

AN OPEN-LABEL PILOT STUDY TO EVALUATE THE EFFICACY  
OF TOFACITINIB IN MODERATE TO SEVERE ALOPECIA  
AREATA, TOTALIS AND UNIVERSALIS

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## PROTOCOL SYNOPSIS

<b>Protocol Title:</b>	An Open-Label Clinical Trial to Evaluate the Efficacy of Tofacitinib in Moderate to Severe Alopecia Areata, Totalis and Universalis
<b>Site Numbers &amp; Names:</b>	Clinical Research Unit Department of Dermatology Columbia University Medical Center
<b>Research Hypothesis:</b>	Among patients with alopecia areata, patients with higher disease burdens are unlikely to have satisfactory outcomes with current therapies. Our hypothesis is that a Janus-Associated Kinase (JAK) inhibitor will be effective therapy in moderate to severe alopecia areata, totalis and universalis by blocking the IFN- $\gamma$ pathway. This pathway is prominent in human alopecia areata lesional tissue, and is associated with IFN- $\gamma$ infiltrate that produces the cytotoxic T cell inflammatory response underlying alopecia areata.

<b>Study Rationale</b>	<p>Alopecia areata (AA) is a common autoimmune disease resulting from autoimmune attack on the hair follicles. The histopathology clearly identifies the “swarm of bees”, comprised of T cells encircling the human hair follicle. Blockade of the IFN-gamma signaling pathway with tofacitinib (inhibits Janus-Associated Kinases (JAK) 1,3, leading to disruption of cytokines and growth factor signaling pathways) has recently emerged as an effective therapy in human T cell mediated diseases. Tofacitinib is FDA-approved for the treatment of moderate to severe rheumatoid arthritis and is under study for many other autoimmune conditions.</p> <p>There is genetic evidence for the importance of the interferon response pathway activating a cytotoxic T-cell response in human AA. Recent GWAS identified 26 genetic susceptibility alleles in the interferon response pathway that included IFN-gamma itself as well as its negative regulator SOCS1 and NKG2D ligands and CD8a.<sup>1</sup> Many of these AA susceptibility alleles are shared by other common autoimmune diseases including Celiac Disease, Type I Diabetes, and Rheumatoid Arthritis. These autoimmune states may share common pathogenic mechanisms and may respond to common treatments.</p> <p>These studies also point to a dominant role for activated NKG2D-bearing CD8 T cells as the likely culprit. Autoaggressive cellular effector CD8<sup>+</sup>NKG2D<sup>+</sup> effector T cells dominate the peribulbar infiltrate in human AA and are intimately associated with dermal sheath cells aberrantly expressing NKG2D ligands. Unpublished data in the mouse has confirmed the human pathological findings<sup>1</sup> identifying activated NKG2D bearing CD8 T cells both adjacent to the hair follicle and in the draining lymph nodes. Thus, the immunopathogenesis of the C3H mouse model faithfully recapitulates human alopecia. Consistent with a primary role for autoimmune T cells in the C3H mouse model of alopecia areata, disease can be induced in normal unaffected mice by transfer of T cells from affected mice or humans.<sup>2-4</sup></p> <p>Based on these pre-clinical proof-of-concept studies in the relevant animal model, the genetic evidence for the relevance of the interferon-<math>\gamma</math> pathway in human alopecia areata, and the lack of existing evidence-based data for any treatment in alopecia areata, there is a strong rationale for testing Jak1,2, &amp; 3 inhibitor treatment in this disease.</p>
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<b>Study Objectives:</b> <b>Primary:</b> <b>Secondary:</b>	<p>The study's <i>primary efficacy endpoint</i> will be the proportion of responders at the end of treatment, which may be up to between 24 weeks/6 months to 72 weeks/18 months of treatment, with response defined as 50% or greater hair re-growth from baseline as assessed by SALT score at the end of treatment - week 24 to 72. This is a relatively strict definition for defining responders and non-responders and was chosen to minimize the potential for spontaneous remission, in which fewer than 10% are expected to achieve this magnitude of hair regrowth spontaneously.</p> <p>As additional secondary endpoints, efficacy will be measured by changes in hair re-growth as a continuous variable as determined by physical exam and Canfield photography, as well as patient and physician global evaluation scores. To assess the durability of responses, patients will continue to be followed for an additional 6 months after treatment is completed. Response at the end of study, week 72-96, will be examined as a secondary outcome. We will compare the efficacy of tofacitinib in promoting hair regrowth in patients with moderate to severe patch type alopecia areata to the efficacy in treating patients with current alopecia totalis (AT) or universalis (AU).</p>
<b>Study Design:</b>	<p>Open-label pilot study of tofacitinib 5mg to 10mg PO BID, for 6 to 18 months in the treatment of moderate to severe AA, and alopecia totalis or universalis, followed by 6 months follow-up off drug to assess for delayed response to treatment and/or the incidence and timing of recurrence of disease.</p> <p>The initial treatment dose will be tofacitinib 5mg PO BID, and may be increased up to 10mg PO BID, if clinically indicated at the discretion of the investigator.</p> <p>The initial treatment duration will be 6 months, and may be extended by up to 12 additional months if clinically indicated with an acceptable lab safety profile, at the discretion of the investigator.</p>
<b>Study Schema</b> <b>Drugs / Doses /</b> <b>Length of Treatment)</b>	<p>Tofacitinib (5 mg to 10 mg) will be taken orally twice a day for up to 24 to 72 weeks.</p> <p>Study drug dose may be reduced and/or temporarily discontinued if indicated based upon laboratory values.</p>
<b>Accrual Goal:</b> <b>(Total number of subjects)</b>	15

<b>Accrual Rate:</b> (Number of subjects expected per month)	1-3
<b>FPFV:</b> <b>LPFV:</b> <b>Follow Up:</b>	October, 1, 2014 December, 31, 2015 12 months
<b>Correlative Studies:</b> (PK/PD, etc.)	<p>Our published work and previous studies in human AA have reported increased NKG2DL expression and augmented expression of IFN response genes in the target hair follicle end organ, and elevated NKG2D expression in circulating CD8 T cells and NK cells (Ito et al, 2008; Petukhova et al, 2010).</p> <p><u>Clinical Correlative Studies</u></p> <p>Our study will include longitudinal assessment of AA biomarkers at baseline and during treatment to correlate treatment and disease status with;</p> <ol style="list-style-type: none"> <li>1) Histological improvement with reduced T cell peribulbar infiltrates.</li> <li>2) Declines in circulating and peribulbar CD8<sup>+</sup>NKG2D<sup>+</sup> infiltration and in follicular hair follicle NKG2DL expression.</li> <li>3) Reduced IFN inflammatory biomarkers in the skin and blood.</li> </ol>
<b>Inclusion Criteria:</b>	<p>Patients between 18 to 65 years of age.</p> <p>Patients with a diagnosis of patch type alopecia areata.</p> <p><b>Patients will have &gt;30% and &lt;95% total scalp hair loss at baseline as measured using the SALT score or patients with 100% scalp hair loss.</b></p> <p><b>Duration of hair loss greater than 3 months without an upper limit of duration as long as there is reason to believe that regrowth is possible in the opinion of the investigator.</b></p> <p>No evidence of significant active/ongoing regrowth in the areas to be assessed present at baseline.</p> <p>Patients may be naïve to treatment or unresponsive to intralesional (IL) steroids or other treatments for alopecia areata.</p>

<b>Exclusion Criteria:</b>	<p>Patients with a history of or active skin disease on the scalp such as psoriasis or seborrheic dermatitis.</p> <p>Patients in whom the diagnosis of alopecia areata is in question.</p> <p>Patients with active medical conditions or malignancies (except adequately treated basal or squamous cell carcinoma) that in the opinion of the investigator would increase the risks associated with study participation, including patients with a history of recurrent infections.</p> <p>Patients with active infection at baseline.</p> <p>Patients undergoing concurrent live vaccination.</p> <p>Women of childbearing potential who are unable or unwilling to use two forms of birth control for the study duration.</p> <p>Women who are pregnant or nursing.</p> <p>Patients known to be HIV or hepatitis B or C positive.</p> <p>Patients with history or evidence of hematopoietic abnormality.</p> <p>Patients with Hgb &lt;9.0 at baseline.</p> <p>Patients with lymphocytes &lt; 500 at baseline.</p> <p>Patients with ANC &lt; 1000 at baseline.</p> <p>Patients with history or evidence of renal or hepatic impairment.</p> <p>Patients with history of immunosuppression or history of recurrent serious infections.</p> <p>Patients with prior history or at risk for GI perforation (i.e. active diverticulitis, inflammatory bowel disease and other risk factors).</p> <p>Patients over the age of 65, as the risk of serious infections is reportedly higher in this population</p> <p>Patients unwilling or unable to discontinue treatments known to affect hair regrowth in AA.</p> <p>Patients taking any medication considered a strong CYP3A4 inhibitor who is unable or unwilling to stop this medication for the duration of the study.</p> <p>Patients receiving treatment deemed to affect alopecia areata within 2 weeks to one month of baseline visit depending on the specific treatment.</p>
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<p><b>Criteria for Evaluation:</b> (Efficacy, safety, stopping rules, etc.)</p>	<p><b>EFFICACY</b></p> <p><u>Primary endpoint:</u> SALT (Severity of Alopecia Tool) Scores: The proportion of scalp involvement is determined by dividing the scalp into 4 quadrants and estimating the percentage of the scalp surface that all the alopecic areas would occupy if placed <b>together</b>.</p> <p><u>Secondary endpoint:</u> Changes in hair re-growth as a continuous variable will be determined using physical exams and Canfield photography, as well as patient and physician global evaluation scores. Additionally, patient reported outcomes, safety measures, incidence and timing of relapse will be important secondary outcomes.</p> <p>To assess safety, frequent and close monitoring of clinical and laboratory findings have been implemented. Adverse events will be captured and analyzed as a secondary endpoint.</p> <p>All physical examination findings, vital sign abnormalities, and clinical laboratory abnormalities, will be captured as AEs when deemed medically significant by the investigator. A qualified physician associated with the study will be available to assess clinical signs and symptoms that may be indicative of an adverse event. Safety will be assessed by summarizing the incidence and type of AEs. The proportion of patients who discontinued treatment will be summarized.</p>
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<b>Statistics:</b>	<p>This is an open-label study to examine the efficacy of 6 to 18 months of tofacitinib in the treatment of moderate to severe AA, and AT/AU.</p> <p>We have chosen our primary endpoint conservatively (the proportion of patients with &gt;50% improvement in SALT index) as a relatively strict criteria that should minimize placebo response rates to 10% or less (0-1 of 10 subjects). This allows a higher degree of confidence that appreciable response rates are attributable to drug rather than spontaneous remission. Historically, patients with AT or AU are even less likely to experience spontaneous remission</p> <p><u>Power analysis.</u> Based on two recently completed randomized trials (Strober et al, 2008; Price et al, 2009) in similar patient populations (moderate to severe AA) we expect placebo response rates to be between 6% (&gt;50% improvement in SALT score<sup>3</sup> (Olsen et al, 1999)) and 12% (&gt;25% improvement). We estimate the likelihood of spontaneous regrowth of <math>\geq 50\%</math> baseline hair loss to be approximately 8% and aim to detect a 40% response rate with tofacitinib treatment.</p> <p><u>With our sample of n=15, we will have greater than 80% power to reject the null hypothesis that the response rate to treatment with tofacitinib is the same as the underlying remission rate of 10%, if we observe a response rate in our sample of 48% (a 38% effect size), holding type I error to 5%. If we assume that the underlying remission rate is 30% (the historical response rate in less severely affected patch type AA patients), then we have greater than 80% power to reject that null hypothesis if the observed rate is 73% or above (an effect size of 43%), again holding type I error to 5%.</u></p> <p><u>Since this is a small open-label proof of concept study, we recognize that it will likely lack adequate power to conclusively demonstrate small efficacy signals. Yet, descriptive summaries of all primary and secondary efficacy outcomes (proportions for binary outcomes, means for continuous outcomes) will provide a preliminary indication of the effect size that will aid in the design of subsequent efficacy trials. To assess safety as a secondary endpoint, we will summarize, via descriptive statistics, the occurrence of adverse events for the study group.</u></p> <p><u>Accrual projections.</u> The Dermatology clinic and private practice at CUMC currently sees 600 AA patients yearly, of which we anticipate 20-30% would be eligible (&gt;30% hair loss). Accrual will be greatly facilitated by targeted recruitment through our existing NAAF registry which includes 500 AA patients in the New York area, the majority of which have patchy type disease. Thus, complete accrual of 15 subjects should be feasible within 1 year from a pool of &gt;100 eligible new subjects yearly.</p>
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# 1 INTRODUCTION

## 1.1 Research Hypothesis

Among patients with alopecia areata, patients with higher disease burdens are unlikely to have satisfactory outcomes with current therapies. Our hypothesis is that the JAK1/JAK3 inhibitor tofacitinib will be effective therapy in moderate to severe alopecia areata by attenuating both IL-15 and IFN-gamma signaling, thereby blocking re-activation of CD8<sup>+</sup>NKG2D<sup>+</sup> memory T cells, and aborting the cytotoxic T cell inflammatory response underlying alopecia areata.

### 1.1.1 Overview of Alopecia Areata

Alopecia areata (AA) is a major medical problem and is the most prevalent autoimmune disease in the US, with a lifetime risk of 1.7%, affecting both males and females across all ethnic groups.<sup>8</sup> Additionally, AA represents the second most common form of human hair loss, second only to androgenetic alopecia, and causes significant disfigurement and psychological distress to affected individuals (Figure 2).

Alopecia areata usually presents with patchy hair loss. Approximately one-third of these patients will experience spontaneous remissions within the first year. However, many patients will develop waxing and waning disease with some progressing to alopecia totalis (total scalp hair loss) or alopecia universalis (loss of all body hair). This population that suffers from a disfiguring disease represents a significant unmet

medical need.<sup>9</sup> Alopecia totalis/universalis seldom, if ever, remits spontaneously or with current treatment. In our targeted population of moderate-severe patch-type alopecia, placebo response rates in other clinical studies have been in the 6-12% range.<sup>6,7</sup>

Clinical development of innovative therapies in AA has lagged far behind other autoimmune conditions. This may be due in part to the perception that AA is merely a cosmetic disorder. In reality, AA carries one of the highest burdens amongst all skin diseases, particularly among children and adolescents whose self-image is so closely linked to their appearance.<sup>10</sup>



**Figure 2.** Clinical manifestations of AA. Patchy disease (a and b), In b, the patient is in a spontaneous regrowth phase. For patients with alopecia universalis, there is a complete lack of body hair and scalp hair (c), while patients with alopecia totalis lack scalp hair but not body hair. In d, the randomness of hair regrowth is observed, with regrowth in the parietal region but not either the occipital or temporal regions.

