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Mark A. Schroeder
Washington University School of Medicine in St. Louis
Jaebok Choi
Washington University School of Medicine in St. Louis
Karl Staser
Washington University School of Medicine in St. Louis
John F. DiPersio
Washington University School of Medicine in St. Louis

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Reviews

The Role of Janus Kinase Signaling in Graft-Versus-Host Disease and Graft Versus Leukemia

Mark A. Schroeder *, Jaebok Choi, Karl Staser, John F. DiPersio

Washington University School of Medicine, Division of Oncology, Section of Stem Cell Transplantation, St. Louis, Missouri

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For patients with hematologic malignancies, allogeneic hematopoietic cell transplantation (alloHCT) offers a potential curative treatment option, primarily due to an allogeneic immune response against recipient tumor cells (ie, graft-versus-leukemia [GVL] activity). However, many recipients of alloHCT develop graft-versus-host disease (GVHD), in which allogeneic immune responses lead to the damage of healthy tissue. GVHD is a leading cause of nonrelapse mortality and a key contributor to morbidity among patients undergoing alloHCT. Therefore, improving alloHCT outcomes will require treatment strategies that prevent or mitigate GVHD without disrupting GVL activity. Janus kinases (JAKs) are intracellular signaling molecules that are well positioned to regulate GVHD. A variety of cytokines that signal through the JAK signaling pathways play a role in regulating the development, proliferation, and activation of several immune cell types important for GVHD pathogenesis, including dendritic cells, macrophages, T cells, B cells, and neutrophils. Importantly, despite JAK regulation of GVHD, preclinical evidence suggests that JAK inhibition preserves GVL activity. Here we provide an overview of potential roles for JAK signaling in the pathogenesis of acute and chronic GVHD as well as effects on GVL activity. We also review preclinical and clinical results with JAK inhibitors in acute and chronic GVHD settings, with added focus on those actively being evaluated in patients with acute and chronic GVHD.

INTRODUCTION

Allogeneic hematopoietic cell transplantation (alloHCT) is potentially curative for a variety of hematologic malignancies [1]. An important component is the graft-versus-leukemia (GVL) response [2], in which alloreactive (antirecipient) donor T cells target recipient tumor cells, inhibiting relapse [3]. Graft-versus-host disease (GVHD) is a potentially fatal complication of alloHCT. Similar to GVL, alloreactive donor T cells are activated during GVHD; however, rather than targeting tumor cells, the immune response attacks healthy recipient tissue. GVHD is a major cause of morbidity [4] and a leading cause of nonrelapse mortality [1] after alloHCT. Developing treatment options that curb GVHD without abrogating GVL is important for improving alloHCT effectiveness.

The pathogeneses of acute GVHD (aGVHD) and chronic GVHD (cGVHD) share similarities, with aGVHD a risk factor for developing cGVHD [5]. Both are inflammatory disorders, initiated by antigen-presenting cell (APC) activation of alloreactive T cells [6]. After activation, T cells infiltrate target tissues [7], and positive feedback loops drive proinflammatory cytokine signaling [6], resulting in inflammation [8], tissue damage, and organ failure [4]. After alloHCT, immune cells and related signaling molecules drive aGVHD in 3 general phases: (1) conditioning treatment, which results in tissue inflammation; (2) the initial interaction between APCs and allogeneic T cells resulting in T cell activation; and (3) immune cell migration and tissue damage. Janus kinases (JAKs) are important effectors of all 3 phases, transducing inflammatory signaling downstream of cytokines and regulating development and function of immune cells including APCs and T cells [9]. cGVHD may be exacerbated by diminished central and peripheral tolerance, with B cells and macrophages playing active roles [10-13]. A variety of tissues may be affected in GVHD, including lung, liver, gastrointestinal tract, skin, and secondary lymphoid organs [4].

The prevalence of GVHD is influenced by several factors, including degree of HLA match between donor and recipient, donor cell source (ie, bone marrow, peripheral blood, or umbilical cord), conditioning regimen, and post-transplant prophylaxis [5]. Among HLA-matched donor-recipient pairs,

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* Correspondence and reprint requests: Mark A. Schroeder, MD, Washington University School of Medicine, Division of Oncology, Section of Stem Cell Transplantation, 4590 Children's Place, St Louis, MO 63110.
E-mail address: markschroeder@wustl.edu (M.A. Schroeder).

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grade II to IV aGVHD is estimated to occur in 25% to 55% of patients within the first 100 days, and cGVHD may occur in 5% to 40% within the first 2 years.

To date, there are limited clinical means to predict with accuracy the development of GVHD and treatment response. Numerous studies have focused on prevention. GVHD prophylaxis generally includes cyclosporine plus methotrexate or mycophenolate mofetil, tacrolimus plus methotrexate, or antithymocyte globulin [14], and no therapy added to this backbone has proven to be of added benefit to prevent the disease. In those that develop GVHD, corticosteroids are the standard first-line systemic treatment option for patients with aGVHD [15] and cGVHD [16], and this has not changed for several decades. As reviewed by Coutinho and Chapman [17], the anti-inflammatory and immunosuppressive effects of corticosteroids primarily signal through the glucocorticoid receptor, repressing expression of cytokines and other inflammatory response genes. Response to corticosteroids may be predicted at diagnosis based on International Bone Marrow Transplant Registry clinical grade or the Ann Arbor score, which is calculated based on plasma levels of TNFR1, ST2, and REG3α [18], but has yet to be routinely used in clinical practice to direct therapy outside of clinical trials. The only drug currently approved for treatment of GVHD is ibrutinib. Ibrutinib, a Bruton tyrosine kinase (BTK) and IL-2 inducible T cell kinase (ITK) inhibitor, was recently approved by the U.S. Food and Drug Administration as second-line treatment for adult patients with cGVHD [19]. Guidelines drafted before the approval of ibrutinib for cGVHD recommended clinical trial enrollment for alloHCT patients after first-line (steroid) failure [15,16].

