounded by histone and granular proteins such as myeloperoxidase and neutrophil elastase.[7] Dysregulation of NET production (NETosis) has been shown to play a role in endothelial injury.[8-10] We propose that abatacept not only decreases the risk of acute GvHD but may also reduce the incidence of endothelial injury syndromes such as engraftment syndrome, transplant-associated thrombotic microangiopathy (TA-TMA), and lung injury, by limiting NETosis and complement activation.

We collected plasma samples from 262 consecutive pediatric HSCT patients who underwent allogeneic HSCT at Cincinnati Children's Hospital Medical Center (CCHMC) and consented to participate in the CCHMC HSCT biorepository, approved by the institutional review board. We reviewed medical records and clinical databases for demographics and clinical outcomes. Patients were divided into three main categories regarding their GvHD prophylaxis - patients who received calcineurin inhibitor-based therapy with either mycophenolic acid or methotrexate (CNI), abatacept in addition to CNI-based therapy with either mycophenolic acid or methotrexate (CNI-ABA), and patients who received an *ex vivo* T-cell depleted graft (TCD). Abatacept was given per recommended guidelines on days -1, +5, +14, and + 28 at 10 mg/kg per dose.[1-3] All patients were prospectively screened for TA-TMA prior to transplantation at baseline and risk-stratified using Jodele criteria.[11] GvHD was graded using the Glucksberg criteria.[12] Engraftment syndrome was

