whom HCT-CI appeared to be crucially important was the group of patients at high risk for disease relapse (beyond CR1/CR2 or with high-risk cytogenetics). A high HCT-CI in these patients resulted in uniformly negative outcomes. This group of patients with very poor prognosis should probably not be transplanted. Lastly, the intermediate-risk group could be the group that would benefit the most from posttransplant interventions to decrease relapse rate.

This analysis is limited by a relatively small number of patients treated with haploidentical transplants only. Factors included in the risk model were selected from significant factors identified in the multivariable model for PFS without weighting of the prognostic impact of each component. This model needs to be validated in a larger number of patients, including HLA-matched transplants, as it could have major implications in treatment decisions.

In conclusion, risk stratification models combining disease and patient characteristics could further refine prognosis for potential ASCT patients, better identify patients who could benefit from maintenance therapies posttransplant, as well as serve as a tool to further compare results among different studies.

*L.S.B. and R.R. contributed equally.

The online version of this article contains a data supplement.

Contribution: L.S.B. and R.R. collected data and wrote the manuscript; R.M.S. analyzed data and reviewed and approved the manuscript; P. Kongtim. collected data, interpreted the results, and wrote the manuscript; J.C. and G.R. contributed with data collection and reviewed and approved the manuscript; W.W., A.A., S.A., C.M.H., S.P., M.Q., I.F.K., Q.B., B.O., U.P., E.J.S., D.M., K.R., P. Kebriaei, and R.E.C. contributed with treatment of patients and reviewed and approved the manuscript; and S.O.C. contributed with study design, data collection and interpretation, and manuscript writing.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

Correspondence: Stefan O. Ciurea, The University of Texas MD Anderson Cancer Center, Unit 423, 1515 Holcombe Blvd, Houston, TX 77030; e-mail: sciurea@mdanderson.org.

References

1. Blood flashback: Thomas ED, Buckner CD, Banaji M, et al. One hundred patients with acute leukemia treated by chemotherapy, total body irradiation, and allogeneic marrow transplantation. *Blood.* 1977;49(4):511-533. *Blood.* 2016;128(20):2373.

2. Cornelissen JJ, Blaise D. Hematopoietic stem cell transplantation for patients with AML in first complete remission. *Blood.* 2016;127(1):62-70.

3. Löwenberg B, Downing JR, Burnett A. Acute myeloid leukemia. *N Engl J Med.* 1999;341(14):1051-1062.

4. Grimwade D, Hills RK, Moorman AV, et al; National Cancer Research Institute Adult Leukaemia Working Group. Refinement of cytogenetic classification in acute myeloid leukemia: determination of prognostic significance of rare recurring chromosomal abnormalities among 5876 younger adult patients treated in the United Kingdom Medical Research Council trials. *Blood.* 2010; 116(3):354-365.

5. Petersdorf EW, Gooley TA, Anasetti C, et al. Optimizing outcome after unrelated marrow transplantation by comprehensive matching of HLA class I and II alleles in the donor and recipient. *Blood.* 1998;92(10):3515-3520.

6. Sorror ML, Maris MB, Storb R, et al. Hematopoietic cell transplantation (HCT)-specific comorbidity index: a new tool for risk assessment before allogeneic HCT. *Blood.* 2005;106(8):2912-2919.

7. Armand P, Gibson CJ, Cutler C, et al. A disease risk index for patients undergoing allogeneic stem cell transplantation. *Blood.* 2012;120(4):905-913.

8. Slovak ML, Kopecky KJ, Cassileth PA, et al. Karyotypic analysis predicts outcome of preremission and postremission therapy in adult acute myeloid leukemia: a Southwest Oncology Group/Eastern Cooperative Oncology Group Study. *Blood.* 2000;96(13):4075-4083.

9. Ciurea SO, Mulanovich V, Saliba RM, et al. Improved early outcomes using a T cell replete graft compared with T cell depleted haploidentical hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant.* 2012;18(12): 1835-1844.

10. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. *J Am Stat Assoc.* 1958;53(282):457-481.

11. Cox D. Regression models and life tables. *J R Stat Soc Ser B.* 1972;34: 187-220.

12. van Putten W. CART: Stata module to perform classification and regression tree analysis. Chestnut Hill, MA: Statistical Software Components; 2006.

To the editor:

Daratumumab (anti-CD38) induces loss of CD38 on red blood cells

Harold C. Sullivan,[1] Christian Gerner-Smidt,[1] Ajay K. Nooka,[2] Connie M. Arthur,[1] Louisa Thompson,[1] Amanda Mener,[1] Seema R. Patel,[1] Marianne Yee,[3] Ross M. Fasano,[1] Cassandra D. Josephson,[1] Richard M. Kaufman,[4] John D. Roback,[1] Sagar Lonial,[2] and Sean R. Stowell[1]

[1]Center for Transfusion Medicine and Cellular Therapies, Department of Pathology and Laboratory Medicine, [2]Department of Hematology and Medical Oncology, and [3]Department of Pediatrics, Emory University School of Medicine, Atlanta, GA; and [4]Department of Pathology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA

The recently approved anti-CD38 monoclonal antibody daratumumab (DARA) provides a unique therapeutic strategy that more selectively targets plasma cells in patients with plasma cell myeloma.[1-6] While DARA recognizes and removes malignant plasma cells, red blood cells (RBCs) also express CD38.[7] However, many patients on DARA unexpectedly display a negative direct antiglobulin test (DAT) result, despite producing pan-reactive indirect antibody test (IAT) results observed on antibody screens.[8-11] Pan-reactive IAT results caused by DARA complicate pretransfusion testing,[8-11] and the discrepancy between positive IAT results and negative DAT results observed in patients on DARA remains a common, yet incompletely understood,

outcome of DARA treatment. Furthermore, while DARA eliminates plasma cell myeloma through a variety of mechanisms,[1-6] this antibody fails to induce detectable RBC hemolysis.[8,9,12,13] The negative DAT result following DARA treatment coupled with the apparent resistance of RBCs to DARA-induced hemolysis led us to hypothesize that DARA may induce removal of CD38 from the RBC surface, resulting in a negative DAT result that may protect RBCs from DARA-induced removal.

