Published in final edited form as:

Bone Marrow Transplant. 2019 March ; 54(3): 490–493. doi:10.1038/s41409-018-0331-1.

Analysis of BMT CTN-0201 and −0901 samples did not reproduce the reported association between recipient *REG3A* rs7588571 and chronic GVHD

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To the Editor:

Regenerating islet-derived protein 3 alpha (REG3A) is a C-type lectin constitutively expressed and apically secreted by Paneth cells at high levels, with powerful bactericidal activity against gram-positive bacteria of the gut[1]. By limiting the number of mucosal adherent bacteria, REG3A separates gut microbiota and epithelium, promoting intestinal tolerance to microbial antigens and reducing bacterial translocation[2]. Circulating REG3A has been used as a biomarker of gastrointestinal GVHD, and has been used to predict response to therapy and non-relapse mortality (NRM)[3–5]. A recent study reported a novel association between a single nucleotide polymorphism (SNP rs7588571; minor allele A with frequency 0.49) in the putative promotor region of *REG3A* and extensive chronic GVHD (ecGVHD)[6]. In that study, performed in 119 evaluable adult Japanese allogeneic HCT recipients, a higher incidence of ecGVHD was observed in patients with the non-GG genotype than in those with the GG genotype (odds ratio in multivariable analysis: 2.6; 95%

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Competing Interests: The authors have no competing interests.

AR and RS designed the study. SLY and BT performed the experiments. RS analyzed the data. AR wrote the paper. CA, EKW, BlS, BRB, and DJW critically evaluated the results and enhanced the manuscript.

confidence interval [95%CI]: 1. 1–6.0; $P = 0.029$). No association was found between this SNP and acute GVHD (overall and grade II-IV). All patients received a bone marrow graft from an HLA-matched sibling and GVHD prophylaxis with cyclosporine and methotrexate. The median age was ~37 years and conditioning was myeloablative (MA) in >95% of transplants. Our goal in the present study was to test the reproducibility of the observed association between rs7588571 and ecGVHD using samples from two Blood and Marrow Transplant Clinical Trials Network (BMT CTN) protocols 0201 and 0901.

BMT CTN-0201 was a phase 3 randomized multicenter clinical trial (2004–2009, 551 patients) comparing bone marrow (BM) vs. peripheral blood (PB) as graft source in HCT from unrelated donors, and with 2-year overall survival as the primary endpoint[7]. The results demonstrated similar rates of acute GVHD between the groups, but higher rates of cGVHD among recipients of PB grafts (2-year cGVHD: 53% vs. 41% in PB vs. BM groups, respectively). BMT CTN-0901 was a phase 3 randomized multicenter clinical trial (2011–2014, 272 patients, matched sibling or unrelated donors) comparing myeloablative (MA) vs. reduced-intensity (RI) conditioning in patients with acute myeloid leukemia or myelodysplastic syndromes, and with the primary endpoint of 18-month overall survival[8]. The graft source was PB in 92% of patients and BM in 8%. The results demonstrated higher rates of acute and chronic GVHD among MA patients (1.5-year cGVHD: 66% vs. 37% in myeloablative vs. RI, respectively).

From BMT CTN-0201, we excluded patients with 1 or more HLA locus mismatch (HLA-A, B, C, or DRB1; n = 142), active disease at the time of HCT (n = 24), no remaining research aliquots (n = 176), only 1 remaining research aliquot (n = 22, reserved for future studies), and no reported cGVHD data (n = 2). Exclusion of cases with HLA mismatch was performed because the effect size of SNPs in transplant outcomes is generally small[9] and we wanted to eliminate major determinants of GVHD risk *(i.e.,* HLA mismatch[10]) to permit visibility of SNP effects. Exclusion of patients with active disease at the time of HCT was due to their expected high relapse risk, which often translates into clinical interventions (both prophylactic and therapeutic) associated with altered risk of GVHD. From BMT CTN-0901, we excluded patients with no available research specimens (n = 21), those without reported cGVHD data (n = 11), those who did not undergo a transplant (n = 7), those who received anti-thymocyte globulin (n = 40), and 1 HLA locus mismatch (HLA-A, B, C, or DRB1; n = 19) grafts. The minority of patients who received BM allografts (n = 22) were also excluded so the graft source remained uniform in the 0901 analyses. All exclusions for 0201 and 0901 samples were pre-specified and performed prior to analysis.

