whether obinutuzumab is best used alone or in combination. Cross-trial comparisons have many limitations, but the 18-month PFS with standard-dose obinutuzumab (59%) reported in the article by Byrd et al appears shorter than what was reported for obinutuzumab plus chlorambucil (~80% at 18 months),[3] and the authors argue that this may favor the use of higher-dose obinutuzumab in which the PFS at 18 months was more similar to that in the data for the chlorambucil combination. However, at later time points, the PFS curves of the 2 obinutuzumab dose regimens merged (see Figure 2 in the article by Byrd et al that begins on page 79), and there was no significant PFS benefit that favored the higher-dose obinutuzumab or that would change current dosing practice. These data indicate that higher-dose obinutuzumab has only limited advantage; it achieves deeper remissions which, after finishing the 6 months of treatment, do not translate into any major PFS benefit when compared with standard-dose obinutuzumab. Accordingly, current trials use standard dosing of obinutuzumab and favor obinutuzumab maintenance strategies over the higher-dose obinutuzumab used in combination trials (see table).

The most obvious combination partners for obinutuzumab are the standard CIT regimen (fludarabine-cyclophosphamide-rituximab or bendamustine-rituximab) in which rituximab is replaced by obinutuzumab to achieve more complete and/or durable responses. We also need to take into account that the use of CIT in CLL patients, especially in patients with high-risk disease, is declining because of superb data and broader availability of kinase inhibitors targeting B-cell receptor signaling (ie, the Bruton's tyrosine kinase inhibitor ibrutinib,[9] the phosphatidylinositol 3-kinase delta inhibitor idelalisib,[10] and the B-cell lymphoma 2 antagonist GDC-0199). These agents are changing the current landscape of CLL therapy; the high number of obinutuzumab trials in combination with novel agents (see table) reflects this ongoing major change in clinical practice. With the addition of these new effective agents, including obinutuzumab, to our therapeutic armamentarium, long-term disease control can be achieved in more and more CLL patients, even those with high-risk features. Conversely, the high costs of long-term treatment with these agents to maintain remissions will increase the burden on our health care systems and our patients.

Combination treatment strategies to eradicate CLL, allowing for treatment discontinuation, would therefore be desirable, and clinical trial efforts with this goal are currently ongoing (eg, NCT02401503).

In summary, the study by Byrd et al[1] highlights the high activity of obinutuzumab as a single agent in patients with CLL and corroborates earlier trial experience showing that there is a dose response with anti-CD20 mAbs in CLL.[2,3] Thus, despite the limitations of the trial by Byrd et al, which does not provide a robust rationale for use of higher-dose obinutuzumab in the short-term because of the lack of any major PFS benefit, we should not discard the possibility of a revival of higher-dose obinutuzumab in the future, for example in combination strategies in which achievement of deep remissions is the goal.

Conflict-of-interest disclosure: J.A.B. received research funding from Pharmacyclics and Gilead. ■

REFERENCES

1. Byrd JC, Flynn JM, Kipps TJ, et al. Randomized phase 2 study of obinutuzumab monotherapy in symptomatic, previously untreated chronic lymphocytic leukemia. *Blood.* 2016;127(1):79-86.

2. Byrd JC, Murphy T, Howard RS, et al. Rituximab using a thrice weekly dosing schedule in B-cell chronic lymphocytic leukemia and small lymphocytic lymphoma demonstrates clinical activity and acceptable toxicity. *J Clin Oncol.* 2001;19(8):2153-2164.

3. O'Brien SM, Kantarjian H, Thomas DA, et al. Rituximab dose-escalation trial in chronic lymphocytic leukemia. *J Clin Oncol.* 2001;19(8):2165-2170.

4. Keating MJ, O'Brien S, Albitar M, et al. Early results of a chemoimmunotherapy regimen of fludarabine, cyclophosphamide, and rituximab as initial therapy for chronic lymphocytic leukemia. *J Clin Oncol.* 2005;23(18):4079-4088.

