

Jak Inhibition Prevents Bleomycin-Induced Fibrosis in Mice and Is Effective in Patients with Morphea



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TO THE EDITOR

Morphea is an autoimmune disease that causes fibrosis of the skin that may result in significant morbidity. Eosinophilic fasciitis (EF) may be considered a deeper form of morphea, and features of both morphea and EF can be present in individual patients. Morphea and EF have been postulated to have pathogenic features that overlap with systemic sclerosis (Laxer and Zulian, 2006). In these diseases, dysregulated immune responses are thought to induce fibrosis (Torok et al., 2019). Treatment of these diseases with nonspecific immunosuppressants such as corticosteroids is often ineffective and associated with toxicity.

IL-4/IL-13, IL-12, and IFN- γ have been implicated in autoimmune fibrosis (Torok et al., 2019). These cytokines signal via the janus kinase–signal transducer and activator of transcription (Jak-STAT) pathway, suggesting inhibition of this pathway might be therapeutic in this group of diseases. Additionally, transforming growth factor- β , a cytokine with well-characterized profibrotic effects, has been reported to activate Jak-STAT in the context of fibrosis (Chakraborty et al., 2017). *STAT4* and *IRF5* polymorphisms are associated with scleroderma (Tsou and Sawalha, 2017), further implicating Jak-STAT signaling.

We previously reported successful treatment of two patients, one with generalized morphea and one with EF, with the Jak inhibitor tofacitinib (Kim et al., 2018). Here, four additional patients with generalized morphea (totaling six consecutive patients) were treated with tofacitinib (inhibits Jak1/3>2) ($n = 3$) or baricitinib (inhibits Jak1/2) ($n = 1$), and long-term follow-up of the previously reported patients is presented. Verbal informed consent for

this off-label treatment with a Jak inhibitor was obtained. These six patients had previously failed multiple therapies typically used for severe morphea, including methotrexate, prednisone, psoralen plus UVA, and extracorporeal photopheresis (Table 1). In some patients, Jak inhibitors were initially used in combination with either prednisone or methotrexate and/or extracorporeal photopheresis.

All six patients treated with Jak inhibitors experienced improvement in signs/symptoms of disease, such as erythema, induration, range-of-motion, and strength. The time to initial response ranged from 1 to 3 months, and the time to meaningful clinical improvement ranged from 5 to 9 months. Patients continued to improve up to 30 months after initiation of Jak inhibitor treatment (Table 1) (Figure 1a and c). Objective improvement in morphea/EF was observed using a morphea severity scale (Cunningham et al., 1998) (Supplementary Material). All patients consented to publication of their clinical images.

Patient 1 had resolution of the prayer sign with tofacitinib treatment, and both patients 1 and 2 had been intermittently requiring a cane for ambulation because of restricted range-of-motion involving the lower extremities (Kim et al., 2018), and the latter resolved in both patients with tofacitinib treatment. Patient 4 had ulcerative morphea and had complete resolution of painful ulcerations with tofacitinib (Figure 1a); stopping tofacitinib led to recurrence of painful ulcerations (Supplementary Figure S1) and restarting it again led to improvement. Patient 5 is the only patient treated with baricitinib; resolution of erythema was noted after 2 months of treatment, and subjective improvement in mobility

was reported after 6 months. Patients 1 and 3 have been able to discontinue treatment without disease recurrence (Table 1), patient 2 is stably improved on low-dose tofacitinib, and the other three patients remain stably controlled on either tofacitinib or baricitinib monotherapy.

To better understand the activity of Jak inhibition, we used a bleomycin-induced murine model of fibrosis with features of human scleroderma/morphea, including a similar inflammatory infiltrate, increase in collagen production, and thickening of the dermis (Yamamoto et al., 2000b). In this model, mice received injections of bleomycin into flank skin for 12 days to induce a morphea-like phenotype (Supplementary Material) characterized by skin thickening and increased collagen production (Figure 1b). Mice were treated with either tofacitinib (30 mg/kg/day) or ruxolitinib (10 mg/kg/day). Mice treated with these inhibitors were protected from dermal thickening (Figure 1b and e). The effect was more apparent in female mice than male mice (Supplementary Figure S2).