JAKs are intracellular signaling components that function downstream of many cytokines [9]. Various JAK inhibitors are approved or under investigation for treating various malignancies, autoimmune disorders, and inflammatory diseases (Table 1) [20-22]. There are 4 members of the JAK family, of which JAK1, JAK2, and JAK3 may be most relevant for GVHD [7,23-27]. TYK2, the fourth member of the JAK family, although less implicated in GVHD using in vitro or in vivo preclinical models, mediates signaling through several inflammatory receptors including IL-6, IL-10, IL-12, IL-23, IL-27, IL-31, type I interferon receptors, and oncostatin M [28]. JAKs regulate the activities of immune cells that underlie GVHD, including APCs [29], T cells [30], and B cells [31,32]. Preclinical and clinical studies suggest that JAK inhibition may disrupt GVHD [7,23-25] without negatively affecting GVL activity [24,25]. This article reviews the pathogenesis of GVHD with a focus on aspects that may be regulated by JAK signaling and provides an overview of findings from preclinical and clinical studies evaluating JAK inhibitors in GVHD settings. For a broader review of GVHD pathogenesis and new treatment strategies, please see recent articles by Betts et al. [33], Cooke et al. [34], MacDonald et al. [35], Im et al. [36], and Zeiser and Blazar [37].

ROLE OF JAKS IN AGVHD PATHOGENESIS AND GVL ACTIVITY

JAKs are key regulators of immune cell development and function [9] and therefore are well positioned to regulate aspects of all 3 phases of aGVHD pathogenesis (Figure 1) [6,7,9,11,23,25,26,29,32,38-49]. Intracellular signaling downstream of multiple cytokines is transduced by JAK family members [9]. Additionally, JAKs function in several cell types involved in GVHD, including dendritic cells (DCs), macrophages, T cells, B cells, and neutrophils, making them ideal targets for aGVHD treatments.

Phase 1: Conditioning Treatment

Conditioning regimens, which eliminate malignant or abnormal hematologic cells in the recipient, establish a receptive environment for GVL and aGVHD. As recently reviewed by Magenau et al. [6], the conditioning regimen may cause tissue damage and increased cytokine expression (eg, TNFα and IL-6), conferring immunologic changes that activate recipient APCs (Figure 1A) [6,29,38,39].

Antigen-presenting cells

Activation of APCs during the development of GVHD is regulated by JAK signaling. In human cell culture experiments, macrophage activation with IFNα, IFNγ, and TNF was diminished by the JAK1/JAK2 inhibitor ruxolitinib or the JAK1/JAK3 inhibitor tofacitinib [38]. Similarly, in cell lines established from patients with melanoma, IFNγ signaling upregulated the expression of TAP1, MHC I, and PD-L1 in a JAK2-dependent pathway [39]. JAK signaling is also required for activation and function of DCs. Addition of the JAK1/JAK2 inhibitor ruxolitinib to murine and human monocyte cell cultures prevented maturation to DCs [29]. Ruxolitinib-treated cells lacked DC morphologic features; remained $CD14^+$; had low expression levels of DC markers, including CD1a, CD80, CD86, MHC II, and IL-12; and were ineffective in promoting proliferation and activation of $CD4^+$ and $CD8^+$ T cells. The JAK1/JAK3 inhibitor tofacitinib similarly inhibited DC activation [40]. Human monocyte–derived DCs cultured with tofacitinib had reduced expression of IFNγ, TNFα, IL-1β, and IL-6; increased expression of IDO1 and IDO2; and reduced type I IFN-induced expression of CD80/CD86.

Phase 2: Allogeneic Donor T Cell Activation after HSCT

After alloHCT, aGVHD, and GVL activity are initiated by interactions between APCs and allogeneic donor T cells [2,3,50,51], which lead to T-cell activation in a JAK-dependent process (Figure 1B) [9,23,40,41].

T cells

In a series of murine cell culture experiments, Spoerl et al. [23] demonstrated that JAK1/JAK2 are required for T-cell activation following interaction with APCs. Pretreatment of mouse $CD4^+$ or $CD8^+$ T cells with ruxolitinib reduced proliferation following exposure to activated APCs or CD3/CD28 beads. Ruxolitinib treatment of APC/T-cell cocultures was also associated with reduced production of IFNγ, IL-17A, and IL-2. In addition, lower concentrations of ruxolitinib were associated with an increased proportion of regulatory T cells (Tregs), compared with reduced levels of $CD4^+$ cells. Similarly, a separate study demonstrated that addition of the JAK2 inhibitor TG101348 to cocultures of DCs and T cells reduced T-cell proliferation and increased the Treg:effector T cell (Teff) ratio [52].

The JAKs primarily regulate T-cell activation, proliferation, and function via transduction of cytokine signaling [9]. Activation of the γ-chain cytokine receptor was required for $CD8^+$ T cell–mediated aGVHD and cGVHD in a murine model [41]. Several cytokines that activate JAK1 and JAK3 signal through γ-chain receptors—including IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21 [9]—and the γ-chain effects on GVHD require JAK3 [41]. Antibody blockade of the γ-chain protein reduced JAK3 phosphorylation, and alloHCT with donor T cells from $JAK3^{-/-}$ mice was associated with less severe GVHD. In cell

Table 1
JAK Inhibitors in Active Clinical Development or With Regulatory Approval in the United States

