patients, whereas NGS detected 266 mutations in 163 patients, including the 161 mutations detected by SS and additional 73 mutations present only at low level (<20%, low-level mutations).[1] Furthermore, NGS was able to find multiple mutations in 23% of the cases compared with only 10% detected by SS (see figure, part A), and a high percentage of these cases were apparently carrying compound mutations.[1] Because it was previously shown that many compound mutations could be artifacts caused by polymerase chain reaction–mediated recombination,[5] Deininger at al developed and applied an algorithm that predicts the frequency of false-positive compounds mutations. They found that 88% of possible compound mutations were most likely false positives and that only 9% of patients (42% of patients with multiple mutations) had true compound mutations at baseline (see figure, part A).[1] True compound mutations involved one mutation classically implicated in TKI resistance, underlining that the presence of particular mutations makes the resistant clones develop an additional mutation on the same allele, and this may occur more frequently in patients treated sequentially with multiple TKIs such as in the PACE trial.

In vitro experiments had previously shown that compound mutations involving the T315I variant were able to confer resistance to ponatinib.[6] Moreover, in a cohort of 64 Ph-positive acute lymphocytic leukemia or CML patients treated in the PACE trial or in the ponatinib expanded access program, compound mutations were reported to occur quite frequently during ponatinib treatment, particularly in more advanced phases of the disease.[6] Quite the opposite, the results of Deininger et al show that the presence of true compound mutations in pluri-resistant CML patients in chronic phase is quite low and that they do not represent a major cause of primary resistance to ponatinib. Irrespective of the method used (SS or NGS) to assess the presence of mutations, the responses observed in chronic-phase CML patients enrolled in the PACE trial and their durability could not be predicted on the basis of the mutational status (presence and type of mutation) determined at baseline (see figure, part B).[1] Furthermore, reanalyzing with the SS method postbaseline samples of 129 patients who discontinued ponatinib treatment of poor response or for adverse events, the expansion of clones harboring new mutations not detected at baseline by NGS, including compound mutations, were found in only 8 patients, suggesting again that mutations, including compound mutations, are not major drivers in determining resistance to ponatinib.

These findings reveal new aspects of the role that mutations and genomic instability have in determining progressive resistance to TKI therapy. During the initial phase of the disease, resistance to TKI therapy seems to be mainly dependent on the selection and expansion of clones harboring BCR-ABL1 mutations that allow the persistence of the BCR-ABL1 tyrosine kinase activity. At later stages of the disease, resistance to TKI therapy seems to rely mainly on the activation of BCR-ABL1–independent mechanisms,[7] which involve oncogenic pathways other than BCR-ABL1 that make BCR-ABL1 TK activity redundant and dispensable. According to this interpretation, the efficacy of ponatinib in the patients resistant to multiple TKIs could be explained not only by its ability to inactivate BCR-ABL1 mutations, but also by its aptitude to suppress other oncogenic routes than those strictly dependent on the BCR-ABL1 TK activity. Indeed ponatinib is known to be a multikinase inhibitor.[8] This consideration may support a more extensive investigation of the efficacy of ponatinib, either in early chronic-phase CML, because of its ability to suppress mutations and controvert genomic instability, as well as in Ph-negative hematologic malignancies, owing to its multitarget activity.[9,10]

