

B-cell targeting in chronic Graft-versus-Host disease

Robert Zeiser¹, Stefanie Sarantopoulos², Bruce R. Blazar³

¹ Department of Hematology, Oncology and Stem Cell Transplantation, Faculty of Medicine, Freiburg University, Germany

² Dept. of Medicine, Division of Hematological Malignancies & Cellular Therapy, Duke Cancer Institute, Duke University Medical Center, Durham, NC, USA

³ Department of Pediatrics, Division of Blood and Marrow Transplantation, University of Minnesota, Minneapolis, Minnesota 55455, USA

Corresponding author:

Robert Zeiser, MD

Department of Hematology,
Oncology and Stem cell transplantation
University Medical Center Freiburg
Freiburg, D-79106 Freiburg
Germany

Tel: +49-761-270-36250, Fax: +49-761-270-36250

robert.zeiser@uniklinik-freiburg.de

Abstract

Over the last decade our understanding of the pathophysiology of chronic graft-versus-host disease (cGVHD) has improved considerably. In this spotlight, we discuss emerging insights into the pathophysiology of cGVHD with a focus on B-cells. First, we summarize supporting evidence derived from mouse and human studies. Next, novel cGVHD therapy approaches that target B-cells will be covered to provide treating physicians with an overview of the rationale behind the emerging armamentarium against cGVHD.

Introduction

Chronic graft-versus-host disease (cGVHD) is a major complication in patients undergoing allogeneic hematopoietic cell transplantation (allo-HCT) leading to reduced patient reported quality of life¹ and nonrelapse mortality (NRM)². Risk factors for cGVHD development include prior acute GVHD (aGVHD), donor peripheral blood stem cell grafts, HLA-disparity, female donors for male recipients and recipient age³. Clinical cGVHD can involve classical aGVHD epithelial target tissues (intestinal tract; liver; skin; lung) and any other organ system, including oral, esophageal, musculoskeletal, joint, fascial, ocular, hair and nails, lymphohematopoietic system and genital tissues⁴. The pleiotropic symptoms resulting from such broad organ involvement made past diagnosis and scoring difficult. The 2005 and revised 2014 NIH criteria have brought greater consistency to terminology and methods for cGVHD diagnosis and staging^{4,5}.

To identify and validate novel targets in cGVHD, numerous mouse models are used. However, individual cGVHD mouse models cannot reproduce all features of cGVHD seen in patients (reviewed in^{6,7}) who present with a heterogeneous disease-spectrum. Most models have one or two dominant cGVHD manifestations involving limited numbers of organs. These different manifestations of cGVHD depend on several factors including the cytokines that are released. Some of these cytokines or their receptors are attractive targets to treat cGVHD. For instance an anti-IL-2 receptor common-gamma chain neutralizing mAb reduced cGVHD⁸ which may be based on a broad inhibitory effect on multiple cytokine receptors. Also targeting of individual cytokines such as IL-17 was active against cGVHD⁹. Additionally, the type and degree of donor and recipient genetic disparity in models suggest that the antigens recognized by B- and T-cells as well as the number of donor T-cells transferred can dictate cGVHD phenotypes. Thus, mechanistic studies of multiple models when feasible are ideal¹⁰.

The role of B-cell in cGVHD based on findings in mice

Under normal conditions B-cells contribute to adaptive immunity by producing antibodies, secreting cytokines and presenting antigen. B-cell activation begins when an antigen is recognized via the B-cell receptor (BCR). Activated B-cells participate in a two-step differentiation process that yields both short-lived plasmablasts for immediate protection against a pathogen and long-lived plasma cells and memory B-cells for persistent protection¹¹. Together with BCR signaling, B-cell activating factor (BAFF) determines B-cell fate/survival. Comparable to the normal B-cell activation process, the first step in the pathogenesis of cGVHD is the recognition of antigen via the BCR (**Figure 1A, step 1**). In contrast to the normal situation, B-cells exhibit BCR-hyper-responsiveness in cGVHD as shown in mouse models¹²⁻¹⁴. After activation pathogenic B-cells expand (**Figure 1A, step 2**) and are strongly impacted by soluble factors in the microenvironment such as IL-4, IL-17⁹, IL-21^{12,15} and BAFF¹⁶ (**Figure 1A, step 3**). This process is connected to the formation of germinal centers (GCs) in cooperation with donor Tfollicular helper cells (Tfhs). GC B-cells undergo somatic hypermutation that can favor cGVHD by increasing the frequency of B-cells capable of producing antibody to antigens that trigger the BCR.