5. Hallek M, Fischer K, Fingerle-Rowson G, et al; International Group of Investigators; German Chronic Lymphocytic Leukaemia Study Group. Addition of rituximab to fludarabine and cyclophosphamide in patients with chronic lymphocytic leukaemia: a randomised, open-label, phase 3 trial. *Lancet.* 2010;376(9747):1164-1174.

6. Fischer K, Cramer P, Busch R, et al. Bendamustine in combination with rituximab for previously untreated patients with chronic lymphocytic leukemia: a multicenter phase II trial of the German Chronic Lymphocytic Leukemia Study Group. *J Clin Oncol.* 2012;30(26):3209-3216.

7. Goede V, Fischer K, Busch R, et al. Obinutuzumab plus chlorambucil in patients with CLL and coexisting conditions. *N Engl J Med.* 2014;370(12):1101-1110.

8. Hillmen P, Robak T, Janssens A, et al; COMPLEMENT 1 Study Investigators. Chlorambucil plus ofatumumab versus chlorambucil alone in previously untreated patients with chronic lymphocytic leukaemia (COMPLEMENT 1): a randomised, multicentre, open-label phase 3 trial. *Lancet.* 2015;385(9980):1873-1883.

9. Byrd JC, Furman RR, Coutre SE, et al. Three-year follow-up of treatment-naïve and previously treated patients with CLL and SLL receiving single-agent ibrutinib. *Blood.* 2015;125(16):2497-2506.

10. Furman RR, Sharman JP, Coutre SE, et al. Idelalisib and rituximab in relapsed chronic lymphocytic leukemia. *N Engl J Med.* 2014;370(11):997-1007.

DOI 10.1182/blood-2015-10-676882

● ● ● IMMUNOBIOLOGY

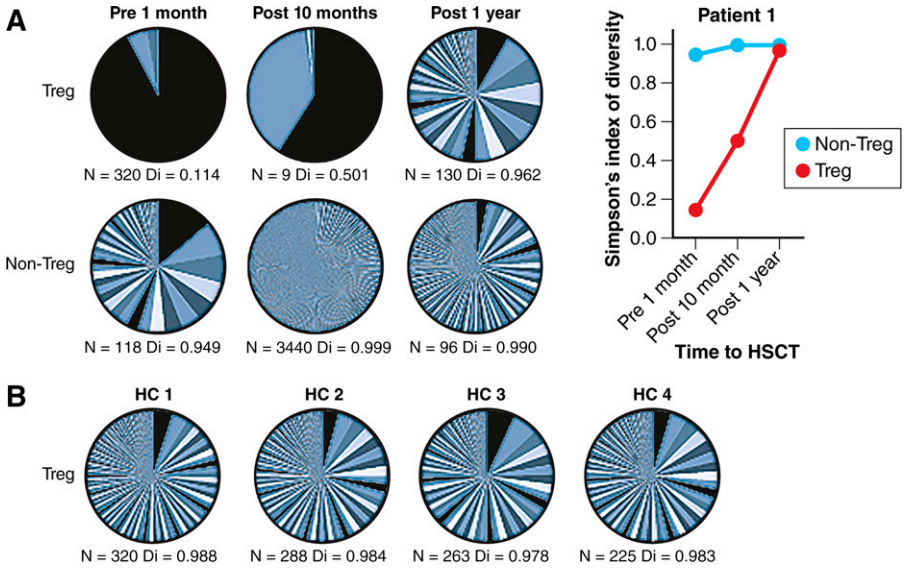
Comment on Delemarre et al, page 91

Rebooting autoimmunity with autologous HSCT

John A. Snowden SHEFFIELD TEACHING HOSPITALS NHS FOUNDATION TRUST; UNIVERSITY OF SHEFFIELD

Autologous hematopoietic stem cell transplantation (HSCT) is increasingly used for severe autoimmune and inflammatory diseases, but the mechanisms involved have yet to be elucidated. In this issue of *Blood*, Delemarre et al report their findings in both animal and human models which provide insights into restoration of functionality and diversity within the regulatory T-cell (Treg) compartment following HSCT.[1]