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

REFERENCES

1. Deininger MW, Hodgson JG, Shah NP, et al. Compound mutations in BCR-ABL1 are not major drivers of primary or secondary resistance to ponatinib in CP-CML patients. *Blood.* 2016;127(6):703-712.
2. O'Hare T, Eide CA, Deininger MWN. Bcr-Abl kinase domain mutations, drug resistance, and the road to a cure for chronic myeloid leukemia. *Blood.* 2007;110(7): 2242-2249.
3. Cortes JE, Kantarjian H, Shah NP, et al. Ponatinib in refractory Philadelphia chromosome-positive leukemias. *N Engl J Med.* 2012;367(22):2075-2088.
4. Cortes JE, Kim DW, Pinilla-Ibarz J, et al; PACE Investigators. A phase 2 trial of ponatinib in Philadelphia chromosome-positive leukemias. *N Engl J Med.* 2013; 369(19):1783-1796.
5. Parker WT, Phillis SR, Yeung DT, Hughes TP, Scott HS, Branford S. Many BCR-ABL1 compound mutations reported in chronic myeloid leukemia patients may actually be artifacts due to PCR-mediated recombination. *Blood.* 2014;124(1):153-155.
6. Zabriskie MS, Eide CA, Tantravahi SK, et al. BCR-ABL1 compound mutations combining key kinase domain positions confer clinical resistance to ponatinib in Ph chromosome-positive leukemia. *Cancer Cell.* 2014;26(3):428-442.
7. Morotti A, Panuzzo C, Fava C, Saglio G. Kinase-inhibitor-insensitive cancer stem cells in chronic myeloid leukemia. *Expert Opin Biol Ther.* 2014;14(3):287-299.
8. Huang W-S, Metcalf CA, Sundaramoorthi R, et al. Discovery of 3-[2-(imidazo[1,2-b]pyridazin-3-yl)ethynyl]-4-methyl-N-4-[(4-methylpiperazin-1-yl)methyl]-3-(trifluoromethyl)phenylbenzamide (AP24534), a potent, orally active pan-inhibitor of breakpoint cluster region-abelson (BCR-ABL) kinase including the T315I gatekeeper mutant. *J Med Chem.* 2010;53(12):4701-4719.
9. Gozgit JM, Wong MJ, Wardwell S, et al. Potent activity of ponatinib (AP24534) in models of FLT3-driven acute myeloid leukemia and other hematologic malignancies. *Mol Cancer Ther.* 2011;10(6):1028-1035.
10. Shah NP, Talpaz M, Deininger MWN, et al. Ponatinib in patients with refractory acute myeloid leukaemia: findings from a phase 1 study. *Br J Haematol.* 2013;162(4):548-552.

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IMMUNOBIOLOGY

Comment on Guo et al, page 735

B cells help CD8$^+$ T-cell responses

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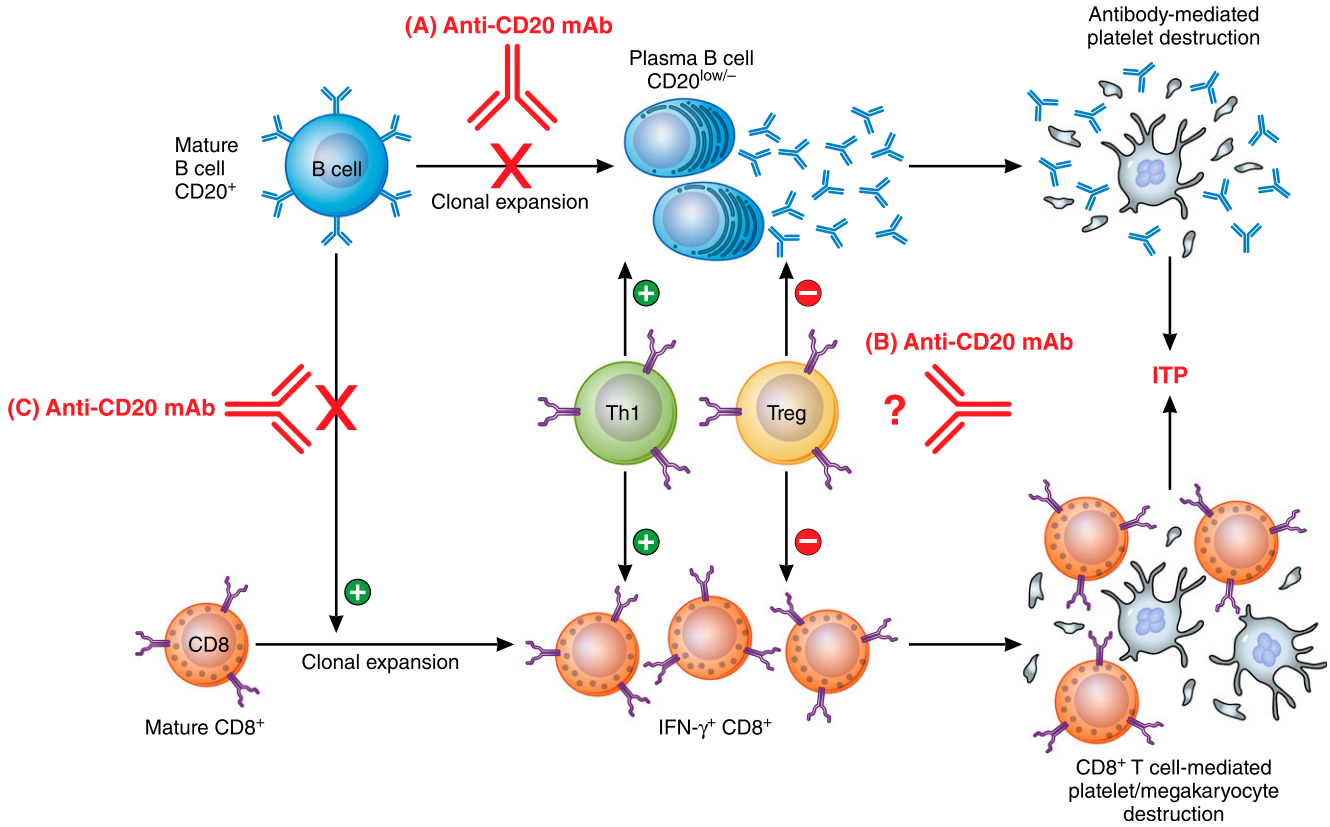
In this issue of *Blood*, Guo et al used an anti-CD20 monoclonal antibody (mAb) in a murine model of immune thrombocytopenia (ITP) to show for the first time that B-cell depletion therapy inhibits splenic CD8$^+$ T-cell proliferation and rescues T-cell–mediated ITP.[1]

