An Open-Label Pilot Study to Evaluate the Efficacy of Tofacitinib in Moderate to Severe Patch-Type Alopecia Areata, Totalis, and Universalis

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Alopecia areata (AA) is a common autoimmune disease with a lifetime risk of ~2%. In AA, the immune system targets the hair follicle, resulting in clinical hair loss. The prognosis of AA is unpredictable, and currently there is no definitive treatment. Our previous whole genome expression studies identified active immune circuits in AA lesions, including common γ-chain cytokine and IFN pathways. Because these pathways are mediated through JAK kinases, we prioritized clinical exploration of small molecule JAK inhibitors. In preclinical trials in mice, tofacitinib successfully prevented AA development and reversed established disease. In our tofacitinib trial in 12 patients with moderate to severe AA, 11 patients completed a full course of treatment with minimal adverse events. Following limited response to the initial dose (5 mg b.i.d.), the dose was escalated (10 mg b.i.d.) for nonresponding subjects. Eight of 12 patients demonstrated ≥50% hair regrowth, while three patients demonstrated <50% hair regrowth, as measured by Severity in Alopecia Tool scoring. One patient demonstrated no regrowth. Gene expression profiles and Alopecia Areata Disease Activity Index scores correlated with clinical response. Our open-label studies of ruxolitinib and tofacitinib have shown dramatic clinical responses in moderate to severe AA, providing strong rationale for larger clinical trials using JAK inhibitors in AA. ClinicalTrials.gov ID NCT02299297.


INTRODUCTION
Alopecia areata (AA) is a common autoimmune disease with a lifetime risk of approximately 2%, affecting an estimated 5.3 million individuals in the United States (McMichael et al., 2007; Safavi et al., 1995). Persistent moderate to severe AA causes significant disfigurement and psychological distress to affected individuals (Colon et al., 1991). Clinical development of innovative therapies in AA has lagged far behind other autoimmune conditions.

AA results from an autoimmune attack on the hair follicles. Using comparative genomics of the transcriptional profiles of skin from both AA model mice and humans with AA, we found that cytotoxic CD8 (+) NKG2D (+) T cells are both necessary and sufficient for the induction of AA in mouse models of disease. On the basis of our preclinical findings (Xing et al., 2014), we initiated a phase 2 efficacy signal-seeking clinical trial in moderate to severe AA, assessing the clinical and immunopathological response to treatment with oral tofacitinib, a JAK1,3 inhibitor that also inhibits JAK2. Presently, tofacitinib is Food and Drug Administration-approved for the treatment of adult patients with moderate to severe rheumatoid arthritis and is under study for many other autoimmune conditions (Schwartz et al., 2016). Tofacitinib has been shown to prevent the onset of, and reverse, AA in the C3H-HeJ animal model of AA. Thus far, several studies have demonstrated clinical efficacy of oral tofacitinib in patients treated with AA (Jabbari et al., 2016) or alopecia universalis (AU) (Kennedy et al., 2016; Liu et al., 2017). In all reported cases, clinical response was achieved with minimal or no adverse events.

RESULTS
Primary Efficacy End Point
This study was an open-label, clinical trial to investigate tofacitinib 5 mg to 10 mg p.o. twice daily in the treatment of moderate to severe AA.

Eight of 12 patients met the study’s primary efficacy end point of ≥50% hair regrowth from baseline as assessed by the Severity in Alopecia Tool (SALT) at the end of treatment. The duration of treatment ranged from 6 to 18 months, at the discretion of the investigator and dependent on the individual subject’s response, as well as safety considerations. The
length of time from baseline for patients to reach the primary efficacy end point was, on average, 32 weeks, with the time period ranging from as little as 8 weeks to as much as 64 weeks (Figures 1 and 2, Table 1).

Four of the five patients with either alopecia totalis (AT) or AU achieved \( \geq 50\% \) response in hair regrowth. All patients who had reached the study’s primary efficacy end point, with the exception of one patient, failed to respond to lower doses, in some cases despite prolonged treatment, but responded with onset of regrowth within 4 weeks of initiation of the higher dose of tofacitinib, at 10 mg b.i.d.

**Secondary Efficacy Outcomes**

As secondary end points, efficacy was measured by changes in hair regrowth as a continuous variable, as determined by physical examination and Canfield photography, as well as patient and physician global evaluation scores.

