DRUG ADVANCES

blood advances



# How ibrutinib, a B-cell malignancy drug, became an FDA-approved second-line therapy for steroid-resistant chronic GVHD

Samantha M. Jaglowski[1] and Bruce R. Blazar[2]

[1]Division of Hematology, Department of Internal Medicine, The Ohio State University, Columbus, OH, and [2]Division of Blood and Marrow Transplantation, Department of Pediatrics, The University of Minnesota, Minneapolis, MN

Allogeneic hematopoietic stem cell transplantation (allo-SCT) is potentially curative for a number of hematologic conditions, both malignant and nonmalignant. However, its success can be limited by the development of acute and chronic graft-versus-host disease (GVHD). Chronic GVHD (cGVHD) is the most common long-term complication following allo-SCT, and patients who develop this condition have significantly higher morbidity and mortality and significantly lower quality of life than patients who do not. Until recently, there were no US Food and Drug Administration (FDA)–approved therapies for cGVHD treatment. In this review article, we describe how ibrutinib was identified as potential cGVHD therapy based on preclinical cGVHD models and clinical studies in B-cell malignancies and elucidation of its mechanisms of action in cGVHD. Results from a phase 2 clinical trial that was designed based on National Institutes of Health Criteria for the grading and staging of cGVHD culminated in the FDA-approval of ibrutinib as second line therapy of steroid-refractory or steroid-resistant cGVHD. Results of ibrutinib studies in phase 3 randomized studies, for cGVHD prophylaxis and as first -line testing along with steroids will be especially important in selecting the preferred indications for ibrutinib in patients at risk for or who have developed cGVHD.

## Introduction

Although allogeneic hematopoietic stem cell transplantation (allo-SCT) is potentially curative for a number of hematologic malignancies, its use is limited by the development of acute[1] and chronic graft-versus-host disease (GVHD).[2] Chronic GVHD (cGVHD) is the most common long-term complication following allo-SCT, affecting 30% to 70% of patients who have received nonmanipulated grafts, a standard calcineurin inhibitor–based regimen, and who survive beyond the first 100 days. Higher rates of cGVHD are seen in recipients of colony-stimulating factor mobilized peripheral blood grafts compared with marrow grafts.[3,4] In vivo T-cell depletion with antithymocyte globulin or with alemtuzumab can reduce the incidence of acute GVHD and cGVHD as reported in some but not all studies, the latter because of possible adverse effects on relapse and virus reactivation.[5-9] cGVHD and its associated immune deficiency has been identified as a leading cause of nonrelapse mortality in allo-SCT survivors who are 4.7 times more likely to develop severe or life-threatening health conditions compared with healthy siblings.[10,11] Patients with active cGVHD are more likely to report adverse general health, mental health, functional impairments, activity limitation, and pain than allo-SCT survivors with no history of cGVHD.[12] Any organ system can be affected.

Morbidity is frequently caused by long-term exposure to the corticosteroids and calcineurin inhibitors required to treat the condition.[13] Nearly 50% of patients with cGVHD require treatment with second-line therapy because of inadequate response, resulting in a ~2.5-fold higher nonrelapse mortality rates; no standard second-line therapy has been embraced.[14,15] Prospective clinical studies evaluating new agents have been hampered by inconsistencies in design, and few multicenter studies have been conducted. The development of National Institutes of Health (NIH) Consensus Criteria for grading and

Submitted 21 February 2018; accepted 22 July 2018. DOI 10.1182/bloodadvances.2018013060.

staging cGVHD represents a significant advance, providing a more clinically useful severity measure, better classifications to assist in developing laboratory correlates,[16,17] and increasing rigor in trial design and response,[18-21] providing a strong platform for bringing new therapies toward US Food and Drug Administration approval.

## cGVHD pathogenic mechanisms

The immunopathology underlying development of cGVHD is incompletely understood and likely multifactorial.[2,22-26] Mouse models typically involve 3 main pathological mechanisms: defective thymic function, auto- or allo-antibody production, and fibrosis.[2,22-24,26] Thymic damage leads to both defective negative selection of auto- and allo-reactive T cells that can support pathogenic B-cell development, a reduction in the number or function of T regulatory cells (Tregs) capable of controlling harmful T- and B-cell responses, and failure to adequately control B-cell expansion.

Following engagement of the antigen-specific B-cell receptor (BCR), signaling through the extracellular signal-regulated kinase, AKT, splenic tyrosine kinase, BCR-NOTCH2, and B-cell linker protein pathways increases, pointing toward a state of constant activation.[27-29] After BCR activation, B cells become potent antigen-presenting cells, mature, and gain the functional capacity to process and present minor histocompatibility antigens for HLA-matched donor-recipient pairs, typically in the context of major histocompatibility complex (MHC) molecules, to donor $CD4^+$ T cells. Following T-cell receptor engagement, donor $CD4^+$ T cells trigger B-cell maturation and production of B-cell survival cytokines (eg, interleukin-4 [IL-4], IL-17, IL-21) known to be critical in various autoimmune disorders.[23,30] In further support of the role of pathogenic role B cells in cGVHD pathogenesis, B cells from cGVHD patients are dysfunctional, resistant to apoptosis as a result of the deficiency of the proapoptotic protein, Bim,[27] and undergo excessive B-cell size and survival increases upon exposure to exogenous B cell-activating factor of the tumor necrosis family (BAFF).[31] High plasma BAFF levels and high BAFF/B-cell levels result in increased numbers of circulating pregerminal center (GC) B cells and post-GC plasmablast-like cells,[31] capable of producing donor-derived alloantibodies without antigen restimulation. Additionally, cGVHD patients have delayed naïve B-cell recovery.[31] In response to antigenic stimulation, costimulatory molecules[32] and cytokine production are upregulated and T and B cells clonally expand, form effector and memory cells, and traffic to cGVHD target organ sites where IL-10, IL-13, IL-22, and IL-33 are released.[2] Macrophages, recruited into cGVHD target organs in response to immune cell infiltration or tissue injury, can secrete transforming growth factor-β that stimulates collagen production from fibroblasts,[23] leading to a scleroderma-like syndrome.[26]