There are **no** evidence-based treatments for AA. A comprehensive Cochrane analysis assessment of seventeen randomized clinical trials (RCTs) involving a total of 540 participants found no proven treatment of AA.<sup>54</sup> Each trial included from 6 to 85 participants and assessed a range of interventions that included topical and oral corticosteroids, topical cyclosporine, photodynamic therapy and topical minoxidil. Overall, **none** of the interventions showed significant treatment benefit in terms of hair growth when compared with placebo. It was concluded that the effectiveness of few (if any) treatments for AA are proven. No RCTs on the use of diphenylcycloprophenone, dinitrochlorobenzene, intralesional corticosteroids or dithranol were found, although these drugs are commonly used for the treatment of AA. Similarly, although topical corticosteroids and minoxidil are widely prescribed and appear to be safe, there is no convincing evidence that they are beneficial in the long-term. Most trials have been poorly reported and/or are so small that any important clinical benefits are inconclusive.

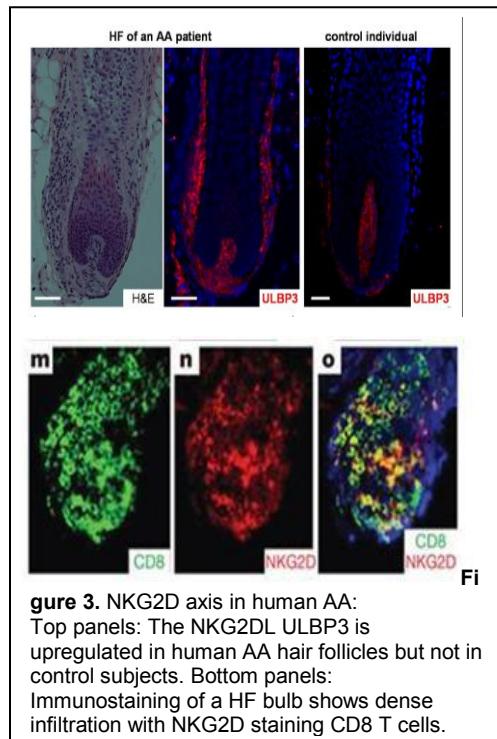
**This study will provide data on the efficacy of tofacitinib in alopecia areata.** One clear advantage of immunotherapeutic studies in the skin is the relative ease of access of the target organ. Shared genetic and common pathological pathways provide strong rationale for increasing the scope of approved pharmaceuticals (such as tofacitinib) for novel indications (such as AA). Studies of tofacitinib in plaque psoriasis and psoriatic arthritis, ankylosing spondylitis, atopic dermatitis (topical), ulcerative colitis, juvenile idiopathic arthritis, and Crohn's disease are underway (Clinical Trials Identifier NCT01710046 and NCT01877668, Clinicaltrials.gov). Indeed, positive studies in any one of these autoimmune diseases that share a common cause could serve as the basis for advancing common treatments.

Currently, tofacitinib is FDA-approved for the treatment of adult patients with moderate-to-severe rheumatoid arthritis (RA) who have had an inadequate response or intolerance to methotrexate. For the treatment of RA, tofacitinib is approved as monotherapy or for combination therapy with methotrexate or other nonbiologic disease-modifying antirheumatic drugs (DMARDs).

### 1.1.2 Pathology and role of T cells in human Alopecia Areata

#### CD8+ Killer T cells in Human AA

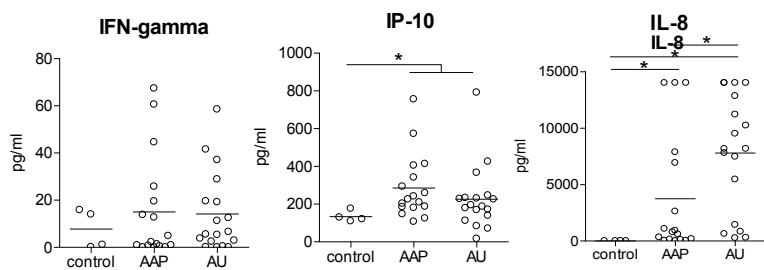
Alopecia areata results from autoimmune attack on the hair follicles. The relevant autoantigens are unknown, but the histopathology clearly identifies the “swarm of bees” encircling the human hair follicle. Infiltrates containing both CD4 and CD8 T cells have been described, but our recent studies pointed to a dominant role for activated NKG2D-bearing CD8 T cells that are the likely culprit autoaggressive cellular effectors<sup>1</sup>. Our collaborators recently demonstrated that CD8<sup>+</sup>NKG2D<sup>+</sup> effector T cells dominate the peribulbar infiltrate in human AA that are intimately associated with dermal sheath cells aberrantly expressing NKG2D ligands (Figure 3).



**Figure 3. NKG2D axis in human AA:**  
Top panels: The NKG2DL ULBP3 is upregulated in human AA hair follicles but not in control subjects. Bottom panels: Immunostaining of a HF bulb shows dense infiltration with NKG2D staining CD8 T cells.

#### Interferon driven Th1-response in human AA

Consistent with a pathogenic cellular immune response, AA has been viewed as a Th1-driven disease<sup>11-13</sup>. For instance, elevated Th1 cytokines/chemokines (Figure 4) are seen in the peripheral blood of AA patients and IFN-inducible gene signatures have been described in the skin of AA patients.



**Figure 4. Elevated Serum Chemokines and Cytokines in Human AA.** Interferon- $\gamma$  and IFN-induced chemokines (IP-10/CXCL10) are elevated in the serum of **human** AA, in some cases correlating with disease severity, i.e. patchy disease (AAP) vs. universalis (AU).

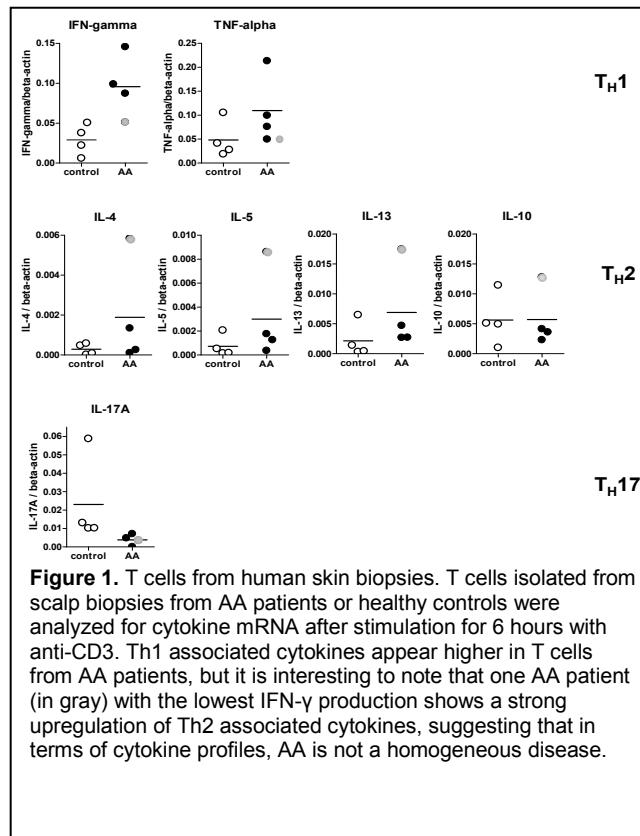
## 2

## RATIONALE FOR USE OF TOFACITINIB IN AA

Interferon gamma and AA

The hair follicle has been considered a relative immunological sanctuary, expressing little or no MHC molecules, and instead expressing inhibitory Class I-like molecules such as HLA-E. In AA, lesional hair follicles express high levels of MHC molecules and NKG2D ligands, permitting recognition by NKG2D-bearing CD8 killer cells found intimately associated with the hair follicle in the human and in mice<sup>1</sup>. Interferons, as key activators of the MHC locus and of the cellular immune response, could play a key role in eliminating immunologic privilege and orchestrating the induction and persistence of the inflammatory response in mouse and human AA.

In the C3H-HeJ mouse model of AA, IFN- $\gamma$  is required for pathogenesis, and administration of IFN- $\gamma$  accelerates disease<sup>3,14</sup>.



**Figure 1.** T cells from human skin biopsies. T cells isolated from scalp biopsies from AA patients or healthy controls were analyzed for cytokine mRNA after stimulation for 6 hours with anti-CD3. Th1 associated cytokines appear higher in T cells from AA patients, but it is interesting to note that one AA patient (in gray) with the lowest IFN- $\gamma$  production shows a strong upregulation of Th2 associated cytokines, suggesting that in terms of cytokine profiles, AA is not a homogeneous disease.

Likewise, occurrence of human AA has been noted in several series as a side-effect of Type I interferon therapy<sup>15-25</sup>. Importantly, administration of IFN- $\gamma$  neutralizing antibodies reverses AA pathogenesis in the C3H-HeJ mouse<sup>26,2</sup>. In our collaborators' transcriptional profiling studies of lesional vs. non-lesional C3H skin, the interferon response was overwhelmingly the dominant signature with 17 of the top 20 upregulated genes being interferon response genes. Interferon gamma itself was upregulated, but not Type I interferons (data not shown). To confirm this finding, our collaborators performed quantitative real-time PCR on cDNA generated from affected and non-affected C3H/HeJ mice, utilizing an interferon signaling and response qPCR array (Stellararray, Lonza Cat #00189608). This array contains primers that measure the expression of genes encoding interferons and their receptors, components of the JAK-STAT signaling pathway, interferon-inducing cytokines and response genes, as well as several housekeeping genes. The results validate the involvement of the IFN- $\gamma$  pathway; the IFN- $\gamma$  receptor 1 and the IFN- $\gamma$  inducible chemokines CXCL9/10/11 (left most red bars up-regulated by <10-fold in Fig. 3), are highly overexpressed in affected mice. In contrast, the IFN Type I response appears to be markedly repressed in affected mice, as evident by the sharp down regulation of Type I interferons (alphaB, alpha 4) and their receptor IFNAR1 (bright blue bars).

In addition to our data on C3H-HeJ mouse skin, in human AA, transcriptional profiles have noted a Type I IFN response in lesional biopsies<sup>45</sup> and Th1 skewing and elevated IFN-response cytokines/chemokines in the peripheral blood<sup>2-31</sup> and reviewed in scalp biopsies<sup>32</sup>.

The cellular source of IFN- $\gamma$  are likely T cells; in the C3H/HeJ mice our collaborators have shown that IFN-gamma producing CD8+NKG2D+ cells dominate the dermal HF infiltrate (unpublished). In human AA, they have shown in Figure 1 that IFN- $\gamma$  producing cells were identified in 4 of 5 dermal crawl-out assays<sup>37</sup>. Our collaborators' unpublished data implicate IL-15 in driving activation of IFN-producing CD8 T cells. **Tofacitinib would be predicted to also inhibit the IL-15 receptor which signals through JAK1 and JAK3.** They have several lines of evidence from our translational research program implicating IFN- $\gamma$  in AA.

#### GWAS in AA Identifies IFN- $\gamma$ Pathway Genes

Since our collaborators initial GWAS publication<sup>1</sup>, we have identified **twenty-six putative susceptibility genes** (9 with p-values  $<10^{-4}$  and additional 17 with p-values  $=10^{-3}$ ) in the IFN/IFN-response pathway, including IFN- $\gamma$  itself as well as its negative regulator SOCS1.

Human		Mouse	
Gene	Fold Upregulated	Gene	Fold Upregulated
CXCL10	6.749738	Cxcl10	37.489223
CXCL9	6.3546677	Cxcl9	75.22101
CD8A	2.8763134	Cd8a	14.440988
STAT1	2.647132	Stat1	14.508814
IFI44	2.504809	Ifi44	20.594664
CCL2	2.4538028	Ccl2	6.1081963
GZMA	2.4454105	Gzma	56.385307
RSAD2	2.1267016	Rsad2	2.905758
PTPRC	2.0699668	Ptprc	2.068105
TLR3	2.0492992	Tlr3	2.5370347
CD274	2.0426362	Cd274	8.396741

Figure 2

manner, our collaborators are investigating whether there are detectable differences of gene expression detected in tissue samples of AA patients compared to healthy controls. Our collaborators have conducted preliminary profiling of the transcriptome comparing mRNA microarray profiles of affected scalp samples from 12 AA patients and normal scalp samples from 6 healthy controls, and we identified approximately 148 differentially expressed, upregulated genes and 120 downregulated genes (**Figure 2**). **This analysis revealed a striking interferon gamma signature**, as well as genes characteristic of **cytotoxic CD8 T cells** (CD8a, granzyme), which is conserved between human AA biopsies and the C3H-HeJ mouse model (**Figure 2**).

## 2.1 Preclinical Studies: JAK1/JAK2 inhibitors effectively treat Alopecia Areata in the C3H-HeJ mouse model.

C3H/HeJ mice develop spontaneous alopecia areata and the histopathological changes are identical to human alopecia including the importance of IFN- $\gamma$  producing CD8 $^{+}$  NKG2D bearing T cells which are present in alopecic skin, and are massively expanded and activated in AA cutaneous lymph nodes (data not shown). As with human alopecia, we have demonstrated that IFN-response genes are highly upregulated in C3H/HeJ alopecic skin but not in non-lesional skin, and is normalized in mice treated with effective therapy (data not shown).

Consistent with a primary role for autoimmune T cells, in C3H mice disease can be induced in normal unaffected mice by transfer of T cells (either or both CD4 and CD8 T cells) from affected mice or humans<sup>2-4</sup>. Thus the immunopathogenesis of the C3H mouse model faithfully recapitulates human alopecia and provides the rationale for preclinical studies in the C3H/HeJ mouse.

**Proof-of-concept has been provided for the JAK1/JAK3 inhibitor tofacitinib as a treatment in the C3H alopecic mouse model.**

### 2.1.1 Genetics of Alopecia Areata

#### *Common Cause Hypothesis in Autoimmune Diseases*

The ‘**Common Cause**’ theory of autoimmunity is well-supported by decades of research using various methodologies and provides strong support for the existence of shared disease mechanisms. Epidemiological studies first demonstrated that some autoimmune diseases cluster within families, suggesting shared pathogenesis. More recently, hypothesis-free research strategies in genetics have shifted away from linkage studies in families, to genome-wide association studies (GWAS) in population-based cohorts. The results of these studies have provided tremendous support to the “common cause hypothesis”<sup>33</sup> and have importantly identified **susceptibility alleles in specific genes that underlie grouped sets of autoimmune diseases, arguing for unifying/general mechanisms that dysregulate tolerance at one of several multiple end organ sites, with autoimmune destruction in the pancreas, joint, or skin, etc., the potential final disease outcome**. In fact, outside of the HLA, at least 23 such genes have been associated with two or more autoimmune diseases in a GWAS<sup>34-35</sup>. The majority of these shared genes can be mapped onto a discrete set of immunological molecular pathways.