B-cell depletion therapy using a chimeric anti-human CD20 mAb, rituximab, is a commonly used treatment of both adults and children with ITP. It is also widely used in several other autoimmune diseases, including systemic lupus erythematosus, rheumatoid arthritis, autoimmune hemolytic anemia, multiple sclerosis, and diabetes. The



Previously reported mechanisms of anti-CD20 mAb: (A) depletion of B cells and subsequent decrease in plasma B-cell population and antibody production; (B) downregulation of Th1 and upregulation of Treg populations for unclear reasons. Work by Guo et al revealed a novel mechanism: (C) inhibition of $CD8^+$ T-cell proliferation and amelioration of $CD8^+$ T-cell–mediated pathogenesis. Professional illustration by Patrick Lane, ScEYEnce Studios.

overall response rate of rituximab in ITP is approximately 60%, with a 20% to 40% treatment-free long-term remission rate.[2,3] Rituximab in combination with other therapies, such as corticosteroids or thrombopoietin receptor agonists, have also shown synergistic effects. Thus, rituximab provides a well-tolerated, long-term remission option for salvage treatment of ITP patients.[2,3]

Since its initial success in ITP management, there has been increased interest in understanding the mechanisms involved in the ameliorating effects of rituximab.[4] The initial explanation was that rituximab inhibits antibody-mediated platelet destruction by removal of B cells. However, antiplatelet antibody titers did not necessarily decrease in patients in remission and antiplatelet antibody-negative patients could also respond, suggesting antibody-independent mechanism(s).[5] In 2007, Stasi et al reported normalized Th1/Th2 (interferon [IFN]-γ^+/interleukin-4^+ of $CD4^+$ T cells) and Tc1/Tc2 (IFN-γ^+/interleukin-4^+ of $CD8^+$ T cells) ratios in the peripheral blood of rituximab responders at 3 and 6 months after treatment but not in patients with active ITP or in rituximab nonresponders, suggesting an immunomodulatory effect of rituximab on both $CD4^+$ and $CD8^+$ T-cell responses.[6] Similarly, Nazi et al reported a decreased number of IFN-γ^+–producing T cells in patients with ITP upon exogenous antigen stimulation 6 months after rituximab administration.[7] Subsequently, further examination of the $CD4^+$ T cells showed restored numbers and function of $CD4^+$ T-regulatory cells (Tregs) after rituximab therapy, which may help explain the restored balance within $CD4^+$ and $CD8^+$ T cells.[8] In contrast, spleens from patients who failed rituximab therapy showed that the number of Tregs remained low and the frequency of memory $CD8^+$ T cells increased.[9,10] Beyond the changes in numbers and frequencies of different cell populations, our knowledge of how B-cell depletion therapy could regulate $CD4^+$ and $CD8^+$ T-cell responses and restore Tregs in ITP still remains a matter of debate. One limitation is the impossibility of spleen sample acquisition from responder patients for more detailed studies. In addition, although it is well accepted that in patients with ITP, there are both B-cell (antibody)–mediated and $CD8^+$ T-cell–mediated mechanisms, it has been difficult to sort the exact contributions of 1 pathway over the other.