Given the beneficial response to Jak inhibition observed in both humans and mice, we evaluated patterns of Jak-STAT activation in fibrotic tissue using immunohistochemistry for phosphorylated (activated) STAT1 and STAT3. An archival series of seven biopsies from different patients with morphea was used (Supplementary Table S1). In all human morphea cases, STAT1 was phosphorylated in lymphocytes and occasionally spindled fibroblasts, and STAT3 was phosphorylated in both lymphocytes and spindled fibroblasts (Figure 1d and g). In murine bleomycin-treated skin, negligible STAT1 phosphorylation was observed; however, STAT3 phosphorylation was common in spindled cells and was reduced with Jak inhibitor treatment (Figure 1f and i).

In patients with scleroderma/morphea, it has been shown that *COL1A1* production is increased through both

Abbreviations: EF, eosinophilic fasciitis; Jak, janus kinase; STAT, signal transducer and activator of transcription

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Table 1. Patients with Morphea and EF Treated with Tofacitinib

Age (y) /Sex	Diagnosis	Skin biopsy findings	Duration of disease before Jak inhibitor	Therapies utilized before Jak inhibitor	Jak inhibitor and dose	Time to: initial response (1), significant clinical improvement (2), maximal response (3)	Therapies weaned off while on Jak inhibitor	Total time on Jak inhibitor	Adverse effects
69, female	Eosinophilic fasciitis	Dermal fibrosis, fascial thickening	12 months	Prednisone	Tofacitinib 5 mg b.i.d	1 month (1) 9 months (2) 26 months (3)	Prednisone	30 months	None
59, female	Generalized deep morphea (postradiation)	Dermal fibrosis	10 months	Prednisone ECP	Tofacitinib 5–10 mg b.i.d	1 month (1) 7 months (2) 16 months (3)	Prednisone ECP Methotrexate	40 months	None
69, female	Generalized morphea	Dermal fibrosis	18 months	Prednisone Methotrexate	Tofacitinib 5 mg b.i.d	2 months (1) 5 months (2) 11 months (3)	Methotrexate	16 months	None
84, female	Generalized morphea with ulceration	Dermal fibrosis	40 months	Prednisone Methotrexate ILT	Tofacitinib 5 mg b.i.d	1 month (1) 7 months (2) 11 months (3)	Methotrexate	17 months	None
54, male	Generalized morphea	Dermal fibrosis	30 months	Prednisone PUVA ECP	Baricitinib 2 mg daily	2 months (1) 6 months (2) NA (3)	ECP	12 months	None
73, female	Generalized morphea	Dermal fibrosis	48 months	Methotrexate Mycophenolate mofetil	Tofacitinib 5–10 mg b.i.d	3 months (1) 4 months (2) 4 months (3)	N/A	10 months	None

Abbreviations: ECP, extracorporeal photopheresis; EF, eosinophilic fasciitis; ILT, intralesional triamcinolone; PUVA, psoralen and UVA phototherapy. Previously published (Kim et al., 2018) with updated follow-up data provided herein.

transcriptional and translational mechanisms and is thought to play a role in fibrosis. Given both the improvement in fibrosis and induration in our patients with morphea with Jak inhibitor treatment and, in murine bleomycin-treated skin, the reduction in STAT activation in fibroblasts during Jak inhibitor treatment, we wondered whether Jak inhibitors might directly inhibit excess collagen production by fibroblasts. Consistent with prior reports (Yamamoto et al., 2000a), we found that normal cultured human fibroblasts upregulated *COL1A1* in response to bleomycin. We found that this effect could be prevented with Jak inhibition (Figure 1h and Supplementary Table S2). In murine bleomycin-treated skin, reduced *Col1a1* expression was also observed with Jak inhibitor treatment (Figure 1j).

In summary, Jak inhibition leads to decreased inflammation in patients with recalcitrant morphea and EF; in some patients, even reversal of fibrosis is observed. We found that both tofacitinib and baricitinib were effective. Our observations in morphea and EF are consistent with what has been reported in patients with chronic graft-versus-host-disease (which can have fibrotic manifestations in the skin) (Zeiser et al., 2015) treated with ruxolitinib and in a single patient with systemic sclerosis treated with tofacitinib (Deverapalli and Rosmarin, 2018). We also demonstrate that Jak inhibition prevents fibrosis in a bleomycin-induced mouse model of morphea and directly decreases collagen production by fibroblasts. Our murine data suggest that ruxolitinib may have slightly more potent antifibrotic effects than tofacitinib. Wang et al. (2019) recently reported that tofacitinib can decrease bleomycin-induced fibrosis in mice but did not evaluate the effects of ruxolitinib, nor did they evaluate efficacy in patients. These data suggest that Jak inhibitors are targeted treatment for morphea and EF and highlight the need for additional study of Jak inhibitors for the treatment of fibrosing disorders in general.