## 2.2 Efficacy of JAK3 inhibitor (tofacitinib) in mouse models of Alopecia Areata

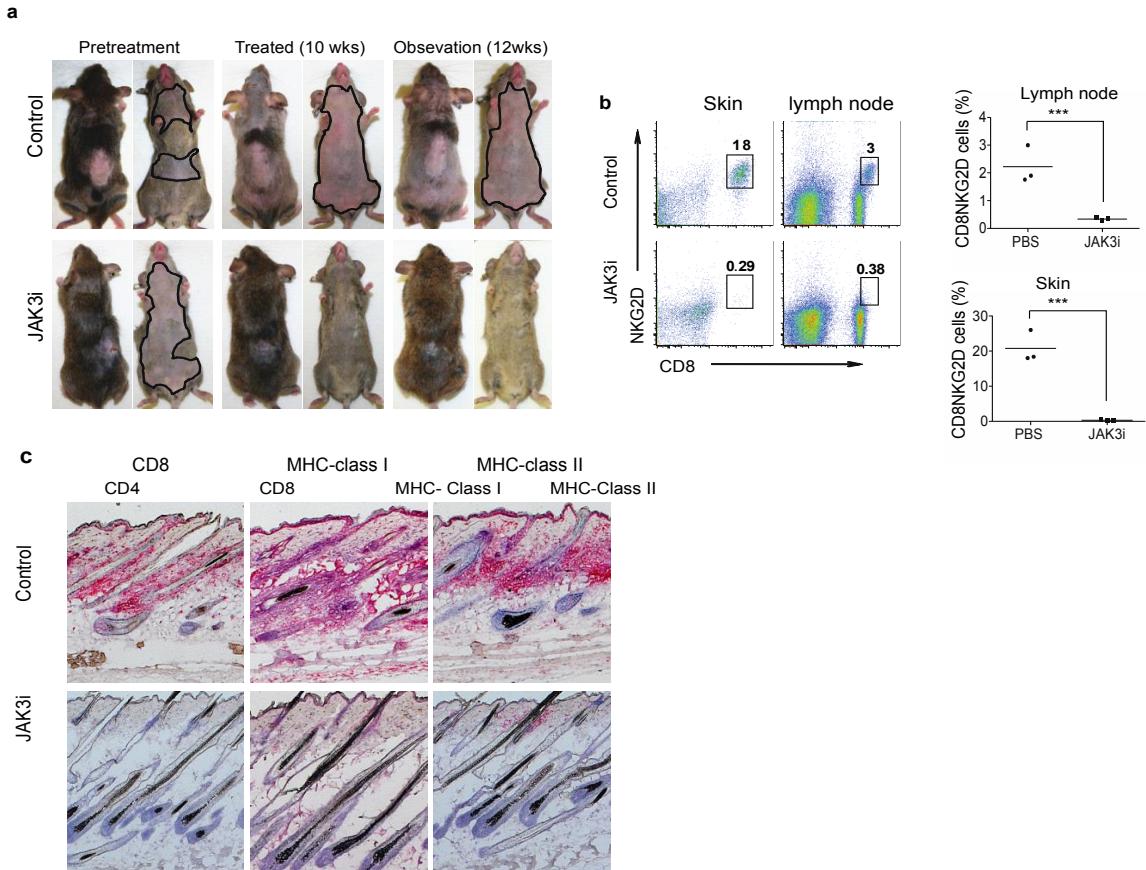
Here, we demonstrated that systemic administration of ruxolitinib (JAK1/2i, Figure 3f-h) and tofacitinib (JAK3i, Figure 3k-m) treatment prevented the development of AA and the expansion of CD8<sup>+</sup>NKG2D<sup>+</sup> T cells in all grafted recipients. Skin of mice treated with either protein tyrosine kinase inhibitor (PTKi) showed no histological signs of inflammation (Figure 3 i,n). Global transcriptional analysis of whole skin biopsies showed that both drugs also blocked the dermal inflammatory signature, as measured by ALADIN (*Alopecia Areata Disease Activity Index*, Figure 3j,o), a transcriptional biomarker instrument that combines the CTL and IFN inflammatory scores, as well as by Gene Expression Dynamic Index (GEDI) analysis.

To evaluate the efficacy of JAK3i in the context of active AA, we next asked whether systemic JAK3i treatment could reverse established disease by initiating therapy 7 weeks after grafting, a time point at which all mice had developed extensive AA. Importantly, systemic therapy induced hair regrowth all over the body, and likewise eliminated the expansion of CD8<sup>+</sup>NKG2D<sup>+</sup> T cells and reversed histological markers of disease (Figure in the middle below, a-c), an effect that persisted 2-3 months after the cessation of treatment.

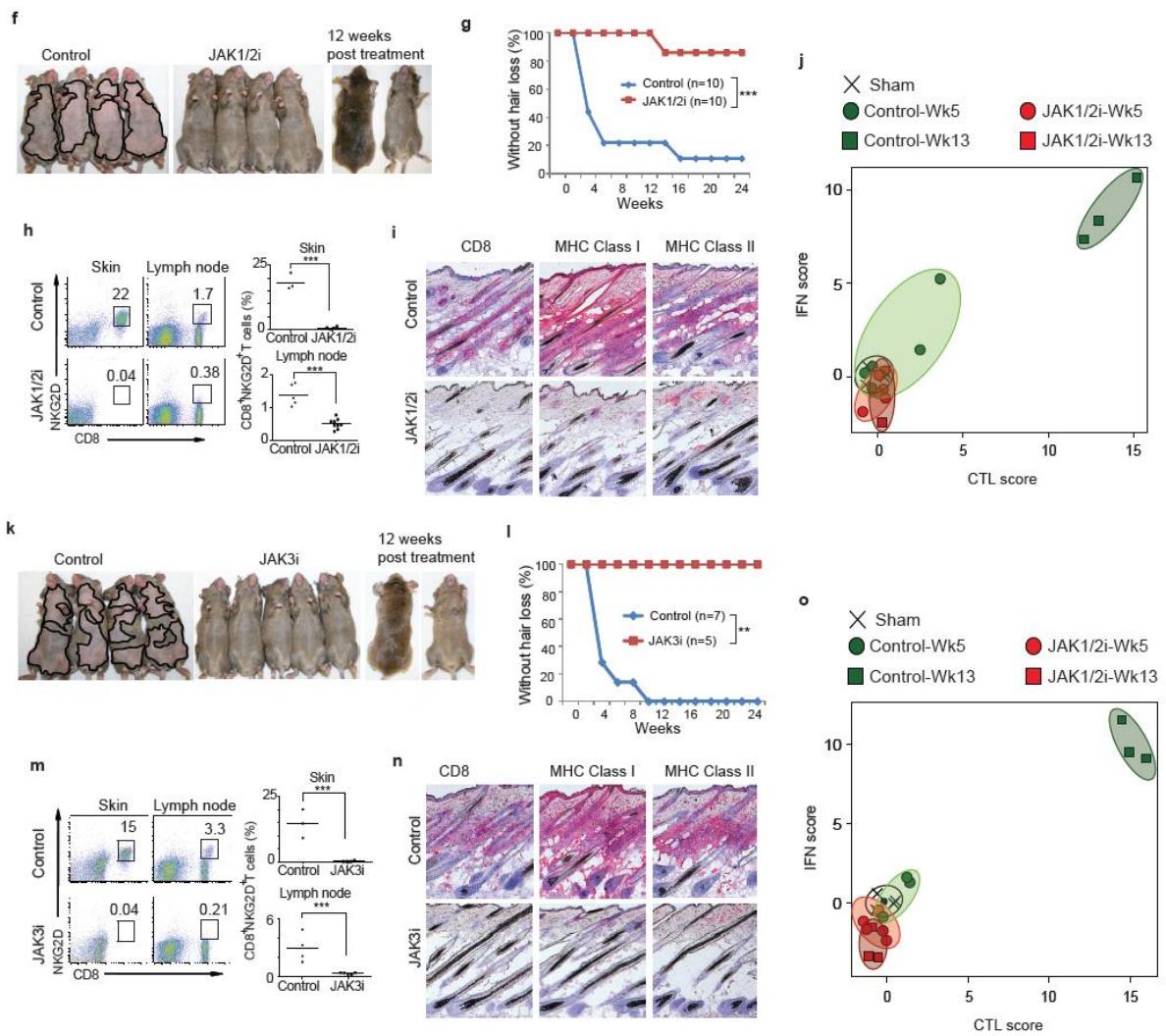
Finally, to test a more clinically relevant route of delivery, we asked if topical administration of PTKis could reverse long-standing alopecia areata with similar kinetics as systemic delivery. In established disease, we found that topical ruxolitinib, as well as topical tofacitinib, were both highly effective in reversing disease in treated lesions (applied to back skin), and complete hair regrowth was observed within 12 weeks following topical therapy (Figure 4a,b). Topical therapy was associated with the disappearance of CD8<sup>+</sup>NKG2D<sup>+</sup> T cells in the treated skin and lymph node (Figure 4c), and the normalization of the ALADIN transcriptional signature (Figure 4d), as well as the reversal of histological markers of disease (Figure 4e) and correction of the GEDI in all treated mice. Notably, untreated areas on the abdomen remained alopecic (Figure 4a, outlined areas) demonstrating that topical therapy was locally effective and that the observed therapeutic effects were not the result of systemic absorption.

Further, these effects were visible as early as 2-4 weeks after onset of treatment (Supplementary Figure 14) and were durable 2-3 months after the cessation of treatment (Figure 4a, 8 week post treatment, right panels).

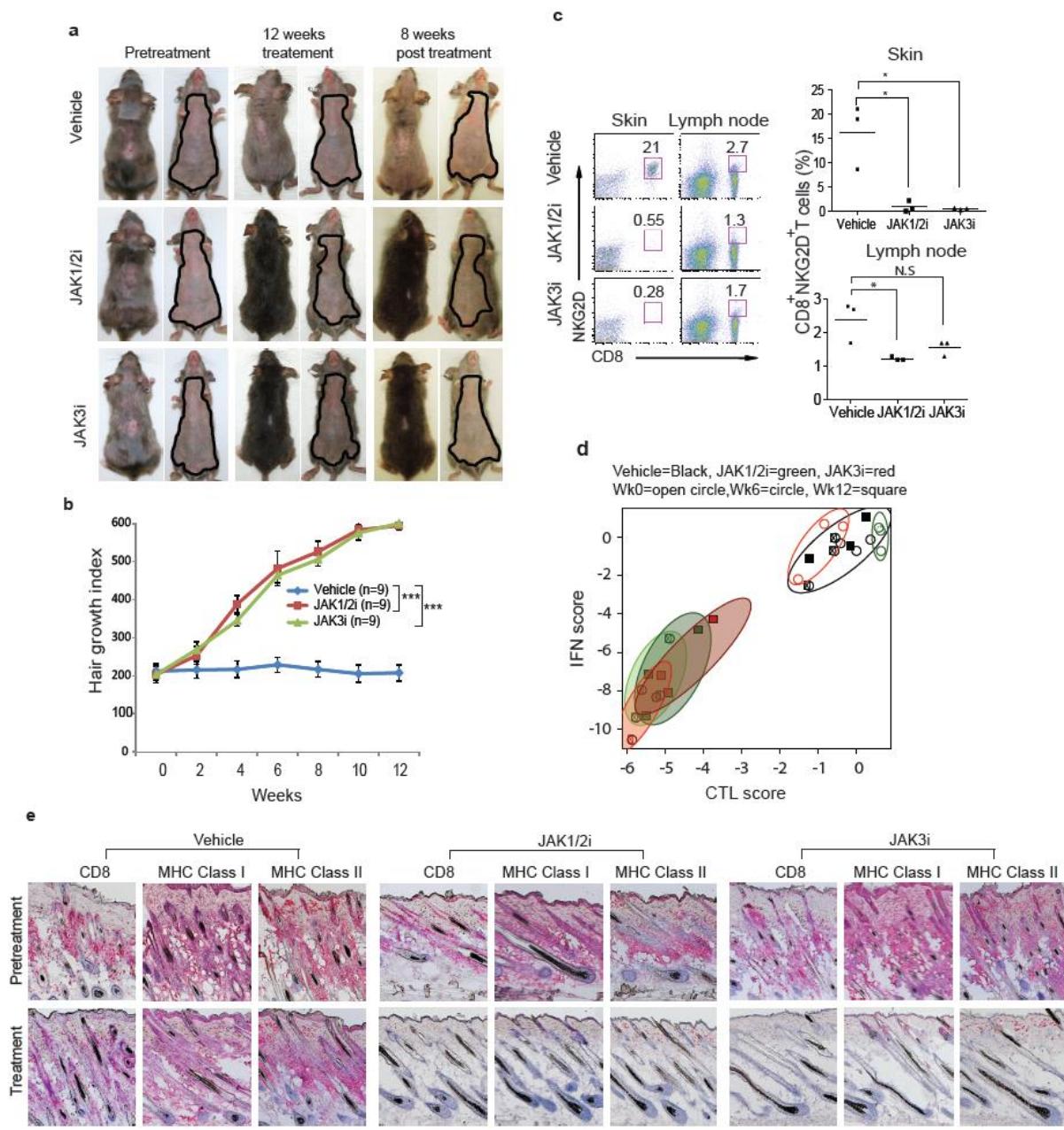
Taken together, our data identify CD8<sup>+</sup>NKG2D<sup>+</sup> T cells as the immune cellular effectors responsible for autoimmune attack of the hair follicle and provide support for a model of AA pathogenesis. We have identified hair follicle expression of NKG2DL/IL-15 as potential proximal triggers that activate and sustain IFN- $\gamma$ -producing CD8<sup>+</sup>NKG2D<sup>+</sup> CTLs. Conversely, we postulate that IFN- $\gamma$  produced by CD8 T cells leads to the collapse of immune privilege in the hair follicle, inducing further production of IL-15 thus completing a feed-forward inflammatory loop that conspires to promote Type I cellular autoimmunity.



**Figure. Systemic treatment of AA mice with JAK3 inhibitor.** C3H/HeJ mice with long-standing alopecia areata were treated with tofacitinib with Alzet osmotic mini-pumps (pumps, model 2004, Durect Corporation) implanted subcutaneously on the back of each mouse to deliver vehicle (poly(ethylene glycol) (PEG)300) or vehicle containing JAK3i tofacitinib (Abmole) at 15 mg/kg/day for 12 wks. **a**, Alopecia areata reversal was complete on both the back and belly. **b**, Flow cytometric analysis of skin and cutaneous lymph node populations shows elimination of the CD8<sup>+</sup>NKG2D<sup>+</sup> T cell population in treated mice (n=3 per group). **c**, Immunostaining of skin from mice treated with tofacitinib or placebo demonstrates elimination of CD8 infiltration and MHC I and II upregulation in tofacitinib treated mice.



**Figure 3**



**Figure 4**

## **Tofacitinib in humans with AA**

A recent publication documents complete regrowth of scalp and body hair in a patient with alopecia universalis treated with tofacitinib for severe psoriasis. JID June 2014 (Killing Two Birds with One Stone: Oral Tofacitinib Reverses Alopecia Universalis in a Patient with Plaque Psoriasis. Brittany G. Craiglow, MD and Brett A. King, MD, PhD. Department of Dermatology, Yale University School of Medicine, New Haven, CT.)