Clinical data and pre-HCT recipient peripheral blood mononuclear cell samples were provided by the BMT CTN. *REG3A* rs7588571 was sequenced at the University of Minnesota Genomics Center by PCR of genomic DNA (iPLEX Gold method, Sequenom, San Diego, CA, USA), as described previously[11]. We built a multivariable, additive allelic model to estimate the effect of each additional copy of the minor allele on 2-year cumulative incidence of cGVHD (overall and ecGVHD). Graft source (PB vs. BM) as the randomization factor in BMT CTN-0201 and conditioning intensity (RI vs. MA) as the randomization factor in BMT CTN-0901 were included as covariates. Death without cGVHD was considered a competing risk in these Fine and Gray regression models[12].

A total of 185 patients from BMT CTN-0201 (BM: 94; PB: 91) and 152 patients from BMT CTN-0901 (all PB) were included (Table 1). The SNP genotypes were in Hardy- Weinberg equilibrium and call rate was 100%. We found no association between rs7588571 and cGVHD (A vs. G: HR 0.94, 95%CI 0.77–1.15, $P = 0.55$) or ecGVHD (HR 1.04, 95%CI 0.84–1.29, $P = 0.69$) (Table 2, Figure 1). Considering the generally small effect size of SNPs and the complexity of GVHD pathogenesis, most SNP/outcome associations in the HCT setting have failed large-scale validation attempts[9,13,14] for reasons such as small sample size, population stratification, and failure to adjust for multiple comparisons[15]. Using samples from two large BMT CTN randomized clinical trials, we could not reproduce the recently reported association between rs7588571 and cGVHD[6]. Patient-, disease-, and transplant-related specifics were different between the studies and may have contributed to the differences in findings.

Acknowledgements:

Support for the primary clinical trials was provided to the Blood and Marrow Transplant Clinical Trials Network by grant #U10HL069294 from the National Heart, Lung, and Blood Institute and the National Cancer Institute. The Department of Navy, Office of Navy Research, and the National Marrow Donor Program also provided support. Enrollment support was provided by DKMS Germany. Research reported in this publication was also supported by NIH grant P30 CA77598 utilizing the Biostatistics and Bioinformatics Core shared resource of the Masonic Cancer Center, University of Minnesota and by the by the National Center for Advancing Translational Sciences of the National Institutes of Health Award Number UL1TR000114. Any views, opinions, findings, conclusions or recommendations expressed in this material are those of the author(s) and do not reflect the views or the official policy or position of the above-mentioned parties. Armin Rashidi was supported by an American Blood and Marrow Transplantation Young Investigator Award.

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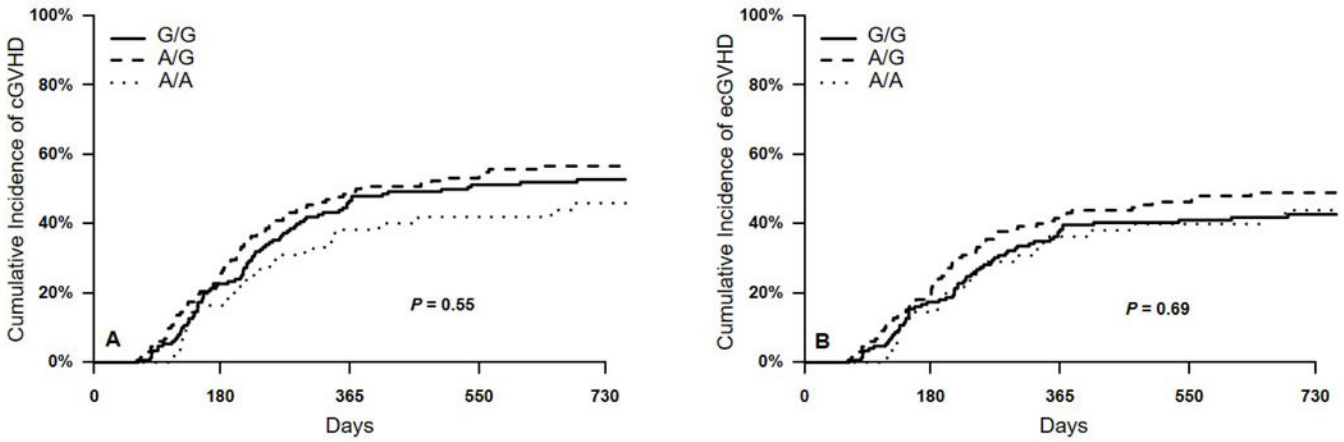