## Rationale for evaluating ibrutinib as a therapy for CGVHD patients

To deplete potentially pathogenic B cells, clinical studies evaluating the use of rituximab, a chimeric anti-CD20 monoclonal antibody, have been performed. A phase 2 rituximab treatment study demonstrated a 70% clinical response rate, permitting a 75% reduction in steroid dose,[33] whereas a phase 2 rituximab prophylaxis study also showed a steroid-sparing effect.[34] Following rituximab treatment, BAFF/B-cell ratios and activated $CD27^+$ B-cell frequencies were decreased in patients who had stable or improved disease.[35] Taken together, these findings provide strong justification for targeting B cells in patients with cGVHD.

Bruton's tyrosine kinase (BTK) is induced by signaling of and is proximal to the BCR and BTK activation is critical for B-cell survival, proliferation, and migration (Figure 1).[36] Individuals lacking functioning BTK do not have circulating B cells and are unable to produce immunoglobulin or mount humoral immune responses.[37] BTK is a nonreceptor tyrosine kinase belonging to the Tec family of kinases, most of which are expressed on hematopoietic cells. BTK is predominantly expressed in B cells but not T or natural killer cells; in plasma cells, BTK is downregulated.[36] In combination with B-cell linker protein, BTK subsequently phosphorylates phospholipase Cγ2 (PLCγ2), triggering a series of downstream events including transcriptional regulation involving NF-κB and NFAT.[36]

Ibrutinib was designed as a selective and irreversible inhibitor of the BTK protein.[38] When added directly to human whole blood, ibrutinib inhibits signal transduction from the BCR and blocks activation of B cells. In human B-cell lymphoma cell lines, ibrutinib arrests cell growth and induces apoptosis. In vivo, ibrutinib inhibits B-cell tumor growth in vivo in xenograft models at doses without overt toxicity.[39] Using a specially designed pharmacodynamic assay, binding of ibrutinib to the active site of BTK has been demonstrated in vivo in rat and dog peripheral blood mononuclear cells, mouse splenocytes, and xenografted tumor cells.[39] Ibrutinib added to human blood ex vivo leads to complete BTK occupancy (50% inhibitory concentration = 100 nM) and inhibition of B-cell activation measured by CD69 expression.[39]

In addition to inhibiting BTK, ibrutinib is an irreversible inhibitor of interleukin-2 inducible kinase (ITK), another Tec family kinase that shares significant sequence and functional homology with BTK.[40] ITK is involved in proximal T-cell receptor signaling, activating PLCγ2, and launching a signaling cascade that includes the NFAT, NF-κB, and MAPK pathways results in T-cell activation, cytokine release, and rapid proliferation.[41] ITK has a dominant role in the activation of Th2 but not Th1 cells, the former linked to cGVHD development in rodents[2,22,23] and late onset cGVHD in patients, in contrast to the latter, which can release interferon-γ (IFN-γ), which induces BAFF.[42] In contrast to Th2 inhibition by ibrutinib, Th1 cell activation is supported by resting lymphocyte kinase, which is resistant to ibrutinib; these properties allow for the activation and proliferation of Th1 and $CD8^+$ T cells[40] that may participate in pathogen and tumor responses. Although the immunopathology underlying cGVHD is complex, alloreactive Th1, Th2, and Th17 T cells have been associated with cGVHD by driving chronic inflammatory responses, pro-fibrotic pathways, and B-cell antihost antibody production.[2,22,23,43] For example, studies in cGVHD patients have pointed an IFN-inducible gene signature[44] along with IFN-γ producing Th1 cells,[42] which can promote cGVHD.[31] Th2 cell release of IL-13 can fuel the fibrogenic contributions of macrophages in cGVHD. ITK has been shown to critically important in Th17 cells, which can support GC formation[45] and B cells outside the GC[46] by upregulating IL17A messenger RNA and protein expression, evidenced by reduced IL17A in vitro and in vivo responses with ITK-deficient T cells.[41,47,48] Because Tregs can use both ITK and resting lymphocyte kinase, the increase in Tregs seen with ITK deficient T cells was expected, also a result of reduced