In addition, our preliminary data to date, from our ongoing trial of ruxolitinib (previously referred to as INCB 18424 - a JAK1/2 inhibitor) CU IRB AAAL7102, suggests a preliminary response rate of 66% at 3 months in patients with moderate to severe AA. The response measure is defined as a  $\geq 50\%$  change in SALT score, the same primary outcome used in the currently proposed study (data unpublished).

## **2.3 Summary of Results of Investigational Program**

In November 2012, the U.S. Food and Drug Administration (FDA) approved the first-in-class Janus Kinase (JAK) inhibitor, tofacitinib, for treatment of rheumatoid arthritis (RA), spearheading the initiation of kinase inhibitors for treatment of rheumatic diseases. Despite advances in RA management over the last twenty years with use of cytokine and target cell inhibitors, a significant proportion of RA patients do not respond or maintain response efficacy to therapy. Rheumatoid arthritis results with the activation of both innate and adaptive immune pathways. Cells implicated in the immune system dysregulation use kinases as gatekeepers to determine their responsiveness to external stimuli such as antigens, cytokines, and immune complexes. New molecule inhibitors targeting cytoplasmic kinases such as the JAK family, splenic tyrosine kinase (SYK), or mitogen-activated phosphokinase p38 (p38MAPK), will amend the resulting dysregulated immune system through distinct paths and further aid our understanding of its complexities.

In addition to tofacitinib, at least three other JAK inhibitors are currently in phase II studies for treatment of RA, psoriasis, and inflammatory bowel disease (see Table 1).

**Table 1 (adapted from *The Rheumatologist*, June 2013)**

Clinical Trials of current Kinases			
Agent	Target	Indication	Developmental Stage
Tofacitinib	Jak3, Jak1	RA	FDA approved
		PsO, IBD	Phase II trial
Baricitinib	Jak1, Jak2	RA, PsO	Phase II trial
GLPG-0634	Jak1	RA	Phase II trial
INCB18424	Jak1, Jak2	PsO	Phase II trial
VX-509	Jak3	RA	Phase II trial
R348	Jak3 + SYK	RA, PsO	Phase I trial
Fostamatinib	SYK	RA	Phase III trial
Pamapimod	p38 MAPK	RA	Phase II trial
VX-702	P38 MAPK	RA	Phase II trial
SCIO-469	P38 MAPK	RA	Phase II trial
Topicals			
R333	Jak/SYK Inhibitor	Discoid Skin Lesions	Phase II trial
R348	Jak3 + SYK Inhibitor	Keratoconjunctivitis sicca	Phase I trial

These drugs are unique from the currently available therapies, due to their effects on the intracellular signaling apparatus including the JAK pathway utilized by inflammatory cytokines and growth factors. JAK kinases are activated after cytokine binding to one or more cytokine receptors, leading to JAK phosphorylated-activation and resulting in activation of signal transducer and activator of gene transcription (STAT) proteins. Certain cytokines, such as interleukin (IL) 6, IL-2, and IL-12/IL-23, are JAK kinase dependent. The JAK family consists of four tyrosine kinases. JAK-1, JAK-2, and TYK2 are expressed ubiquitously, whereas JAK 3 is limited to lymphoid tissues.<sup>1</sup> All JAKs possess two similar phosphate-transferring domains, one with actual kinase activity and a pseudokinase domain, which in turn downregulates the activating kinase domain.<sup>2</sup> Thus JAKs maintain dual regulatory functions. The JAK-STAT interaction is responsible for the signal transduction of over 50 members of the 4-helix bundle cytokine family, including interferons and growth factors such as leptin, prolactin, GM-CSF, and erythropoietin (EPO).

### **2.3.1 Pharmacokinetics of Oral Formulation**

Following oral administration of tofacitinib (XELJANZ), peak plasma concentrations are reached within 0.5-1 hour, elimination half-life is ~3 hours and a dose-proportional increase in systemic exposure was observed in the therapeutic dose range. Steady state concentrations are achieved in 24-48 hours with negligible accumulation after twice daily administration.

#### **Absorption**

The absolute oral bioavailability of tofacitinib is 74%. Coadministration of XELJANZ with a high-fat meal resulted in no changes in AUC while Cmax was reduced by 32%. In clinical trials, XELJANZ was administered without regard to meals.

#### **Distribution**

After intravenous administration, the volume of distribution is 87 L. The protein binding of tofacitinib is ~40%. Tofacitinib binds predominantly to albumin and does not appear to bind to  $\alpha$ 1-acid glycoprotein. Tofacitinib distributes equally between red blood cells and plasma.

#### **Metabolism and Elimination**

Clearance mechanisms for tofacitinib are approximately 70% hepatic metabolism and 30% renal excretion of the parent drug. The metabolism of tofacitinib is primarily mediated by CYP3A4 with minor contribution from CYP2C19. In a human radiolabeled study, more than 65% of the total circulating radioactivity was accounted for by unchanged tofacitinib, with the remaining 35% attributed to 8 metabolites, each accounting for less than 8% of total radioactivity. The pharmacologic activity of tofacitinib is attributed to the parent molecule.

#### **Pharmacokinetics in Rheumatoid Arthritis Patients**

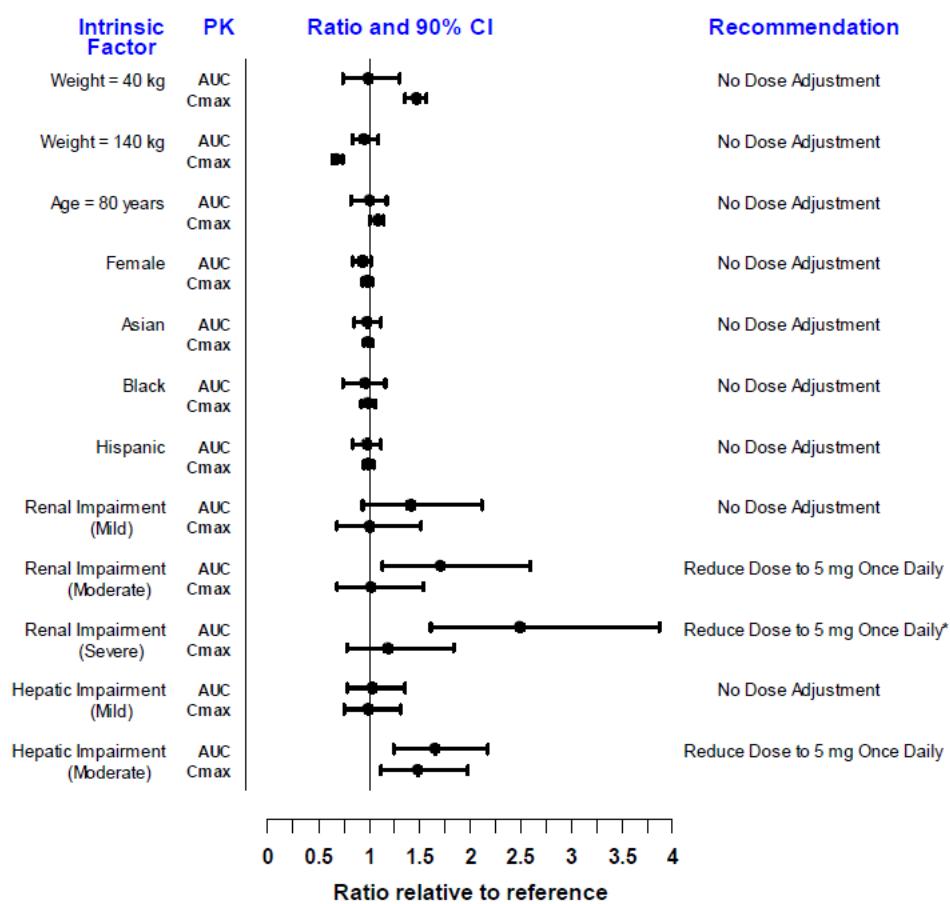
Population PK analysis in rheumatoid arthritis patients indicated no clinically relevant change in tofacitinib exposure, after accounting for differences in renal function (i.e. creatinine clearance) between patients, based on age, weight, gender and race (Figure 1). An approximately linear relationship between body weight and volume of distribution was

observed, resulting in higher peak (C<sub>max</sub>) and lower trough (C<sub>min</sub>) concentrations in lighter patients. However, this difference is not considered to be clinically relevant. The between-subject variability (% coefficient of variation) in AUC of tofacitinib is estimated to be approximately 27%.

## Specific Populations

The effect of renal and hepatic impairment and other intrinsic factors on the pharmacokinetics of tofacitinib is shown in Figure 1.

**Figure 1: Impact of Intrinsic Factors on Tofacitinib Pharmacokinetics**



\* Supplemental doses are not necessary in patients after dialysis

Reference values for weight, age, gender, and race comparisons are 70 kg, 55 years, male, and White, respectively; Reference groups for renal and hepatic impairment data are subjects with normal renal and hepatic function.

## 2.3.2 Drug Interactions

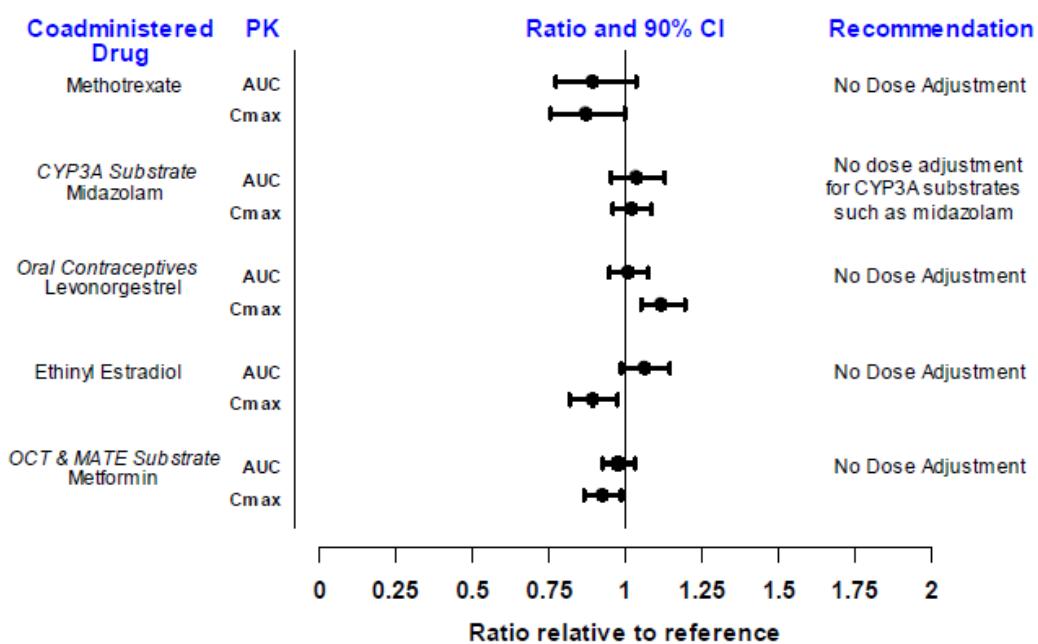
### Potential for XELJANZ to Influence the PK of Other Drugs

*In vitro* studies indicate that tofacitinib does not significantly inhibit or induce the activity of the major human drug-metabolizing CYPs (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4) at concentrations exceeding 185 times the steady state Cmax of a 5 mg twice daily dose. These *in vitro* results were confirmed by a human drug interaction study showing no changes in the PK of midazolam, a highly sensitive CYP3A4 substrate, when coadministered with XELJANZ.

In rheumatoid arthritis patients, the oral clearance of tofacitinib does not vary with time, indicating that tofacitinib does not normalize CYP enzyme activity in rheumatoid arthritis patients. Therefore, coadministration with XELJANZ is not expected to result in clinically relevant increases in the metabolism of CYP substrates in rheumatoid arthritis patients.

*In vitro* data indicate that the potential for tofacitinib to inhibit transporters such as P-glycoprotein, organic anionic or cationic transporters at therapeutic concentrations is low. Dosing recommendations for coadministered drugs following administration with XELJANZ are shown in Figure 2.

**Figure 2. Impact of XELJANZ on PK of Other Drugs**

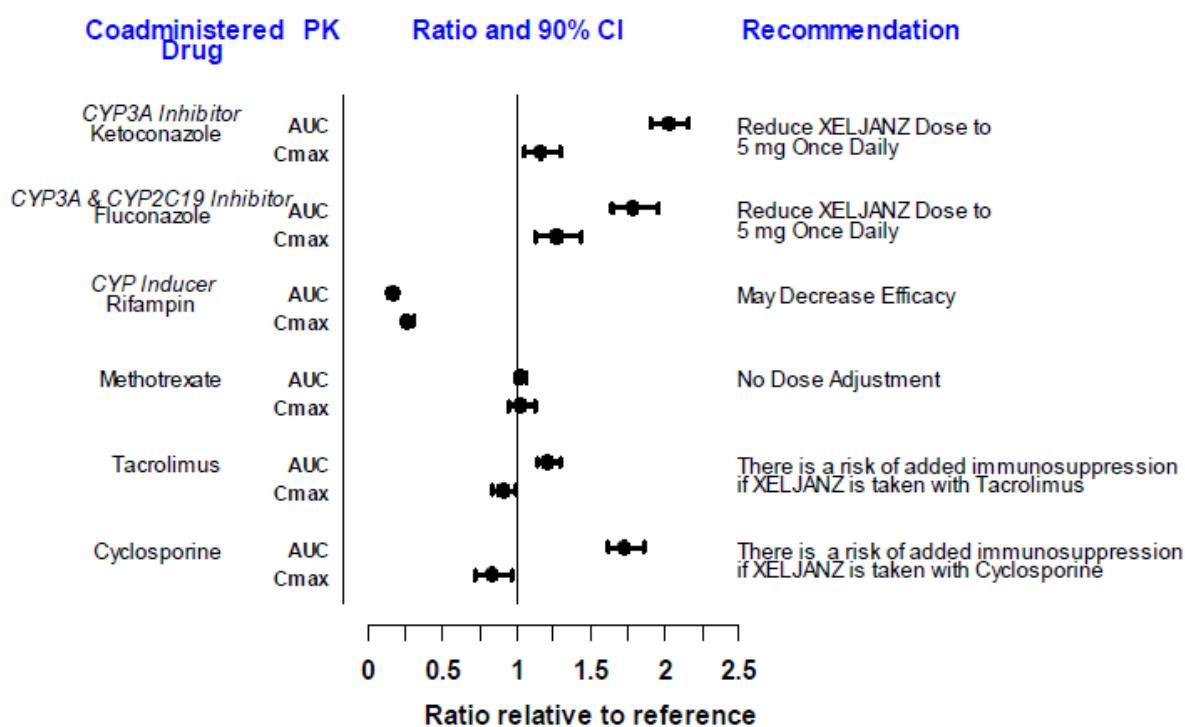


Note: Reference group is administration of concomitant medication alone; OCT = Organic Cationic Transporter; MATE = Multidrug and Toxic Compound Extrusion

### 2.3.3 Potential for Other Drugs to Influence the PK of Tofacitinib

Since tofacitinib is metabolized by CYP3A4, interaction with drugs that inhibit or induce CYP3A4 is likely. Inhibitors of CYP2C19 alone or P-glycoprotein are unlikely to substantially alter the PK of tofacitinib. Dosing recommendations for XELJANZ for administration with CYP inhibitors or inducers are shown in Figure 3.