To examine the impact of DARA on RBC CD38 levels, 24 samples were obtained from 13 DARA-treated patients enrolled in a phase 2 efficacy and safety trial (www.clinicaltrials.gov identifier #NCT01985126).
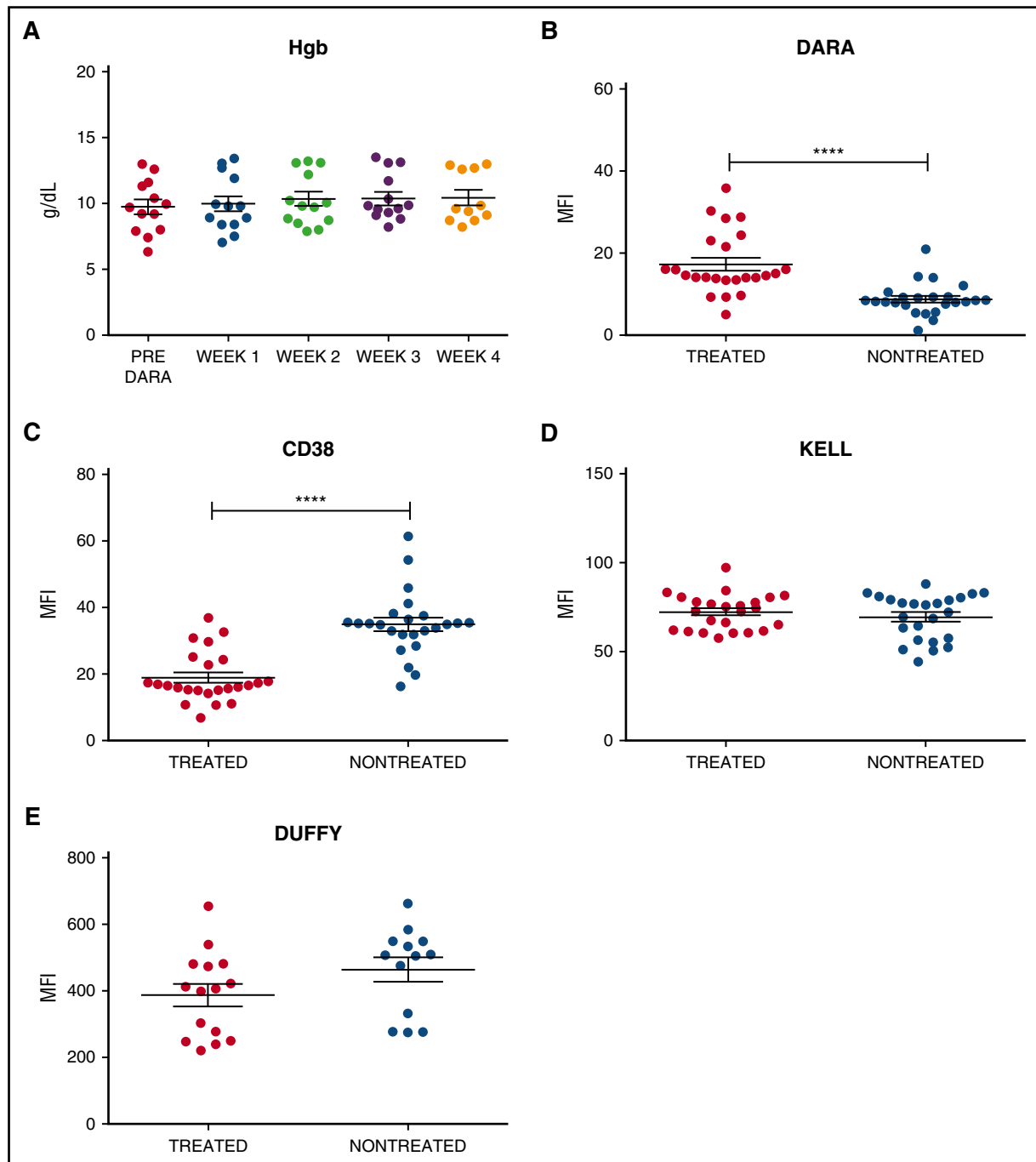
Figure 1. Antibody and CD38 antigen levels are decreased on RBCs isolated from DARA treated individuals. (A) Hemoglobin (Hgb) levels of DARA-treated patients before DARA exposure and at weeks 1, 2, 3, and 4 of treatment (week 4 n = 11, as hemoglobin data were not available for 2 patients). (B-E) Flow cytometric detection of in vivo DARA binding (B), CD38 antigen (C), Kell antigen (D), and Duffy antigen (E) on RBCs isolated from DARA-treated and nontreated patients. Significance was determined by Student *t* test (****$P \leq .0001$). MFI, mean fluorescence intensity.

Samples from 24 nontreated controls were obtained from the clinical laboratory. Similar to previous reports,[8,9] the 24 samples from DARA-treated patients demonstrated anti-RBC antibodies in the serum (antibody screen reactivity: weak positive to 2+; supplemental Table 1, available on the *Blood* Web site). In contrast, none of the samples from DARA-treated patients demonstrated a positive DAT, indicating that standard clinical tests could not detect binding of serum DARA to the patients' own RBCs (supplemental Table 2). Consistent with these and previous results,[8,9,12,13] DARA also failed to cause significant anemia, suggesting minimal (if any)