Data availability statement

No new large-scale datasets were created as part of this work.

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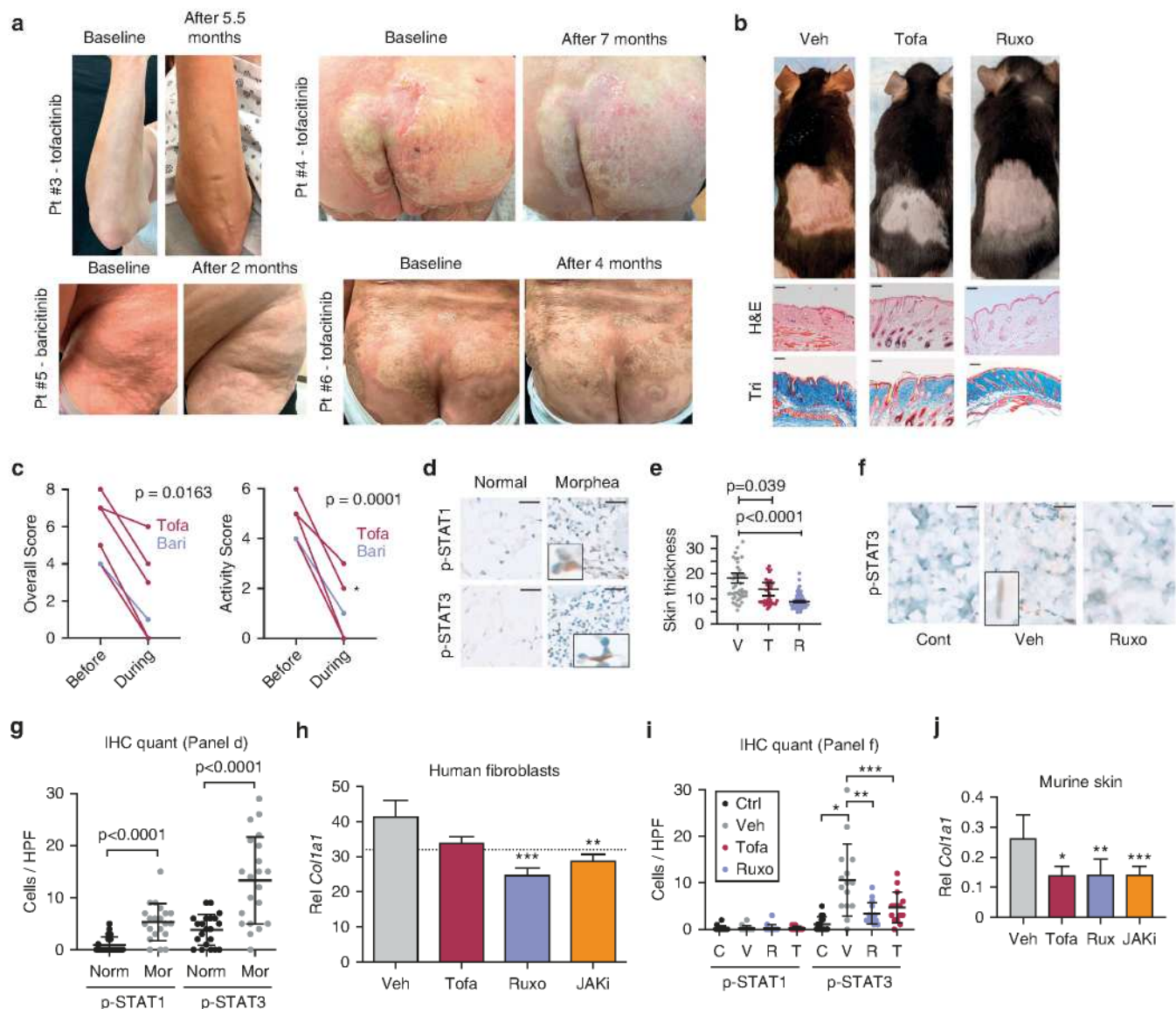