In this issue, a murine model of ITP was used that allows for the study of antibody- and $CD8^+$ T-cell–mediated ITP separately.[1] CD61 (GPIIIa) knockout (KO) mice were immunized with wild-type platelets and when their splenocytes were transferred to severe combined immunodeficient (SCID, $CD61^+$) mice, significant thrombocytopenia was induced. By transferring splenocytes that were first B cell–depleted in vitro, $CD8^+$ T-cell–mediated ITP independent of B cells could be induced in the SCID mice. Of interest, administration of a mouse anti-CD20 mAb into the CD61 KO mice before or after platelet immunization significantly inhibited the splenocyte's ability to induce thrombocytopenia in the SCID mice. It appeared that the $CD8^+$ T-cell defect was associated with decreased $CD8^+$ T-cell proliferation in vitro, which raises the possibility of a novel mechanism of B-cell depletion therapy that limits $CD8^+$ T-cell

responses. Although rituximab has been reported to be effective in $CD8^+$ T cell–dominated autoimmune diseases, such as diabetes, this study provides the first mechanistic explanation for the regulation of $CD8^+$ T-cell responses by B cells (see figure).[1]

Overall, inhibition of $CD8^+$ T-cell proliferation and prevention of the development of ITP by a mouse anti-CD20 mAb suggests a novel role for B cells in maintaining $CD8^+$ T-cell responses. This provides us with an explanation for the therapeutic effect of B-cell depletion therapy for ITP and potentially for other T-cell–mediated autoimmune diseases as well. This may not only improve our understanding of disease pathogenesis, but may also provide helpful information for developing new strategies for ITP management.

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

REFERENCES

1. Guo L, Kapur R, Aslam R, et al. $CD20^+$ B-cell depletion therapy suppresses murine $CD8^+$ T-cell–mediated immune thrombocytopenia. *Blood.* 2016;127(6):735-738.
2. Khellaf M, Charles-Nelson A, Fain O, et al. Safety and efficacy of rituximab in adult immune thrombocytopenia: results from a prospective registry including 248 patients. *Blood.* 2014;124(22):3228-3236.
3. Patel VL, Mahévas M, Lee SY, et al. Outcomes 5 years after response to rituximab therapy in children and adults with immune thrombocytopenia. *Blood.* 2012;119(25):5989-5995.
4. Stasi R, Pagano A, Stipa E, Amadori S. Rituximab chimeric anti-CD20 monoclonal antibody treatment for adults with chronic idiopathic thrombocytopenic purpura. *Blood.* 2001;98(4):952-957.
5. Cooper N, Stasi R, Cunningham-Rundles S, Cesarman E, McFarland JG, Bussel JB. Platelet-associated antibodies, cellular immunity and FCGR3a genotype influence the response to rituximab in immune thrombocytopenia. *Br J Haematol.* 2012;158(4):539-547.
6. Stasi R, Del Poeta G, Stipa E, et al. Response to B-cell–depleting therapy with rituximab reverts the abnormalities of T-cell subsets in patients with idiopathic thrombocytopenic purpura. *Blood.* 2007;110(8):2924-2930.
7. Nazi I, Kelton JG, Larché M, et al. The effect of rituximab on vaccine responses in patients with immune thrombocytopenia. *Blood.* 2013;122(11):1946-1953.
8. Stasi R, Cooper N, Del Poeta G, et al. Analysis of regulatory T-cell changes in patients with idiopathic thrombocytopenic purpura receiving B cell–depleting therapy with rituximab. *Blood.* 2008;112(4):1147-1150.
9. Audia S, Samson M, Guy J, et al. Immunologic effects of rituximab on the human spleen in immune thrombocytopenia. *Blood.* 2011;118(16):4394-4400.
10. Audia S, Samson M, Mahévas M, et al. Preferential splenic $CD8^+$ T-cell activation in rituximab-nonresponder patients with immune thrombocytopenia. *Blood.* 2013;122(14):2477-2486.