The findings of Delemarre et al come some 25 years after their fellow Dutch investigator, the late Prof Dirk van Bekkum (1925-2015), started to lay down the preclinical foundations for using HSCT in autoimmune diseases with an elegant series of experiments.[2,3] Over the last 2 decades the field has developed gradually in the clinical setting in a variety of diseases. International databases now have several thousand patients and there is an increasing evidence base of large series and randomized controlled trials to support the use



Successful autologous HSCT leads to a renewed and more diverse Treg TCR repertoire. (A) Patient with severe autoimmune disease pre- and postautologous HSCT. The pie charts show TCR β-chain sequencing results in Treg and non-Treg cells collected from a patient with severe autoimmune disease before and after autologous HSCT. N represents the number of different TCR sequences per sample and Di indicates diversity (0 = no diversity, 1 = maximal diversity). Changes in Di before and after HSCT are also shown in the graph. (B) Four healthy controls (HC). For comparison, TCR β-chain sequencing results in Treg cells from 4 HCs are shown in a similar fashion. The figure has been adapted from Figure 4 in the article by Delemarre et al that begins on page 91. Professional illustration by Patrick Lane, ScEYEnce Studios.

of autologous HSCT, particularly in multiple sclerosis (MS), connective tissue diseases, and Crohn's disease. Consensus clinical guidelines are now available to assist with patient selection and treatment schedules.[4,5]

To date, a variety of mechanisms have been proposed to explain the clinical effects (and associated immune "reboot") of autologous HSCT in severe autoimmune diseases. Whereas a "debulking of inflammation" is an instantaneous and predictable effect of any high-dose cytotoxic conditioning regimen, sustained clinical responses are best explained by long-term alterations in immune reconstitution via thymic and/or extrathymic pathways. Shifts in T- and B-cell subpopulations from memory to naive cell dominance, with restoration of polyclonal T-cell receptor (TCR) diversity, correction of immune gene expression abnormalities, and other changes in T cells, B cells, plasmablasts, and natural killer cells support immune re-education and tolerization with autologous HSCT. Like clinical responses, the ability to reconstitute varies between diseases and patients.[5-10] However, 1 finding common to several diseases, including MS, systemic lupus erythematosus, systemic sclerosis, and juvenile idiopathic arthritis, is enhanced posttransplant levels of Treg cells.[5,6,8-10]

Delemarre and coworkers provide a further step toward a better understanding of the resetting of the Treg compartment. First, they report the reconstitution of host and donor-derived Treg compartments following congenic HSCT in a proteoglycan-induced mouse arthritis model. Although initially both populations expand, the naive, donor-derived Treg pool predominates to reconstitute a stable, functional, and tolerizing donor-derived Treg compartment.

Second, they use baseline and follow-up samples taken from pediatric patients receiving autologous HSCT for juvenile idiopathic arthritis and dermatomyositis. Prior to HSCT, patients displayed characteristics of a restricted oligoclonal Treg receptor repertoire. Subsequent clinical improvements (with 1 patient out to more than a decade) were associated with a striking resetting of Treg TCR diversity. TCR diversity of other non-Treg compartments also increased, although this was nowhere near as pronounced as changes to the Treg compartment (see figure).

Finally, returning to their animal model, they attempt to augment the therapeutic effect with additional Treg infusions. However, the biology in this area is clearly not so straightforward. Despite decreasing proinflammatory cytokine production, there was none of the desired improvement in clinical indices. Instead, there was a delay in immune reconstitution of the graft-derived T-cell compartment and, in view of the potential for risk, the authors highlight caution in extrapolating a similar approach for clinical studies.

What implications does this study have for other diseases? Clearly, autoimmune diseases are diverse in both their etiologies, pathogenesis, and manifestations, and the clinical studies were performed in relatively rare pediatric diseases. Similar studies are warranted in autoimmune diseases that are more commonly transplanted, such as MS, systemic sclerosis, and Crohn's disease, and across all age groups given the variation in thymic involution and rebound.