Name	JAK Target	Disease Setting	Clinical Phase or Regulatory Approval (ClinicalTrials.gov Identifier)
Regulatory approval in humans			
Ruxolitinib [20]	JAK1/JAK2	Myelofibrosis, polycythemia vera	FDA approval
		GVHD	Expanded access program (NCT03147742)
			Phase 3 (NCT02913261, NCT03112603)
			Phase 2 (NCT02953678, NCT02396628, NCT02997280, NCT02806375)
		Other hematologic malignancies	Phase 2 (NCT00726232, NCT01751425, NCT03041636, NCT02257138, NCT00639002, NCT02723994, NCT01431209, NCT02164500)
			Phase 1 (NCT01895842)
		Alopecia areata	Phase 2 (NCT02553330)
		Atopic dermatitis	Phase 2 (NCT03011892)
		Plaque psoriasis	Phase 2 (NCT00820950)
		Rheumatoid arthritis	Phase 2 (NCT00550043)
		Vitiligo	Phase 2 (NCT02809976)
Tofacitinib [21]	JAK1/JAK3	Rheumatoid arthritis	FDA approval
		Juvenile idiopathic arthritis	Phase 3 (NCT02592434)
		Psoriatic arthritis	Phase 3 (NCT01976364)
		Ulcerative colitis	Phase 3 (NCT01465763)
		Alopecia areata	Phase 2 (NCT02299297)
		Ankylosing spondylitis	Phase 2 (NCT01786668)
		Atopic dermatitis	Phase 2 (NCT02001181)
		Crohn disease	Phase 2 (NCT01393899)
		Keratoconjunctivitis sicca	Phase 2 (NCT01226680)
		Kidney transplant	Phase 2 (NCT00263328)
		Plaque psoriasis	Phase 2 (NCT01831466)
		Dermatomyositis	Phase 1 (NCT03002649)
		Systemic lupus erythematosus	Phase 1 (NCT02535689)
Regulatory approval in dogs			
Oclacitinib [22]	JAK1/JAK3	Allergic and atopic dermatitis (canine)	FDA approval
Currently without regulatory approval			
Baricitinib	JAK1/JAK2	Rheumatoid arthritis	Phase 3 (NCT02265705)
		Atopic dermatitis	Phase 2 (NCT02576938)
		Diabetic kidney disease	Phase 2 (NCT01683409)
		Giant cell arteritis	Phase 2 (NCT03026504)
		Chronic GVHD	Phase 2(NCT02759731)
		Psoriasis	Phase 2 (NCT01490632)
		Systemic lupus erythematosus	Phase 2 (NCT02708095)
BMS-911543	JAK2	Myelofibrosis	Phase 1/2 (NCT01236352)
Filgotinib	JAK1	Crohn disease	Phase 3 (NCT02914600)
		Rheumatoid arthritis	Phase 3 (NCT03025308)
		Ulcerative colitis	Phase 3 (NCT02914535)
INCB52793	JAK1	Advanced malignancies	Phase 1 (NCT02265510)
Itacitinib	JAK1	Hematologic malignancies	Phase 2 (NCT02456675, NCT02018861, NCT01633372)
		Plaque psoriasis	Phase 2 (NCT01634087)
		Pruritus	Phase 2 (NCT02909569)
		Rheumatoid arthritis	Phase 2 (NCT01626573)
		Solid tumors	Phase 2 (NCT02917993, NCT01858883)
			Phase 1 (NCT02646748)
		GVHD	Phase 1 (NCT02614612)
Momelotinib	JAK1/JAK2	Myelofibrosis	Phase 3 (NCT01969838)
		Pancreatic ductal adenocarcinoma	Phase 3 (NCT02101021)
		NSCLC	Phase 1 (NCT02258607)
NS018	JAK2	Myelofibrosis	Phase 2 (NCT01423851)
Peficitinib	pan-JAK	Rheumatoid arthritis	Phase 3 (NCT01638013)
		Psoriasis	Phase 2 (NCT01096862)
		Ulcerative colitis	Phase 2 (NCT01959282)
PF-04965842	JAK1	Atopic dermatitis	Phase 2 (NCT02780167)
PF-06651600	JAK3	Alopecia areata	Phase 2 (NCT02974868)
		Rheumatoid arthritis	Phase 2 (NCT02969044)
		Ulcerative colitis	Phase 2(NCT02958865)
PF-06700841	JAK1/TYK2	Alopecia areata	Phase 2 (NCT02974868)
		Plaque psoriasis	Phase 2 (NCT02969018)
		Ulcerative colitis	Phase 2 (NCT02958865)
SHR0302	JAK1	Rheumatoid arthritis	Phase 1 (NCT02665910)
Upadacitinib	JAK1	Rheumatoid arthritis	Phase 3 (NCT02706847)
		Ulcerative colitis	Phase 3 (NCT03006068)
		Atopic dermatitis	Phase2 (NCT02925117)
		Crohn's disease	Phase 2 (NCT02782663)
WP1066	JAK2	Glioma and brain metastases from melanoma	Phase 1 (NCT01904123)

For drugs indexed in multiple clinical trials at ClinicalTrials.gov for the same indication, only 1 trial in the highest phase of development is listed, with the exception of GVHD (all indexed trials are listed).
FDA indicates U.S. Food and Drug Administration; NSCLC, non-small-cell lung cancer; TYK, tyrosine kinase.