**Figure 3. Impact of Other Drugs on PK of XELJANZ**



Note: Reference group is administration of tofacitinib alone

### 2.3.4 Overall safety of Tofacitinib

Safety analyses of pooled data of tofacitinib-treated patients show a similar serious infection rate to biologics of 2.7/100 patient-years with opportunistic infections including tuberculosis, fungal infections, and pneumocystis reported. Screening for latent tuberculosis is recommended. The frequency of serious infections was increased in patients over the age of 65. In long-term extensions, a slightly higher rate of infections and nonmelanoma skin cancers was seen in the 10-mg p.o. b.i.d. studies, and this dose was therefore not approved by the FDA. However, a higher incidence ratio (4.5) of herpes zoster was seen compared to other biologics (1.32).<sup>7</sup> There was no association with neutropenia, lymphopenia, or concomitant

nonbiologic DMARDs. No safety data are available on impact of tofacitinib on patients with chronic viral hepatitis reactivation, as these patients were excluded from the trials. Prophylactic vaccination for herpes zoster and hepatitis B should be updated prior to treatment whenever possible, and live virus vaccination during treatment should be avoided.

Malignancies including lymphoma have been reported in conjunction with treatment with tofacitinib, and Epstein-Barr virus lymphoproliferative disorders were seen in renal transplant studies.

GI perforations occurred in 10 of 5,043 tofacitinib-treated patients in analysis of pooled study data from ORAL trials with long-term extensions resulting in 0.144 events per 100 patient-years. Six of these cases were diverticular in nature, three were appendiceal perforations, and one an isolated gastric ulcer.

Laboratory-test monitoring should include initially a complete blood count (CBC), comprehensive metabolic panel (CMP), and fasting lipid panel. Minor decreases in absolute lymphocyte, neutrophil, and red blood cell counts were seen in less than 1% of patients.

Transaminitis was also reported, which usually responded to a reduction in dose or discontinuation of therapy with only 0.5% reaching three times the upper limit of normal on the 5-mg p.o. b.i.d. A dose-response change in creatinine (mean <0.1 mg/dl) was seen, which generally peaked at six weeks and then plateaued with a return to baseline four to six weeks after discontinuation. However, in long-term extensions, discontinuation of therapy only occurred due to renal insufficiency in 0.4% of patients in the 5-mg b.i.d. studies. The cause of this change is not understood. Mean LDL cholesterol increased by 15% and mean HDL increase by 10% with no change in LDL/HDL ratio. This effect was generally present within six weeks. A repeat fasting lipid panel is recommended at two to three months of therapy. Initial monitoring of CBC and CMP monthly for the first three months and then every two to three months thereafter would be prudent as this agent is used in larger, more heterogeneous patient populations.

### **3           OVERALL RISK/BENEFIT ASSESSMENT**

The efficacy of tofacitinib in alopecia areata is uncertain and determination of any activity in this disease is the objective of this study. The long term safety profile of tofacitinib is reassuring and confirms the potential for a favorable positive benefit/risk of tofacitinib in the treatment of alopecia areata.

There were no adverse effects reported in the one publication to date regarding tofacitinib in the treatment of alopecia universalis. 1 healthy patient with psoriasis and alopecia universalis was treated with tofacitinib for 8 months.

### 3.1 Current therapeutic options for Alopecia Areata

**There is no FDA approved drug for alopecia areata.** A recent Cochrane report (Delamere et al, 2008) concluded that there was no evidenced based support for any intervention in this disease. Standard of care remains observation for mild disease, and lesional/oral steroids for more advanced cases (Alkhailifah et al, 2010a,b). **In moderate-severe patch-type alopecia placebo response rates have been in the 6-12% range** (Price et al, 2008; Strober et al, 2009). Limited data is available for the response rate in AT/AU. Intralesional steroid responses are unpredictable and occur less frequently in subjects with higher disease burdens. This population that suffers from a disfiguring disease represents a significant unmet medical need (Alkhailifah et al, 2010a,b). Evidence-based and steroid-sparing interventions are needed.

Use of tofacitinib carries potential risks, including treatment-associated infection. **Alopecia areata patients as a whole are young (median age 23-30) and otherwise healthy, which should mitigate risk of adverse effects.** The target population in this proposed study is subjects with greater than 30% hair loss which represents unusually extensive disease, affecting fewer than 30% of all alopecia subjects, with disfiguring consequences and severe psychosocial morbidity. Although AA does not cause physical pain, several studies have noted that AA seriously impairs quality of life (QoL), mainly by altering self-perception and self-esteem, both of which interfere with social life. **Because AA is chronic, visible to others, and often difficult to treat, its impact on quality of life is underappreciated (Bickers et al, 2006; Hanan et al, 2008; Dubois et al, 2010)** and comparable to psoriasis (Bickers et al, 2006). Patient advocacy groups, including National Alopecia Areata Foundation have developed an active disease registry of over 10,000 subjects to document the impact on quality of life and encourage clinical investigation in this disease.

## 4 STUDY RATIONALE

Alopecia areata is a prevalent disease with high unmet need with significant psychosocial morbidity and no current evidence-based treatments. Preclinical studies have set the stage for evaluation of tofacitinib in alopecia areata. Tofacitinib is a Janus Kinase inhibitor that specifically targets JAK1/JAK3. JAKs are intracellular enzymes that conduct signals from inflammatory cytokines or growth factor interactions on cell membranes that influence hematopoiesis and immune cell function. After cytokines or growth factors bind their respective receptors, JAKs phosphorylate and activate Signal Transducers and Activators of Transcription (STATs), which modulate intracellular activity such as gene expression. Tofacitinib interferes the signaling pathway via JAK inhibition, preventing phosphorylation and activation of STATs. Inflammatory cytokines such as Interleukin (IL) 6, IL-2, and IL-12/IL-23 are dependent on Janus Kinases.

Treatment results with tofacitinib has shown dose-dependent reduction of circulating CD16/CD56 + natural killer cells (NKC), with a maximal reduction 8-10 weeks after treatment

initiation. These changes generally usually resolved within 2-6 weeks after discontinuation. Tofacitinib was also associated with dose-dependent increases in B cell counts.

Preclinical studies have set the stage for evaluation of targeted inhibition of the IFN- $\gamma$  pathway with Janus Kinase (JAK) inhibitors in alopecia areata. Importantly, ruxolitinib, a JAK1/2 inhibitor, reverses AA pathogenesis in the C3H-HeJ mouse model, preventing development of disease. Furthermore based on GWAS studies conducted by our collaborators<sup>1</sup> and an independent replication study we know that the IFN- $\gamma$  pathway is relevant in human AA. Based on this clear rationale and the relative safety of tofacitinib in this young, otherwise-healthy, motivated population, we propose the following RCT pilot study to evaluate tofacitinib as a therapeutic modality in AA.

To our knowledge, tofacitinib has never been examined in human AA except for a single case report of the treatment of a patient with alopecia universalis and psoriasis with tofacitinib. That patient reportedly regrew all scalp and body hair. We propose an open-label clinical trial to test the safety and efficacy of tofacitinib 5mg to 10mg orally, twice-daily dosing for up to 6 to 12 months, in the treatment of 15 subjects diagnosed with moderate to severe patch-type AA and/or AT/AU. This initial clinical experience with tofacitinib, if positive, would provide the basis for considering and designing subsequent larger and appropriately powered RCTs.

The study proposed here targets the identical moderate-severe AA patient population as two other preceding RCT studies that also evaluated therapeutic effects of immune biologics (alefacept and efalizumab) (Price et al, 2008; Strober et al, 2009). In addition, we propose an exploratory substudy in a small group of patients with AT/AU.

Since the initial writing of this protocol new preliminary findings have noted significant hair regrowth in patients who are treated with tofacitinib at a dose of 10mg PO BID. Based on previously a published report of successful treatment of alopecia areata with tofacitinib (JID June 2014. Killing Two Birds with One Stone: Oral Tofacitinib Reverses Alopecia Universalis in a Patient with Plaque Psoriasis. Brittany G. Craiglow, MD and Brett A. King, MD, PhD. Department of Dermatology, Yale University School of Medicine, New Haven, CT.) and subsequent unpublished information from Dr. King and other investigators using tofacitinib in the treatment of alopecia areata, it is evident that a longer course of treatment may be needed, for some patients to achieve full response. In addition, there have not been serious adverse effects noted in this study, or per the unpublished reports of other investigators who have used tofacitinib for extended periods in patients with alopecia areata. Therefore it appears that increasing the duration of treatment may be beneficial in some patients and is well tolerated.

**2. Patients with mild disease are excluded since natural remission is considerably more likely in the early stages of disease or in mild disease (involving less than 25% hair loss) where spontaneous successful regrowth has been reported in up to 50% of individuals<sup>34</sup>. In more extensive disease, spontaneous remissions are less likely and are usually restricted to incomplete hair regrowth involving a minority of scalp areas. Thus to minimize placebo response rates this study is specifically focused on subjects with moderate to severe**

**patch type alopecia areata with greater than 30% hair loss or alopecia totalis or universalis and disease duration greater than 3 months.**

We have adopted the following study plan to identify a treatment “signal” in alopecia areata:

The standard dosing schedule of tofacitinib 5mg orally twice daily, currently used in rheumatoid arthritis treatment, will also be used here to begin treatment of moderate to severe alopecia areata or AT/AU. This dose may be increased up to 10mg orally twice daily, if clinically indicated and at the investigator’s discretion, for a total treatment duration of up to 6 to 18 months.

## **5 STUDY OBJECTIVES**

### **5.1 Primary Objective**

The study’s *primary efficacy endpoint* will be the proportion of responders at the end of treatment, after up to 24 weeks/6 months to 72 weeks/18 months of treatment, defined as 50% or greater hair re-growth from baseline as assessed by SALT at week 24 to 72. This is a relatively strict definition for defining responders and non-responders and was chosen to minimize the potential for spontaneous remission, in which fewer than 10% are expected to achieve this magnitude of hair regrowth spontaneously.

The duration of treatment will be up to 6 to 18 months, at the discretion of the investigator, dependent on individual subject response as well as safety considerations.

To assess the durability of responses, patients who achieve 50% regrowth from baseline during the first 6 to 18 months, will continue to be followed for an additional 6 months off treatment or until it is determined that relapse has occurred.

### **5.2 Secondary Objectives**

As secondary endpoints, safety and additional efficacy outcomes will be assessed. Additional efficacy outcomes will be measured by changes in hair re-growth as a continuous variable as determined by physical exam and Canfield photography, as well as patient and physician global evaluation scores. To assess the durability of responses, patients who achieve 50% reduction in baseline SALT score will continue to be followed for an additional 6 months off treatment or until it is determined that relapse has occurred.

#### *Secondary efficacy outcomes in detail*

1. Percent hair regrowth from baseline determined by SALT measurements following end of treatment (at 24 to 72 weeks) and during the observational phase at 8, 16, and 24 weeks after end of treatment.

2. Comparison of the proportion of responders in the experimental group with the historically known placebo response rate (<10%), with response defined as 50% change in SALT score (50% regrowth) from baseline, at end of treatment (week 24 to 72), and at the end of study (8, 16, and 24 weeks after end of treatment).
3. Comparison of the proportion of subjects in each group attaining global overall improvement SALT score of A5 (100% coverage) supported by ≤ SALT 25 at the end of treatment (week 24 to 72), and at the end of study (8, 16, and 24 weeks after end of treatment).
4. Change in PGA (Physician Global Assessment) based on live evaluations and evaluation of standardized photographs between baseline, and each subsequent visit during the treatment period and the follow up period.
5. Change in patient global assessment between baseline, and each subsequent visit during the treatment period and the follow up period.
6. Change in patient quality of life assessment from baseline compared to subsequent visits during the treatment period and the follow up period.
7. Frequency of occurrence and timing of relapse (as defined above) in responders followed for 6 months off therapy.
8. Safety will be evaluated as a secondary endpoint using descriptive statistics to summarize the cumulative incidence and types of AEs.
9. The proportion of patients who discontinued treatment will be summarized.

## **6 ETHICAL CONSIDERATIONS**

### **6.1 Good Clinical Practice**

This study will be conducted in accordance with Good Clinical Practice (GCP), as defined by the International Conference on Harmonization (ICH) 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).

The study will be conducted in compliance with the protocol. The protocol, any amendments, and the subject informed consent will receive Institutional Review Board/Independent Ethics Committee (IRB) approval/favorable opinion before initiation of the study.

All potential serious breaches must be reported immediately. A serious breach is a breach of the conditions and principles of GCP in connection with the study or the protocol, which is likely to affect, to a significant degree, the safety or physical or mental integrity of the subjects of the study or the scientific value of the study.

Study personnel involved in conducting this study will be qualified by education, training, and experience to perform their respective tasks. This study will not use the services of study personnel where sanctions have been invoked or where there has been scientific misconduct or fraud (e.g., loss of medical licensure; debarment). Systems with procedures that ensure the quality of every aspect of the study will be implemented.

## **6.2      Institutional Review Board/Independent Ethics Committee**

Before study initiation, the investigator must have written and dated approval/favorable opinion from the IRB for the protocol, consent form, subject recruitment materials/process (e.g., advertisements), and any other written information to be provided to subjects. The investigator should also provide the IRB with a copy of the Investigator Brochure or product labeling, information to be provided to subjects, and any updates. The investigator should provide the IRB with reports, updates, and other information (e.g., expedited safety reports, amendments, and administrative letters) according to regulatory requirements or institution procedures.

## **6.3      Informed Consent**

Investigators must ensure that subjects are clearly and fully informed about the purpose, potential risks, and other critical issues regarding clinical studies in which they volunteer to participate. Freely given written informed consent must be obtained from every subject before clinical study participation, including informed consent for any screening procedures conducted to establish subject eligibility for the study.

The rights, safety, and well-being of the study subjects are the most important considerations and should prevail over interests of science and society.

# **7            INVESTIGATIONAL PLAN**

## **7.1      Study Design and Duration**

**Screening:** A complete medical history will be taken. Patients will be screened for pre-existing renal (basic metabolic and urine analysis), hematologic (CBC with differential and reticulocytes) or hepatic dysfunction; lipid panel; prior exposure to tuberculosis (PPD and or quantiferon test) or hepatitis; pregnancy tests will be done in women of childbearing potential.