DARA-mediated hemolysis (Figure 1A). To more specifically investigate the interaction between DARA and RBCs, we examined antibody reactivity on the RBC surface using a more sensitive flow cytometry–based DAT in which antibody engagement of RBCs is assessed using fluorescently labeled anti–human immunoglobulin G.[14,15] RBCs isolated from DARA-treated patients demonstrated weak antibody positivity when compared with RBCs isolated from nontreated patients (Figure 1B). To determine whether low antibody binding to RBCs from DARA-treated individuals reflected incomplete DARA engagement of CD38, we next
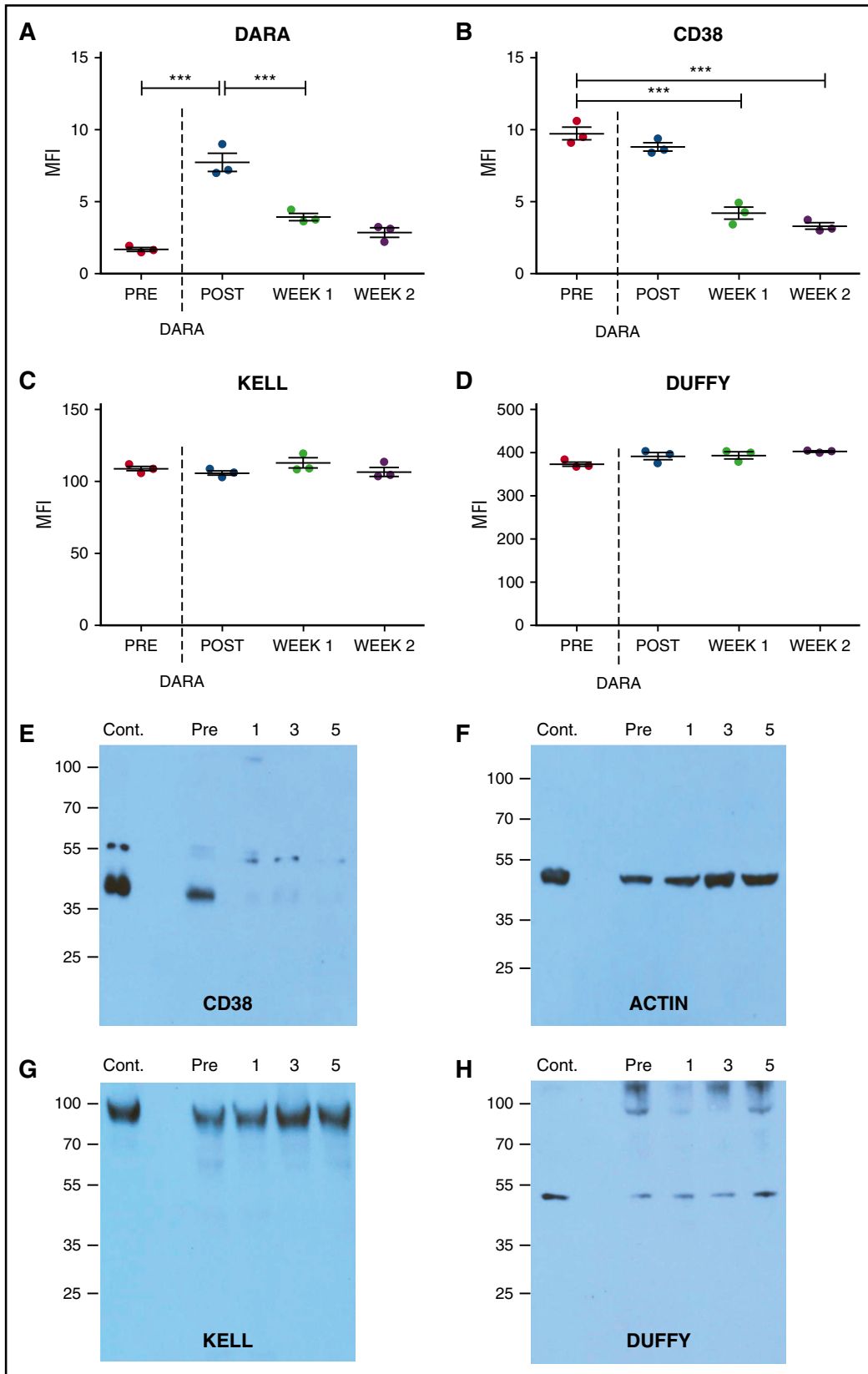
Figure 2. CD38 antigen and bound antibody specifically decline following DARA treatment. (A-D) Bound DARA (ie, flow cytometry–based DAT) (A), CD38 antigen (B), Kell antigen (C), and Duffy antigen (D) before and after initial DARA administration (post, same day as administration; week 1, 1 week after initial administration; week 2, 2 weeks after initial administration). (E-H) Western blot analysis of the CD38 antigen (E), β-actin (F), Kell antigen (G), and Duffy antigen (H) before and after DARA administration (1, 1 week following initial administration; 3, 3 weeks following initial administration; 5, 5 weeks following initial administration). Each time point consists of 3 repeat runs of the same sample. Dotted line represents first DARA administration. Significance was determined in panels A-D by 1-way analysis of variance with Tukey posttest (***$P \leq .001$).