IL-4 produced by CD4 T-cells promotes B-cell immunoglobulin (Ig)-isotype switching¹⁷⁻¹⁹, allowing daughter cells from the same activated B-cell to produce secreted pathogenic IgG in cGVHD mice^{12,20}. Tfhs produce IL-21 which can promote auto- and allo-reactive B-cell activation and survival along with increased local BAFF levels in cGVHD¹⁶. While the role of GCs in cGVHD initiation is likely to be important in many cGVHD mouse models, GCs were found not to be required for disease development in a recent report²¹, possibly reflecting the wide clinical spectrum of cGVHD in patients. In a consecutive step activated B-cells can promote tissue injury via antibody and cytokine production and release, leading to the clinical manifestations of cGVHD (**Figure 1A, step 4**). IgG-induced macrophage activation may contribute to cGVHD via secretion of pro-inflammatory cytokines such as IL-6 and IL-22²², which maintain inflammation. Tissue stiffness in cGVHD can be enhanced by copious Ig production and deposition together with fibroblast-derived extracellular matrix molecules including collagen and proteoglycans (**Figure 1A, step 4**).

The role of B-cells in cGVHD - evidence from studies on human tissues

Pathogenic B-cell activation is found in various autoimmune diseases including systemic lupus erythematosus, multiple sclerosis, rheumatoid arthritis, type 1 diabetes, and others as well as in cGVHD^{19,20}. During cGVHD donor B-cells and T-cells mount a coordinated response to both allogeneic and autologous antigens which leads to their expansion (**Figure**

1A, step 1 and 2). Allogenic antigens include minor histocompatibility antigens (mHA)^{23,24} that are typically expressed or processed intracellularly and presented as peptides by MHC molecules. These include Y-chromosome proteins/peptides in male recipients of female donor grafts, as well as cell membrane antigens, the former correlating with cGVHD by multivariable logistic regression analysis²⁵. Autoantigens are antigens on donor hematopoietic cells, which can be found for example on megakaryocytes or platelets. In agreement with the concept of recognition of autoantigens, patients can develop autoimmune thrombocytopenia following allo-HCT which are mediated by antibodies produced by donor B cells and are directed against donor platelets.

BAFF promotes B-cell survival and activation (**Figure 1A**, step 3) and is significantly increased in cGVHD patient plasma^{26,27}. BAFF and BCR-associated signaling work in concert to promote activation and survival of B-cells from cGVHD patients²⁸. In cGVHD patients, B-cells exhibit increased BCR-responsiveness²⁸ via increased proximal BCR intracellular signaling molecules splenic tyrosine kinase (SYK) and B-cell-linker (BLNK)²⁹. In that context it is important to understand which cell-intrinsic mechanisms enhance BCR responses. A novel observation here is that BCR responses to surrogate antigen were markedly increased when NOTCH2 was also activated³⁰. Intrinsic differences in important transcription factors like IRF4, contributed to NOTCH2 expression and responsiveness. How extrinsic factors like BAFF and intrinsic molecular pathways like NOTCH promote BCR-activated B-cells is currently not clear but is an area of active investigation.