**Global overall improvement in SALT score at end of treatment.** Eleven of 12 patients attained a global overall improvement in SALT score at the end of treatment, with results ranging from 12.1\% to 100\% regrowth, with an average 56.8\% regrowth. Baseline SALT scores for the 12 patients ranged from 46\% to 100\%, and at the end of treatment SALT scores ranged from 0\% to 99\%. The average baseline SALT score of 81.3\% decreased to 40.8\% at the end of treatment. Only one subject had no response to the study medication after 36 weeks of administration, having experienced a negligible decrease in SALT score, with approximately 1\% hair regrowth that consisted of 0.5- to 1-mm depigmented fine terminal hairs throughout the scalp and facial area. Vellus hair growth was not used in SALT score calculations. No patients experienced worsening of AA from baseline at the time of treatment discontinuation, with 11 patients exhibiting varying degrees of hair regrowth.

**Time to regrowth of scalp and body hair.** Regrowth was seen in responders as soon as 4 weeks after the effective dose of study medication was initiated. All 12 patients experienced between 0 and <25\% of hair regrowth by week 4, as assessed by Physician Global Assessment, a static evaluation of scalp regrowth rated as “worse,” “same,” or “improved.” The degrees to which hair regrowth presented itself at 4 weeks were highly varied and individual responses ranged from <1\% with the introduction of few fine terminal hairs to at most approximately 45\% regrowth with depigmented and pigmented, terminal and fine terminal hairs. Three patients displayed mild shedding of scalp hair while on tofacitinib, but at end of treatment, remained improved compared to baseline.

All 12 enrolled patients exhibited varying degrees of body hair regrowth. Regrowth of body hair was documented as soon as 4 weeks after effective dose of study medication was initiated. Body hair regrowth was mixed, with some patients experiencing minimal to full facial hair regrowth, including eyelashes and eyebrows. Other areas of body hair regrowth noted in patients included the arms, legs, axillary, and groin area.

**Durability of responses.** To assess the durability of responses, patients who achieved 50\% regrowth from baseline during the first 6 to 18 months were followed for an additional 6 months off treatment or until it was determined that relapse had occurred. Of the eight patients who achieved 50\% regrowth, one patient dropped out of the observation period in order to continue the medication outside of the study. Of the seven patients who were followed observationally, six patients exhibited variable hair shedding after completion of the study treatment, with two patients showing initial signs of shedding approximately 3–4 weeks after end of treatment, and four patients showing initial signs of shedding approximately 8 weeks after end of treatment. Hair shedding was initially slight, but accelerated at 4–6 months off tofacitinib. The final patient did not exhibit any hair shedding throughout the observational period (24 weeks/6 months off tofacitinib). Excellent durability of response was seen in three of the eight responders, maintaining lower SALT scores compared to baseline SALT scores at nearly 24 weeks off tofacitinib. Four patients experienced worsening of AA compared to baseline at the conclusion of the study, at nearly 24 weeks off the study medication. Shedding of body hair coincided with the timeline of scalp hair loss.

Overall, 11 of the 12 patients who were initially enrolled in this study completed the intended 24 to 72 weeks of study treatment. One patient underwent early termination of the study treatment at week 12 due to experiencing hypertensive urgency as an adverse event.

**Change in patient quality of life assessment.** Change in patient quality of life assessment was compared from baseline to selected visits during the treatment period (weeks 12 and 24). Quality of life measures were based on changes in the Dermatology Life Quality Index (Basra et al., 2008). Seven of the 12 patients experienced a decrease in their Dermatology Life Quality Index score, as measured from baseline to week 24 (Supplementary Table S1 online). The mean baseline Dermatology Life Quality Index score of 6.5 ± 5 decreased to 5.2 ± 6.7 at 3 months of treatment and later increased to 6 ± 6.9 at the end of 6 months of treatment.

**Differences in regrowth between patients with patch-type AA versus patients with AT or AU.** At the end of treatment, the five subjects who had either AT or AU had experienced hair regrowth ranging from 1.0\% to 84\%, with an average of 52.2\% regrowth. This is in comparison to subjects with moderate to severe patchy AA who, at the end of treatment, experienced hair regrowth ranging from 12.1\% to 100\%, with an average 52.1\% regrowth. Overall, patients with patch-type AA or AT or AU had, on average, very similar percentage hair regrowth at the end of study treatment. Given the small sample of these patients, it is not known if the observation of similar regrowth rates among AA, AT, and AU patients will continue to hold in future studies.