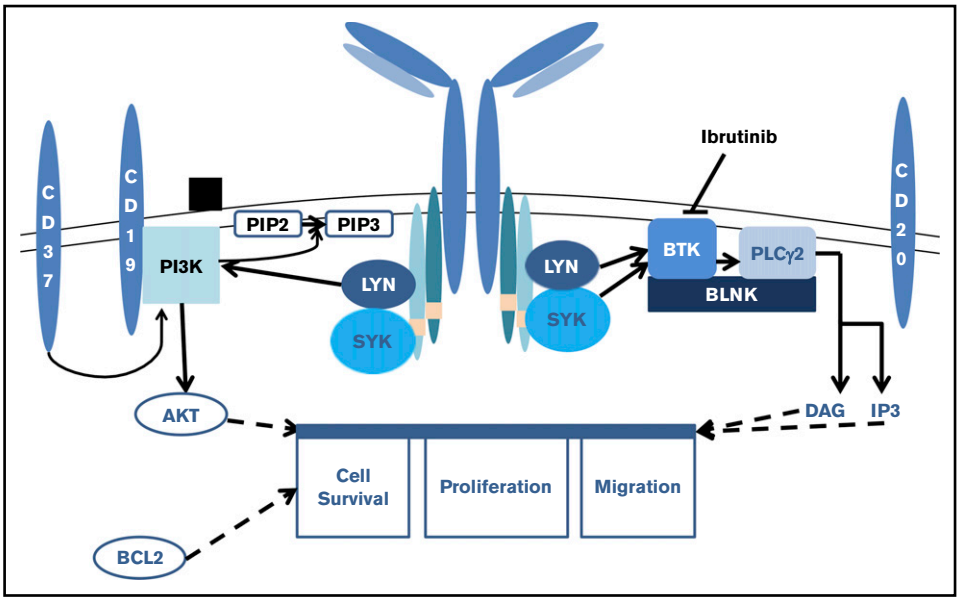


**Figure 1. The role of BTK in B-cell survival, proliferation, and migration.**

IL17A production that can hinder peripheral Treg generation.[48] However, contrary to the direct effects of ibrutinib on suppressing Th17 responses,[49,50] ibrutinib-treated lipopolysaccharide (LPS)-activated dendritic cells promoted T-cell proliferation and enhanced IL-17 production upon T-cell coculture.[51] Chronic lymphocytic leukemia patients have an abnormal immune system with high Tregs and decreased Th17 cells; ibrutinib-treated cGVHD patients had a more normal immune system, as shown by reduced Treg/CD4 ratios and Th17 frequencies.[52] In an analysis of patients treated at Stanford and on multi-institution studies of ibrutinib in patients who relapsed with B-cell malignancies following allo-SCT, the absolute number of $CD3^+CD8^+$ T cells was unchanged after 6 months of ibrutinib treatment, although $CD4^+$ T cells were 50% reduced.[53] There was a 75% reduction of GATA3 expressing Th2 $CD4^+$ cells without a significant change Tbet-expressing Th1 $CD4^+$ cells. Interestingly, these effects persisted long after discontinuation of ibrutinib. The extent to which the resolution of cGVHD in patients is dependent upon shifts in Th1, Th2, or Th17 cytokines or direct inhibition of the survival of B cells that leads to immunoglobulin production remains unknown.

## Preclinical development of ibrutinib in cGVHD

With these desirable biological properties of ibrutinib, we proceeded to testing ibrutinib therapy in 2 established in vivo allogeneic bone marrow transplant (allo-BMT) model systems. In 1 model of MHC disparate grafts (C57BL/6→B10.BR), recipients are given 2 daily doses of cyclophosphamide before total body irradiation, followed by the infusion of bone marrow plus a small number of mature T cells.[54] As a result of chronic alloantigen stimulation with a markedly reduced level of inflammation compared with acute GVHD and several chronic GVHD model systems, donor B cells and pathogenic anti-host reactive antibodies that are generated cause multiorgan system disease with bronchiolitis obliterans syndrome.[54] Ibrutinib given by intraperitoneal injection (15 mg/kg per day in methylcellulose) starting on day 28 post-BMT proved highly effective in reversing bronchiolitis obliterans syndrome, as evidenced by pulmonary function tests and histopathology when initiated 1 month post-BMT when cGVHD was active.[55] GC reactions, tissue immunoglobulin deposition, and fibrosis were normalized compared with the bone marrow only (no cGVHD) controls. By using donor mice deficient in BTK in B cells or ITK in T cells in this model, both BTK and ITK were found to be required for optimal cGVHD induction, suggesting that the combined BTK and ITK effects of ibrutinib may have been especially efficacious for cGVHD treatment in this setting. In an MHC- matched, minor histocompatibility–mismatched model (LP/J→C57BL/6) in which recipients are lethally irradiated and given modest doses of mature spleen cells, sclerodermatous cGVHD develops. Approximately one-third of recipients survived to day 25 posttransplant and began to develop classic external signs of cGVHD, including scleroderma, hair loss, hunched posture, weight loss, and dermal fibrosis. At this time, mice were randomly assigned to treatment with vehicle, cyclosporine, or ibrutinib administered via drinking water (25 mg/kg per day). As compared with vehicle and cyclosporine treatment groups, ibrutinib improved the overall intensity of cGVHD, with amelioration of scleroderma, alopecia, and weight loss. In both models, the maximal therapeutic benefit occurred with prolonged administration. In contrast to its benefit as a therapy for established cGVHD, ibrutinib was ineffective when given as cGVHD prophylaxis (day −2 until cGVHD onset, ~28 days) in the LP/J→C57BL/6 model that has scleroderma as a prominent feature along with other organ manifestations.

Although the studies described here showed a profound response to ibrutinib for treating established cGVHD, other studies focused on prophylaxis by daily oral gavage administration in 4 different murine models.[56] In a DBA/2→BALB/c model with sclerodermatous manifestations of cGVHD, recipients given prophylactic ibrutinib had an improved survival rate compared with vehicle controls and showed delayed onset and overall reduction of proteinuria development. The length of treatment was critical to fully suppress cGVHD induction; 2 weeks of treatment was not effective, whereas 4 weeks was. When treatment was delayed by 1 to 2 weeks after allo-BMT, recipients treated with ibrutinib