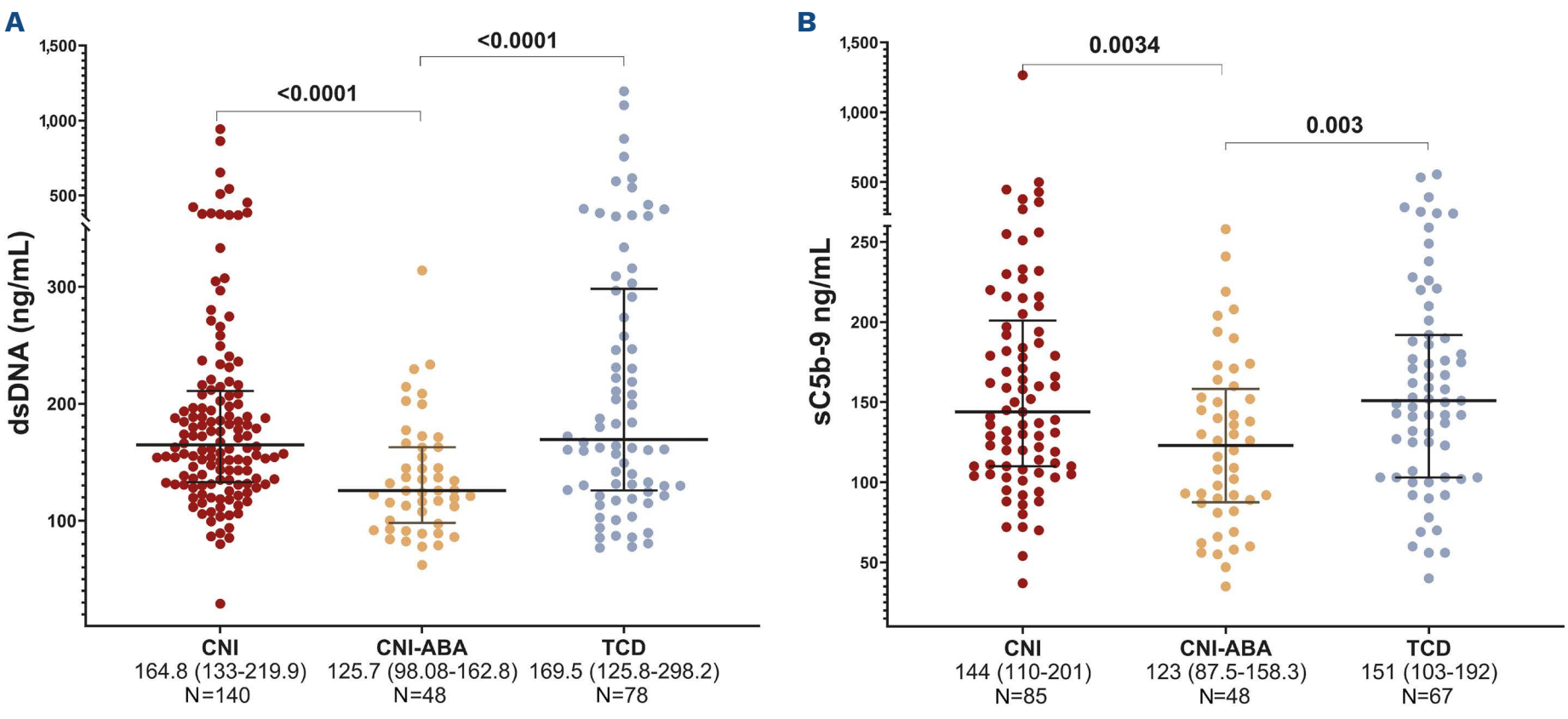

Figure 1. dsDNA and sc5b9 levels according to graft-*versus*-host disease prophylaxis. (A) Graft-*versus*-host disease (GvHD) prophylaxis and day 14 dsDNA levels (median, interquartile range). The x axis shows type of GvHD prophylaxis, with values from patients treated with only a calcineurin inhibitor (CNI) represented as red dots (N=140), values from patients treated with a calcineurin inhibitor and abatacept (CNI-ABA) represented by yellow dots (N=48) and values from patients in whom T-cell depletion was used (TCD) represented by light blue dots (N=78). Day 14 dsDNA levels were significantly lower in patients who received CNI-ABA for GvHD prophylaxis (164.8 *vs*. 125.7 vs. 169.5 ng/mL). (B) GvHD prophylaxis and day 7 sC5b9 levels (median, interquartile range) CNI (red dots) (N=85), CNI-ABA (yellow dots) (N=48), TCD: (light blue dots) (N=67). Day 7 sc5b9 levels were significantly lower in patients who received CNI-ABA for GvHD prophylaxis (144 *vs*. 123 *vs*. 151 ng/mL). Unpaired samples were compared using the Wilcoxon rank sum test (equivalent to the Mann-Whitney test).

diagnosed using Center for International Blood and Marrow Transplant Research (CIBMTR) criteria. We recorded any supplemental oxygen requirement for more than 1 hour, with particular attention to the peri-engraftment (day +7 to +21) period as a marker of early pulmonary injury.

The patients' demographics are shown in *Online Supplementary Table S1*. Fifty-three percent of patients (N=140) received CNI GvHD prophylaxis without abatacept, 26% (N=48) received CNI-ABA and 28% a TCD graft without additional immune suppression (N=74). Thirty-three percent of HSCT recipients (N=87) required supplemental oxygen in the early transplant period (from the start of conditioning to day +26): simple mask/blow-by, 25% (N=22/87); nasal cannula, 53% (N=46/87); non-invasive pressure support, 3% (N=7/87); mechanical ventilation, 14% (N=12/87) at a median of day +8 (range, -13 to +26), for a median of 4 days (range, 1-57). TA-TMA was diagnosed at a median of 25.5 days (interquartile range: 15-48 days) after HSCT.

On day +14 we measured double-stranded (ds)DNA levels as a surrogate for NETosis using the Quant-iT PicoGreen dsDNA reagent and kit, as previously described.[13] Patients who received CNI-ABA GvHD prophylaxis had lower median day +14 dsDNA compared to patients who received CNI or TCD (126 ng/mL *vs*. 165 ng/mL *vs*. 170 ng/mL, *P*<0.0001) (Figure 1A). Underlying diagnosis, stem cell source, conditioning regimen, and GvHD prophylaxis all significantly modified dsDNA levels in univariate analysis (*Online Supplementary Table S1*). We and others have shown that NETosis activates complement, leading to endothelial injury and TA-TMA.[10,13,14] sC5b9 levels are used clinically as a marker of complement activation. Patients who received CNI-ABA had a lower median sC5b9 level compared to patients who received CNI or TCD (123 ng/mL *vs*. 144 ng/mL *vs*. 151 ng/mL, *P*=0.005) (Figure 1B). We also measured transforming growth factor-beta (TGFβ) levels after observing the effect of abatacept on NETosis to see if reduced endothelial injury and complement activation could be related to the effect of abatacept on residual host antigen-presenting cells such as macrophages. TGFβ at day 14 was significantly lower in patients who received CNI-ABA compared to CNI or TCD (369 ng/mL *vs*. 598 ng/mL *vs*. 833 ng/mL, *P*=0.001).