**Biomarker and Clinical Correlative Studies**

Gene expression profiling was performed on skin biopsies taken at baseline and up to 24 weeks of treatment, with additional optional biopsies performed if indicated by clinical considerations.

We applied both naı¨ ve and supervised clustering to this data set in order to assess two features: the overall molecular effect of tofacitinib treatment on patient samples and the concordant molecular response of the disease. The former was assessed by an unbiased, unsupervised differential
expression analysis, and the latter was measured by response to previously published gene expression signatures defining AA pathology. To ensure parity of the data, for this analysis we only included patient samples that had matched pretreatment (Tx-00) and 24 weeks of treatment (Tx-24) samples.

Figure 3a represents the overall molecular impact of tofacitinib treatments observed in the patient biopsies. Molecular response in this instance was defined as molecular divergence between patient samples taken at pretreatment and >24 weeks of treatment. Unsupervised clustering was able to produce a biomarker panel that robustly segregated pre- and post-treatment samples with no crossover in the clustering (Figure 3a, dendrogram). The gene list that comprises this signature is available in the Supplementary Materials and Methods online.

These molecular effects coincide with significant shifts in Alopecia Areata Disease Activity Index (ALADIN) score in most patients (Figure 3b, heatmap). In this instance, unaffected control patient biopsies were included as a reference (indicated as AAB-N-Cx). All initial patient biopsies cluster away from unaffected controls using ALADIN. Treated patient samples that cluster with the control samples indicate significant suppression of AA molecular pathology and suggest response to tofacitinib, while patient samples that remain clustered with pretreated samples suggest resistance or recalcitrance to treatment. This is more simply represented in the 3-dimensional ALADIN plot. Prior to treatment, patients start at a steady state with significant distance from healthy, unaffected samples (T0 vs. normal control). During treatment, patients that respond will shift in this space toward the normal control samples (T24). One patient (a clinical nonresponder) showed virtually no molecular shift following treatment (T0 and T24 data points are proximal to each other). The pharmacodynamic reduction in ALADIN score during treatment in the favorable responders and lack thereof in the patients in whom there was little or no clinical response, confirm the utility of the ALADIN scores as a physiological relevant dynamic biomarker that positively correlates with clinical response.

Adverse Events
In this study, an adverse event is defined as any new untoward medical occurrence or worsening of a pre-existing medical condition in a patient or clinical investigation subject administered an investigational (medicinal) product and may or may not have a causal relationship with this

Figure 1. Clinical progression of therapeutic response to tofacitinib and relapse after discontinuation. (a) Relapse after treatment was stopped on week 24. (b) Relapse after treatment was stopped on week 50.
patients remained on 5 mg b.i.d. for 4 to 6 months prior to respond to 5 mg b.i.d. after at least 1 month of therapy. Some C3H-HeJ animal model of AA (Xing et al., 2014). (Kennedy et al., 2016), the majority of our patients did not mg b.i.d. In contrast to previous work on this subject was 10 mg b.i.d. Only one subject had a full response to 5 12 patients. The effective dose for the majority of responders improvement in SALT score at the end of treatment for 11 of (81.3%) decreased to 40.8% at the end of treatment. Overall, patients with either patch-type AA, or AT or AU had on average very similar percentage hair regrowth at the end of study treatment. This suggests that the severity of disease may not be the determining factor for response versus no response. It may be possible that a long duration of current episode of AA decreases the probability of therapeutic response, but based on our observations, longer duration of disease does not appear to preclude response to therapy.

Tofacitinib was well tolerated in all 12 patients. One patient discontinued the study due to hypertension. There were no reported serious adverse events. Observed adverse effects were infrequent and clinical laboratory abnormalities were uncommon.

This study adds to the existing literature supporting the potential of JAK inhibitors to halt hair loss and allow regrowth in some patients with AA. Although this therapy is not a cure, as evidenced by relapse of hair loss after treatment ended, tofacitinib may potentially be a therapeutic option for the treatment of hair loss in some AA patients. Further work is needed to elucidate optimal treatment strategies for maintenance of response and minimization of risks. Limitations in this study consist of those inherent in an open-label study. This includes observer bias as a result of physicians and study participants being unblinded during treatment. To limit this bias, objective tools, such as SALT scoring and photography, were used to measure the primary and secondary efficacy of treatment. Another limitation of this study is the small sample size. This decreases the study’s external validity and, as a result, may not be generalizable to

treatment. Specific frequencies for adverse events are noted in Table 2. Tofacitinib was discontinued for one patient with a history of hypertension who experienced hypertensive urgency while on the study medication.