maintained a significantly higher rate of survival and significantly lower proteinuria development compared with controls. In contrast to data in the LP/J→C57BL/6 model, prophylactic treatment of ibrutinib in the DBA/2→BALB/c model was more effective in suppressing proteinuria development, and treated recipients displayed significantly lower B-, but not T-cell, proliferation. Similarly, in an autoantibody-mediated cGVHD model (DBA/2→B6D2F1), recipients given prophylactic treatment developed significantly less proteinuria compared with vehicle controls, with reduction in immunoglobulin G (IgG) and IgG2a serum autoantibodies. In a different model of predominantly sclerodermatous cGVHD (B10.D2→BALB/c), prophylactic administration of ibrutinib (10 mg/kg per dose; by mouth) reduced cGVHD symptoms compared with vehicle controls, improved B-cell reconstitution, and reduced the percentage of $CD4^+CXCR5^+$ pharmacodynamic-$1^{high}$ T follicular helper cells. Five milligrams per kilogram did not affect the onset or decrease severity of cGVHD compared with the vehicle control group. In a model in which whole splenocytes from B6 donors transferred into conditioned BALB/c recipients recapitulates a transition from acute GVHD to a scleroderma-like form of cGVHD with salivary gland involvement and serum antibodies, prophylactic ibrutinib also led to increased survival compared with vehicle controls. The reason for the seemingly discordant results with prophylaxis in the LP/J→C57BL/6 model compared with the 4 models described previously is not certain, although, as suggested, daily oral gavage may have ensured higher levels of drug than providing ibrutinib in the drinking water.

In all models reported to date in published manuscripts, B-cell activation and differentiation was affected by ibrutinib administration, whereas the effects on T cells were not uniform. Models using ibrutinib in both prophylactic and treatment settings demonstrated efficacy. Across models, the clinical score was generally improved, and ibrutinib was demonstrated to be well-tolerated in a posttransplant setting with reduction in Th2 $CD4^+$ cells, providing the rationale for moving ibrutinib into clinical cGVHD treatment trials.

## Clinical development in cGVHD

Based on these preclinical data, we conducted a phase 1b/2, open-label, multicenter study to determine the safety and efficacy of ibrutinib in patients who failed at least 1 line of therapy for cGVHD.[57] The phase 1b portion was conducted using a modified 3+3+3 design to evaluate the safety of daily oral ibrutinib and determine the recommended phase 2 dose. In the phase 2 portion, patients were treated with ibrutinib at the recommended phase 2 dose and followed for signs of progression or improvement of cGVHD. The primary efficacy end point for phase 2 was the best overall cGVHD response rate, defined as the proportion of all patients who achieved a complete response or partial response. Response criteria were based on the 2005 NIH cGVHD Consensus Panel[21] with modifications based on the 2014 NIH response criteria.[17]

Six patients were enrolled in the phase 1b portion at a dose of 420 mg. There were no dose-limiting toxicities reported. An additional 36 patients were treated in the phase 2 portion, for a total of 42 patients. Because either skin or mouth involvement was an enrollment requirement, mouth and skin were the most frequently involved organs, and 85% of patients showed evidence of cGVHD in ≥2 organs. The median Karnofsky Performance Status score was 80, with the majority of patients between 60 and 80. Patients were allowed to remain on their previous immunosuppressant regimen throughout the trial. At a median follow-up of 13.9 months (range, 0.5-24.9 months), 12 patients (29%) were still receiving ibrutinib and 30 (71%) had discontinued treatment. The most common reasons for treatment discontinuation were adverse events (AEs) (n = 14), cGVHD progression (n = 5), or patient decision (n = 6); 2 patients discontinued after resolution of cGVHD symptoms. Most AEs were grade 1 or 2, and were most commonly fatigue, diarrhea, muscle spasms, nausea, and bruising. The most common grade 3 of higher AEs were pneumonia, fatigue, and diarrhea. Twenty-nine (69%) patients developed infectious complications of any grade, including 13 (31%) grade ≥3 events. Two patients had a relapse of their underlying malignancy. Seven patients died during study follow-up: 2 occurred while on ibrutinib and the other 5 deaths occurred after discontinuation of ibrutinib, with 3 deaths attributed to cGVHD and 2 to unknown causes. Given known toxicities of ibrutinib, no major hemorrhage events observed; atrial fibrillation was reported in 1 patient. About one-third of patients required dose reductions, most commonly for fatigue. AEs led to treatment discontinuation in 14 patients (33%), with the most common reasons being fatigue (n = 3) and pneumonia (n = 2).

For the 7 patients with progression of cGVHD, the median time to progression was 5.6 months (range, 1.7-15.7). The overall response rate was 67%, with a complete response rate of 21% and a partial response rate of 45%. Excluding the patients who discontinued treatment before a response assessment, the overall response rate was 76%. Seventy-one percent of patients who responded showed a sustained response for ≥20 weeks. The median time to initial response was 87 days; however, for the 4 responders who were enrolled after a protocol amendment changing the timing of first response assessment to 5 weeks, the median time to initial response was 30 days. There were similar rates of response in the skin (88%), mouth (88%), and gastrointestinal organs (91%). Of 25 responders with ≥2 involved organs, 20 (80%) showed a response in ≥2 organs. The median corticosteroid dose among responders decreased from 0.29 mg/kg per day (range, 0.06-1.30 mg/kg per day) at baseline (n = 42) to 0.12 mg/kg per day (range, 0.00-0.18 mg/kg per day) at week 49 (n = 12), and 5 responders completely discontinued corticosteroids. Overall, 26 patients (62%) reached a corticosteroid dose of <0.15 mg/kg per day during the study. These results were accompanied by improvement in patient-reported symptoms, with at least a 7-point decrease in Lee cGVHD Symptom Scale[58] and overall summary score in 10 of the 42 (24%) treated patients on at least 2 consecutive visits.