**Assessments:** Baseline assessments of disease severity will be done. This will include SALT score (Severity of Alopecia Tool, Addendum 1), Physician global assessment (PGA), patient global assessment and patient quality of life assessment. In order to minimize or eliminate inter-rater variability, every effort will be made to have the same investigator evaluate an individual subject at each visit. Whenever possible the same investigator (the PI or her designee) will examine the patient's scalp, determine the SALT score, and grade the photographic images.

### **Visit Schedule and Assessments**

Screening assessments and all scheduled study visits are outlined in the Table of Study Assessments, Addendum 2.

#### **Baseline to Week 24 (period on study medication)**

Patients will be seen at baseline, and at weeks 4, 8, 12, 16, 20 and 24 weeks. Assessments of disease severity will be performed via physical exam and photography and will be objectively quantified using the SALT, Physician global assessment (PGA) at baseline, and weeks 4, 8, 12, 16, 20, and 24, and patient global assessment and patient quality of life assessment at baseline and weeks 12 and 24.

PGA and patient evaluations will be based on similar 100mm visual analog scales with 0 labeled as no scalp hair loss and 100 labeled as complete scalp hair loss. Patients and physicians will place a vertical mark along the horizontal line depending on their assessment of the degree of hair loss present at the time of assessment.

Patient quality of life assessments will be based on changes in the Dermatology Life Quality Index DLQI, which we have previously used for patient evaluation in the AA registry, and the Skindex-29 Questionnaire.

Photography will be performed at baseline and weeks 4, 8, 12, 16, 20, and 24; skin biopsies will be performed at baseline and weeks 4 and 24; blood draws for safety parameters and immune studies will be done based on the attached table of study procedures.

Optional additional biopsies and blood draws may be obtained at the investigator's discretion and with the subject's permission as indicated by clinical considerations such as variation in the timeline to regrowth from one subject to another, focal apparent resistance to treatment, as well as other potential unpredictable clinical occurrences. Unscheduled biopsies and blood draws will ensure that tissue and blood samples can be obtained at optimal time points to evaluate the changes occurring in response (or lack of response) to treatment. The patient will be given the option to agree to, or decline, the additional biopsies and blood draws.

Adverse events will be assessed at every visit.

#### Week 28 to 72 (Optional treatment extension)

The treatment duration may be extended by up to 12 additional months beyond the initially scheduled 6 months of treatment, if clinically indicated and at the discretion of the investigator. Patients will continue to be seen monthly while on extended tofacitinib treatment. Assessments of disease severity will continue to be performed via physical exam and photography, and will be objectively quantified using the SALT and Physician global assessment (PGA). Patient global assessment and patient quality of life assessment will also be completed.

PGA and patient evaluations will be based on similar 100mm visual analog scales with 0 labeled as no scalp hair loss and 100 labeled as complete scalp hair loss. Patients and physicians will place a vertical mark along the horizontal line depending on their assessment of the degree of hair loss present at the time of assessment.

Patient quality of life assessments will be based on changes in the Dermatology Life Quality Index DLQI, which we have previously used for patient evaluation in the AA registry, and the Skindex-29 Questionnaire.

Photography will be performed at all visits.

Optional additional biopsies and blood draws may be obtained at the investigator's discretion and with the subject's permission as indicated by clinical considerations such as variation in the timeline to regrowth from one subject to another, focal apparent resistance to treatment, as well as other potential unpredictable clinical occurrences. Unscheduled biopsies and blood draws will ensure that tissue and blood samples can be obtained at optimal time points to evaluate the changes occurring in response (or lack of response) to treatment. The patient will be given the option to agree to, or decline, the additional biopsies and blood draws.

Adverse events will be assessed at every visit.

#### 8, 16, and 24 weeks after end of treatment (follow-up period off study medication)

All patients will be seen at 8, 16, and 24 weeks after completion of study drug, to determine durability of response or evidence for a delayed effect. Importantly patients who achieved  $\geq 50\%$  regrowth as measured by SALT score (responders) will be seen at 8, 16, and 24 weeks after end of study treatment in order to assess the frequency and timing of relapse or to capture the occurrence of further regrowth.

Relapse will be defined as any recurrence of hair loss in responders (subjects achieving greater than 50% decrease in SALT score from baseline during the first 6 to 18 months of the study) who had achieved stable regrowth without continued loss for at least 2 months; and loss of  $\geq 25\%$  of regrowth in patients who had achieved 50% regrowth during treatment but still had chronic low grade hair loss.

Nonresponders will also be offered the option to continue follow-up in order to assess for delayed response. Efficacy evaluations: SALT, PGA, Patient global assessment and patient quality of life assessment will be performed.

Blood draws for safety parameters will be conducted at 8 weeks after end of treatment if deemed necessary to assess normalization of any previously abnormal values.

Adverse events will be assessed at every visit.

Standardized photographs of the subject's scalp will be taken at every visit; blood draws for safety parameters and immune studies will be done based on the attached table of study procedures. Photographs will be used to support determination of percent hair regrowth. Photography will be performed using a high resolution digital camera with Intellistudio system from Canfield Scientific, Inc., Patients will be positioned using a laser guide, after which photographs of the entire scalp will be taken at standardized locations and at a fixed distance. Close-up photographs may also be taken using an epiluminescent attachment in order to assess for the presence of early regrowth.

#### Unscheduled Visits

An unscheduled visit can occur at any time during the study for instance as prompted by signs of infection or local injection site reactions. A source document must be maintained for these unscheduled visits. The date for the visit and any data generated must be recorded on the

appropriate CRF. At treatment discontinuation/early termination subjects will undergo end of study evaluations per the Schedule of Assessments.

#### Telephone contact/retention

Patients will be contacted on a monthly basis, between the planned study visits at 8, 16, and 24 weeks, in the observational phase of the study to ascertain the occurrence of adverse events as well as to assess the status of hair growth or loss.

Any concerns or questions the patient may have will also be addressed. If deemed necessary, the subject may be asked to come in for an unscheduled visit.

#### Duration of Treatment

After the screening period, subjects will begin tofacitinib 5mg twice-daily and will continue treatment for 6 months. The treatment may be extended by up to 12 additional months if clinically indicated at the discretion of the investigator.

The 18-month treatment period is expected to provide adequate time to assess the short-term efficacy and safety of tofacitinib in patients with moderate to severe AA, AT or AU. Responders will then be followed for 6 months off drug.

#### Study Population

For entry into the study, the following criteria MUST be met. Any exceptions must be approved by the Principal Investigator and/or IRB before enrollment.

## **7.2 Inclusion Criteria**

### **1) Signed Written Informed Consent**

Before any study procedures are performed, subjects will have the details of the study described to them, and they will be given a written informed consent document to read. Subjects will have the opportunity to ask questions. They may also take the Informed Consent document home for discussion with family or friends. If subjects consent to participate in the study, they will indicate that consent by signing and dating the informed consent document in the presence of study personnel. A copy of the signed informed consent will be given to the subject.

- 2) Men and women between the ages of 18 and 65 years of age.
- 3) Patients with a diagnosis of moderate to severe patch type alopecia areata
- 4) Patients with alopecia totalis or universalis may be enrolled
- 5) Patients will have >30% and <95% total scalp hair loss at baseline as measured using the SALT score to qualify as moderate to severe patch type AA; and 95-100% scalp hair loss to qualify as alopecia totalis or universalis
- 6) Duration of hair loss greater than 3 months without an upper limit of duration as long as there is reason to believe that regrowth is possible in the opinion of the investigator.
- 7) No evidence of significant active ongoing regrowth present at baseline.

- 8) Patients with a history of alopecia totalis/universalis can be included as long as the current episode of hair loss meets the criteria of 30 to 95% hair loss (i.e. they are not currently AT or AU), and as long as in the opinion of the investigator there does appear to be potential for regrowth. Patients with current episodes of alopecia totalis/universalis may be included in this study.
- 9) Vaccinations should be up to date in agreement with current immunization guidelines prior to start of tofacitinib. The patient will be asked to obtain verbal verification from their primary care provider that this is the case.
- 10) Patients may be naïve to treatment or unresponsive to intralesional (IL) steroids or other treatments for alopecia areata.
- 11) Women of childbearing potential (WOCBP) must use highly effective methods of birth control [for at least 12 weeks after the last dose of investigational product] to minimize the risk of pregnancy. WOCBP must follow instructions for birth control for the entire duration of the study including a minimum of 90 days after dosing has been completed.

Acceptable methods of highly effective birth control include:

- Condom with spermicide
- Diaphragm and spermicide
- Cervical cap and spermicide

The use of intrauterine devices, (IUDs) shall be at the discretion of the investigator.

- a) Women must have a negative serum or urine pregnancy test (minimum sensitivity 25 IU/L or equivalent units of HCG) within 24 hours prior to the start of investigational product.
- b) Women must not be breastfeeding

## 7.3 Exclusion Criteria

### 1) Sex and Reproductive Status

- a) WOCBP who are **unwilling or unable** to use an acceptable method to avoid pregnancy for the entire study period and for up to 12 weeks after the last dose of study drug.
- b) WOCBP using a prohibited contraceptive method.
- c) Women who are pregnant or breastfeeding.
- d) Women with a positive pregnancy test on enrollment or before administration of tofacitinib.
- e) Sexually active fertile men not using effective birth control if their partners are WOCBP.

### 2) Target Disease Exceptions

- a) Patients with a history of or active skin disease on the scalp such as psoriasis or seborrheic dermatitis.
- b) Patients in whom the diagnosis of alopecia areata is in question or in whom the pattern of hair loss is such that quantification of hair loss and assessment of regrowth is difficult.

E.g. patients with diffuse alopecia areata. This assessment is at the investigator's discretion.

- c) Patients with active medical conditions or malignancies (except adequately treated basal or squamous cell carcinoma) that in the opinion of the investigator would increase the risks associated with study participation, including patients with a history of recurrent infections.
- d) Patients with hemoglobin levels <9 g/dL, lymphocyte count <500 cells/mm<sup>3</sup>, absolute neutrophil count (ANC) < 1000 cells/mm<sup>3</sup>, at baseline.
- e) Patients known to be HIV or hepatitis B or C positive.
- f) Patients with history or evidence of moderate or severe hepatic and/or renal impairment.
- g) Patients with history of immunosuppression or history of recurrent serious infections.

### 3) Coexisting disease or concurrent medications

- a) Patients taking potent inhibitors of Cytochrome P450 3A4 (e.g. ketoconazole)
- b) Patients receiving one or more concomitant medications that result in both moderate inhibition of CYP3A4 and potent inhibition of CYP2C19 (e.g., fluconazole).
- c) Patients known to be HIV or hepatitis B or C positive.
- d) Patients with evidence of infection or active/untreated skin cancer.
- e) Patients who have been treated with intralesional steroids, systemic steroids, anthralin, squaric acid, DPCP (diphenylcycloprophenone), protopic, minoxidil or other medication which in the opinion of the investigator may affect hair regrowth within one month of the baseline visit.
- f) Patients who are impaired, incapacitated, or incapable of completing study-related assessments.
- g) Patients with current symptoms of severe, progressive, or uncontrolled renal, hepatic, hematologic, gastrointestinal, pulmonary, cardiac, neurologic, or cerebral disease, which, in the opinion of the investigator, might place a subject at unacceptable risk for participation in the study.
- h) Female patients who have had a breast cancer screening that is suspicious for malignancy and in whom the possibility of malignancy cannot be reasonably excluded by additional clinical, laboratory, or other diagnostic evaluations.
- i) Patients with a history of cancer in the last 5 years, other than non-melanoma skin cell cancers cured by local resection or carcinoma in situ. Existing non-melanoma skin cell cancers should be removed, the lesion site healed, and residual cancer ruled out before administration of the study drug.
- j) Patients who currently abuse drugs or alcohol.
- k) Patients with evidence (as assessed by the investigator) of active or latent bacterial or viral infections at the time of potential enrollment, including subjects with evidence of human immunodeficiency virus (HIV) detected during screening.
- l) Patients with herpes zoster or cytomegalovirus (CMV) that resolved less than 2 months before the informed consent document was signed.

- m) Patients who have received any live vaccines within 3 months of the anticipated first dose of study medication. Subjects may not receive live vaccine concurrently with tofacitinib.
- n) Patients with a history or symptoms suggestive of gastrointestinal perforation or disorders that might increase the risk of GI perforation such as gastric ulcers or diverticulitis
- o) Patients who take NSAIDs at high dose or on a frequent basis, which in the investigator's opinion might increase the risks of gastrointestinal perforation
- p) Patients with any serious bacterial infection within the last 3 months, unless treated and resolved with antibiotics, or any chronic bacterial infection (e.g., chronic pyelonephritis, osteomyelitis, or bronchiectasis).
- q) Patients at risk for tuberculosis (TB). Specifically excluded from this study will be subjects with a history of active TB within the last 3 years, even if it was treated; a history of active TB greater than 3 years ago, unless there is documentation that the prior anti-TB treatment was appropriate in duration and type; current clinical, radiographic, or laboratory evidence of active TB; and latent TB that was not successfully treated ( $\geq 4$  weeks).

#### 4) Physical and Laboratory Test Findings

- a) Patients must not be positive for HIV, Hepatitis B or C.
- b) Patients who are positive for hepatitis C antibody if the presence of hepatitis C virus was also shown with polymerase chain reaction or recombinant immunoblot assay.
- c) Patients with any of the following laboratory values
  - i) Hemoglobin  $<9.0$  g/dL
  - ii) Lymphocytes  $<500/\text{mm}^3$
  - iii) ANC  $<1000 \text{ cells/mm}^3$
  - iv) Serum creatinine  $>2$  times the ULN
  - v) Serum ALT or AST  $>2$  times the ULN

Any other laboratory test results that, in the opinion of the investigator, might place a subject at unacceptable risk for participation in the study.

#### 7.4 Prohibited Treatments and/or Therapies

- a) Patients who have at any time received treatment with any investigational drug within 28 days (or less than 5 terminal half-lives of elimination) of the Day 1 dose.
- b) Any concomitant Disease Modifying Anti-Rheumatic Drugs (DMARDs) or immunosuppressants, such as anakinra, methotrexate, adalimumab, etanercept, azathioprine, cyclosporine, corticosteroids, etc.
- c) Patients who have been treated with intralesional steroids, systemic steroids, anthralin, squaric acid, DPCP (diphenylcycloprophenone), protopic, minoxidil or other medication which in the opinion of the investigator may affect hair regrowth within one month of the baseline visit.

- d) Patients receiving potent inhibitors of Cytochrome P450 3A4 (CYP3A4) (e.g., ketoconazole)
- e) Patients receiving one or more concomitant medications that result in both moderate inhibition of CYP3A4 and potent inhibition of CYP2C19 (e.g., fluconazole).

Eligibility criteria for this study have been carefully considered to ensure the safety of the study patients and to ensure that the results of the study can be used. It is imperative that patients fully meet all eligibility criteria.