Figure 1. Jak inhibition leads to improvement in patients with morphea and prevents bleomycin-induced fibrosis in mice. (a) Patients treated with baricitinib (lower left) or tofacitinib. (b) Gross, H&E, and Tri stained sections. Bar = 50 μ m. (c) Morphea severity scores. *Indicates that two patients had identical scores. (d) Phospho-STAT1/3 staining in morphea. Bar = 25 μ m. (e) Skin thickness (mean $\pm$ SEM) in bleomycin-treated mice. V: vehicle, T: tofacitinib, R: ruxolitinib. (f) Normal (Cont) and bleomycin-treated murine skin stained with phospho-STAT3. Bar = 25 μ m. (g) Quantification of (d). (h) *Col1a1* expression in human fibroblasts treated with bleomycin $\pm$ Jak inhibitors (10 μ M), normalized to *HPRT1* expression. Dotted line: baseline *COL1A1*. $***P = 0.004$ (vs Veh), $**P = 0.038$ (vs Veh). (i) Quantification of (f). (j) *Col1a1* expression (mean $\pm$ SEM) in skin from bleomycin-treated mice, normalized to *Actb*. $*P = 0.07$ vs Veh, $**P = 0.11$ vs Veh, $***P = 0.04$ vs Veh. All patients consented to publication of their clinical images. H&E, hematoxylin and eosin; JAKi, Jak inhibitor; phospho-, phosphorylated; Ruxo, ruxolitinib; SEM, standard error of the mean; STAT, signal transducer and activator of transcription; Tri, trichrome; Tofa, tofacitinib; Veh, vehicle.

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CONFLICT OF INTEREST

WD has research funding from Pfizer, the manufacturer of tofacitinib; however, this was not used to support this work, which was initiated before the research funding. WD is also a consultant for Eli Lilly. BK is an investigator for Concert Pharmaceuticals Inc, Eli Lilly and Company, and Pfizer Inc; he is a consultant to and/or has served on advisory boards for Aclaris Therapeutics, Arena Pharmaceuticals, Concert Pharmaceuticals Inc, Dermavant Sciences, Eli Lilly and Company, and Pfizer Inc; and he is on speakers' bureau for Pfizer Inc, Regeneron, and Sanofi Genzyme. JMR and JEH are inventors on patent application #62489191, "Diagnosis and Treatment of Vitiligo," which covers targeting IL-15 and Trm for the

treatment of vitiligo. JEH is scientific founder of Villar Therapeutics, Inc, which is focused on developing treatments for vitiligo. JMR and JEH are inventors on patent application #15/851,651, "Anti-human CXCR3 antibodies for the Treatment of Vitiligo," which covers targeting CXCR3 for the treatment of vitiligo.

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Career Development Awards from the Dermatology Foundation (to JMR and WD), Target Identification in Lupus Award from the Lupus Research Alliance (to JMR), NIH awards AR09114 and AR07302 (to JEH), and the Ranjini and Ajay Poddar Resource Fund for Dermatologic Diseases Research (to BK). Microscopy equipment used for this study is maintained by the Morphology Core Facility, and tissue sectioning and pathology services are maintained by the University of Massachusetts Medical School Diabetes and Endocrinology Research Center Morphology Core. Experiments involving animals were approved by the University of Massachusetts Medical School Institutional Animal Care and Use Committee. Use of human materials was approved by the University of Massachusetts Medical School Institutional Review Board. For mouse model inquiries, correspondence should be addressed to: Dr. Jillian Richmond, UMass Medical School 364 Plantation St LRB 270E Worcester, MA 01605, jillian.richmond@umassmed.edu, @TCCellTracker.

AUTHOR CONTRIBUTIONS

Conceptualization: WD, JR, BK; Data Curation: WD, JR, BK; Formal Analysis: WD, JR, BK; Funding Acquisition: JH, JR, BK; Investigation: WD, DP, CG, MG, AW, KD, AD, JH, JR, BK; Resources: KD, AD, JH; Supervision: JR, BK; Writing - Original Draft Preparation: WD, JR, BK.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at <https://doi.org/10.1016/j.jid.2019.12.019>.