The mean steady-state occupancy levels of BTK and ITK were 93% (range, 46%-99%; n = 36) and 37% (range, 0%-71%; n = 38), respectively, on day 8 of treatment. BTK occupancy was sufficient to effectively block 91% of BTK-driven basophil activation in an ex vivo IgE stimulation assay. Furthermore, measurement of ITK-mediated activation of PLCγ1-Y783 in CD4 T cells revealed that ITK kinase function was inhibited by a mean of 73% (range, 52%-86%) on day 8. There was also a significant reduction in soluble plasma factors that are markers of inflammation and lymphocyte activation, including tumor necrosis factor-α and soluble CD25, and chemotactic factors, including C-X-C motif chemokine ligand 9 and C-X-C motif chemokine ligand 10.[59] Forty-two-parameter mass cytometry single-cell analysis showed a 10-fold reduction in

absolute numbers of cGVHD-implicated pre-GC B cells ($CD19^+$, $CD27^+$, $CD38^+$, $IgD^+$) and diminished T follicular helper cells, Th17, and total B cells by 2.6-, 1.5-, and 1.4-fold, respectively, over the first 90 days following ibrutinib.[60] Relative numbers of CD4 and CD8 T cells, class-switched B cells, and $CD14^+$ monocytes remained unchanged, and invariant natural killer T cells, Th1, and Treg cells increased incrementally by 1.8-, 1.2-, and 1.1-fold at day 90, respectively. Plasma IgG levels persisted, whereas IgM significantly decreased, corroborating an ibrutinib GC effect that did not deplete long-lived plasma cells. T-cell reactivity against influenza virus increased and antibodies against Epstein-Barr virus and tetanus toxoid remained unchanged. Single-cell phosphorylation analysis showed that BTK and ITK signaling was attenuated following ibrutinib treatment in defined B- and T-cell subsets. PLC$\gamma$1/2 activation was simultaneously diminished in the pre-GC and plasmablast B-cell subsets, highlighting ibrutinib's multifactorial mechanism of action.

## Future directions

A phase 3 study to evaluate the role of ibrutinib in combination with corticosteroids in treating patients with newly diagnosed moderate-to-severe cGVHD is currently enrolling (NCT02959944). Ongoing studies are evaluating ibrutinib following allo-SCT for relapsed or refractory lymphoma (NCT02869633) or acute leukemia (NCT03267186). Clinical trials including ibrutinib for cGVHD prophylaxis or combined with steroids for first-line therapy, outcome and immunological analyses focused on antitumor and antipathogen responses, and understanding of the frequency and severity of side effects as the drug becomes more widely used to treat or possibly prevent cGVHD will be critical to achieve the optimal benefits of ibrutinib.

Inflammation is an important hallmark of early cGVHD, and BTK may play a significant role in its development. Better understanding of ibrutinib's role in mitigating inflammation will inform rational studies of combinations of drugs to combat cGVHD. In addition to its effects on lymphocytes,[61] ibrutinib has been shown to modulate the recruitment and cytokine responses of myeloid cells in complex immune disease.[62] LPS strongly induces inflammation by inducing polarization of macrophages to the classic inflammatory M1 and away from the anti-inflammatory, potentially fibrogenic M2 population. BTK also is a critical signal transducer downstream of LPS-triggered TLR4; in BTK-deficient mice, there is markedly reduced recruitment of M1 macrophages following administration of LPS coupled with an induction of immunosuppressive M2-associated markers.[63] Consistent with ibrutinib's inhibitory effects on TLR4 signaling in macrophages, upon treatment of bone marrow–derived dendritic cells (DCs) with ibrutinib, LPS-treated DCs displayed lower synthesis of TNF-$\alpha$ and nitric oxide and higher induction of IL-6, transforming growth factor-$\beta$, and IL-10 and IL-18. Although ibrutinib dampened MHC-II and CD86 expression on DCs, CD80 expression was upregulated.

Macrophages and polymorphonuclear cells typically die during response to microbial and immune inflammatory stimuli. BTK-deficient macrophages show enhanced susceptibility to apoptotic death upon exposure to LPS and interferon-$\gamma$ in vitro. The lack of pro-survival signaling through the BTK-phosphatidylinositol 3-kinase-Akt pathway, and persistent MEK signaling, led to enhanced death in BTK-deficient macrophages downstream of inflammatory triggers. BTK is rapidly phosphorylated in murine macrophages upon NLRP3 activation, supporting inflammasome-dependent IL-1$\beta$ release, which may fuel tissue injury and fibrosis.[64,65] Thus BTK plays a complex role in the regulation of the survival and function of macrophages that can clear pathogens, but has been shown to be critical for initiating or propagating cGVHD in multiple preclinical models.[66-68] Additional studies to discern the potential roles of these inflammatory pathways, other pathogenic mechanisms and influence on macrophage number and function in ibrutinib-treated cGVHD patients is warranted.

We expect that the development of new biomarkers with validated prognostic value in cGVHD onset or therapy response,[69] along with new insights as to the mechanisms responsible for ibrutinib inhibition of cGVHD pathogenesis, will help guide the targeted use of ibrutinib and inform future rational combination studies. As with all cGVHD therapies, ongoing and future ibrutinib studies must carefully monitor outcome parameters including relapse, infections, toxicities, quality of life, overall survival, and GVHD-free, relapse-free survival using currently available NIH Consensus Criteria for diagnosis, staging, trial design, and response assessment facilitating outcome comparisons between ibrutinib and other cGVHD therapies.