## **7.5 Women of Childbearing Potential**

Women of childbearing potential (WOCBP) include any female who has experienced menarche and who has not undergone successful surgical sterilization (hysterectomy, bilateral tubal ligation, or bilateral oophorectomy), and who is not postmenopausal. Post menopause is defined as:

- Amenorrhea  $\geq$  12 consecutive months without another cause, and a documented serum follicle stimulating hormone (FSH) level  $> 35$  mIU/mL or
- Women with irregular menstrual periods and a documented serum follicle stimulating hormone (FSH) level  $> 35$  mIU/mL or

NOTE: FSH level testing is not required for women  $\geq$  62 years old with amenorrhea of  $\geq$  1 year

- Women on hormone replacement therapy (HRT)

Women who are using oral or other hormonal contraceptives, such as vaginal products, skin patches, or implanted or injectable products, or mechanical products, such as an intrauterine device or barrier methods (diaphragm, condoms, spermicides), to prevent pregnancy or who are practicing abstinence or who have a sterile (e.g., vasectomy) partner should be considered to be of childbearing potential.

## **7.6 Concomitant Treatments**

### **7.6.1 Prohibited and/or Restricted Treatments**

Any medication known to affect hair growth in alopecia areata including but not limited to topical, intralesional or systemic steroids, squaric acid, anthralin, protopic, minoxidil, diphenylcyclopropenone, cyclosporine and any other medications, which in the judgment of the investigator, may affect hair growth in patients with alopecia areata.

Any medications known to be potent inhibitors of cytochrome P450 3A4 (CYP3A4) (e.g., ketoconazole), or one or more concomitant medications that result in both moderate inhibition of CYP3A4 and potent inhibition of CYP2C19 (e.g., fluconazole), due to loss or reduced clinical response to tofacitinib.

Any immunosuppressive drugs (e.g., azathioprine, tacrolimus, cyclosporine), that can potentiate risk for immunosuppression.

### **7.6.2 Other Restrictions and Precautions**

Not applicable.

## **7.7 Discontinuation of Subjects from Treatment**

Subjects MUST discontinue study treatment and withdraw from the study for any of the following reasons:

- Withdrawal of informed consent (subject's decision to withdraw for any reason)
- Any clinical adverse event (AE), laboratory abnormality, or intercurrent illness which, in the opinion of the investigator, indicates that continued participation in the study is not in the best interest of the subject
- Pregnancy
  - Instruct WOCBP to contact the investigator or study staff immediately if they suspect they might be pregnant (e.g., missed or late menstrual period) at any time during study participation. Institutional policy and local regulations should determine the frequency of on-study pregnancy tests for WOCBP enrolled in the study.
- Loss of ability to freely provide consent through imprisonment or involuntarily incarceration for treatment of either a psychiatric or physical (e.g., infectious disease) illness.

## **8 TREATMENTS**

### **8.1 Study Treatment: Tofacitinib**

An investigational product, also known as investigational medicinal product in some regions, is defined as follows: A pharmaceutical form of an active substance or placebo being tested or used as a reference in a clinical study, including products already with a marketing authorization but used or assembled (formulated or packaged) in a way different from the authorized form, or used for an unauthorized indication, or when used to gain further information about the authorized form. In this protocol, the investigational product is tofacitinib.

Other medications used in the study as support or escape medication for preventative, diagnostic, or therapeutic reasons as components of a given standard of care are considered non-investigational products.

#### **8.1.1 Identification**

Tofacitinib is provided as 5 mg tofacitinib (equivalent to 8 mg tofacitinib citrate) tablets: White, round, immediate-release film-coated tablets, debossed with "Pfizer" on one side, and "JKI 5" on the other side.

### **8.1.2 Storage, Handling, and Dispensing**

The investigational product should be stored in a secure area according to local regulations. The investigator is responsible for ensuring that it is dispensed only to study subjects and only from official study sites by authorized personnel, as dictated by local regulations.

All investigational product supplies that will be used in the study must be maintained securely under the direct responsibility of the investigator or delegated by the investigator to the hospital pharmacist, or other personnel licensed to store and dispense drugs. All drugs shall be dispensed in accordance with the investigator's responsibility to ensure that an accurate record of drugs issued and returned is maintained.

The investigator is responsible for ensuring that the investigational product is stored under the appropriate environmental conditions (temperature, light, and humidity), as determined by the sponsor and defined by the Investigator Brochure or SmPC/ reference label. If concerns regarding the quality or appearance of the investigational product arise, do not dispense the investigational product and contact the sponsor immediately. Refer to the Investigator Brochure for additional information regarding handling, preparation, and storage of tofacitinib.

### **8.1.3 Additional Information for the Handling, Dispensing, and Storage of Tofacitinib**

White, round, immediate-release film-coated tablets, debossed with "Pfizer" on one side, and "JKI 5" on the other side, and available in:

Bottles of 28:	NDC 0069-1001-03
Bottles of 60:	NDC 0069-1001-01
Bottles of 180:	NDC 0069-1001-02

#### **Storage and Handling**

Store at 20°C to 25°C (68°F to 77°F). [See USP Controlled Room Temperature].  
Do not repackage.

### **8.2 Selection and Timing of Dose for Each Subject**

Patients will be treated with tofacitinib 5 mg to 10mg twice-daily. Treatment will be continued for up to 6 to 18 months to provide adequate time to assess the short-term efficacy and safety of tofacitinib in patients with alopecia areata. Study drug dose may be modified or held at the discretion of the investigator. Patients will then be followed for an additional 6 months after completion of treatment to assess the timing and incidence of relapse or to monitor for delayed or continued response after end of treatment.

### **8.2.1 Dose Modifications for Adverse Events**

If there is evidence of toxicity, as determined by laboratory tests or by clinical assessment that could place the subject at increased risk in the judgment of the investigator, administration of tofacitinib will be decreased or interrupted. Subjects may be considered eligible to continue with tofacitinib treatment only if full resolution of the adverse event is documented.

## **8.3 Concomitant Treatments**

### **8.3.1 Prohibited and/or Restricted Treatments**

The following medications are prohibited throughout the complete study period:

- Any medications known to affect hair growth in alopecia areata including but not limited to topical, intralesional or systemic steroids, anthralin, squaric acid, diphenylcyclopropenone, protopic, cyclosporine or any other medications which in the opinion of the investigator may affect hair growth in AA.
- Live vaccines.
- Use of any investigational drug other than study medication.
- Corticosteroid use at unstable dose and/or superior to the equivalent of prednisone 10 mg/day is not permitted.

### **8.3.2 Other Restrictions and Precautions**

## **8.4 Immunizations**

There is limited information available regarding the effectiveness of immunizations in non-human primates and humans that have been treated with tofacitinib. Limited data are available on the effect of therapeutic vaccinations in subjects receiving tofacitinib.

Due to the risk of infection, vaccination of subjects with any live vaccine is absolutely contraindicated during the treatment phase of the study (that is, at any time after entry into the induction period), as is the administration of LIVE oral polio vaccine to household contacts. The Centers for Disease Control and Prevention Advisory Committee on Immunization Practices (CDC-ACIP) recommends that subjects should not be administered a live virus vaccination for at least 3 months after discontinuing high-dose corticosteroid therapy (defined as more than 20 mg of prednisone per day for more than 2 weeks). In view of the long half-life of tofacitinib, study subjects should not be administered a live virus vaccine for a minimum of 3 months following the last dose of tofacitinib.

## **8.5 Treatment Compliance**

Compliance will be documented via the use of patient diaries to record the date and time of self-administration of medication at home. Study drug reconciliation will be performed at each visit during the treatment period.

## **9 STUDY ASSESSMENTS AND PROCEDURES**

### **9.1 Time and Events Schedule**

The Time and Events Schedule (Section 6, Table 3) summarizes the frequency and timing of various measurements.

#### Pre-screening procedures

Written informed consent will be obtained for this study by the principal investigator or his/her designee from all patients prior to any protocol-specific procedures. The study will be conducted in accordance with the Food and Drug Administration (FDA) approved revision of the Declaration of Helsinki, current FDA regulations, and International Conference on Harmonization (ICH) guidelines.

## Procedures Performed Prior to Treatment

### Screening (Day -28 to -1):

The following screening evaluations will be performed within 4 weeks prior to enrollment:

- Informed consent, inclusion/exclusion criteria, prior concomitant medications
- Medical history, physical examination, including a dermatological exam
- Vital signs, body weight
- Assessments of disease severity (amount of hair loss present) will be conducted including SALT (Severity of Alopecia Tool) and Physician's global assessment (PGA)
- Clinical laboratory evaluation (complete blood count, basic metabolic profile, hepatic panel and urinalysis), hepatitis B and C screening panel, HIV test, fasting lipid profile, serum pregnancy test for women of child bearing potential.
- Tuberculosis testing (PPD skin test or quantiferon test)

A visit will be scheduled 48-72 hours after placement of the PPD for reading of the test results. If the subject is unable to return for PPD, the results may be interpreted and documented by a qualified medical professional. If possible, the PPD reading visit may be combined with the baseline visit. The results of all assessments/tests listed above must be reviewed prior to enrollment to ensure that the patient meets entry criteria and that no exclusion criteria are present.

### Baseline (Day 0) Procedures Performed Prior to treatment

- Review of inclusion/exclusion criteria
- Vital signs, body weight
- Adverse events reporting, concomitant medication review
- Photography
- Physician evaluation utilizing the PGA and SALT scale
- Patient global assessment and quality of life assessment
- Urine pregnancy testing for WOCBP
- Blood collection for immunological studies
- Scalp biopsy (4mm punch biopsy)

### Weeks 4, 8, 12, 16, and 20 (Days 28, 56, 84, 112, and 140)

Visit days will have an acceptable window of  $\pm 3$  days.

- Vital signs, body weight
- Adverse events reporting, concomitant medication review
- Photography
- Physician evaluation utilizing the PGA and SALT scale
- Patient global assessment and quality of life assessment (week 12)
- Urine pregnancy testing for WOCBP
- Clinical laboratory evaluation (weeks 4, 8, 12, 16, and 20 weeks and as otherwise deemed necessary at the discretion of the investigator to monitor for abnormal values and for normalization of those values). In general, labs will be drawn ranging from weekly to every 4 weeks, depending on whether they are in or out of range, and to

monitor for resolution of abnormalities. Fasting lipid profiles will be repeated at months 1 and 2, end of treatment and as needed. Scalp biopsy (4mm punch biopsy) (week 4)

- Blood collection for immunological studies (week 4)
- Optional Scalp biopsy (4mm punch biopsy) in addition to weeks 4 and 24. Optional additional biopsies and blood draws may be obtained at the investigator's discretion and with the subject's permission as indicated by clinical considerations such as variation in the timeline to regrowth from one subject to another, focal apparent resistance to treatment, as well as other potential unpredictable clinical occurrences. Unscheduled biopsies and blood draws will ensure that tissue and blood samples can be obtained at optimal time-points to evaluate the changes occurring in response (or lack of response) to treatment. The patient will be given the option to agree to, or decline, the additional biopsies and blood draws.
- Optional Blood collection for immunological studies, at the discretion of the investigator as outlined above.

#### Week 24 (day 168±7) – End of Treatment

- Vital signs, body weight
- Physical and dermatological examination
- Adverse events reporting, concomitant medication review
- Photography
- Physician evaluation utilizing the PGA and SALT scale
- Patient global assessment and quality of life assessment
- Urine pregnancy testing for WOCBP
- Clinical laboratory evaluation
- Scalp biopsy (4mm punch biopsy)
- Blood collection for immunological studies

#### Weeks 28, 32, 36, 40, 44, 48, 52, 56, 60, 64, 68, and 72 (Optional Treatment Extension)

The treatment duration may be extended up to 12 additional months if clinically indicated at the discretion of the investigator. Visit days will have an acceptable window of ±7 days.

- Vital signs, body weight
- Adverse events reporting, concomitant medication review
- Photography
- Physician evaluation utilizing the PGA and SALT scale
- Patient global assessment and quality of life assessment
- Urine pregnancy testing for WOCBP
- Clinical laboratory evaluation (as scheduled and as otherwise deemed necessary at the discretion of the investigator to monitor for abnormal values and for normalization of those values).
- Optional Scalp biopsy (4mm punch biopsy). Optional additional biopsies and blood draws may be obtained at the investigator's discretion and with the subject's permission as indicated by clinical considerations such as variation in the timeline to regrowth from one subject to another, focal apparent resistance to treatment, as well as other potential

unpredictable clinical occurrences. Unscheduled biopsies and blood draws will ensure that tissue and blood samples can be obtained at optimal time-points to evaluate the changes occurring in response (or lack of response) to treatment. The patient will be given the option to agree to, or decline, the additional biopsies and blood draws.

- Optional Blood collection for immunological studies, at the discretion of the investigator as outlined above.

#### 8, 16, and 24 Weeks after End of Treatment – Observational Period

- Vital signs, body weight
- Physical and dermatological examination (only at End of Study)
- Adverse events reporting, concomitant medication review
- Photography
- Physician evaluation utilizing the PGA and SALT scale
- Patient global assessment and quality of life assessment (weeks 32 and 48)
- Clinical laboratory evaluation at 8 weeks after End of Treatment for safety parameters will be conducted if deemed necessary to assess for normalization of any previously abnormal values
- Optional Scalp biopsy as indicated
- Optional Blood collection as indicated

#### Early Termination

- Vital signs, body weight
- Physical and dermatological examination
- Adverse events reporting, concomitant medication review
- Photography
- Physician evaluation utilizing the PGA and SALT scale
- Patient global assessment and quality of life assessment
- Clinical laboratory evaluation if indicated
- Optional scalp biopsy and blood collection for immunological studies
- The reason for early withdrawal/study drug discontinuation must be documented

#### Unscheduled Visits

An unscheduled visit can occur at any time during the study. A source document must be maintained for these unscheduled visits. The date for the visit and any data generated must be recorded on the appropriate CRF. At treatment discontinuation/early termination subjects will undergo study evaluations per the Schedule of Assessments.

#### Duration of Treatment

Treatment will be continued for up to 6 to 18 months to provide adequate time to assess the short-term efficacy and safety of tofacitinib in patients with alopecia areata. Patients will then be followed for an additional 6 months to assess the timing and incidence of relapse of AA.

## **9.2 Study Completion or Early Discontinuation Visit**

At the time of study early withdrawal, the reason for early withdrawal and any new or continuing adverse events should be documented.

### **9.2.1 Study Drug Discontinuation**

If study drug administration is discontinued, the reason for discontinuation will be recorded.