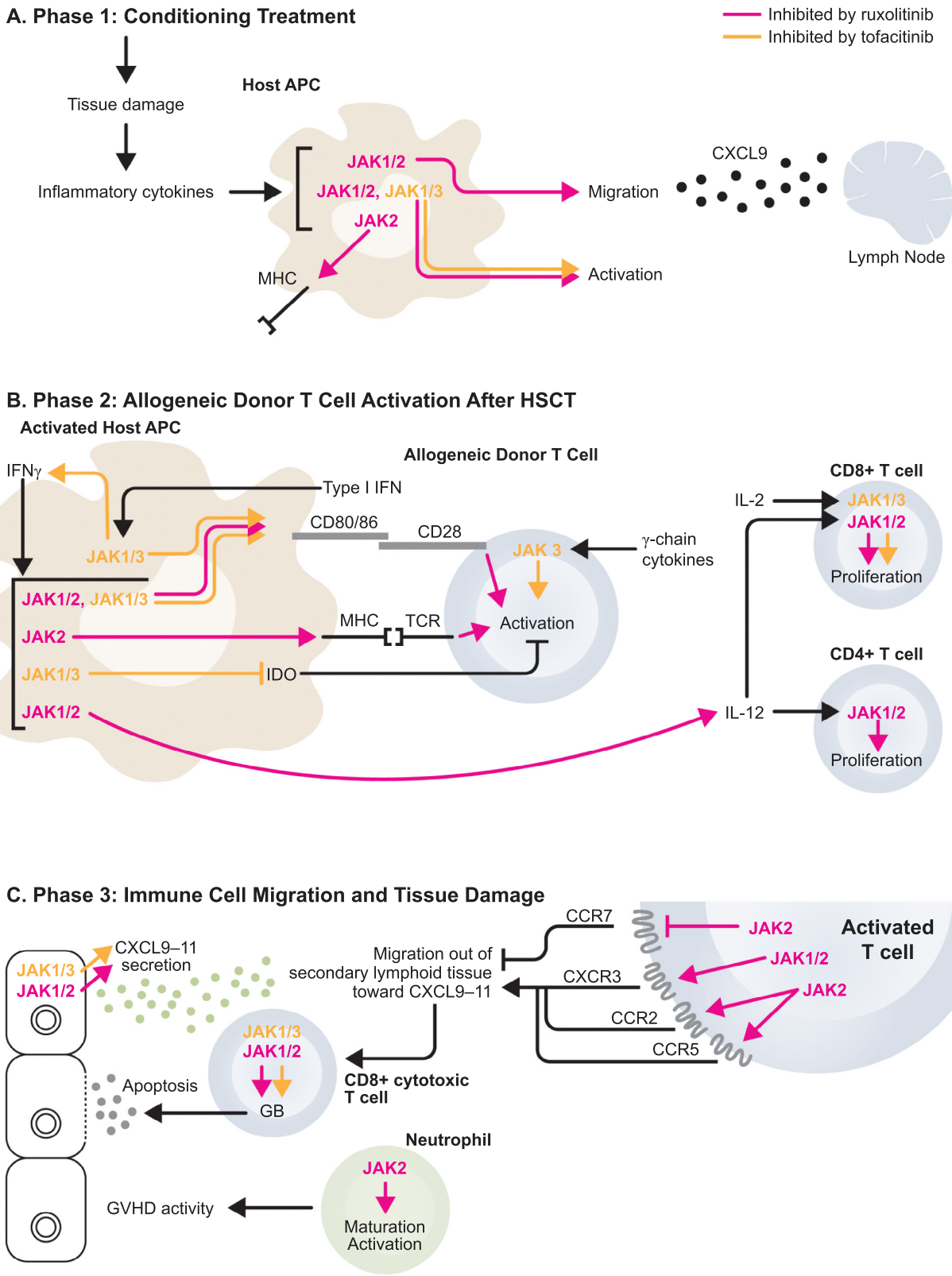

Figure 1. JAK Activity in aGVHD. (A) The conditioning regimen may cause the release of inflammatory cytokines [6], which signal through JAKs to activate APCs [29,38,39]; activated macrophages migrate toward CXCL9 secreted from lymph nodes in a JAK1/JAK2-dependent process [29]. (B) After HCT, JAKs regulate allogenic donor T-cell activation through secondary signals in APCs, such as CD80/86 [40], IDO, and IFN signaling [40], and in T cells downstream of the γ-chain cytokine receptor [9,41]. JAK activity in $CD4^+$ and $CD8^+$ T cells also promotes proliferation, whereas JAK signaling inhibits proliferation of Tregs [23]. (C) After T-cell activation, migration out of the secondary lymphoid tissue is regulated by chemokine receptors, which are in turn regulated by JAK signaling [7,26,42-47]. T-cell cytotoxic activity, including granzyme B (GB) production, is promoted by JAK activity [23,26]. Neutrophils, which may participate in GVHD [48], use JAK signaling pathways to regulate their development and activity [49]. DAMP, damage-associated molecular pattern; IDO, indoleamine 2,3 dioxygenase; PAMP, pathogen-associated molecular pattern; PD-1, programmed cell death protein 1; PD-L1, programmed cell death protein ligand 1.

culture experiments, the JAK2 inhibitor tyrphostin B42 blocked IL-12–induced proliferation of murine T cells [30]. Addition of tofacitinib to human CD8+ T-cell cultures reduced cell proliferation induced by IL-2 and prevented upregulation of IFNγ [26].

Phase 3: Immune Cell Migration and Tissue Damage

In the third phase of aGVHD, activated immune cells migrate to target tissue, causing tissue damage. Evidence from murine models suggests that chemokines act as tissue-specific migration signals for T cells [7,42,45]. Once recruited to the target tissue, cytotoxic CD8+ and CD4+ T cells provide apoptotic signals leading to tissue damage in murine models [53]. Apoptosis initiated by cytotoxic CD4+ T cells may be cell-cell contact independent and mediated through cytotoxic cytokines (eg, TNFα/TRAIL, IFNγ, IL-1) or cell-cell contact dependent and mediated via the FAS/FAS-L pathway [54] (Figure 1C) [7,11,23,25,26,32,38,42-49].