incubated RBCs from DARA-treated or nontreated patients with saturating levels of DARA. RBCs from nontreated patients displayed higher DARA engagement than RBCs from DARA-treated individuals (Figure 1C), strongly suggesting that the reduced DAT level following DARA treatment did not reflect incomplete CD38 binding but instead possibly reflected an actual loss of the CD38 target antigen. In contrast to CD38, DARA treatment failed to alter the levels of Kell or Duffy RBC antigens (Figure 1D-E), indicating that the effects of DARA were CD38 specific.

While this cross-sectional analysis of the impact of DARA on CD38 levels suggests that DARA treatment may alter CD38 detection, it remained possible that the reduced DAT observed following DARA treatment might simply reflect an inability of DARA to fully engage RBCs in vivo. To test this, we examined the impact of DARA treatment on RBC CD38 levels before and after infusion. Antibody (DAT) reactivity on the RBC surface immediately following DARA infusion was robust and occurred at a level similar to that observed following DARA incubation with RBCs from nontreated individuals in vitro (Figure 2A). Additional DARA incubation failed to increase antibody engagement, strongly suggesting that DARA infusion led to complete saturation of CD38-binding sites on RBCs in vivo. To determine whether DARA engagement persists over time, we examined RBCs 1 week following DARA infusion (Figure 2A). Unlike the DAT level observed immediately following DARA exposure, DAT levels dropped considerably 1 week later, largely matching the levels observed in our cross-sectional analysis of patients on active DARA therapy (Figure 1). Incubation of RBCs with saturating levels of DARA failed to increase antibody reactivity, consistent with the excess DARA detected in serum samples when conducting IATs (supplemental Table 1), strongly suggesting that decreases in DATs reflected loss of CD38. DARA-induced decreases in detectable CD38 could be observed within 6 hours of initiating treatment and also exhibited reversibility, as CD38 was again detectable 6 months after discontinuing treatment and corresponded with a loss of serum DARA detectable by IATs (supplemental Figures 1 and 2; supplemental Table 3). Similar to our cross-sectional analysis, DARA-induced changes over time also appeared to be CD38 specific (Figure 2B), as no alterations in Kell or Duffy antigens were detected (Figure 2C-D).

Detection of CD38 on RBCs following DARA treatment could be obscured by the masking of CD38 by degraded DARA fragments and/or complement split products that prevent typical anti–immunoglobulin G antibodies from recognizing bound DARA or the CD38 antigen. As a result, we next determined whether the reduced DATs observed following DARA treatment actually reflected masking of CD38 by DARA fragments and/or complement split products. To accomplish this, we first examined whether DARA induced complement fixation on the RBC surface. No complement was detected on RBCs from DARA-treated individuals (supplemental Figure 3), signifying that complement-mediated masking of CD38 was not likely responsible for decreased CD38 detection. We then directly examined CD38 levels using western blot analysis. While CD38 protein was clearly present on RBCs before DARA infusion, it became undetectable 1 week after treatment (Figure 2E). DARA-induced changes in CD38 were once again antigen specific, as DARA treatment failed to induce similar changes in Kell or Duffy antigens (Figure 2F-G). Taken together, these results demonstrate that DARA possesses the ability to uniformly remove CD38 from the RBC surface without inducing detectable hemolysis.