Clinical laboratory evaluation was performed on all subjects during the screening period, at baseline, and as otherwise deemed necessary to monitor for abnormal values and for normalization of those values. Clinical laboratory evaluation consisted of complete blood count, basic metabolic profile, hepatic panel, and urinalysis with microscopic examination, hepatitis B and C screening panel, HIV test, fasting lipid profile, tuberculosis, and serum pregnancy test for women of child-bearing potential. No patient met protocol-defined clinical laboratory discontinuation criteria. Overall, there were no major abnormalities in laboratory evaluations. The laboratory adverse events that were observed are listed in Table 2. Notably, tofacitinib was discontinued for one patient after experiencing persistent 1+ blood on urinalysis. The patient’s primary care doctor was concerned and requested that we discontinue the study drug.

**DISCUSSION**

Tofacitinib is known to effectively treat rheumatoid arthritis by modulating the IFN response inflammatory pathway by inhibition of JAK1/JAK3 (Keisuke et al., 2012). AA and rheumatoid arthritis share the same IFN-γ response pathway, which provided the rationale for selecting tofacitinib for evaluation in AA (Xing et al., 2014). Already, tofacitinib has been shown to prevent the onset of, and reverse, AA in the C3H-HeJ animal model of AA (Xing et al., 2014).

Tofacitinib was effective in achieving a global overall improvement in SALT score at the end of treatment for 11 of 12 patients. The effective dose for the majority of responders was 10 mg b.i.d. Only one subject had a full response to 5 mg b.i.d. In contrast to previous work on this subject (Kennedy et al., 2016), the majority of our patients did not respond to 5 mg b.i.d. after at least 1 month of therapy. Some patients remained on 5 mg b.i.d. for 4 to 6 months prior to dose escalation, yet had absolutely no hair growth. However, once the dose was increased to 10 mg b.i.d., regrowth onset was seen within 1 month. Further studies are needed to explore the difference in response between our studies versus prior reported studies. Baseline SALT scores for the 12 patients ranged from 46% to 100% and at the end of treatment SALT scores ranged from 0% to 99%. The mean baseline SALT score of 81.3% decreased to 40.8% at the end of treatment. Overall, patients with either patch-type AA, or AT or AU had on average very similar percentage hair regrowth at the end of study treatment. This suggests that the severity of disease may not be the determining factor for response versus no response. It may be possible that a long duration of current episode of AA decreases the probability of therapeutic response, but based on our observations, longer duration of disease does not appear to preclude response to therapy.

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Table 1. Characteristics of enrolled patients and treatment course outcomes

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patient No.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Age, y</td>
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<tr>
<td>Sex</td>
<td>F</td>
</tr>
<tr>
<td>Race</td>
<td>Caucasian</td>
</tr>
<tr>
<td>Duration of current episode of scalp hair loss, y</td>
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</tr>
<tr>
<td>Total duration patient has had AA, y</td>
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</tr>
<tr>
<td>Total duration of AT/AU (if patient has AT/AU), y</td>
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</tr>
<tr>
<td>Weeks of dose escalation(s)</td>
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</tr>
<tr>
<td>Week treatment was stopped</td>
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</tr>
<tr>
<td>Baseline SALT score, %</td>
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</tr>
<tr>
<td>Lowest SALT score achieved, %</td>
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</tr>
<tr>
<td>Week lowest SALT score was achieved, %</td>
<td>48</td>
</tr>
<tr>
<td>Greatest regrowth percentage of SALT score achieved, %</td>
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</tr>
<tr>
<td>Patient outcome</td>
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<tr>
<td>Clinical durability of response, 1 wk</td>
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</tr>
<tr>
<td>Absolute durability of response, 2 wk</td>
<td>NA</td>
</tr>
</tbody>
</table>

Abbreviations: AA, alopecia areata; AT, alopecia totalis; AU, alopecia universalis; F, female; M, male; NA, not applicable; SALT, Severity in Alopecia Tool.