Chemokine signaling

Chemokine migration cues for immune cells, including APCs and T cells, signal through JAK signaling. Several analyses in murine models have demonstrated that JAK1/JAK2 are required for normal migration of DCs, macrophages, and T cells. The involvement of JAK signaling in chemokine-mediated T cell trafficking to GVHD target organs was first demonstrated in a preclinical mouse model of GVHD [7]. CXCR3 is a chemokine receptor, positively regulated by IFNγ receptor-JAK1/JAK2 signaling [7]. Ruxolitinib and the JAK1/JAK2 inhibitor momelotinib blocked upregulation of CXCR3 in wild-type T cells to a similar degree as deletion of IFNγ receptor [7]. Furthermore, ruxolitinib mitigated GVHD by altering T-cell trafficking to GVHD organs in a major MHC-mismatch mouse model of alloHCT as a result of CXCR3 downregulation [7]. In addition, ruxolitinib treatment in murine models inhibited in vitro DC migration toward the CXCR3 ligand CXCL9 and in vivo migration of DCs to draining lymph nodes [29]. In agreement with these original findings, in a murine aGVHD model, ruxolitinib treatment was associated with reduced expression of the chemokine receptor CXCR3 on splenic CD4+ and CD8+ T cells and reduced T-cell and macrophage infiltration in the skin, small intestines, and liver [25]. The extent to which JAK1, JAK2, or JAK3 are involved in this process of chemokine signaling and migration is still not clear given the inherent off target effect potential of small-molecule inhibitors used in these studies. Nonetheless, interrogation of specific JAKs has been reported. In vitro blockade of JAK2 prevented CCR7 downregulation following activation of murine-naive T cells [42], and JAK2 signaling may facilitate signaling downstream of other chemokine receptors involved in T-cell migration, including CCR2 [46] and CCR5 [44]. In a murine model of skin GVHD, topical ruxolitinib treatment reduced CXCL9 expression and T-cell skin infiltration [55]. JAK3 may also regulate chemokine-directed cell migration. Experiments in a cell culture system of murine keratinocyte target tissue demonstrated that IFNγ and Toll-like signaling induction of CXCL10 was inhibited by tofacitinib [26]. Finally, the pan-JAK inhibitors PF956980 and PF1367550 inhibited IFNγ-driven release of CXCL9, CXCL10, and CXCL11 from primary human airway epithelial cells in vitro [47].

T cells

Preclinical analyses in murine and human model systems have demonstrated that JAK signaling is required for T cell cytotoxic activity in GVHD. In vitro analyses found that ruxolitinib pretreatment of murine APC/T-cell cocultures was associated with reduced granzyme B levels in CD8+ T cells [23] and addition of tofacitinib to human CD8+ T-cell cultures prevented upregulation of perforin and granzyme B [26]. In a cell culture model of keratinocyte target tissue, tofacitinib inhibited IFNγ-induced cell death [26]. In a murine model, activation of the γ-chain cytokine receptor was required for production of granzyme B in CD8+ T cell–mediated GVHD [41].

Natural killer cells

Natural killer (NK) cells are immediate effector cells of the innate immune system that play a complex role in aGVHD and GVL. As reviewed by Simonetta et al. [56], NK cells are associated with aGVHD protective and promoting effects. Patients with elevated NK cell counts or alloreactivity had reduced risk of aGVHD [56], elevated NK cell counts and NK cell to myeloid-derived suppressor cell ratio were associated with increased response in patients with aGVHD treated with the JAK1 inhibitor itacitinib (INCB039110) [57], and adoptive transfer of NK cells in alloHCT patients has been associated with reduced aGVHD [56]. However, in some cases, activated NK cells may promote aGVHD through the secretion of inflammatory cytokines such as IFNγ and TNFα. The specific effects of JAK inhibition on this cellular subset and their role in GVHD and GVL is an area of ongoing investigation.

Neutrophils

Specifics surrounding the pathway by which neutrophils influence GVHD pathogenesis has not been fully elucidated, but some evidence suggests there may be a JAK-mediated role. Work in a murine model demonstrated that neutrophils migrated to the ileum after alloHCT [48]. Mice receiving alloHCT from donors lacking the ability to develop neutrophils had prolonged survival and decreased GVHD severity compared with wild-type donor tissue [48]. Finally, cell culture experiments have shown that granulocyte macrophage colony-stimulating factor activation of neutrophils can be disrupted by JAK2 inhibition with AG490 [49].

ROLE OF JAKS IN CGVHD PATHOGENESIS

Although cGVHD and aGVHD are recognized as distinct complications of alloHCT [4], they are similar immunologic disorders driven by APC interactions with alloreactive T cells that illicit an immune response against recipient tissue [6]. Distinguishing aspects of cGVHD pathogenesis include insufficient central tolerance resulting from thymic or peripheral lymph node dysfunction, inadequate peripheral tolerance resulting from Treg dysfunction or reduced numbers, reduced ratios of Tregs to Teff cells, and tissue fibrosis.

Insufficient Central Tolerance

The thymus plays an important role in preventing autoimmune activity through the removal of autoreactive T cells [12], and aberrant function may promote cGVHD. Damage to the thymus by alloreactive CD4+ and CD8+ T cells soon after transplant may lead to de novo thymic generation of alloreactive CD4+ T cells via defective clonal deletion or central tolerance [58] and induction of cGVHD [59]. In this model, aGVHD is mediated by the passive transfer of alloreactive donor T cells present in the bone marrow or peripheral blood stem cell allograft. In contrast, cGVHD is mediated by donor T cells generated from donor stem cells that are inadequately clonally deleted by a thymus damaged via passively transferred T cells that are also responsible for aGVHD. This

results in the accumulation of alloreactive T cells that contribute to the clinical syndrome of cGVHD. JAKs are well positioned to modulate central tolerance during cGVHD pathogenesis given their roles in T-cell proliferation, activation, and function, as described in the previous aGVHD/GVL section.