REFERENCES

- Chakraborty D, Šumová B, Mallano T, Chen CW, Distler A, Bergmann C, et al. Activation of STAT3 integrates common profibrotic pathways to promote fibroblast activation and tissue fibrosis. *Nat Commun* 2017;8:1130.
- Cunningham BB, Landells ID, Langman C, Sailer DE, Paller AS. Topical calcipotriene for morphea/linear scleroderma. *J Am Acad Dermatol* 1998;39:211–5.
- Deverapalli SC, Rosmarin D. The use of JAK inhibitors in the treatment of progressive systemic sclerosis. *J Eur Acad Dermatol Venereol* 2018;32:e328.
- Kim SR, Charos A, Damsky W, Heald P, Girardi M, King BA. Treatment of generalized deep morphea and eosinophilic fasciitis with the Janus kinase inhibitor tofacitinib. *JAAD Case Rep* 2018;4:443–5.
- Laxer RM, Zulian F. Localized scleroderma. *Curr Opin Rheumatol* 2006;18:606–13.
- Torok KS, Li SC, Jacobs HM, Taber SF, Stevens AM, Zulian F, et al. Immunopathogenesis of pediatric localized scleroderma. *Front Immunol* 2019;10:908.
- Tsou PS, Sawalha AH. Unfolding the pathogenesis of scleroderma through genomics and epigenomics. *J Autoimmun* 2017;83:73–94.
- Wang W, Bhattacharyya S, Marangoni RG, Carns M, Dennis-Aren K, Yeldandi A, et al. The JAK/STAT pathway is activated in systemic sclerosis and is effectively targeted by tofacitinib. *J Scleroderma Relat Disord* 2020;5:40–50.
- Yamamoto T, Eckes B, Krieg T. Bleomycin increases steady-state levels of type I collagen, fibronectin and decorin mRNAs in human skin fibroblasts. *Arch Dermatol Res* 2000a;292:556–61.
- Yamamoto T, Kuroda M, Nishioka K. Animal model of sclerotic skin. III: histopathological comparison of bleomycin-induced scleroderma in various mice strains. *Arch Dermatol Res* 2000b;292:535–41.
- Zeiser R, Burchert A, Lengerke C, Verbeek M, Maas-Bauer K, Metzelder SK, et al. Ruxolitinib in corticosteroid-refractory graft-versus-host disease after allogeneic stem cell transplantation: a multicenter survey. *Leukemia* 2015;29:2062–8.

Albinism: An Underdiagnosed Condition

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TO THE EDITOR

Albinism is a clinically and genetically heterogeneous condition characterized by variable degrees of hypopigmentation extending from a complete absence of pigmentation to normal pigmentation and by ophthalmological anomalies, including nystagmus, foveal hypoplasia, chiasmic misrouting of the optic nerves, iris transillumination, retinal hypopigmentation, and reduced visual acuity. Nineteen genes are involved in the oculocutaneous, ocular, and syndromic (Hermansky-Pudlak Syndrome and Chediak-Higashi Syndrome) forms of the disease and in the more recently characterized foveal hypoplasia, optic nerve decussation defects and anterior

segment dysgenesis (Arveiler et al., 2017; Montoliu et al., 2014).

The molecular diagnosis of albinism relies on the systematic analysis of the 19 genes in order to search for single nucleotide variants or gene rearrangements (Lasseaux et al., 2018).

The wide phenotypic heterogeneity observed in patients impedes the establishment of phenotype-genotype correlations (Table 1). Strikingly, on the one hand, patients with variants in a given gene can have very different clinical presentations, and on the other hand, a similar phenotype can be owing to variants in different genes. This is observed in nonsyndromic, as well as in syndromic forms of the disease. Hence, for example, a patient with a clinically

classified “ocular” form may in fact have an oculocutaneous or a syndromic form that can remain clinically unsuspected until adulthood. In addition, ophthalmologic signs may be incomplete and/or atypical (Table 1). As a consequence, the diagnosis of albinism may not be raised, if not discarded.