Tfh cells can support anti-host antibody production³¹. This process typically takes place in GCs an area of the lymph node where B-cells are activated in mice³¹, but where this occurs in cGVHD in patients remains unknown. Akin to autoimmune disease patients, this process also may occur in extrafollicular locations. Antigen targets of B-cell responses in cGVHD remain largely unknown, but ultimately, both auto- and allo-immune B-cell responses can occur. Lack of sufficient T regulatory cells (Tregs) in cGVHD patients can contribute to impaired peripheral tolerance³². Tregs are capable of selectively killing B-cells³³ and their deficiency would predispose to a failure to control pathogenic B-cells. While human memory Tregs expand after allo-HCT they cannot compensate for the lack of naïve Treg due to short telomeres and increased apoptosis³⁴. CGVHD tissue stiffness and organ dysfunction are likely supported by cooperation between B-cells and macrophages leading to fibroblast activation, however so far there is no direct evidence for this interaction (**Figure 1A**, step 4).

Impaired central and peripheral tolerance mechanisms in cGVHD

Under homeostatic conditions, multiple mechanisms prevent pathogenic B-cell function via central (thymic) and peripheral tolerance. In patients undergoing allo-HCT, uncontrolled expansion and Ig production by B-cells is possibly because of thymic dysfunction. Impaired thymic function is caused by aging, conditioning regimen toxicity, calcineurin inhibitors, alloreactive T-cells, and Ig deposition^{19,35}. Alloreactive T-cells contribute to the process by depleting thymic dendritic cells, medullary thymic epithelial cells (mTECs) and cortical TECs (cTECs)^{35,36}. A recent report also suggests pathologic antibodies target TECs in a cGVHD model^{15,18}. cGVHD affects both positive selection by cTECs and negative selection by thymic B-cells and cTECs^{35,37} that allows potentially pathogenic CD4⁺ T-cells to escape from tolerization or deletion before peripheral export^{38,39} and impedes the development of Tregs that contribute to peripheral tolerance.

Mouse studies revealed that peripheral immune tolerance to recipient tissues after transplantation is mediated by Tregs, Tfollicular regulatory cells (Tfrs) representing Tregs that migrate to the GCs¹², regulatory B-cells (Bregs)⁴⁰, type-1 regulatory T-cells (Tr1)⁴¹ and invariant NKT-cells (iNKTs)⁴²⁻⁴⁴. Tregs and Tfrs negatively regulate B-cell responses and cGVHD⁴⁴ and Bregs that release IL-10 have been shown to ameliorate sclerodermatous cGVHD severity⁴⁵. In agreement with these mouse studies, analysis in cGVHD patients suggest that B-cells with a regulatory phenotype are both decreased and inactive^{40,46}. Increased T-cell 'help' decreases self-regulation by B-cells by promoting aberrant B-cell generation. Additionally, the absence of robust recovery of the peripheral B-cell compartment results in excess BAFF and promotion of autoreactive B-cells that can cooperate to overwhelm peripheral tolerance mechanisms in cGVHD patients⁴⁷. Additionally, thymic T cell generation, negative selection of anti-host reactive T-cells, thymic Treg production and peripheral Treg survival are severely reduced in cGVHD patients^{32,48,49}.

Novel and early phase therapeutic strategies that target B-cell in cGVHD

B-cell depletion with anti-CD20 antibodies was performed in preclinical models and patients^{12,50,51}. Anti-CD20 mAbs given in the prophylactic setting reduced murine cGVHD, while established cGVHD was non-responsive^{12,50}. In the clinical setting the anti-CD20 mAb rituximab conferred some efficacy in steroid-refractory (SR)-cGVHD patients⁵² with attenuation of cGVHD in those patients who robustly recovered B-cells^{47,53}. A prospective phase-II-trial showed that naïve B-cells (PD-L1^{hi}) were significantly reduced at cGVHD diagnosis, but increased after rituximab-treatment⁵¹. To target plasma cells, different drugs that have been successfully used in the treatment of multiple myeloma such as pomalidomide⁵⁴ were tested in cGVHD (**Table 1, Figure 1B**). IL-6 was shown to contribute to

cGVHD. Because IL-6 is known to promote plasmablast and plasma cell survival⁵⁵, further study of IL-6 and B-cells is warranted. The anti-IL-6 receptor mAb tocilizumab is being investigated in a clinical trial as cGVHD-therapy⁵⁶. In other diseases, IL-6 also has a known role in promotion of collagen deposition and extracellular matrix production by fibroblasts⁵⁷.