Inadequate Peripheral Tolerance

In addition to the central tolerance role performed by the thymus, Tregs perform similar roles inhibiting cGVHD in the periphery. Treg IL-10 production was required for the cGVHD inhibitory effect in a murine model [60] and patients with cGVHD may have reduced Treg counts in relation to the total peripheral blood lymphocyte pool [61]. In preliminary clinical evaluations, Treg adoptive transfer during alloHCT is associated with low cGVHD rates [62]. Similarly, low-dose IL-2 treatment for cGVHD was associated with Treg expansion, increased Treg:Teff ratios, and amelioration of cGVHD signs and symptoms in some patients [63]. As discussed in the previous aGVHD/GVL section, JAK1/JAK2 signaling regulates development and proliferation of Tregs [23] and the Treg:Teff ratio, with JAK2 inhibition promoting Treg proliferation [52].

Tissue Fibrosis

Tissue fibrosis is a common characteristic of cGVHD [4], which may result from target tissue infiltration by macrophages, TGFβ secretion, and B cell activity. In addition, cytokine signaling is required for cGVHD fibrosis. Activation of the γ-chain cytokine receptor, which signals through JAK1 and JAK3 [9,41], was required for development of fibrosis in the lung and liver in a murine model [41].

Macrophages

Tissue-infiltrating macrophages may promote fibrosis through TGFβ secretion. In murine cGVHD models, macrophages infiltrated target tissues, including lung and skin, in a process promoted by IL-17 and colony-stimulating factor 1 signaling [13]. Inhibition of IL-17 or colony-stimulating factor 1 signaling was associated with reduced cGVHD symptoms in the skin and lungs. Moreover, activated macrophages secrete TGFβ, which is an important profibrotic signal [13]. As discussed in the previous aGVHD/GVL section, JAKs are key regulators of macrophage activation [38].

B cells

Alloreactive B cells are important to the pathogenesis of cGVHD. In retrospective analyses of patients who underwent alloHCT, elevated plasma levels of B-cell activating factor and detection of antibodies against the H-Y antigen were positively correlated with cGVHD [10,11] and GVL activity [11]. B-cell depletion with rituximab prophylaxis following alloHCT was associated with modestly reduced cGVHD risk compared with a contemporaneous control group in a single-arm phase 2 clinical trial [64]. The B- and T-cell signaling factors ITK and BTK are both required for cGVHD development, as indicated in murine models receiving ITK- or BTK-deficient alloHCT [65]. Furthermore, treatment with ibrutinib blocked germinal cell formation, Ig deposition, and development of bronchiolar obliterans.

Preclinical evidence suggests that the development and activation of B cells may be regulated by JAK signaling. IFNγ signaling promoted expansion of $CD38^{+}$ $C27^{-}$ germinal cell B cells in a process that was completely blocked by ruxolitinib and partially blocked by tofacitinib [31]. In addition, tofacitinib treatment blocked B cell receptor– and sCD40L-induced expression of AICDA and XBP-1, and inhibited antibody production induced by B-cell stimulation with BCR, sCD40L, and IL-4 [32].

PRECLINICAL ACTIVITY OF JAK INHIBITORS

Preclinical evidence indicates that inhibition of JAK1, JAK2, or JAK3 diminishes GVHD responses without abrogating GVL activity in murine models.

JAK1/JAK2 Inhibition

Our group was the first to demonstrate that JAK1/JAK2 inhibition using ruxolitinib mitigates GVHD while preserving GVL in murine models of major mismatch alloHCT [7,24]. Ruxolitinib treatment was associated with lower grade aGVHD in the skin, liver, and intestines compared with placebo [7]. In addition, T-cell trafficking was altered with ruxolitinib, in which T cells remained concentrated in the spleen rather than migrating to gastrointestinal organs, the liver, and other parts of the body. Other groups have recently reported that ruxolitinib improved survival, lowered GVHD scores, increased measures of intact intestines, and reduced TNFα and IL-12 levels compared with placebo [23]. Overall, allogenic T-cell and central memory T-cell levels were reduced with ruxolitinib, whereas the frequency of Tregs was increased in the spleen and gastrointestinal tract [23], although these effects may be strain dependent [25]. In a murine model of skin GVHD, topical ruxolitinib treatment was associated with reduced T-cell infiltration of the skin, protection from GVHD-associated reductions in $LGR5^{+}$ hair follicle stem cells, and improved pathologic skin GVHD scores [55]. In addition to ruxolitinib, the JAK1/JAK2 inhibitor baricitinib was effective in GVHD prophylaxis and treatment [66]. In a murine model, prophylactic baricitinib treatment reduced GVHD, increased Treg counts, and decreased markers for Th1 and Th2 cells. Furthermore, baricitinib treatment was associated with a reduction in disease severity and a survival benefit in mice with established GVHD.

In murine models of GVHD and leukemia or lymphoma relapse, treatment with ruxolitinib improved survival and reduced GVHD in the skin, liver, and gastrointestinal organs while preserving GVL activity [24,25]. In contrast, among mice receiving T-cell replete alloHCT with a leukemia cell line, overall survival (OS) was similar between ruxolitinib and placebo, and leukemia cell infiltration of the spleen and bone was similarly inhibited in the presence or absence of ruxolitinib [25]. Ruxolitinib was superior to the JAK2 inhibitors TG101348 and AZD1480 for OS [24], suggesting that both JAK1 and JAK2 are important in the pathogenesis of GVHD.

JAK1/JAK3 Inhibition

In a murine model of skin and mucosal GVHD, JAK1/JAK3 inhibition was associated with reduced measures of aGVHD and improved survival [26]. Mice receiving prophylaxis with tofacitinib experienced less weight loss and prevention of skin and mucosal lesions compared with placebo. In addition, serum levels of IFNγ and TNFα were lower in tofacitinib-treated mice. In mice with established aGVHD, treatment with tofacitinib was associated with prevention of further weight loss and skin or mucosal lesions and a trend toward prolonged survival.