Illustratively, variants of the *TYR* gene responsible for oculocutaneous albinism type 1 (OCA1) can elicit either severe forms with a complete absence of pigmentation or forms with a pigmentation that appears normal, particularly in populations with a light phototype. Among 268 patients diagnosed with OCA1 in our recent study, 122 (45.5%) were compound heterozygotes for a known pathogenic *TYR* variant and the hypomorphic variant c.1205G>A/p.Arg402Gln (hereafter called R402Q) (Lasseaux et al., 2018). The R402Q allele is common, with a worldwide prevalence of 17.7%, and 26.48% in Europe (7.01%

Abbreviation: OCA1, oculocutaneous albinism type 1

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SUPPLEMENTARY MATERIALS AND METHODS

Study subjects

The diagnosis of morphea or eosinophilic fasciitis (EF) was confirmed by skin biopsy in all patients, all of whom had been referred to our clinic for severe morphea and EF recalcitrant to standard therapies. These patients had severe, recalcitrant skin disease and were worsening with standard-of-care therapies; off-label use of Jak inhibitors was clinically indicated based on the judgment of the investigators. We discussed with all patients that tofacitinib or baricitinib were being used off-label for the treatment of their cutaneous disease. Only after verbal informed consent was obtained was Jak inhibitor therapy initiated at FDA-approved doses.

Initial response was defined as the time to any detectable clinical improvement and includes subjective (patient history) and/or objective (physical examination) determinations. Maximal response was defined as the time required to reach the best effect (based on objective physical exam determination such as improvements in erythema and induration). Maximal responses were sustained on the medication.

Historical morphea biopsies used for immunohistochemistry

A historical series of biopsies from patients with morphea was assembled by searching the University of Massachusetts Medical School (UMMS) database. Research involving human biopsies was approved by the UMMS Institutional Review Board (Protocol 14848). The biopsies included in the immunohistochemical analysis are described in [Supplementary Table S1](#). Normal skin samples included tips of ellipses from skin cancer excisions and mastoplasty and abdominoplasty specimens.

Mice

All mice were housed in pathogen-free facilities at UMMS and procedures were approved by the UMMS Institutional Animal Care and Use Committee (Protocols 2266 and 2657) and in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Mice used for these studies were on the C57BL/6J (B6)

background or a mixed 129 × C57BL/6 background that had been backcrossed to B6 for more than 10 generations. Age- and sex-matched mice were used, and both male and female mice of all strains were tested to avoid gender bias; sex-specific effects are reported in [Supplementary Figure S2](#). Replicate experiments were performed two to five times.

Bleomycin-induced mouse model of localized cutaneous fibrosis

Bleomycin (Sigma-Aldrich, St. Louis, MO) solution was prepared by dissolving bleomycin in phosphate buffered saline at 1 mg/ml. Two locations on the dorsal flank were shaved and depilated for subcutaneous injections. Mice received injections of 50 μ l of the bleomycin treatment solution or phosphate buffered saline vehicle at each site for a total of 100 μ g/mouse for 12 days. At the end of the experiment, all the mice were killed and half of each site was frozen for collagen and RNA analysis, and the other half was prepared for histological analysis.

Jak inhibitor treatment in mice

Each mouse was pretreated with 30 mg/kg tofacitinib ([Okiyama et al., 2014](#)) in 200 μ l of vehicle (ultrapure water with 0.5% methyl cellulose and 0.025% Tween 20) or vehicle alone via daily gavage for 5 days before use in the morphea model. Similarly, for experiments involving ruxolitinib, mice were pretreated with 10 mg/kg ruxolitinib ([Shuey et al., 2016](#)) in 200 μ l vehicle or vehicle alone via daily gavage for 5 days before use in morphea model. Treatments were continued throughout the model, and mice were killed on day 12 for analysis.

Histological analysis of fibrosis in mice

The skin from the injection sites was fixed using 4% paraformaldehyde before paraffin embedding at the UMMS Diabetes and Endocrinology Research Center core facility. Samples were sectioned and stained with trichrome to visualize collagen deposition or hematoxylin and eosin to visualize inflammatory infiltrates. Images were taken with an Olympus BX51 microscope (Olympus, New Haven, CT) with $\times 10$ and $\times 40$ objective lenses, and epidermal and dermal

thicknesses were measured with ImageJ software. The ratio of the dermal to epidermal thickness was calculated for each skin sample to control for variances in skin sampling.