The ability of DARA to induce antigen loss explains conflicting results that surfaced shortly after the introduction of DARA: the ability of DARA to cause a pan-reactive antibody screen in a patient with a negative DAT result and no detectable hemolysis. While loss of CD38 on the RBC surface may impact RBC interactions with endothelial cells or alter other RBC activities,[16] DARA-induced removal of CD38 appears to prevent further DARA engagement, possibly protecting RBCs from continual removal in the face of ongoing DARA treatment. DARA-induced CD38 removal is consistent with alterations in target antigens observed in murine models of incompatible transfusion and correlative findings in patients with autoimmune hemolytic anemia.[17,18] Similar to DARA, in murine models, antibody engagement can induce loss of target antigen, which typically occurs through an Fcγ receptor–dependent pathway.[15,19-21] In these settings, even partial loss of antigen can render RBCs resistant to antibody-mediated hemolysis,[15] suggesting that a threshold effect may exist for antibody-induced clearance. As a result, even incomplete early loss of CD38 might impact the sensitivity of RBCs to possible DARA-induced removal, although the initial density of CD38 may also play a role in the relative resistance of RBCs to DARA. While the precise mechanisms and factors that regulate antibody-induced antigen loss on the RBC surface remain to be determined, understanding factors that dictate antibody-induced antigen loss versus RBC removal may provide an opportunity to selectively enhance antigen loss over RBC clearance in the setting of autoimmune hemolytic anemia and immune-mediated hemolytic transfusion reactions.[15,19-22] Future studies will explore these intriguing possibilities.

The online version of this article contains a data supplement.

Correspondence: Sean R. Stowell, Center for Transfusion Medicine and Cellular Therapies, Department of Pathology and Laboratory Medicine, 105M Whitehead Building, 615 Michael St, Atlanta, GA 30322; e-mail: srstowe@ emory.edu.

References

1. Lokhorst HM, Plesner T, Laubach JP, et al. Targeting CD38 with daratumumab monotherapy in multiple myeloma. *N Engl J Med.* 2015;373(13):1207-1219.

2. van der Veer MS, de Weers M, van Kessel B, et al. Towards effective immunotherapy of myeloma: enhanced elimination of myeloma cells by combination of lenalidomide with the human CD38 monoclonal antibody daratumumab. *Haematologica.* 2011;96(2):284-290.

3. Usmani SZ, Weiss BM, Plesner T, et al. Clinical efficacy of daratumumab monotherapy in patients with heavily pretreated relapsed or refractory multiple myeloma. *Blood.* 2016;128(1):37-44.

4. Overdijk MB, Verploegen S, Bögels M, et al. Antibody-mediated phagocytosis contributes to the anti-tumor activity of the therapeutic antibody daratumumab in lymphoma and multiple myeloma. *MAbs.* 2015;7(2):311-321.

5. Overdijk MB, Jansen JH, Nederend M, et al. The therapeutic CD38 monoclonal antibody daratumumab induces programmed cell death via Fcγ receptor-mediated cross-linking. *J Immunol.* 2016;197(3):807-813.

6. de Weers M, Tai YT, van der Veer MS, et al. Daratumumab, a novel therapeutic human CD38 monoclonal antibody, induces killing of multiple myeloma and other hematological tumors. *J Immunol.* 2011;186(3):1840-1848.

7. Deaglio S, Mehta K, Malavasi F. Human CD38: a (r)evolutionary story of enzymes and receptors. *Leuk Res.* 2001;25(1):1-12.