JAK3 Inhibition

In a murine model for multiorgan aGVHD, prophylactic JAK3 inhibition with WHI-P131 reduced aGVHD severity and prolonged survival compared with control mice [27]. WGI-

P131 treatment was also associated with a survival advantage in mice that received treatment after aGVHD was established.

CLINICAL EVIDENCE WITH JAK1/JAK2 INHIBITORS

Clinical evaluation of JAK inhibitors in GVHD has been limited to the JAK1/JAK2 inhibitor ruxolitinib and the JAK1 inhibitor itacitinib. Results to date suggest promising findings that warrant further evaluation, including activity against aGVHD and cGVHD.

Acute GVHD

Preliminary studies support the efficacy of JAK1/JAK2 inhibitors for the treatment of patients with aGVHD (Table 2) [23,67-72]. In a retrospective analysis that included patients with grade 3/4 corticosteroid refractory–aGVHD (n = 54), ruxolitinib treatment was associated with improvements in aGVHD severity in the intestines, liver, and skin, as well as prolonged survival [67]. At a median follow-up duration of 26.5 weeks, the overall response rate (ORR) was 82%, with a median time to response of 1.5 weeks. Importantly, malignancy relapse was only observed in 9% of patients. Adverse events were primarily cytomegalovirus (CMV) reactivation (33%), grade 3/4 cytopenia (33%), and grade 1/2 cytopenia (22%). In a long-term follow-up analysis (median follow-up duration, 19 months; median ruxolitinib treatment duration, 5 months), median OS was 18 months [68]. Response was ongoing at data cutoff in 41% (22 of 54) of patients, and response to retreatment with ruxolitinib or immunosuppressive therapy after GVHD relapse or progression after achieving partial response/complete response was observed in 78% (11 of 14) of patients.

Several smaller studies have also reported promising results with ruxolitinib. A retrospective analysis of pediatric patients with corticosteroid refractory–aGVHD in the eyes, gastrointestinal tract, liver, and skin (N = 13; efficacy evaluable, n = 11) reported an ORR of 45% (5 of 11) [69]. The most common adverse events were elevated alanine aminotransferase (n = 7), bacterial infections (n = 6), neutropenia (n = 3), and thrombocytopenia (n = 3). In a small pilot study that included 4 patients with grade 3 or 4 corticosteroid refractory–aGVHD in the intestines, liver, or skin, all patients had partial response or better after ruxolitinib treatment, with time to response ranging from 1 to 1.5 weeks [23]. In addition, ruxolitinib treatment was associated with reductions in serum IL-6 and sIL-2R levels. No ruxolitinib-related adverse events were reported. In another small retrospective analysis, a patient treated with ruxolitinib for grade 3 aGVHD in the skin and gastrointestinal tract following HCT for myelofibrosis achieved complete response [70]. The patient ultimately developed cGVHD 2 months after tapering and discontinuing ruxolitinib. Finally, in a case study, ruxolitinib was associated with complete resolution of grade 2 aGVHD in the gastrointestinal tract and skin after 21 days of treatment, as confirmed by histology [71].

Prophylactic treatment with ruxolitinib plus cyclosporine A and antilymphocyte globulin was evaluated in a retrospective study of patients with myelofibrosis undergoing alloHCT (n = 12 of 171 treated with ruxolitinib) [74]. One of 12 (8%) patients developed grade III aGVHD, and all patients were alive after a median follow-up of 112 days. There were no major adverse events during conditioning therapy. Among CMV-positive patients, CMV reactivation was observed in 4 (67%) patients. Ruxolitinib was discontinued in 2 patients because of cytopenias. It should be noted that no other studies have evaluated JAK inhibitors as prophylaxis for GVHD. Even in this very small study, the low rates of aGVHD (1 of 8 patients) cannot be specifically attributed to prophylactic ruxolitinib treatment, because patients also received antilymphocyte globulin as GVHD prophylaxis.

To date, there has only been 1 prospective clinical trial testing the effects of a JAK inhibitor on patients with steroid refractory aGVHD [72]. This trial tested the effect of 2 different doses (200 mg and 300 mg PO daily) of a JAK1-selective inhibitor, itacitinib (INCB039110) on responses in patients with steroid refractory aGVHD. Phase 1 clinical results with itacitinib suggest an acceptable safety profile in patients with aGVHD. In a preliminary safety analysis that included 30 evaluable patients, 1 dose-limiting toxicity was observed (grade 3 thrombocytopenia). The most common adverse events were thrombocytopenia or platelet count decrease (27%), diarrhea (23%), peripheral edema (20%), fatigue (17%), and hyperglycemia (17%). Grade 3 or 4 adverse events occurred in 77% of patients. Of note, the preliminary ORRs were 83% for first-line therapy patients and 64% for steroid refractory patients.

Chronic GVHD

Ruxolitinib treatment has also demonstrated clinical benefit in patients with cGVHD (Table 2). In a retrospective analysis that included patients with grade 3 or 4 corticosteroid refractory–cGVHD in the intestines, liver, lungs, musculoskeletal tissue, and skin (n = 41), ruxolitinib treatment was associated with improvements in cGVHD and prolonged survival [67]. ORR was achieved by 85% of patients at a median follow-up of 22.4 weeks. Median time to response was 3 weeks; malignancy relapse occurred in 2% of patients. Adverse events were primarily CMV reactivation (15%), grade 3 or 4 cytopenia (7%), and grade 1 or 2 cytopenia (10%). Following long-term treatment (median follow-up, 24 months; median ruxolitinib treatment duration, 10 months), response was ongoing in 24% (10 or 41) of patients, and response to retreatment with ruxolitinib or immunosuppressive therapy occurred in 85% (11 of 13) of patients [68]. Median OS had not been reached in the long-term analysis. Similar results were reported in a small retrospective study (n = 19) by Khoury et al. [73].

