TO THE EDITOR

Vitiligo is an autoimmune skin disease presenting with distinct, patchy areas of depigmentation that can appear anywhere on the body. These white patches occur when autoreactive, cytotoxic CD8+ T cells promote the elimination of melanocytes, the pigment-producing cells of the epidermis (Frisoli et al., 2020). Vitiligo affects approximately 1% of the global population equally across races and sexes and frequently presents before the age of 20 years (Taieb and Picardo, 2009).

Currently, there are no U.S. Food and Drug Administration–approved medical treatments to promote repigmentation in vitiligo, whereas topical immunosuppressants and phototherapy are moderately effective when used off-label (Frisoli et al., 2020). Recent studies report the efficacy of the Jak inhibitors (JAKis) tofacitinib (tofa) and ruxolitinib (rux) in patients with vitiligo through case reports and case studies as well as ongoing clinical trials (Craiglow and King, 2015; Harris et al., 2016; Kim et al., 2018; Liu et al., 2017). However, studies also reported a rapid relapse of the disease after stopping both conventional treatments (Cavalié et al., 2015) and JAKis (Harris et al., 2016; Liu et al., 2017).

We and others recently reported that autoreactive resident memory T cells (Trms) form in the lesions of patients with vitiligo and are responsible for disease relapse (Boniface et al., 2018; Cheuk et al., 2017; Richmond et al., 2018). Trms function to recruit autoreactive T cells from the circulation, which participate in killing pigmenting melanocytes (Richmond et al., 2019). Treatments that inhibit Trm function but do not remove them from the skin are not durable (Riding and Harris, 2019). Therefore, we hypothesized that Trm numbers are largely unaffected by JAKi treatment on the basis of the lack of durable responses in patients.

To test our hypothesis, we used the JAKis in our mouse model of vitiligo, which was induced by the adoptive transfer of melanocyte-specific CD8+ T cells, also known as premelanosome protein-specific T cells, into recipient hosts with pigmented skin (Harris et al., 2012). We administered tofa or rux orally once daily to hosts before disease onset (prevention) or after stabilization (reversal) and measured their effect on the extent of the disease as well as T-cell populations within the epidermis and dermis by flow cytometry (see Supplementary Materials).

In the prevention model, hosts were treated daily with vehicle or JAKis from week 2 to week 7 after induction of vitiligo (Figure 1a). We observed a mild but statistically insignificant decrease in the vitiligo score in hosts treated with rux (P = 0.4255) and a larger, statistically significant decrease in vitiligo score with tofa treatment (Figure 1b and c; n = 36 vehicle, 21 tofa, and 13 rux mice; P = 0.0037). Whether this indicates that rux will be less effective at controlling active disease in humans is not clear but should be monitored in clinical studies.

To understand how the JAKis were impacting the disease, we examined the number of immune cells in the skin using flow cytometry. We found that hosts treated with either JAKis had fewer, although not statistically significant, premelanosome protein-specific T cells in the epidermis (Figure 1d; tofa vs. vehicle, P = 0.1076; rux vs. vehicle, P = 0.2779). Rux, but not tofa, appeared to reduce premelanosome protein-specific T cell in the dermis as well (Figure 1e; tofa vs. vehicle, P = 0.5861; rux vs. vehicle, P = 0.0655).

In the reversal model, we treated hosts 12 weeks after induction with JAKis for 8 more weeks (Figure 2a). Both tofa and rux significantly reversed disease compared with vehicle controls (Figure 2b and c; P = 0.0010 and P = 0.0071, respectively, n = 13 vehicle, 14 tofa, and 8 rux). Interestingly, in contrast to the prevention model, the JAKis did not decrease the overall number of premelanosome protein-specific T cells in the epidermis (Figure 2d, P = 0.2032 and P = 0.3958) or dermis (Figure 2e, P = 0.1539 and P = 0.5399) or specifically the Trm subpopulation, marked by the expression of CD103 and CD69 (Figure 2f, P = 0.1189 and P = 0.228). Thus, Trm populations remained in the skin during the reversal, even after significant repigmentation was observed.

Taken together, our data suggest that JAKis may prevent the accumulation of T cells in the skin during disease progression but do not affect T cell numbers once established. The observed efficacy of treatments to promote reversal of disease may instead be through inhibition of T cell function within the skin rather than by decreasing their numbers. Both tofa and rux inhibit signaling through IFN-γ (Damsky and King, 2017), a cytokine produced by Trms and required for the recruitment of cytotoxic cells from circulation (Richmond et al., 2019). Although IFN-γ signaling through Jak3 appears to be necessary for depigmentation, it is dispensable for Trm maintenance in the skin, which supports clinical observations that Jak inhibition does not result in durable treatment responses.

Data availability statement
Primary data are available upon request.

Ethics Statement
All procedures were approved by the University of Massachusetts Medical School Institutional Animal Care and Use Committee in accordance with the...
Figure 1. JAKis reduce vitiligo and prevent epidermal accumulation of autoreactive T cells in mice. (a) Prevention experiments were completed according to the schedule above. (b) Sample photographs from mouse tails after 5 weeks of treatment. (c) Clinical scores of vitiligo in vehicle, tofa-treated, and rux-treated mice. Total number of PMEL in the (d) epidermis and (e) dermis by flow cytometry (each dot represents one animal; n = 36 vehicle, 21 tofa, and 13 rux pooled from at least three separate experiments). JAKi, Jak inhibitor; PMEL, premelanosome protein-specific T cell; rux, ruxolitinib; tofa, tofacitinib.
References


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.2020.04.027.