Immunohistochemical analysis of Jak—signal transducer and activator of transcription (STAT) pathway activation

Immunohistochemistry was performed using heat mediated antigen retrieval with pH 6.0 citrate buffer. Phosphorylated STAT1 Tyr701 and phosphorylated STAT3 Tyr705 antibodies (Cell Signaling Technology, Danvers, MA) were incubated on the tissue according to the manufacturer's instructions. Signal was detected using anti-rabbit horseradish peroxidase—conjugated secondary antibody (Cell Signaling Technology) and a DAB chromogen (Vector Labs, Burlingame, CA). Slides were counterstained with hematoxylin. For quantification of mouse immunohistochemistry, 5 biologic replicates were analyzed for each phosphorylated STAT1 and phosphorylated STAT3. Three high power fields of dermis were selected at random and the number of positive cells was quantified (absolute number of cells per high power field). For quantification of human immunohistochemistry, 7 morphea biopsies were analyzed. Five high power fields of dermis were analyzed and the number of positive cells was quantified (absolute number of cells per high power field).

Mouse fibroblast isolation, human fibroblast culture, and *in vitro* treatments

Dermal tissue was isolated from eight-week-old (or older) mice which were age- and sex-matched as previously described. Briefly, epidermis was removed by incubating skin with 5 (tail) or 50 (ear and back) mg/ml Dispase II enzyme (Roche, Basel, Switzerland) 1 hour at 37 °C or overnight at 4 °C. Dermis tissue was digested for 45 minutes to 1 hour using 1 mg/ml collagenase IV and 2 mg/ml DNase I (Sigma-Aldrich), and tissue was mechanically dissociated using the blunt end of a syringe. Samples were filtered through 70- μ m mesh and were centrifuged at 330g for 10 minutes in FACS buffer (1% fetal bovine serum in

phosphate buffered saline) containing 1 mg/ml DNase I.

For cell sorting of fibroblasts, dermis pellets were stained with anti-CD45 APC and anti-CD31 PE-Cy7, and all double negative singlets were collected by cell sorting with a FACS Aria. These cells, essentially all four phenotypes of mouse skin fibroblasts, were cultured in DMEM containing 10% fetal bovine serum and 1X penicillin/streptomycin for 5–7 days with one split. Cells were grown to 80% confluency and were used in collagen production assays.

Human fibroblasts were obtained from a patient panniculectomy specimen and were isolated using 50 mg/ml Dispase II enzyme (Roche) overnight at 4 °C, and epidermis was removed with a razor blade. Dermis tissue was digested for 45 minutes to 1 hour using 1 mg/ml collagenase IV and 2 mg/ml DNase I (Sigma-Aldrich), and tissue was mechanically dissociated using the blunt end of a syringe. Sample was filtered through 70-µm mesh and centrifuged at 330g for 10 minutes in FACS buffer (1% fetal bovine serum in phosphate buffered saline) containing 1 mg/ml DNase I. The resulting cell suspension was plated and passaged three times using 10% fetal bovine serum and 1% penicillin/streptomycin in DMEM

(cDMEM, Sigma-Aldrich) so that only fibroblasts remained in culture. Two additional human donor fibroblast specimens were obtained from ATCC (cat# PCS-201-012 lot# 80124171 and #70023966) and were cultured in cDMEM. Cells were grown to 80% confluency and were used in collagen production assays.

For collagen mRNA assays, 1.8 µg/ml bleomycin (Sigma-Aldrich) (Nicolay et al., 2016) with 10 µM ruxolitinib or tofacitinib (LC Laboratories, Woburn, MA) (Heine et al., 2013) or vehicle control (water) were added to wild-type fibroblasts for 3–4 hours, and cells were harvested by trypsinization and centrifugation. Pellets were snap frozen and were assessed RNA analysis as described subsequently.

Quantitative PCR

RNA was isolated from mouse back skin or fibroblast cell pellets using RNeasy kits (Qiagen, Hilden, Germany). cDNA was prepared using iScript kits (Bio-Rad, Hercules, CA), and quantitative PCR was performed with SYBR green kits (Bio-Rad).

Statistics

All statistical analyses were performed with GraphPad Prism software. Pairwise comparisons were analyzed using an

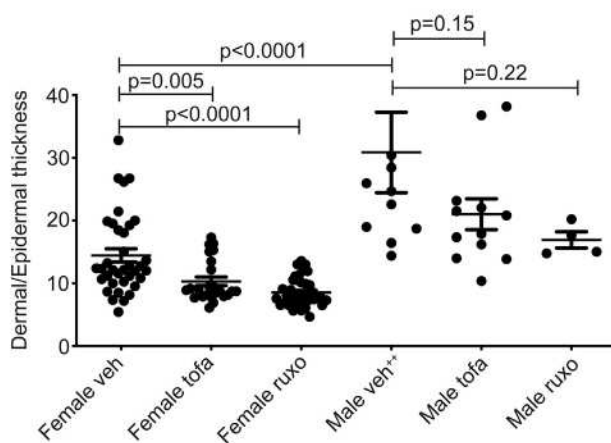
unpaired Student's *t*-test. *P*-values < 0.05 were considered significant.