Several small molecule inhibitors are now in the pipeline building upon the observation that cGVHD patients have hyperreactive BCR signaling via the BCR proximal tyrosine kinase SYK. SYK was found to be up-regulated in cGVHD B-cells in mice^{12,13} and patients²⁹. SYK inhibition reduced established murine cGVHD, associated with reduced GC- and activated CD80/86⁺DCs-responses¹⁰, and induced apoptosis in cGVHD patient B-cells^{10,13,29}. Based on these promising findings, the SYK-inhibitor entospletinib, recently granted FDA orphan drug status, is being studied as first-line treatment with steroids⁵⁸. Further downstream of the BCR is Bruton's tyrosine kinase (BTK). In cGVHD patient B-cells, phosphor-BTK was present in the absence of in vitro stimulation by anti-IgM¹⁴. In agreement with a role of BTK, cGVHD severity was reduced in murine recipients given donor B-cells lacking BTK or ibrutinib that targets BTK¹⁴. Ibrutinib additionally inhibits IL-2-inducible kinase (ITK)¹⁴ and in a cGVHD model where T-cells lacked ITK cGVHD was reduced¹⁴. Based on these findings it is likely, but not formally proven, that both BTK- and ITK-inhibition are critical to the efficacy of ibrutinib in cGVHD. In cGVHD patients, ibrutinib reduced murine sclerodermatous and multi-organ system cGVHD as well as T- and B-cell activation^{14,59}. Guided by these preclinical data, an open-label phase-2-study evaluated the safety and efficacy of ibrutinib in active cGVHD patients with SR-cGVHD⁶⁰. At a median follow-up of 13.9 months, best overall response was 67% (sustained ≥ 20 weeks in 71% of responders)⁶⁰. Based on these clinical data and upon the foundations of the applied NIH consensus criteria from 2005, ibrutinib was FDA-approved for SR-cGVHD.

With better understanding of the role of B-cells in cGVHD pathogenesis, multiple additional strategies have been developed that deplete B-cells, reduce their activation via manipulation of BCR-downstream events or inhibit their migration towards inflammatory sites. Other agents also potentially target cytokine-mediated B-cell differentiation or survival. In normal mice and healthy volunteers, in vitro Tfh generation depends upon the Rho-GTPase kinase-2 (ROCK2)⁶¹. In both murine sclerodermatous and multi-organ system cGVHD models, ROCK2 inhibition with KD025 ameliorated ongoing cGVHD, associated with reduced Tfh's due to inhibiting pSTAT3 and IL-21-production and increased Tfrs due to augmenting pSTAT5 signaling⁶². A phase-2a KD025 trial to treat SR-cGVHD⁶³ is ongoing. BCR stimulation also activates JAK2/STAT3 signalling⁶⁴. In mice, JAK1/2 blockade with ruxolitinib inhibited multiple murine cGVHD features⁶⁵. Clinical responses were reported in a survey of SR-cGVHD patients treated with ruxolitinib⁶⁵. Based on these promising results, a phase-3-

multi-center ruxolitinib trial for treating SR-cGVHD⁶⁶ is in progress. How the B-cell compartment is affected by these agents is unclear.

Pirfenidone inhibits TGF- β receptor-signaling, downregulates NLRP3-inflammasomes, growth factors, and procollagen-I and II, and is FDA-approved for treating idiopathic pulmonary fibrosis. Pirfenidone treatment of established murine cGVHD restored pulmonary function and reversed lung fibrosis, associated with reduced pulmonary macrophage infiltration and TGF- β production⁶⁷. How B-cells are affected by agents that block fibrotic pathways requires further investigation.