## Conclusions

Ibrutinib is the first US Food and Drug Administration–approved drug for the treatment of GVHD, which was originally described as an allo-SCT complication in the 1970s.[70] Although its pathophysiology is not yet completely understood, advancements in both preclinical research and in defining cGVHD, as well as refinement of grading, staging, and response criteria, have allowed for the development of a clinical trial based on solid preclinical data with rational, empiric end points that ultimately led to approval. This in turn creates opportunities to learn more about the pathophysiology of this disease through analysis of molecular characteristics of responders compared with nonresponders. Because the ibrutinib clinical study was accomplished through multi-institution collaboration and pharmaceutical support, its success will hopefully lead to the development and approval of other drugs to treat this potentially devastating condition. Finally, further studies are needed to determine the best approach to employ this drug in patients who have or are at risk for cGVHD.

## Authorship

Contribution: S.M.J. and B.R.B. performed literature searches and prepared the manuscript.

Conflict-of-interest disclosure: S.M.J. receives research funding from Pharmacyclics. B.R.B. is on advisory boards for Kadmon Pharmaceuticals Inc, Kymab, Five Prime Therapeutics, Regimmune, Dr. Reddy, and Equillium Inc; is a founder of Tmunity Therapeutics Inc; and receives funding from Fate Therapeutics, RXi Pharmaceuticals, Alpine Immune Sciences, Kadmon Corporation, Equillium Inc., and Tmunity Therapeutics Inc.

ORCID profiles: S.M.J., 0000-0002-4335-2554; B.R.B., 0000-0002-9608-9841.

Correspondence: Bruce R. Blazar, University of Minnesota, 420 Delaware St SE, MMC 109, Minneapolis, MN 55455; e-mail: blaza001@umn.edu.

## References


1. Zeiser R, Blazar BR. Acute graft-versus-host disease - biologic process, prevention, and therapy. *N Engl J Med*. 2017;377(22):2167-2179.
2. Zeiser R, Blazar BR. Pathophysiology of chronic graft-versus-host disease and therapeutic targets. *N Engl J Med*. 2017;377(26):2565-2579.
3. Anasetti C, Logan BR, Lee SJ, et al. Increased incidence of chronic graft-versus-host disease (GVHD) and no survival advantage with filgrastim-mobilized peripheral blood stem cells (PBSC) compared to bone marrow (BM) transplants from unrelated donors: results of Blood and Marrow Transplant Clinical Trials Network (BMT CTN) Protocol 0201, a phase III, prospective, randomized trial [abstract]. *Blood*. 2011;118(21). Abstract 1.
4. Chang YJ, Weng CL, Sun LX, Zhao YT. Allogeneic bone marrow transplantation compared to peripheral blood stem cell transplantation for the treatment of hematologic malignancies: a meta-analysis based on time-to-event data from randomized controlled trials. *Ann Hematol*. 2012;91(3):427-437.
5. Pérez-Simón JA, Kottaridis PD, Martino R, et al; Spanish and United Kingdom Collaborative Groups for Nonmyeloablative Transplantation. Nonmyeloablative transplantation with or without alemtuzumab: comparison between 2 prospective studies in patients with lymphoproliferative disorders. *Blood*. 2002;100(9):3121-3127.
6. Hale, G.A., et al. Alternate donor hematopoietic cell transplantation (HCT) in non-Hodgkin lymphoma using lower intensity conditioning: a report from the CIBMTR. *Biol Blood Marrow Transplant*. 2012;18(7):1036-1043.
7. Finke J, Bethge WA, Schmoor C, et al; ATG-Fresenius Trial Group. Standard graft-versus-host disease prophylaxis with or without anti-T-cell globulin in haematopoietic cell transplantation from matched unrelated donors: a randomised, open-label, multicentre phase 3 trial. *Lancet Oncol*. 2009;10(9):855-864.
8. Kröger N, Solano C, Wolschke C, et al. Antilymphocyte globulin for prevention of chronic graft-versus-host disease. *N Engl J Med*. 2016;374(1):43-53.
9. Soiffer RJ, Kim HT, McGuirk J, et al. Prospective, randomized, double-blind, phase III clinical trial of anti-T-lymphocyte globulin to assess impact on chronic graft-versus-host disease-free survival in patients undergoing HLA-matched unrelated myeloablative hematopoietic cell transplantation. *J Clin Oncol*. 2017;35(36):4003-4011.
10. Sun CL, Francisco L, Kawashima T, et al. Prevalence and predictors of chronic health conditions after hematopoietic cell transplantation: a report from the Bone Marrow Transplant Survivor Study. *Blood*. 2010;116(17):3129-3139, quiz 3377.
11. Lee JW, Deeg HJ. Prevention of chronic GVHD. *Best Pract Res Clin Haematol*. 2008;21(2):259-270.
12. Fraser CJ, Bhatia S, Ness K, et al. Impact of chronic graft-versus-host disease on the health status of hematopoietic cell transplantation survivors: a report from the Bone Marrow Transplant Survivor Study. *Blood*. 2006;108(8):2867-2873.
13. Mohty M, Apperley JF. Long-term physiological side effects after allogeneic bone marrow transplantation. *Hematology Am Soc Hematol Educ Program*. 2010;2010:229-236.
14. Flowers ME, Storer B, Carpenter P, et al. Treatment change as a predictor of outcome among patients with classic chronic graft-versus-host disease. *Biol Blood Marrow Transplant*. 2008;14(12):1380-1384.
15. Wolff D, Schleuning M, von Harsdorf S, et al. Consensus Conference on Clinical Practice in Chronic GVHD: second-line treatment of chronic graft-versus-host disease. *Biol Blood Marrow Transplant*. 2011;17(1):1-17.
16. Filipovich AH, Weisdorf D, Pavletic S, et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report. *Biol Blood Marrow Transplant*. 2005;11(12):945-956.
17. Jagasia MH, Greinix HT, Arora M, et al. National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: I. The 2014 Diagnosis and Staging Working Group report. *Biol Blood Marrow Transplant*. 2015;21(3):389-401.
18. Pavletic SZ, Martin P, Lee SJ, et al; Response Criteria Working Group. Measuring therapeutic response in chronic graft-versus-host disease: National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: IV. Response Criteria Working Group report. *Biol Blood Marrow Transplant*. 2006;12(3):252-266.
19. Lee SJ, Wolff D, Kitko C, et al. Measuring therapeutic response in chronic graft-versus-host disease. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: IV. The 2014 Response Criteria Working Group report. *Biol Blood Marrow Transplant*. 2015;21(6):984-999.
20. Lee SJ. Classification systems for chronic graft-versus-host disease. *Blood*. 2017;129(1):30-37.
21. Martin PJ, Lee SJ, Przepiorka D, et al. National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: VI. The 2014 Clinical Trial Design Working Group Report. *Biol Blood Marrow Transplant*. 2015;21(8):1343-1359.
22. Cooke KR, Luznik L, Sarantopoulos S, et al. The biology of chronic graft-versus-host disease: A Task Force Report from the National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease. *Biol Blood Marrow Transplant*. 2017;23(2):211-234.
23. MacDonald KP, Blazar BR, Hill GR. Cytokine mediators of chronic graft-versus-host disease. *J Clin Invest*. 2017;127(7):2452-2463.
24. MacDonald KP, Hill GR, Blazar BR. Chronic graft-versus-host disease: biological insights from preclinical and clinical studies. *Blood*. 2017;129(1):13-21.
25. Socié G, Ritz J. Current issues in chronic graft-versus-host disease. *Blood*. 2014;124(3):374-384.
26. Schroeder MA, DiPersio JF. Mouse models of graft-versus-host disease: advances and limitations. *Dis Model Mech*. 2011;4(3):318-333.
27. Allen JL, Fore MS, Wooten J, et al. B cells from patients with chronic GVHD are activated and primed for survival via BAFF-mediated pathways. *Blood*. 2012;120(12):2529-2536.