Patients who received CNI-ABA were significantly less likely to develop peri-engraftment supplemental oxygen requirement, engraftment syndrome, or TA-TMA in univariate analysis (Table 2). Patients who received CNI-ABA had a lower incidence of acute GvHD ≥ grade 2 compared to the CNI group, similar to the TCD group (Table 2). There were no statistical differences in 1-year overall mortality

Table 1. Demographic and transplant characteristics according to graft-versus-host disease prophylaxis.

Characteristic	CNI-based N=140	CNI-based with abatacept N=48	T-cell depletion N=74	*P*
Age at transplant, years, median	9.03	6.87	9.57	0.07
Male, N (%)	83 (59)	27 (56)	42 (57)	0.9
Race, N (%) Caucasian African-American	 131 (94) 6 (4)	 45 (94) 2 (6)	 62 (84) 11 (16)	0.034
Diagnosis, N (%) Malignancy Immune deficiency Marrow failure Hematologic disorder Metabolic disorder	 47 (34) 43 (31) 25 (18) 14 (10) 11 (7)	 17 (35) 0 2 (4) 27 (56) 2 (4)	 9 (12) 16 (22) 47 (64) 2 (2) 0	<0.001
Donor type, N (%) Unrelated Related	 90 (64) 50 (36)	 30 (63) 18 (38)	 56 (76) 18 (24)	0.04
HLA type, N (%) Matched Mismatched	 110 (79) 30 (21)	 36 (75) 12 (25)	 40 (54) 34 (46)	0.001
Stem cell source, N (%) Bone marrow PBSC Cord blood	 114 (81) 13 (9) 13 (9)	 44 (92) 3 (6) 1 (2)	 4 (5) 70 (95) -	<0.001
Conditioning regimen type, N (%) Myeloablative Reduced intensity	 77 (55) 63 (45)	 41 (85) 7 (15)	 47 (64) 27 (36)	<0.001

CNI: calcineurin inhibitor; PBSC: peripheral blood stem cells.

and non-relapse mortality (Table 2). We then performed a multivariate analysis adjusted for diagnosis (malignancy/no malignancy) and conditioning regimen intensity (myeloablative/reduced intensity) using CNI-ABA recipients as the reference group. The odds ratios (OR) for peri-engraftment oxygen requirement (CNI OR=3.78, 95% confidence interval [95% CI]: 1.65-9.83, *P*=0.003; TCD OR=3.15, 95% CI: 1.27-8.68, *P*=0.018) and engraftment syndrome (CNI OR=3.43, 95% CI: 1.23-12.24, *P*=0.031; TCD OR=5.53, 95% CI: 1.86-20.7, *P*=0.004) were significantly higher for patients who received CNI and TCD. The OR for TA-TMA was higher in patients who received CNI (CNI OR=2.57, 95% CI: 1.2-5.90, *P*=0.019) but similar in TCD and CNI-ABA recipients (TCD OR=1.31, 95% CI: 0.55-3.28, *P*=0.55).