Figure 1: Association between rs7588571 and chronic GVHD in BMT CTN-0201 and −0901.

P values are from multivariable, additive allelic models. cGVHD: Chronic graft-versus-host disease (overall); ecGVHD: Extensive chronic GVHD

Table 1:

Patient-, disease-, and transplant-related characteristics

	G/G	A/G	A/A
N	**150**	**132**	**55**
Age (years), mean (SD)	48 (15)	48 (13)	47 (14)
Gender, n (%)			
Male	86 (57)	69 (52)	31 (56)
Female	64 (43)	63 (48)	24 (44)
Donor			
Matched sibling	40 (27)	40 (30)	10 (18)
Matched unrelated	110 (73)	92 (70)	45 (82)
GVHD prophylaxis, n (%)			
Cyclosporine + Methotrexate	24(16)	16(12)	6 (11)
Tacrolimus + Methotrexate	114(6)	102(77)	43 (78)
Other	12(8)	14 (11)	6 (11)
Conditioning, n (%)			
Cy/TBI	36(24)	27(20)	14(25)
Bu/Cy	39(26)	39(30)	15(27)
Flu/Bu	51(34)	44(33)	14(25)
Flu/Mel	15(10)	9(7)	6(11)
Flu/Bu/ATG	9(6)	13(10)	6(11)
Conditioning intensity, n (%)			
Myeloablative	94(63)]	91(69)	38(79)
Reduced intensity	56(37)	41(31)	17(31)
Disease risk[1], n (%)			
Good risk	94(63)	86(65)	37(67)
Poor risk	56(37)	43(33)	18(33)
Unknown	0	3(2)	0
Recipient CMV serostatus, n (%)			
Positive	80(53)	72(55)	40(73)
Negative	69(46)	60(45)	15(27)
Unknown	1(1)	0	0
Underlying disease, n (%)			
AML	94(63)	83(63)	27(49)
ALL	14(9)	9(7)	7(13)
MDS	30(20)	27(20)	14(25)
MPN	12(8)	13(10)	7(13)

[1]In BMT CTN-0201, poor risk was defined as AML in third remission or not in remission, ALL not in remission, MDS with excess blasts in transformation, chronic myeloid leukemia in blast phase, and chronic myelomonocytic leukemia in any stage. In BMT CTN-0901, poor risk was defined as AML with unfavorable risk cytogenetics, *FLT3* mutation, or complete remission ≥3, and intermediate-II or high risk MDS. GVHD: Graft-versus-host disease; ALL : Acute lymphoblastic leukemia; AML: Acute myeloid leukemia; ATG: Anti-thymocyte globulin; Bu: Busulfan; CMV: Cytomegalovirus; CsA: Cyclosporine; Cy: Cyclophosphamide; MDS: Myelodysplastic syndrome; Mel: Melphalan; MPN: Myeloproliferative neoplasm; TBI: Total body irradiation

Table 2:

Multivariable analysis of chronic GVHD

	Chronic GVHD		Extensive chronic GVHD	
	HR (95%CI)	***P***	**HR (95%CI)**	***P***
rs7588571:A vs. G	0.94 (0.77–1.15)	0.55	1.04 (0.84–1.29)	0.69
Graft source: PB vs. BM	1.72 (1.19–2.48)	<0.01	1.71 (1.14–2.54)	0.01
Conditioning: RI vs. MA	0.79 (0.58–1.09)	0.16	0.73 (0.51–1.04)	0.08

BM: bone marrow; CI: confidence interval; GVHD: Chronic graft-versus-host disease; HR: Hazard ratio; MA: Myeloablative; PB: Peripheral blood; RI: Reduced intensity