28. Allen JL, Tata PV, Fore MS, et al. Increased BCR responsiveness in B cells from patients with chronic GVHD. *Blood*. 2014;123(13):2108-2115.
29. Poe JC, Jia W, Su H, et al. An aberrant NOTCH2-BCR signaling axis in B cells from patients with chronic GVHD. *Blood*. 2017;130(19):2131-2145.
30. Shimabukuro-Vornhagen A, Hallek MJ, Storb RF, von Bergwelt-Baildon MS. The role of B cells in the pathogenesis of graft-versus-host disease. *Blood*. 2009;114(24):4919-4927.
31. Sarantopoulos S, Stevenson KE, Kim HT, et al. Altered B-cell homeostasis and excess BAFF in human chronic graft-versus-host disease. *Blood*. 2009; 113(16):3865-3874.
32. Sarantopoulos S, Blazar BR, Cutler C, Ritz J. B cells in chronic graft-versus-host disease. *Biol Blood Marrow Transplant*. 2015;21(1):16-23.
33. Cutler C, Miklos D, Kim HT, et al. Rituximab for steroid-refractory chronic graft-versus-host disease. *Blood*. 2006;108(2):756-762.
34. Cutler C, Kim HT, Bindra B, et al. Rituximab prophylaxis prevents corticosteroid-requiring chronic GVHD after allogeneic peripheral blood stem cell transplantation: results of a phase 2 trial. *Blood*. 2013;122(8):1510-1517.
35. Sarantopoulos S, Stevenson KE, Kim HT, et al. Recovery of B-cell homeostasis after rituximab in chronic graft-versus-host disease. *Blood*. 2011;117(7): 2275-2283.
36. Mohamed AJ, Yu L, Bäckesjö CM, et al. Bruton's tyrosine kinase (Btk): function, regulation, and transformation with special emphasis on the PH domain. *Immunol Rev*. 2009;228(1):58-73.
37. Satterthwaite AB, Witte ON. The role of Bruton's tyrosine kinase in B-cell development and function: a genetic perspective. *Immunol Rev*. 2000;175(1): 120-127.
38. Pan Z, Scheerens H, Li SJ, et al. Discovery of selective irreversible inhibitors for Bruton's tyrosine kinase. *ChemMedChem*. 2007;2(1):58-61.
39. Honigberg LA, Smith AM, Sirisawad M, et al. The Bruton tyrosine kinase inhibitor PCI-32765 blocks B-cell activation and is efficacious in models of autoimmune disease and B-cell malignancy. *Proc Natl Acad Sci USA*. 2010;107(29):13075-13080.
40. Dubovsky JA, Beckwith KA, Natarajan G, et al. Ibrutinib is an irreversible molecular inhibitor of ITK driving a Th1-selective pressure in T lymphocytes. *Blood*. 2013;122(15):2539-2549.
41. Gomez-Rodriguez J, Kraus ZJ, Schwartzberg PL. Tec family kinases Itk and Rlk/Txk in T lymphocytes: cross-regulation of cytokine production and T-cell fates. *FEBS J*. 2011;278(12):1980-1989.
42. Rozmus J, Schultz KR, Wynne K, et al. Early and late extensive chronic graft-versus-host disease in children is characterized by different Th1/Th2 cytokine profiles: findings of the Children's Oncology Group Study ASCT0031. *Biol Blood Marrow Transplant*. 2011;17(12):1804-1813.
43. Forcade E, Paz K, Flynn R, et al. An activated Th17-prone T cell subset involved in chronic graft-versus-host disease sensitive to pharmacological inhibition. *JCI Insight*. 2017;2(12):e92111.
44. Hakim FT, Memon S, Jin P, et al. Upregulation of IFN-inducible and damage-response pathways in chronic graft-versus-host disease. *J Immunol*. 2016; 197(9):3490-3503.
45. Mitsdoerffer M, Lee Y, Jäger A, et al. Proinflammatory T helper type 17 cells are effective B-cell helpers. *Proc Natl Acad Sci USA*. 2010;107(32): 14292-14297.
46. Patakas A, Benson RA, Withers DR, et al. Th17 effector cells support B cell responses outside of germinal centres. *PLoS One*. 2012;7(11): e49715.
47. Gomez-Rodriguez J, Sahu N, Handon R, et al. Differential expression of interleukin-17A and -17F is coupled to T cell receptor signaling via inducible T cell kinase. *Immunity*. 2009;31(4):587-597.
48. Gomez-Rodriguez J, Wohlfert EA, Handon R, et al. Itk-mediated integration of T cell receptor and cytokine signaling regulates the balance between Th17 and regulatory T cells. *J Exp Med*. 2014;211(3):529-543.
49. Li D, Rebecca P, Nurieva R, et al. Ibrutinib treatment modulates T cell activation and polarization in immune response. *Blood*. 2015;126:3435.
50. Yang CS, Dubovsky JA, Chappell DL, et al. Immune modulating agent ibrutinib blocks T-helper 17 activation and release of IL-17A while preserving T-regulatory cell function [abstract]. *Cancer Res*. 2014;74(19 suppl). Abstract 2557.
51. Natarajan G, Terrazas C, Oghumu S, et al. Ibrutinib enhances IL-17 response by modulating the function of bone marrow derived dendritic cells. *OncoImmunology*. 2015;5(1):e1057385.
52. Long M, Beckwith K, Do P, et al. Ibrutinib treatment improves T cell number and function in CLL patients. *J Clin Invest*. 2017;127(8):3052-3064.
53. Ryan CE, Sahaf B, Logan AC, et al. Ibrutinib efficacy and tolerability in patients with relapsed chronic lymphocytic leukemia following allogeneic HCT. *Blood*. 2016;128(25):2899-2908.
54. Flynn R, Du J, Veenstra RG, et al. Increased T follicular helper cells and germinal center B cells are required for cGVHD and bronchiolitis obliterans. *Blood*. 2014;123(25):3988-3998.
55. Dubovsky JA, Flynn R, Du J, et al. Ibrutinib treatment ameliorates murine chronic graft-versus-host disease. *J Clin Invest*. 2014;124(11):4867-4876.
56. Schutt SD, Fu J, Nguyen H, et al. Inhibition of BTK and ITK with ibrutinib is effective in the prevention of chronic graft-versus-host disease in mice. *PLoS One*. 2015;10(9):e0137641.
57. Miklos D, Cutler CS, Arora M, et al. Ibrutinib for chronic graft-versus-host disease after failure of prior therapy. *Blood*. 2017;130(21):2243-2250.
58. Lee S, Cook EF, Soiffer R, Antin JH. Development and validation of a scale to measure symptoms of chronic graft-versus-host disease. *Biol Blood Marrow Transplant*. 2002;8(8):444-452.
59. Yu J, Storer BE, Kushekhar K, et al. Biomarker panel for chronic graft-versus-host disease. *J Clin Oncol*. 2016;34(22):2583-2590.