1Clinical durability of response is defined as the minimum observed time a responder maintained 50% regrowth after the treatment drug has been discontinued. This accounts for the amount of durability that is clinically significant, as it only considers duration in which a patient would still qualify as a “responder” upon discontinuation of drug.

2Absolute durability of response is defined as the minimum observed time a responder maintained a SALT score that was below the patients’ baseline SALT score after the treatment drug has been discontinued. In some cases, there is a range of dates that reflect that this was an outcome that occurred while the patient was between visits.
the population of interest, that is, patients with moderate to severe AA.

MATERIALS AND METHODS

Study Design, Oversight, and Participants

The study was conceived and conducted by the investigative team at Columbia University. This study was conducted in accordance with Good Clinical Practice, as defined by the International Conference on Harmonization and in accordance with the ethical principles underlying European Union Directive 2001/20/EC and the United States Code of Federal Regulations, Title 21, Part 50 (21CFR50). Monitoring for regulatory compliance and adherence to the Institutional Review Board–approved protocol was performed by the Columbia University Clinical Trials Office and the Department of Surgery Regulatory Team. The study was registered on ClinicalTrials.gov prior to initiation. Institutional Review Board approval was granted for all experiments performed in this study and written informed patient consent was obtained for all experiments, procedures, and treatments involved in this study. In addition, permission was granted from patients for the publication of their photographs.

This was an open-label pilot study of tofacitinib 5 mg b.i.d. to 10 mg orally b.i.d., for 6 to 18 months in the treatment of moderate to severe AA, and AT or AU, followed by 6 months follow-up off the drug to assess for delayed response to treatment and/or the incidence and timing of recurrence of disease. The initial treatment dose was tofacitinib 5 mg p.o. b.i.d. for at least 1 month, which was increased to 10 mg + 5 mg q.d. for at least 1 month, and then to 10 mg p.o. b.i.d. if the patient had absence of any terminal hair regrowth on the scalp. End of treatment was defined as at least 6–12 months on a dose that appeared to be having a positive response, that is, an effective dose or ending of treatment if no regrowth occurred within 3 months of the highest tolerable dose, and at least 6 months total tofacitinib treatment. A full course of treatment was defined as at least 6–12 months on a dose that appeared to be having a positive response, that is, an effective dose or ending of treatment if no regrowth occurred within 3 months of the highest tolerable dose and at least 6 months total tofacitinib treatment. We enrolled 12 adult patients, including 7 patients with moderate to severe AA (30–95% hair loss) and 5 patients with AT or AU.

Study Assessments and Outcomes

The study’s primary efficacy end point was the proportion of responders at the end of treatment, (6 to 18 months of treatment),
with response defined as ≥50% hair regrowth from baseline as assessed by the SALT score, a standardized, validated method for estimating hair loss in AA (Olsen et al., 2004). Secondary efficacy end points included hair regrowth as a continuous variable, as determined by physical examination and Canfield photography, as well as patient and physician global evaluation scores. Additionally, quality of life measures (Dermatology Quality of Life Index) were done at regular prespecified intervals. To assess the durability of patient recovery during treatment (see Figure 3) using gene expression and the ALADIN signature. Moreover, we were able to leverage these data to construct biomarkers corresponding to patient response to treatment and tofacitinib mechanism of action (see Figure 3). Rather than being derived from the pathogenic definitions of the disease, this signature is specifically derived to measure patients’ individual molecular responses in their scalp skin biopsies as a function of tofacitinib treatment over time, facilitated by the sequential time points. Statistically significant overlap of this signature and the pathogenic signature provides a quantitative framework for the a priori prediction of drug efficacy (Petukhova et al., 2011). Furthermore, these quantitative molecular profiles can be used to define the molecular regulatory mechanism of response to tofacitinib using network analysis (Chen et al., 2014; Margolin et al., 2006), and to predict nonresponders a priori—patients that exhibit low phenotypic response to treatment also show incomplete or impartial molecular response based on the tofacitinib mechanism of action exhibited in responder patients (see Figure 3).

CONFLICT OF INTEREST
Columbia University has filed patent applications around the use of JAK inhibitors in alopecia areata, which have been licensed to Aclaris Therapeutics, Inc. RC, JMW, and AMC are consultants to Aclaris Therapeutics, Inc. AMC has received grant support from Pfizer, Inc (unrelated to the context of this trial). The remaining authors state no conflict of interest.

ACKNOWLEDGMENTS
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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.2018.01.032.

REFERENCES