Abatacept limits T-cell activity so we looked at viremia within 1 year from transplant. We found a higher frequency of Epstein-Barr virus (EBV) viremia in patients who received CNI-ABA both in univariate analysis (*Online Supplementary Table S2*) as well as multivariate analysis when CNI-ABA was used as the reference group (CNI OR=0.48, 95% CI: 0.24-0.95, *P*=0.04; TCD OR=0.32, 95% CI: 0.14-0.68, *P*=0.003). However, the number of patients who received EBV-directed treatment was similar in each group, suggesting EBV reactivation was mild (*Online Supplementary Table S2*). None of the patients with EBV reactivation in the CNI-ABA group developed idiopathic pneumonia syndrome. There was no statistical difference in rate of cytomegalovirus (CMV) reactivation in the CNI-ABA group compared to the other groups in a univariate analysis (*Online Supplementary Table S2*). However, in multivariate analysis adjusted for age and diagnosis, patients who received CNI and TCD had a lower risk of CMV viremia than patients in the CNI-ABA group (CNI OR=0.45, 95% CI: 0.22-0.92, *P*=0.03; TCD OR=0.45, 95% CI: 0.2-1, *P*=0.05). Patients who received CNI-ABA had lower rates of BK viremia compared to patients in the CNI and TCD groups (*Online Supplementary Table S2*) in univariate analysis. The TCD recipients had a higher OR for BK viremia compared to the CNI-ABA and CNI recipients in multivariate analysis (TCD OR=3.68, 95% CI: 1.63-8.83, *P*=0.002; CNI OR=2.05, 95% CI: 0.97-4.61, *P*=0.07). The rates of BK cystitis were not statistically different between the three groups (*Online Supplementary Table S2*). Patients who received CNI-ABA and CNI regimens had lower rates of adenovirus reactivation than patients who received TCD grafts in univariate analysis (*Online Supplementary Table S2*) but not in multivariate analysis (CNI OR=3.42, 95% CI: 1.12-14.98, *P*=0.06; TCD OR=61.83, 95% CI: 0.49-8.83, *P*=0.4). Patients who received CNI-ABA had fewer bloodstream infections in the first 100 days compared to patients in the other groups (*Online Supplementary Table S2*).

The significant decrease in NETosis we observed in patients who received CNI-ABA made us consider the hypothesis that abatacept may have a direct effect on neutrophils. We analyzed RNA expression in neutrophils isolated from day 14 blood in an exploratory study. We compared three patients who received a CNI-based regimen to three patients who received CNI-ABA (*Online Supplementary Table S1C*). This preliminary analysis suggested potentially important effects of abatacept on neutrophils. Thirty genes were differentially expressed in neutrophils of patients who received CNI-ABA compared to patients who did not (*Online Supplementary Figure S1A*). MHC class II molecules, HLA-DQB1 and HLA-DRB5, showed reduced expression in neutrophils of abatacept recipients, suggesting reduced neutrophil activation. Gene set enrichment pathway analysis using Enrichr (KEGG2021 pathway library) indicated important changes in pathways associated with inflammatory processes, including asthma, antigen presentation, GvHD, T-cell differentiation, and ferroptosis, suggesting possible biologically important modifications of neutrophil function in addition to T cells (*Online Supplementary Figure S1B*).[15] In a separate experiment, we measured dsDNA levels in the supernatant of *ex-vivo* neutrophils stimulated with phorbol myristate acetate (PMA) with and without abatacept (*Online Supplementary Figure S1D*). Neutrophils were isolated (Mitenyi, 130-104-434) from a healthy control and four pa-

Table 2. Graft-*versus*-host disease prophylaxis and hematopoietic stem cell transplantation outcomes: univariate analysis.

Variables	CNI-based N=140	CNI based with abatacept N=48	T-cell-depleted, N=74	*P*
Oxygen requirement, N (%)	55 (39)	7 (15)	25 (34)	0.005
Engraftment syndrome, N (%)	29 (21)	4 (8)	20 (27)	0.035
GvHD ≥ grade 2, N (%)	35 (25)	1 (2)	2 (3)	<0.001
TA-TMA, N (%)	55 (39)	10 (21)	18 (24)	0.018
Mortality at 1 year, N (%)	18 (13)	2 (4)	10 (14)	0.21
NRM at 1 year, N (%)	13 (9)	1 (2)	10 (14)	0.074
ANC >500 cells/μL, median (range).	13 (11-17)	12.5 (11-17)	10.5 (9-11)	<0.001

CNI: calcineurin inhibitor; GvHD: graft-versus-host disease; TA-TMA: transplant-associated thrombotic microangiopathy; NRM: non-relapse mortality; ANC: absolute neutrophil count.

tients' baseline samples and incubated with or without 300 μg/mL abatacept for 1 hour at 37°C before stimulation with 50 nM PMA at 37°C for 4 hours. The addition of abatacept did not decrease NET formation as measured by dsDNA in the culture supernatant. These preliminary results suggest that abatacept likely does not directly modulate PMA-induced NETosis *in vitro*. The effects of abatacept on NETosis observed *in vivo* are likely mediated through more complex immunological interactions that cannot be recapitulated in a simplified PMA-driven *in vitro* system. Further targeted studies are in process to understand this complicated balance that takes place in nascent neutrophils in the setting of HSCT and various types of GvHD prophylaxis. Taken together, our results show that abatacept may have broad benefits in patients undergoing allogeneic HSCT, and this effect may be associated with decreased NETosis and complement activation. Further prospective studies are needed to assess the role of abatacept in patients at risk of endothelial and pulmonary injury.