8. Oostendorp M, Lammerts van Bueren JJ, Doshi P, et al. When blood transfusion medicine becomes complicated due to interference by monoclonal antibody therapy. *Transfusion.* 2015;55(6 Pt 2):1555-1562.

9. Chapuy CI, Nicholson RT, Aguad MD, et al. Resolving the daratumumab interference with blood compatibility testing. *Transfusion*. 2015;55(6 Pt 2):1545-1554.

10. McCudden C, Axel AE, Slaets D, et al. Monitoring multiple myeloma patients treated with daratumumab: teasing out monoclonal antibody interference. *Clin Chem Lab Med*. 2016;54(6):1095-1104.

11. De Vooght KM, Oostendorp M, van Solinge WW. Dealing with anti-CD38 (daratumumab) interference in blood compatibility testing. *Transfusion*. 2016;56(3):778-779.

12. Dimopoulos MA, Sonneveld P, Sun H. Daratumumab and blood-compatibility testing. *N Engl J Med*. 2016;375(25):2497-2498.

13. Dimopoulos MA, Oriol A, Nahi H, et al; POLLUX Investigators. Daratumumab, lenalidomide, and dexamethasone for multiple myeloma. *N Engl J Med*. 2016;375(14):1319-1331.

14. Arthur CM, Patel SR, Sullivan HC, et al. CD8+ T cells mediate antibody-independent platelet clearance in mice. *Blood*. 2016;127(14):1823-1827.

15. Stowell SR, Liepkalns JS, Hendrickson JE, et al. Antigen modulation confers protection to red blood cells from antibody through Fcγ receptor ligation. *J Immunol*. 2013;191(10):5013-5025.

16. Deaglio S, Mallone R, Baj G, et al. CD38/CD31, a receptor/ligand system ruling adhesion and signaling in human leukocytes. *Chem Immunol*. 2000;75:99-120.

17. Zimring JC, Cadwell CM, Spitalnik SL. Antigen loss from antibody-coated red blood cells. *Transfus Med Rev*. 2009;23(3):189-204.

18. Chown B, Kaita H, Lowen B, Lewis M. Transient production of anti-LW by LW-positive people. *Transfusion*. 1971;11(4):220-222.

19. Zimring JC, Hair GA, Chadwick TE, et al. Nonhemolytic antibody-induced loss of erythrocyte surface antigen. *Blood*. 2005;106(3):1105-1112.

20. Girard-Pierce KR, Stowell SR, Smith NH, et al. A novel role for C3 in antibody-induced red blood cell clearance and antigen modulation. *Blood*. 2013;122(10):1793-1801.

21. Zimring JC, Cadwell CM, Chadwick TE, et al. Nonhemolytic antigen loss from red blood cells requires cooperative binding of multiple antibodies recognizing different epitopes. *Blood*. 2007;110(6):2201-2208.

22. Zimring JC, Spitalnik SL. Pathobiology of transfusion reactions. *Annu Rev Pathol*. 2015;10:83-110.

To the editor:

Idelalisib is effective in patients with high-risk follicular lymphoma and early relapse after initial chemoimmunotherapy

Ajay K. Gopal,[1] Brad S. Kahl,[2] Christopher R. Flowers,[3] Peter Martin,[4] Stephen M. Ansell,[5] Esteban Abella-Dominicis,[6] Brian Koh,[6] Wei Ye,[6] Paul M. Barr,[7] Gilles A. Salles,[8] and Jonathan W. Friedberg[7]