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Comment on Szydlowski et al, page 739

FOXO discriminates tonic from chronic in DLBCL

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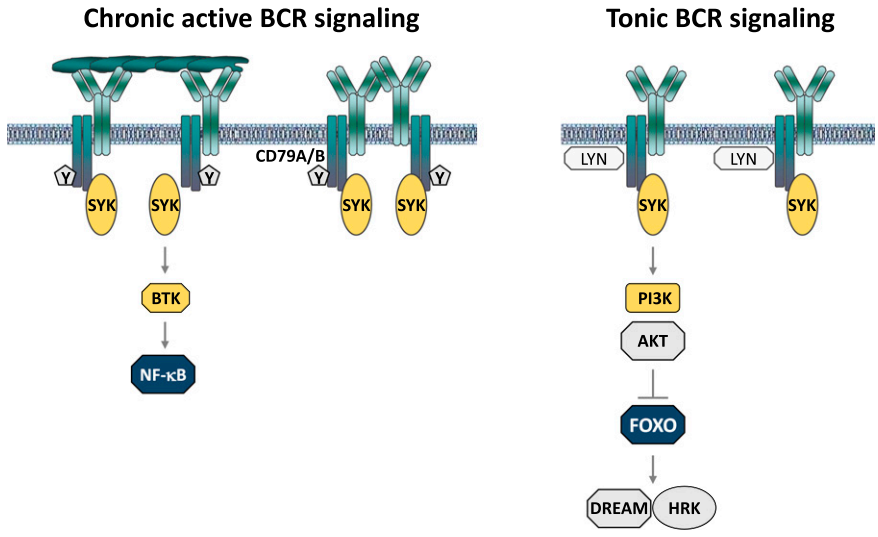
In this issue of *Blood*, Szydlowski et al provide evidence for a major role of the transcription factor subgroup O of forkhead (FOXO1) in mediating the cytotoxic and antiproliferative effects of the spleen tyrosine kinase (SYK) inhibitor R406 in tonic B-cell receptor (BCR) signal-dependent diffuse large B-cell lymphoma (DLBCL).[1]

The BCR pathway has emerged as a major therapeutic target in various B-cell malignancies, including DLBCL. In a subset of patients with activated B cell–like (ABC) DLBCL, this pathway is activated by interactions of the tumor immunoglobulins with self-antigens located on the same or surrounding cells (see figure).[2] These interactions induce the formation of BCR microclusters, which generate a constitutive signal termed *chronic active BCR signal*.[3] In some patients this signal is further amplified by mutations in the CD79A or CD79B subunits of the BCR, which prevent the recruitment of negative regulators of BCR signaling. A major feature of this signal is the constitutive activation of Bruton tyrosine kinase (BTK) and the NF-κB pathway.[3] Importantly, patients with CD79A or CD79B mutations frequently respond to treatment with the BTK inhibitor ibrutinib, providing further evidence that chronic active BCR signaling plays an important role in the pathogenesis of ABC DLBCL.[4]

In addition to patients with chronic active BCR signaling, a second subset of DLBCL has been identified that is also characterized by activation of the BCR pathway, but in the absence of BCR microclusters or CD79A/CD79B mutations. Activation of the BCR pathway in these cases is presumed to be antigen-independent and to occur as a consequence of a tonic BCR signal that is enhanced by genetic alterations of BCR signaling pathway components, such as SYK amplification or phosphatase and tensin homolog (PTEN) deletion.[5] These lymphomas typically belong to the germinal center B cell–like (GCB) DLBCL subset and are characterized by low baseline NF-κB activity but increased activity of the PI3K/AKT pathway. Importantly, inhibition of BCR



Mechanisms of BCR pathway activation in DLBCL. Mutations in CD79A and CD79B are indicated with Y.