Autoreactive B-cell regulation is mediated via Tregs. Treg have the capacity to control recipient-reactive B-cells with their expansion and survive dependent upon IL-2 production by Teffectors⁶⁸. Thus, low-dose IL-2 infusion has been tested as cGVHD treatment. A phase-1/2-study showed that exogenous IL-2 increased Tregs and improved cGVHD^{69,70}. Based on the defects in Tregs reported for cGVHD patients^{32,49} a clinical study analyzed the feasibility and efficacy of human expanded Tregs given to cGVHD patients⁷¹. The study reported that 2 of 5 treated patients achieved a complete remission.

Summary and outlook

Recent advances in our understanding of the role of B-cells in cGVHD pathogenesis have paved the way for novel strategies that target activation, expansion, survival and Ab-production of B-cells. Studies are urgently needed because the first-line “gold standard” for cGVHD therapy remains steroids, which have multiple severe side effects. Both mouse and human studies of B-cell pathways have been a major driver in testing the aforementioned novel therapies. These drugs were in some instances already clinically applied in other diseases. In spite of their potential clinical benefit, an important clinical consideration is that cGVHD is connected to overall reduction in relapse⁷². Thus, too intensive cGVHD prevention may lead to reduced graft-versus-leukemia activity. Clinical judgment, the application of the NIH criteria for cGVHD diagnosis and scoring^{4,73}, novel cGVHD biomarkers⁷⁴ and measurement tools will be essential to make a clinical meaningful progress in cGVHD treatment via B-cell targeting.

Conflict of Interest Disclosure:

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Figure legend:

Figure 1: The role of B-cells in cGVHD

A: Different steps of cGVHD development

Step 1. Antigen presenting cells (APC) present auto- and allo-antigens and prime B-cells. Direct activation of B-cells via Ag or Ag/Ab complexes. APCs prime B-cells against MHC/peptides or neoantigens (e.g. Y chromosome encoded genes). This is enhanced in certain B-cell subgroups by hyperreactive BCR signaling. Besides B-cells activation by APCs there is likely also direct BCR activation via antigen (Ag) or antibody/antigen (Ab/Ag) complexes.

Step 2. Expansion of auto- and allo-reactive B-cells.

Step 3. Activated Tfollicular helper (Tfh) cells produce IL-21 and cell surface costimulatory molecules that lead to germinal center formation, which is not counterbalanced by sufficient Tfollicular regulatory (Tfr) cells. CD4 T helper cells produce IL-4 which promotes Ab class switch in autoreactive B-cells. Stroma cells produce BAFF, which promotes B-cell activation.

Step 4. Plasma cells and plasmablasts produce high amounts of Ig. Deposition of IgG can lead to macrophage activation and organ damage. IgG-induced macrophage activation may contribute to cGVHD via secretion of pro-inflammatory cytokines by macrophages such as IL-6 which promotes B-cell survival and maintains inflammation.

B: Strategies to target B-cells in cGVHD

The sketch shows a B-cell and the mode of action of multiple immunosuppressive strategies that directly act on B-cells or plasma cells in the context of chronic GVHD. The summary of translation of each approach is provided in Table 1.