SUPPLEMENTARY REFERENCES

- Groom JR, Richmond J, Murooka TT, Sorensen EW, Sung JH, Bankert K, et al. CXCR3 chemokine receptor-ligand interactions in the lymph node optimize CD4+ T helper 1 cell differentiation. *Immunity* 2012;37:1091–103.
- Heine A, Held SA, Daecke SN, Wallner S, Jayanarayana SP, Kurts C, et al. The JAK-inhibitor ruxolitinib impairs dendritic cell function in vitro and in vivo. *Blood* 2013;122:1192–202.
- Ito Y, Toriuchi N, Yoshitaka T, Ueno-Kudoh H, Sato T, Yokoyama S, et al. The Mohawk homeobox gene is a critical regulator of tendon differentiation. *Proc Natl Acad Sci USA* 2010;107:10538–42.
- Nicolay NH, Ruhle A, Perez RL, Trinh T, Sisombath S, Weber KJ, et al. Mesenchymal stem cells are sensitive to bleomycin treatment. *Sci Rep* 2016;6:26645.
- Okuyama N, Furumoto Y, Villarroel VA, Linton JT, Tsai WL, Gutermuth J, et al. Reversal of CD8 T-cell-mediated mucocutaneous graft-versus-host-like disease by the JAK inhibitor tofacitinib. *J Invest Dermatol* 2014;134:992–1000.
- Rashighi M, Agarwal P, Richmond JM, Harris TH, Dresser K, Su MW, et al. CXCL10 is critical for the progression and maintenance of depigmentation in a mouse model of vitiligo. *Sci Transl Med* 2014;6:223ra23.
- Shuey DL, Oliver J, Zhou G, Roberts A. Results from oral gavage carcinogenicity studies of ruxolitinib in Tg.rasH2 mice and Sprague-Dawley (CrI:CD) rats. *Regul Toxicol Pharmacol* 2016;81:305–15.



Supplementary Figure S1. Patient 2 had relapse of morphea with recurrence of ulcerative lesions after discontinuation of tofacitinib.



Supplementary Figure S2. Sex-specific effects of bleomycin and JAKi treatment in mice. Two outlier values are outside the axis of the data displayed for male vehicle (87.2 and 51.7). JAKi, Jak inhibitor; Ruxo, ruxolitinib; Tofa, tofacitinib; Veh, vehicle.

Supplementary Table S1. Histologic Case Series of Other Patients with Morphea

Case Number	Age	Sex	Anatomic site	Diagnosis based on clinicopathologic correlation
1	49	F	Left breast	Morphea
2	37	F	Right abdomen	Morphea
3	44	F	Left breast	Morphea
4	46	F	Back	Morphea
5	24	F	Left upper arm	Morphea
6	66	F	Right upper abdomen	Morphea
7	23	F	Left upper arm	Morphea

Abbreviation: F, female.

Supplementary Table S2. Primers Used for PCR

Primer name	Sequence
Mouse primers	
<i>Col1a1</i> -F	GAGCGGAGAGTACTGGATCG
<i>Col1a1</i> -R	GCTTCTTTTCCTTGGGGTTC (Ito et al., 2010)
B-actin-F	GGCTGTATCCCTCCATCG
B-actin-R	CCAGTTGGTAACAATGCCATGT (Rashighi et al., 2014)
<i>B2M</i> -F	CCGAACATACTGAACGTACGTAA
<i>B2M</i> -R	CCCGTTCTTCAGCATTTGGA (Groom et al., 2012)
Human primers	
<i>COL1A1</i> -F	AGACAGTGATTGAATACAAAACCA
<i>COL1A1</i> -R	GGAGTTTACAGGAAGCAGACA
<i>HPRT1</i> -F	AGGGATTTGAATCATGTTTGTG
<i>HPRT1</i> -R	GGCTTTGTATTTTGCTTTTCCA

Abbreviations: F, forward; R, reverse.