Response to autologous HSCT also varies within the same disease and has been related to patient-related factors, such as stage of disease, and there is scope for improving responses and selecting the best patients. In MS, restoration of TCR diversity has been reported as a potential predictor of clinical response.[9] Outcomes in juvenile idiopathic arthritis have also been related to the degree of thymic processing in reconstituting the T-cell compartment.[10] TCR characterization may enable monitoring of pathogenic or protective T-cell clones following autologous HSCT for autoimmune diseases. Whether more specific assessment of Treg TCR diversity, as described by Delemarre et al, is a better predictor remains to be established. It is possible that such testing may assist with the selection of patients most likely to respond from HSCT and in identifying patients who may benefit from early interventions, including low-dose maintenance or salvage treatments or additional cellular therapies. However, in the current study, despite the reasonable logic, no benefit was achieved through additional Treg infusions. Clearly, this area requires more investigation.

Mechanistic studies may have been historically challenging to coordinate across a range of autoimmune diseases, perhaps because autologous HSCT has most often been used sporadically as an exceptional treatment in refractory patients, and sometimes as an emergency salvage procedure. With a greater evidence base the numbers of patients being treated are increasing substantially in some diseases, such as MS. Moreover, patients are being treated at an earlier stage in their disease, when both end organ damage and immune defects are potentially more reversible. Recent publication of international biobanking guidelines may facilitate access of samples to laboratories for specialized immune reconstitution studies before and after HSCT.[6]

Destroying dysfunctional immune systems with autologous HSCT and then closely observing how they are rebuilt from scratch via thymic and other pathways not only helps us to ameliorate intractable disabling and life-threatening states, but also has also provided useful insights into the pathogenesis of autoimmune diseases. By highlighting the ability of autologous HSCT to generate a naive, functional, and diverse donor-derived Treg compartment, Delemarre and colleagues add a further piece to van Bekkum's visionary jigsaw.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

REFERENCES

1. Delemarre EM, van den Broek T, Mijnheer G, et al. Autologous stem cell transplantation aids autoimmune patients by functional renewal and TCR diversification of regulatory T cells. *Blood*. 2016;127(1):91-101.

2. van Bekkum DW, Bohre EP, Houben PF, Knaan-Shanzer S. Regression of adjuvant-induced arthritis in rats following bone marrow transplantation. *Proc Natl Acad Sci USA*. 1989;86(24):10090-10094.

3. Snowden JA, Brooks PM, Biggs JC. Haemopoietic stem cell transplantation for autoimmune diseases. *Br J Haematol*. 1997;99(1):9-22.

4. Tyndall A, Gratwohl A. Blood and marrow stem cell transplants in auto-immune disease: a consensus report written on behalf of the European League against Rheumatism (EULAR) and the European Group for Blood and Marrow Transplantation (EBMT). *Bone Marrow Transplant*. 1997;19(7):643-645.

5. Snowden JA, Saccardi R, Allez M, et al; EBMT Autoimmune Disease Working Party (ADWP); Paediatric Diseases Working Party (PDWP). Haematopoietic SCT in severe autoimmune diseases: updated guidelines of the European Group for Blood and Marrow Transplantation. *Bone Marrow Transplant*. 2012; 47(6):770-790.

6. Alexander T, Bondanza A, Muraro PA, et al. SCT for severe autoimmune diseases: consensus guidelines of the European Society for Blood and Marrow Transplantation for immune monitoring and biobanking. *Bone Marrow Transplant*. 2015;50(2):173-180.

7. Ponchel F, Verburg RJ, Bingham SJ, et al. Interleukin-7 deficiency in rheumatoid arthritis: consequences for therapy-induced lymphopenia. *Arthritis Res Ther*. 2005; 7(1):R80-R92.

8. Baraut J, Grigore EI, Jean-Louis F, et al. Peripheral blood regulatory T cells in patients with diffuse systemic sclerosis (SSc) before and after autologous hematopoietic SCT: a pilot study. *Bone Marrow Transplant*. 2014;49(3): 349-354.

9. Muraro PA, Robins H, Malhotra S, et al. T cell repertoire following autologous stem cell transplantation for multiple sclerosis. *J Clin Invest*. 2014;124(3): 1168-1172.