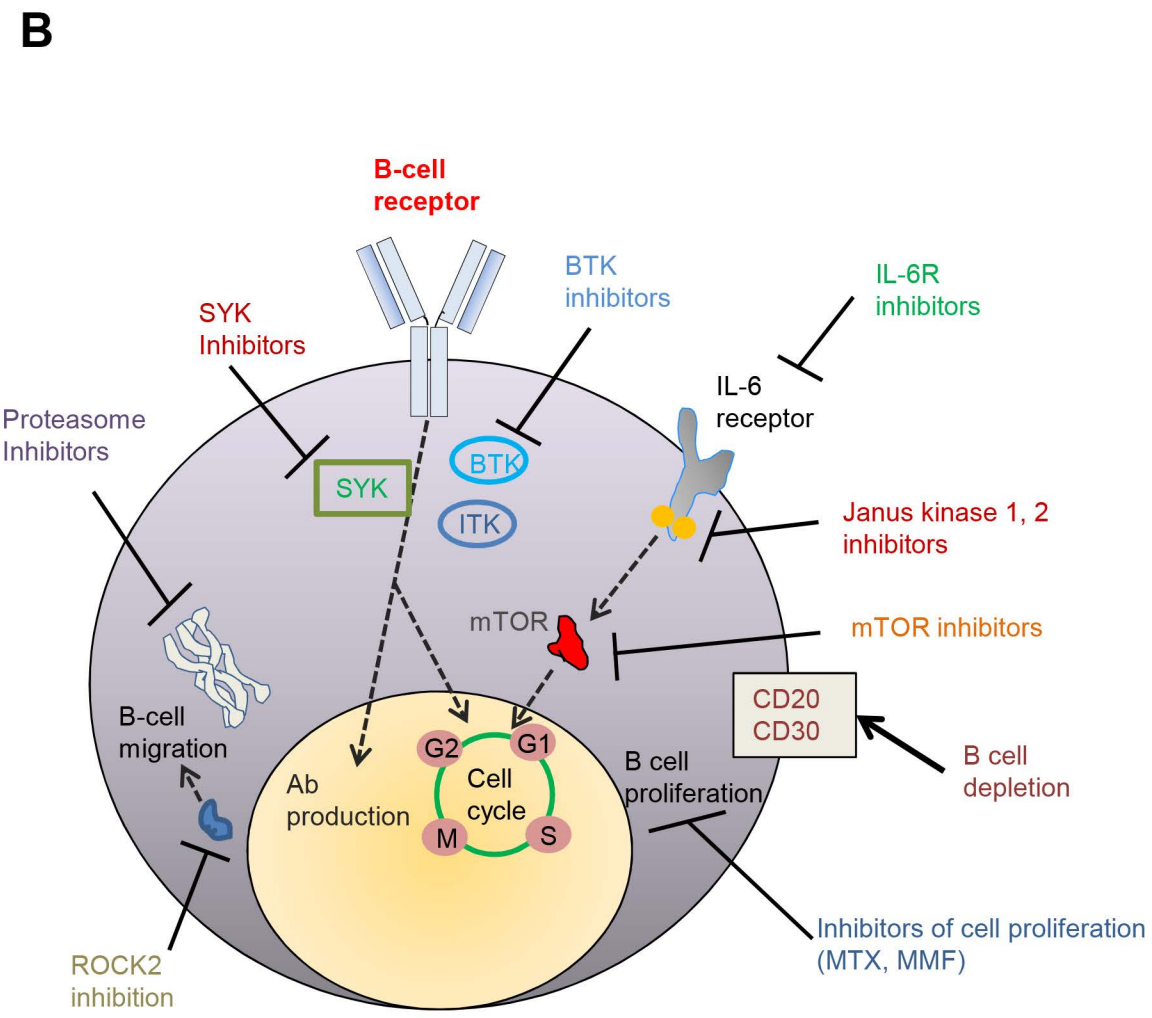
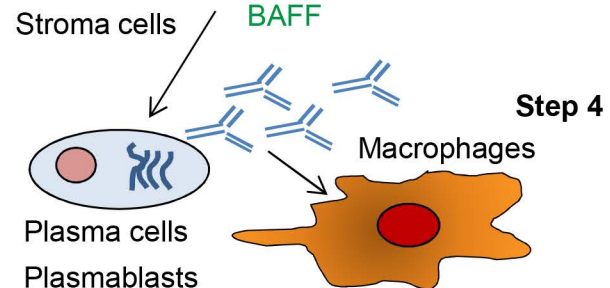
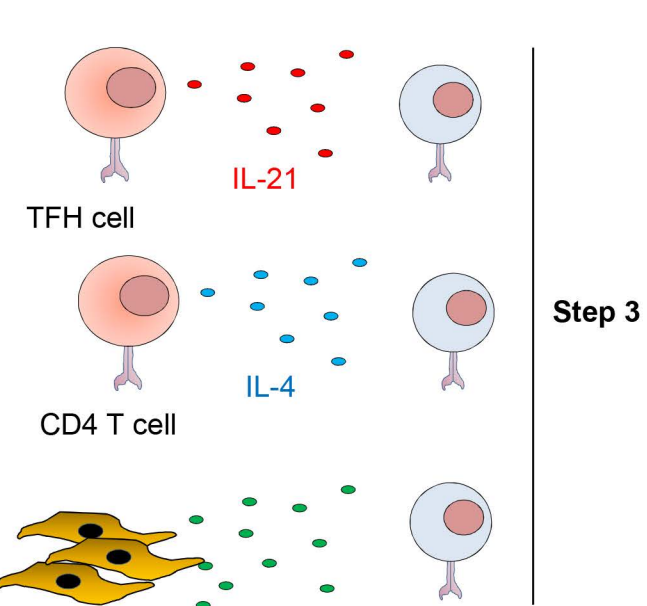
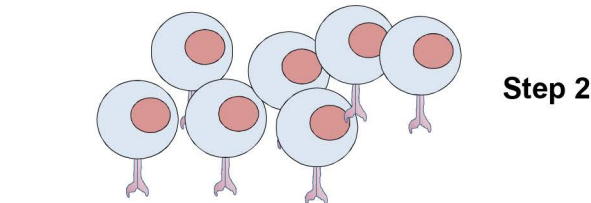
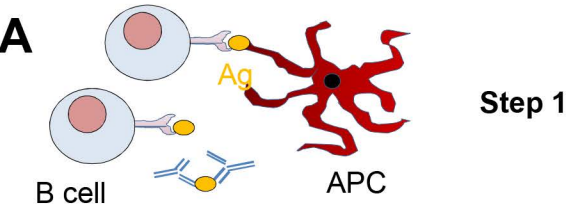
Table 1: Targeting B-cells in cGVHD (alphabetical order)