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Contributions

AI, NL, LL, SA, LA and NG performed experiments. AI, NL, SMD and AL, analyzed results and created the figures. AI, NL, SMD, KM, SJ, PK, CT and CD contributed to the design of the study and writing the paper.

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Data-sharing statement

For original data, please contact *azada.ibrahimova@cchmc.org*.

References

1. Koura DT, Horan JT, Langston AA, et al. In vivo T cell costimulation blockade with abatacept for acute graft-versus-host disease prevention: a first-in-disease trial. Biol Blood Marrow Transplant. 2013;19(11):1638-1649.
2. Watkins B, Qayed M, McCracken C, et al. Phase II trial of costimulation blockade with abatacept for prevention of acute GVHD. J Clin Oncol. 2021;39(17):1865-1877.
3. Khandelwal P, Yeh RF, Yu L, et al. Graft-versus-host disease prophylaxis with abatacept reduces severe acute graft-versus-host disease in allogeneic hematopoietic stem cell transplant for beta-thalassemia major with busulfan, fludarabine, and thiotepa. Transplantation. 2021;105(4):891-896.
4. Abrams JR, Kelley SL, Hayes E, et al. Blockade of T lymphocyte costimulation with cytotoxic T lymphocyte-associated antigen 4-immunoglobulin (CTLA4Ig) reverses the cellular pathology of psoriatic plaques, including the activation of keratinocytes, dendritic cells, and endothelial cells. J Exp Med. 2000;192(5):681-694.
5. Benucci M, Bandinelli F, Damiani A, et al. Factors correlated with the improvement of endothelial dysfunction during abatacept therapy in patients with rheumatoid arthritis. J Inflamm Res. 2018;11:247-252.
6. Tardella M, Di Carlo M, Carotti M, Giovagnoni A, Salaffi F. abatacept in rheumatoid arthritis-associated interstitial lung disease: short-term outcomes and predictors of progression. Clin Rheumatol. 2021;40(12):4861-4867.
7. Brinkmann V, Reichard U, Goosmann C, et al. Neutrophil extracellular traps kill bacteria. Science. 2004;303(5663):1532-1535.
8. Saffarzadeh M, Juenemann C, Queisser MA, et al. Neutrophil extracellular traps directly induce epithelial and endothelial cell death: a predominant role of histones. PLoS One. 2012;7(2):e32366.
9. Hudock KM, Collins MS, Imbrogno M, et al. Neutrophil

extracellular traps activate IL-8 and IL-1 expression in human bronchial epithelia. Am J Physiol Lung Cell Mol Physiol. 2020;319(1):L137-L147.
10. Gloude NJ, Khandelwal P, Luebbering N, et al. Circulating dsDNA, endothelial injury, and complement activation in thrombotic microangiopathy and GVHD. Blood. 2017;130(10):1259-1266.
11. Schoettler ML, Carreras E, Cho B, et al. Harmonizing definitions for diagnostic criteria and prognostic assessment of transplantation-associated thrombotic microangiopathy: a report on behalf of the European Society for Blood and Marrow Transplantation, American Society for Transplantation and Cellular Therapy, Asia-Pacific Blood and Marrow Transplantation Group, and Center for International Blood and Marrow Transplant Research. Transplant Cell Ther. 2023;29(3):151-163.
12. Glucksberg H, Storb R, Fefer A, et al. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors. Transplantation. 1974;18(4):295-304.
13. Arai Y, Yamashita K, Mizugishi K, et al. Serum neutrophil extracellular trap levels predict thrombotic microangiopathy after allogeneic stem cell transplantation. Biol Blood Marrow Transplant. 2013;19(12):1683-1689.
14. Yuen J, Pluthero FG, Douda DN, et al. NETosing neutrophils activate complement both on their own NETs and bacteria via alternative and non-alternative pathways. Front Immunol. 2016;7:137.
15. Chen EY, Tan CM, Kou Y, et al. Enrichr: interactive and collaborative HTML5 gene list enrichment analysis tool. BMC Bioinformatics. 2013;14:128.