10. de Kleer I, Vastert B, Klein M, et al. Autologous stem cell transplantation for autoimmunity induces immunologic self-tolerance by reprogramming autoreactive T cells and restoring the CD4+CD25+ immune regulatory network. *Blood*. 2006;107(4):1696-1702.

DOI 10.1182/blood-2015-11-678607

Comment on Melenotte et al, page 113

Identifying risk factors for B-cell lymphoma

Christopher R. Flowers[1] and Christine F. Skibola[2] [1]EMORY UNIVERSITY; [2]THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

In this issue of *Blood*, Melenotte and colleagues provide an interesting and provocative analysis of a potential novel risk factor for B-cell non-Hodgkin lymphoma (NHL).[1]

This is one of the first studies to focus on *Coxiella burnetii* (the infectious agent associated with Q fever) as an inciting factor for lymphomas. Building on the identification of an incident case, the authors examined the incidence of lymphoma among individuals within a cohort of patients with Q fever. These analyses provide clinically meaningful insights that may aid in the identification of a novel risk factor for diffuse large B-cell lymphoma (DLBCL) and other B-cell NHLs and support the development of a comprehensive understanding of factors associated with lymphoma incidence. Utilizing a French National Referral Center for Q fever database of 1468 consecutive patients diagnosed from 2004 to 2014, and accounting for differences in age and sex distribution between the Q fever cohort and the general French population, the authors identified an increase in the incidence of DLBCL and follicular lymphoma (FL) in individuals who had Q fever compared with the general population, with standardized incidence rates of 25.4 (95% confidence interval [CI], 11.4-56.4) and 6.7 (95% CI, 0.9-47.9), respectively. Moreover, a diagnosis of Q fever with a persistent focal infection was noted to have a greater risk of lymphoma with a hazard ratio of 9 over the period of observation. For these analyses, acute Q fever was defined by the association of clinical symptoms (fever, hepatitis, and/or pneumonia) with the serological criteria of a phase 2 immunoglobulin G (IgG) titer ≥200 and a phase 2 IgM titer ≥50, seroconversion or a positive polymerase chain reaction (PCR), and no endocarditis. Supporting these epidemiological data were findings that interleukin-10 production was significantly increased in patients with lymphoma, particularly those with Q fever. Moreover, *C burnetii* was detected in $CD68^+$ macrophages within lymphoma and lymphadenitis tissues in patients with and without lymphoma, but infection was localized in plasmacytoid dendritic cells in lymphoma tissues only providing early evidence for a possible pathway to lymphomagenesis. Although the selection process for assembly of the cohort of patients and serological samples for the Q fever database may introduce biases that could influence the strength of association between *C burnetii*/Q fever and the incidence of B-cell NHLs, this analysis provides insight for a new infectious disease linked to lymphoma. Further validation of these observations would add Q fever to the list of infectious agents such as Epstein-Barr virus, *Helicobacter pylori*, and hepatitis C infection involved in the pathogenesis of B-cell lymphomas.

These findings must be considered in the context of numerous other analyses examining infectious and other risk factors for B-NHL, most notably the recent large international pooled InterLymph Subtypes Project.[2-5] The International Lymphoma Epidemiology Consortium (InterLymph) was formed in 2001 to perform pooled case-control studies that maximize statistical power to identify common as well as distinct risk factors among NHL subtypes. This group has identified numerous demographic, medical history, environmental, and genetic risk factors for lymphoma, exposing the common threads and heterogeneity in etiology across NHL subtypes. Of relevance to the present study, the subtype study of DLBCL performed a pooled analyses of 4667 cases and 22 639 controls from epidemiological studies in Australia, Europe, and North America and

2016 127: 8-10
doi:10.1182/blood-2015-11-678607

Rebooting autoimmunity with autologous HSCT

John A. Snowden

Blood (print ISSN 0006-4971, online ISSN 1528-0020), is published weekly by the American Society of Hematology, 2021 L St, NW, Suite 900, Washington DC 20036.