Target name	Normal function	Name of drugs tested	Species analyzed	Evidence for a role in cGVHD	Reference
Bruton's tyrosine kinase (BTK) and interleukin-2 inducible T-cell kinase (ITK)	- Downstream of B-cell receptor (BCR) activation	Ibrutinib	mouse	yes	14,59
			human	yes	60
CD20	- B-cell surface antigen	Rituximab	mouse	yes (effective only in prevention)	50
			human	yes	47,51-53
CD30	- B-cells express CD30	Brentuximab	human	clinical trials ongoing	75
IL-6R	- IL-6 induces proliferation of pre-B-cells	Tocilizumab	human	clinical trials ongoing	56
Janus kinase 1/2 (JAK 1/2)	- JAK1/2 mediate downstream effects of cytokine and chemokine receptors in B-cells ⁶⁴	Ruxolitinib	mouse	yes	65
			retrospective clinical data	yes	65
			prospective phase III trial ongoing	clinical trials ongoing	66
Plasma cells	- Production of Ig that cause organ damage in cGVHD	Pomalidomide	human	clinical trials ongoing	54
Proteasome	- Activation of the proteasome is	Bortezomib	mouse	yes	76

	important in plasma cells		human	clinical trials ongoing	77
Proteasome	- Activation of the immunoproteasome is important in plasma cells	Carfilzomib	human	clinical trials ongoing	78
Rho associated coiled-coil containing protein kinase 2 (ROCK2)	- T cell activation with pSTAT3 and pSTAT5 effects - B-cell migration	KD025	mouse	yes decreases Tfh cells	62
			human	clinical trials ongoing	63
Spleen tyrosine kinase (SYK)	- Downstream of BCR activation - Cell Migration - Endocytosis	Entospletinib	mouse	yes	10,13
		Fostamatinib		clinical trials ongoing	58

Abbreviations: BCR, B-cell receptor; BTK, Bruton's tyrosine kinase;; ITK, interleukin-2 inducible T-cell kinase; JAK1/2, Janus- kinase 1/2; pSTAT-3, -5, phosphorylated Signal transducer and activator of transcription 3, 5; SYK, spleen tyrosine kinase; Tfh, T follicular helper cells; Tfr, T follicular regulatory cells;

Figure 1





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B-cell targeting in chronic Graft-versus-Host disease

Robert Zeiser, Stefanie Sarantopoulos and Bruce R. Blazar

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