In a prospective analysis of 2 patients with skin cGVHD treated with ruxolitinib, both patients had a response within 1 week of treatment [23]. Serum levels of IL-6 and sIL-2R decreased after treatment with ruxolitinib. No ruxolitinib-related adverse events were reported.

In a retrospective analysis of 3 patients treated with ruxolitinib for severe cGVHD of the liver, lung, mouth, serosa, or skin after HCT for myelofibrosis, all patients achieved partial response or better, with a time to response ranging from 1 to 28 weeks [70]. Two patients remained on ruxolitinib and free of cGVHD after 340 days and 14 months of treatment, respectively. The final patient died from cGVHD 21 days after discontinuing ruxolitinib because of gastrointestinal bleeding.

CONCLUSIONS

Available evidence suggests that JAK inhibition may be useful for treating patients with aGVHD or cGVHD; however, further data from prospective trials will be important to confirm effectiveness. JAKs are important for the intracellular transduction of many cytokines [9] that regulate the development and function of immune cells involved in GVHD, including DCs [29], macrophages [25,38], T cells [30], B cells [32], and neutrophils [49]. Preclinical and clinical studies have demonstrated that JAK inhibition is associated with

Table 2
Clinical Reports of JAK Inhibitor Treatment for GVHD

Reference	JAK Inhibitor	Study Type	GVHD Severity	Patients	Prior Treatments, Median (Range)	Follow-Up Duration, Median (Range)	Response*	OS (95% CI)
aGVHD								
Zeiser etal. [67]; Zeiser et al. [68]	Ruxolitinib	Retrospective	Grade 3/4	54	3 (1-7)	26.5 (3-106) wk 19 (NA) mo	ORR, 82% (CR, 46%) Ongoing, 41%	6 mo, 79% (67%-91%) 12 mo, 62% (49%-75%)
Khandelwal et al. [69]	Ruxolitinib	Retrospective (pediatric)	Grades 2-4	13 (11 efficacy evaluable)	4 (1-6)	2-306 d†	ORR, 45% (CR, 9%)	13 mo,‡ 54%
Spoerl et al. [23]	Ruxolitinib	Pilot	Grade 3/4	4	4.5 (4-7)	18.5 (15-21) wk	ORR, 100% (CR, 25%)	NA
Mori et al. [70]	Ruxolitinib	Retrospective	Grade 3/4	1	2	NA	ORR, 100% (CR, 100%)	NA
Maffini et al. [71]	Ruxolitinib	Case report	Grade 2	1	3	22.3 wk	ORR, 100% (CR, 100%)	NA
Schroeder et al. [72]	Itacitinib	Phase 1 prospective	Grades IIB–IVD	30	NA	56.5-60.8 d§	NA	NA
cGVHD								
Khoury et al. [73]	Ruxolitinib	Retrospective	Severe¶	19	NA	18 (6-27) mo†	ORR, 89%	NA
Zeiser etal. [67]; Zeiser et al. [68]	Ruxolitinib	Retrospective	Moderate or severe	41	3 (1-10)	22.4 (3-135) wk 24 (NA) mo	ORR, 85% (CR, 7%) Ongoing, 24%	6 mo, 97% (92%-100%) 12 mo, 93% (85%-100%)
Spoerl et al. [23]	Ruxolitinib	Pilot	Grade 3	2	4 (3-5)	23.5 (10-37) wk	Response, 100%	NA
Mori et al. [70]	Ruxolitinib	Retrospective	Severe	3	2 (1-2)	NA	ORR, 100% (CR, 57%)#	NA

CR indicates complete response; NA, not available; PR, partial response.

* Zeiser et al. [67]: aGVHD: CR, absence of any symptoms related to aGVHD; PR, improvement of ≥1 stage in severity of aGVHD in 1 organ without deterioration in another organ; cGVHD: CR, absence of any cGVHD symptoms; PR, discontinuation of or ≥4-week reduction of all systemic immunosuppressive therapy by ≥50%. Khandelwal et al. [69]: aGVHD: CR, resolution of aGVHD symptoms at 4 weeks of ruxolitinib treatment; PR, improvement by ≥1 stage in severity of aGVHD in 1 organ without deterioration in another organ until 4 weeks of ruxolitinib treatment. Spoerl et al. [23]: aGVHD: same as Zeiser et al. [67]; cGVHD: response, discontinuation or ≥4-week reduction of all systemic immunosuppressive therapy by ≥50%. Mori et al. [70], Maffini et al. [71], Khoury et al. [73]: response criteria were not defined.

† Ruxolitinib treatment duration.

‡ Median follow-up, 401 days.

§ Median treatment duration of 200- or 300-mg daily itacitinib.

¶ Fifteen of 19 patients were reported as severe per National Institutes of Health scoring; severity of the remaining 4 patients was not reported.

Responses in individual tissues were reported separately; in 7 different tissues across all 3 patients with cGVHD, PR = 3 and CR = 4.

reductions in the severity of aGVHD and cGVHD and prolonged survival [7,23-25], without interfering with beneficial GVL activity [24,25]. Based on the mechanism of action of JAK inhibitors, prophylactic use will need to be explored to optimally impact both acute and chronic GVHD after alloHCT. Given that aGVHD is a risk factor for cGVHD [5], prophylaxis against aGVHD, including protection against subclinical immune organ damage, may greatly reduce the incidence or severity of cGVHD. As clinical exploration of JAK inhibitors in the GVHD setting continues, it will be important to evaluate outcomes in different patient subgroups stratified by treatment history and affected tissues in an effort to identify patients who are most likely to benefit from JAK-targeted therapy.

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