Figure 2. JAKis reverse depigmentation in vitiligo but do not clear Trm in the skin. (a) Reversal experiments were completed according to the schedule above. (b) Photographs of mouse tails at the beginning and end of treatment after 8 weeks. (c) Change in tail pigment was calculated using before and after photos of each mouse. PMEL count within the (d) epidermis and (e) dermis was assessed at the end of the treatment (each dot represents one animal; n = 13 vehicle, 14 tofa, and 8 rux). (f) Trms were stained in the epidermis and quantified after treatment (n = 6 vehicle, 8 tofa, and 8 rux pooled from two separate experiments). JAKi, Jak inhibitor; PMEL, premelanosome protein-specific T cell; rux, ruxolitinib; tofa, tofacitinib; Trm, resident memory T cell.
SUPPLEMENTARY MATERIALS AND METHODS

Mice

KRT14-Ki67*4XTG2BjL (Krt14-Ki67*) mice originated from B.J. Longley, University of Wisconsin, Madison, WI. B6.129-Cg-Thy1^+Cy Tg (TcraTcrb) 8Rest/J (premelanosome protein-specific T cell TCR transgenic) mice are Thy1.1+ and were purchased from The Jackson Laboratory (Bar Harbor, ME), stock number 005023. All mice were on a C57BL/6j background and were housed in pathogen-free facilities at the University of Massachusetts Medical School, Worcester, MA. All procedures were approved by the University of Massachusetts Medical School Institutional Animal Care and Use Committee in accordance with the National Institute of Health Guide for the care and use of laboratory animals.

Flow cytometry

For each experiment, tail skin was harvested as previously described (Rashighi et al. 2014; Richmond et al. 2018, 2017a, 2017b). Briefly, tail skin samples were incubated with 5 U/ml Dispase II (Roche, Basel, Switzerland) in 1 ml of PBS for 1 hour at 37 °C. The epidermis was removed and crushed over 70 μm filters with syringe plungers. The dermis was digested with 1 mg/kg collagenase IV and 2 mg/ml deoxyribonuclease I (Sigma-Aldrich, St. Louis, MI) in 1 ml RPMI for 1 hour at 37 °C. Skin cells were filtered through 70 μm filters using 1 ml of 1 mg/ml deoxyribonuclease I in FACS-staining buffer. Samples were stained at 4 °C in the dark using the following antibodies at specified dilutions: anti-mouse CD45 (30-F11), CD3 (17A2 and 145-2C11), CD8b (YTS156.7.7), Thy1.1 OX-7 (1:200, Biolegend, San Diego, CA), L/D DAPI (1:1,000, Invitrogen, Waltham, MA). Data were collected using a BD LSR II (BD Bioscience, Franklin Lakes, NJ) and analyzed with FlowJo software v. 10.

Jak inhibitors formulation

Tofacitinib was purchased from Selleckchem, Houston, TX (catalog #S5001) and LC Laboratories, Woburn, MA (catalog #T1399). Ruxolitinib was purchased from LC Laboratories (catalog #R-6688). Jak inhibitors were dissolved in vehicle of 0.5% methylcellulose, 2% tween 80 in deionized water. All compounds were stored according to the manufacturers’ specifications.

Vitiligo induction and scoring

Vitiligo was induced as previously described (Harris et al. 2012; Riding et al. 2018). Krt14-Ki67* mice were irradiated with 500 rads 24 hours before adoptive retro-orbital intravenous transfer of 1 × 10^6 premelanosome protein-specific T cell CD8^+ T cells and intraperitoneal injection of an attenuated 1 × 10^6 plaque-forming unit of recombinant vaccinia virus that expresses gp100. Vitiligo scores were calculated on a point scale from 0 to 5 (Figure 2, Riding et al., 2019) for four distinct skin locations (including the tail, rear footpads, ears, and nose). A blinded investigator scored the mice at week 7.

Prevention studies

Mice were gavaged once daily with tofacitinib (30 mg/kg), ruxolitinib (50 mg/kg), or vehicle in volumes of 100 μl per host for 35 days beginning 2 weeks after induction with vitiligo. On week 7 of the experiment, the disease severity was quantified by determining their vitiligo score. Mice were killed for harvesting of tail skin including dermis and epidermis as described above for sample processing.

Repigmentation studies

A total of 10–12 weeks after vitiligo induction, mice with at least 75% depigmentation on the tail (score of four or five) were assigned to vehicle or treatment groups so that each group had equal severity of vitiligo. Mice were photographed before receiving treatment to document baseline tail pigmentation. Mice were gavaged once daily with tofacitinib (30 mg/kg), ruxolitinib (50 mg/kg), or vehicle for 8 weeks. ImageJ software (National Institute of Health) was used to calculate the total percentage of pigmented tail skin as previously described (Agarwal et al. 2015; Rashighi et al. 2014; Richmond et al. 2019, 2018, 2017b; Riding et al. 2018). The highlighted pixels were quantified and used to calculate the average level of pigmentation for each tail at both the beginning and end of the experiment, and the overall change in percent pigmentation was calculated by subtracting initial pigmentation from the final pigmentation as previously described (Agarwal et al. 2015).

Statistical analysis

All statistical analyses were performed using GraphPad Prism. Dual comparisons for before—after were analyzed with paired Student’s t-test. P-values < 0.05 were considered statistically significant.

SUPPLEMENTARY REFERENCES