60. Sahaf B, Tebaykin D, Hopper M, et al. Ibrutinib inhibits cGVHD pathogenic pre-germinal center B-cells and follicular helper cells while preserving immune memory and Th1 T-cells. *Blood*. 2017;130:4481.

61. Tissino E, Benedetti D, Herman SEM, et al. Functional and clinical relevance of VLA-4 (CD49d/CD29) in ibrutinib-treated chronic lymphocytic leukemia. *J Exp Med*. 2018;215(2):681-697.

62. Chang BY, Huang MM, Francesco M, et al. The Bruton tyrosine kinase inhibitor PCI-32765 ameliorates autoimmune arthritis by inhibition of multiple effector cells. *Arthritis Res Ther*. 2011;13(4):R115.

63. Ni Gabhann J, Hams E, Smith S, et al. Btk regulates macrophage polarization in response to lipopolysaccharide. *PLoS One*. 2014;9(1):e85834.

64. Ito M, Shichita T, Okada M, et al. Bruton's tyrosine kinase is essential for NLRP3 inflammasome activation and contributes to ischaemic brain injury. *Nat Commun*. 2015;6(1):7360.

65. Liu X, Pichulik T, Dang TM, et al. Human NACHT, LRR, and PYD domain-containing protein 3 (NLRP3) inflammasome activity is regulated by and potentially targetable through Bruton tyrosine kinase. *J Allergy Clin Immunol*. 2017;140:1054-1067.

66. Alexander KA, Flynn R, Lineburg KE, et al. CSF-1-dependant donor-derived macrophages mediate chronic graft-versus-host disease. *J Clin Invest*. 2014; 124(10):4266-4280.

67. Du J, Paz K, Flynn R, et al. Pirfenidone ameliorates murine chronic GVHD through inhibition of macrophage infiltration and TGF-β production. *Blood*. 2017;129(18):2570-2580.

68. Khare A, Viswanathan B, Gund R, et al. Role of Bruton's tyrosine kinase in macrophage apoptosis. *Apoptosis*. 2011;16(4):334-346.

69. Du J, Flynn R, Paz K, et al. Murine chronic graft-versus-host disease proteome profiling discovers CCL15 as a novel biomarker in patients. *Blood*. 2018;131(15):1743-1754.

70. 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.