[1]Division of Medical Oncology, Department of Medicine, University of Washington, Clinical Research Division, Fred Hutchinson Cancer Research Center, and Seattle Cancer Care Alliance, Seattle, WA; [2]Oncology Division, Department of Medicine, University of Wisconsin–Madison, Madison, WI; [3]Department of Hematology and Medical Oncology, Winship Cancer Institute of Emory University, Atlanta, GA; [4]Division of Hematology and Oncology, Department of Medicine, Weill Cornell Medicine, New York, NY; [5]Division of Hematology, Mayo Clinic, Rochester, MN; [6]Gilead Sciences, Inc., Foster City, CA; [7]Department of Medicine, James P. Wilmot Cancer Center, University of Rochester, Rochester, NY; and [8]Department of Hematology, University Claude Bernard Lyon 1, Hospices Civils de Lyon, Pierre-Benite, France

Follicular lymphoma (FL) is the most common type of indolent non-Hodgkin lymphoma (NHL), accounting for ~22% of all patients with NHL.[1] Several studies identified a high-risk population of patients, comprising ~20% of all patients with NHL, who had FL that progressed within 24 months from initiation of the first-line chemoimmunotherapy and were at increased risk of death.[2-4]

Idelalisib, a highly selective, orally bioavailable inhibitor of the δ isoform of phosphoinositide 3-kinase δ, is indicated in the United States and Europe as monotherapy for relapsed FL.[5,6] A phase 2, multicenter, open-label, single-arm study evaluated the efficacy and safety of idelalisib in 125 patients previously treated with first-line chemoimmunotherapy who had relapsed indolent NHL that was refractory to both alkylators and rituximab (NCT01282424).[7]

The primary efficacy end point was the overall response rate (ORR), assessed using the standard criteria for lymphoma[8] by an independent review committee. Key secondary end points included duration of response (DOR), lymph node response rate, overall survival (OS), progression-free survival (PFS), and safety.[7] After median duration of treatment with idelalisib monotherapy of 6.6 months, 90% of patients in this study achieved tumor reductions in the size of lymph nodes. The ORR was 57%, with 7 (6%) complete responses and 63/125 (50%) partial responses. The median DOR was 12.5 months and the median PFS was 11 months.[7]

To examine whether idelalisib improved clinical outcomes in patients with FL experiencing early progressive disease (PD) after initial chemoimmunotherapy, we performed a retrospective post hoc analysis in a subgroup of patients with FL. Histological types of FL permitted in the study included grades 1, 2, and 3A (with specific exclusion of grade 3B).

Of the 72 patients with FL,[7] 46 received first-line chemo-immunotherapy. Nine of these patients had late PD after 24 months from the initiation of treatment, and 37 had early PD within ≤24 months from the start of treatment. This high-risk population was well represented in the trial (37/125 [30%]), and all 37 patients received idelalisib treatment (supplemental Figure 1, available on the *Blood* Web site). A total of 19 (51.4%) of these patients were male and 18 (48.6%) were female; 89.2% had grade 1 or 2 FL, and 56.8% had high-risk Follicular Lymphoma International Prognostic Index[9] scores. The majority of patients (21/37 [56.8%]) received rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone-based therapies (R-CHOP-like [R-CHOP, R-CHEP, R-CHO]) as first-line therapy (supplemental Table 1; supplemental Figure 1), and were heavily pretreated, with median number of 3 prior therapies. The median (range) time from the start of first-line therapy to the start of idelalisib treatment was 30.3 (8.9–94.7) months and the mean (standard deviation) intertreatment interval between first- and second-line treatments was 12.5 (6.12) months.

Idelalisib had an acceptable safety profile in patients with early PD. Overall, 34 (91.9%) and 19 (51.4%) early PD patients received oral idelalisib 150 mg twice daily for ≥2 months and ≥6 months, respectively. In total, 13 out of 37 patients (35.1%) and 4 out of 37 patients (10.8%) had to reduce idelalisib dose to 100 mg and 75 mg twice daily, respectively, mostly due to increased levels of alanine aminotransferase and aspartate aminotransferase, followed by diarrhea and neutropenia, consistent with the similar trend observed in the overall study population.[7] The most commonly occurring AEs were diarrhea (51.4%), cough (40.5%), pyrexia (35.1%), nausea (32.4%), neutropenia (24.3%), night sweats (24.3%),