**Table 3: Schedule of Assessments**

Visit	1 Screening	2 Base- line	3 Week 4	4 Week 8	5 Week 12	6 Week 16	7 Week 20	8 Week 24	± Weeks 28, 32, 36, 42, 44, 48, 52, 56, 60, 64, 68, 72	8W þ End of Tx	16W þ End of Tx	24W þ End of Tx
Day	-28 to -1	0	28±3	56±3	84±3	112 ±3	140 ±3	168 ±7				
Informed Consent	X											
Inclusion/Exclusion Criteria	X	X										
Medical History	X											
Body Weight	X	X	X	X	X	X	X	X	X	X	X	X
Physical Exam	X							X				X
Vital Signs	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant Medications	X	X	X	X	X	X	X	X	X	X	X	X
Adverse events		X	X	X	X	X	X	X	X	X	X	X
Photography		X	X	X	X	X	X	X	X	X	X	X
SALT evaluation	X	X	X	X	X	X	X	X	X	X	X	X
Physician global assessment	X	X	X	X	X	X	X	X	X	X	X	X
Patient quality of life assessment, – DLQI & Skindex-29		X			X			X	X <sup>c</sup>	X		X
Patient global assessment		X			X			X	X <sup>c</sup>	X		X
Clinical laboratory evaluation*	X		X	X	X	X	X	X	X	X <sup>a</sup>		
Hepatitis & HIV testing	X											
Fasting Lipid Profile	X		X	X				X	X <sup>c</sup>			
PPD test and reading or quantiferon testing	X											
Serum pregnancy test	X											
Urine pregnancy test		X	X	X	X	X	X	X	X			
Scalp Biopsy		X	X	X <sup>b</sup>	X <sup>b</sup>	X <sup>b</sup>	X <sup>b</sup>	X	X <sup>b</sup>	X <sup>b</sup>	X <sup>b</sup>	X <sup>b</sup>
Phlebotomy for immunological studies		X	X	X <sup>b</sup>	X <sup>b</sup>	X <sup>b</sup>	X <sup>b</sup>	X	X <sup>b</sup>	X <sup>b</sup>	X <sup>b</sup>	X <sup>b</sup>

<sup>a</sup>Clinical laboratory evaluation consists of CBC w/diff, BMP, hepatic panel, fasting lipid panel and urinalysis. Additional evaluations may be performed (including after the end of treatment visit) if needed to monitor or to verify normalization of previously abnormal lab values.

<sup>b</sup>Optional additional biopsies and blood draws may be obtained at the investigator's discretion and with the subject's permission as indicated by clinical considerations such as variation in the timeline to regrowth from one subject to another, focal apparent resistance to treatment, as well as other potential unpredictable clinical occurrences.

Unscheduled biopsies and blood draws will insure that tissue and blood samples can be obtained at optimal time points to evaluate the changes occurring in response (or lack of response) to treatment. The patient will be given the option to agree to, or decline, the additional biopsies and blood draws.

<sup>c</sup>at end of treatment

### **9.3 Study Materials**

Tofacitinib will be purchased at an approved commercial pharmacy and will be managed and dispensed by the Columbia University Research Pharmacy.

## **10 SAFETY ASSESSMENTS**

Analysis of safety is included as a secondary endpoint. All subjects who receive a dose of tofacitinib will be evaluated for safety. Safety outcomes include adverse events, clinically significant changes in vital signs, laboratory test abnormalities, and clinical tolerability of the drug. The investigator will determine the severity of each adverse event as mild, moderate, severe, or very severe. Laboratory findings that the investigator feels are clinically relevant should be recorded as adverse events. In addition, the investigator will determine the relationship of the adverse event to the administration of the study drug. Any occurrence of a SAE from time of consent forward, up to and including follow-up visits will be reported. See Section 6.5 for the SAE reporting procedures.

### **10.1 Physical Examinations**

During the treatment period, the physical examination is to be performed before administration of tofacitinib. While the interim physical examination may not be as comprehensive as the complete physical examination, important body systems should be included as deemed clinically indicated by the investigator. These body systems may include lymph nodes, liver, spleen, and breast. An interim physical examination may note any changes in the subject's condition since the last assessment and does not preclude examination of any of the body systems as clinically indicated.

### **10.2 Tuberculin Skin Testing**

A tuberculin skin test (PPD test: purified protein derivative tuberculosis skin test) should be performed and interpreted according to the applicable local Health Authority and/or Medical Society guidelines (those that provide recommendations for tuberculin skin testing for subjects who are to receive biologics, who are immunosuppressed, who have a prior history of BCG vaccinations<sup>(viii,ix)</sup>, or who have a prior positive test). Tuberculin skin testing is not contraindicated for persons who have been vaccinated with BCG.

QuantiFERON® testing is an acceptable alternative when tuberculin skin testing is not appropriate. A tuberculin skin test is not required if one was performed within 1 month of screening and documentation of testing is on file. If tuberculin skin testing is performed at screening, then the 48-72-hour reading must be completed before administration of tofacitinib.

If the results of Tuberculin skin testing are equivocal or positive, quantiferon testing may be done to provide additional clinical information.

## 11 EFFICACY ASSESSMENT

### 11.1 Primary Efficacy Assessment

The study's *primary efficacy endpoint* will be the proportion of responders after 6 months of treatment, with response defined as 50% or greater hair re-growth from baseline as assessed by SALT score (Figure 6) at week 24. This is a relatively strict definition for defining responders and non-responders and was chosen to minimize the potential for spontaneous remission, in which approximately 8% or less are expected to achieve this magnitude of hair regrowth spontaneously.

#### SALT – Severity of Alopecia Tool

A. The proportion of scalp involvement is determined by dividing the scalp into 4 quadrants and estimating the percentage of the scalp surface that all the alopecic areas would occupy if placed together. The following groups will be used:

##### S: Scalp hair loss

<u>S0</u> = No hair loss	<u>S4</u> = 76%-99% hair loss
<u>S1</u> = <25% hair loss	<u>a</u> = 76%-95% hair loss
<u>S2</u> = 26%-50% hair loss	<u>b</u> = 96%-99% hair loss
<u>S3</u> = 51%-75% hair loss	<u>S5</u> = 100% hair loss

Percentage change from baseline (% regrowth) =

$$[(\text{SALT BL} - \text{SALT F/U}) / \text{SALT BL}] \times 100\% = \% \text{ change from baseline}$$

Absolute regrowth = SALT BL – SALT F/U = absolute change from BL

##### Body hair loss

B0 = No body hair loss

B1 = some body hair loss

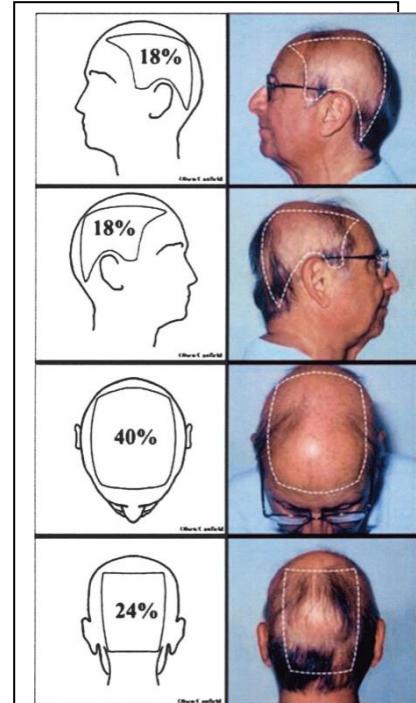
B2 = total (100%) body hair loss

##### Nail involvement

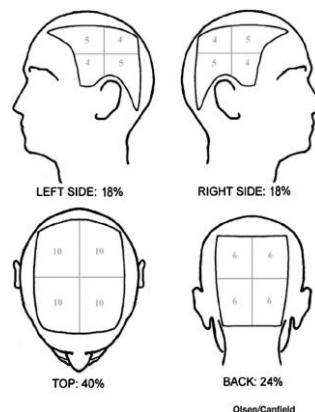
N0 = No nail involvement

N1 = some nail dystrophy

N1a = 20 nail dystrophy



**Figure 6. SALT score.** The percentage of hair loss in any one of the four views (areas) of the scalp = the percentage hair loss x percent surface area of the scalp in that area. The SALT score then equals the sum of the scalp hair loss in each area.



### 11.1.1 Secondary Efficacy Assessments

As secondary endpoints, efficacy will be measured by changes in hair re-growth as a continuous variable as determined by physical exam and Canfield photography, as well as patient and physician global evaluation scores. To assess the durability of responses, patients will continue to be followed for an additional 6 months off treatment. To assess the durability of response and the incidence of relapse, patients who achieve 50% regrowth from baseline (50% reduction in baseline SALT score) will continue to be followed for an additional 6 months off treatment or until it is determined that relapse has occurred. Relapse will be defined as any recurrence of hair loss in responders (subjects achieving greater than 50% decrease in SALT score from baseline during the first 6 to 18 months of the study) who had achieved stable regrowth without continued loss for at least 2 months; and loss of  $\geq 25\%$  of regrowth in patients who had achieved 50% regrowth during treatment but still had chronic low grade hair loss. Nonresponders to 6 months study treatment (defined as failure to achieve 50% improvement in SALT score compared to baseline) will not be required to participate in the 6 month follow-up period but may be offered open-label tofacitinib if deemed appropriate by the investigator. Partial responders will continue to be followed to assess for delayed achievement of complete response. Analysis of safety is included as a secondary endpoint. All subjects who receive a dose of tofacitinib will be evaluated for safety.

#### Secondary efficacy outcomes in detail

1. Percent hair regrowth from baseline determined by SALT measurements following end of treatment (at 24 to 72 weeks) and during the observational phase at 8, 16, and 24 weeks after end of treatment.
2. Comparison of the proportion of responders in the experimental group with the historically known placebo response rate (<10%), with response defined as 50% change in SALT score (50% regrowth) from baseline, at end of treatment (week 24 to 72), and during the observational phase at 8, 16, and 24 weeks after end of treatment.
3. Comparison of the proportion of subjects in each group attaining global overall improvement SALT score of A5 (100% coverage) supported by  $\leq$  SALT 25 at the end of treatment (week 24 to 72), and during the observational phase at 8, 16, and 24 weeks after end of treatment.
4. Change in PGA (Physician Global Assessment) based on live evaluations and evaluation of standardized photographs between baseline, and each subsequent visit during the treatment period and the follow up period.
5. Change in patient global assessment between baseline, and selected subsequent visits during the treatment period and the follow up period.
6. Change in patient quality of life assessment from baseline compared to selected subsequent visits during the treatment period and the follow up period.
7. Frequency of occurrence and timing of relapse (as defined above) in responders followed for 6 months off therapy.
8. Safety will be evaluated as a secondary endpoint using descriptive statistics to summarize the cumulative incidence and types of AEs.
9. The proportion of patients who discontinued treatment will be summarized.

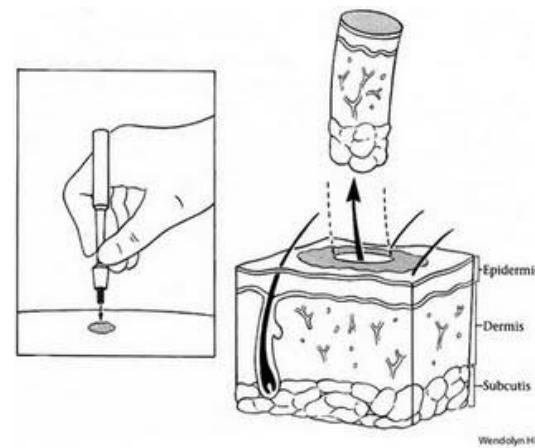
## 11.2 Other Assessments

### Photography

Photography will be performed using a high-resolution digital camera with Intellistudio system from Canfield Scientific, Inc. Patients will be positioned using a laser guide, after which photographs of the entire scalp will be taken at standardized locations and at a fixed distance. Close-up photographs may also be taken using an epiluminescent attachment in order to assess for the presence of early regrowth.

### Biopsy

All punch biopsies will be performed by the study doctor at a specific location and time point according to the schedule of study assessments or at unscheduled time points based on clinical indications. The biopsy site will be anesthetized with an injection of 1% lidocaine with epinephrine. After approximately 1 minute, the physician applies pressure to the biopsy site using a 4mm diameter skin punch (a sterile cylindrical tube with a sharp edge). The punch is twisted until the blade of the skin punch has pierced the epidermis and dermis of the skin and enters the subcutaneous fat. Depending on the thickness of the skin in the area being biopsied, the cylindrical blade may be buried to the hub (approximately 6mm in depth). After the blade has sufficiently cored or carved out a cylinder of skin, the skin punch is removed. Nontraumatic forceps are used to gently grasp the cored skin, pulling upward to remove the core and reveal the subcutaneous fat. Scissors are used to cut the cored tissue free from the underlying subcutaneous fat. The specimen is placed immediately into the appropriate media. Once the specimen has been removed, pressure is applied to the biopsy site with a sterile 2 x 2 gauze. The biopsy site is then closed with several simple interrupted sutures. Either an absorbable or nonabsorbable suture may be used at the investigator's discretion. Antibiotic ointment is applied and the area is covered with a standard BandAid or sterile gauze and paper tape. If the presence of adjacent hair makes adhesion of a bandage difficult, antibiotic ointment will be used without a covering. When necessary, a small pressure dressing may be applied. Subjects will be instructed in wound care and will be advised to call the research unit if they have any concerning signs or symptoms during healing.



### Biosamples

A recent histological/gene expression study of synovial biopsies from RA patients treated with tofacitinib noted only mild changes in cellular synovial infiltrates during treatment. However, marked changes in IFN- $\gamma$  expression was identified, with significant reductions only seen in the clinically responding patients (Buch et al. 2009). The decrease in IFN- $\gamma$  production in the responders implies that functional inhibition of T cell activity is important for therapeutic outcome. Our proposed studies will include longitudinal biopsies that could provide additional support for interferon- $\gamma$  modulation and tofacitinib treatment outcome.

Our procurement of serial biopsies, sera and PBMCs obtained at baseline and during treatment will provide the opportunity to correlate resolution of inflammatory biomarkers with treatment response in AA.

Biosamples will be obtained upon entry and again after 4 weeks, and 24 weeks of treatment. Biospecimens at each time point will include: 1) a 4 mm skin biopsy; 2) 5 ml of blood for serum; 3) 60 ml of blood for PBMCs.

Blood volumes for research: 60 ml of blood is required to insure that sufficient viable cells are available after thawing of cryopreserved PBMC aliquots to enable functional T cell studies in triplicate. Our experience is that viable recovery of PBMC in freeze-thawed specimens is highly variable and range from  $0.1-1.0 \times 10^6$  per ml of blood. Our blood volume obtained for research is 65 ml at one time, a maximum of 130 ml in a 4 week period and 260 ml in total over 6 months.

#### Biomarker Assessment and Clinical Correlative Studies

Our mechanistic studies are focused on correlating treatment and disease status with:

- 1) histological improvement of T cell infiltrates;
- 2) reduced IFN responses in the skin and blood;
- 3) reduced HF NKG2DL expression; and
- 4) declines in circulating and peribulbar CD4<sup>+</sup> and CD8<sup>+</sup>NKG2D<sup>+</sup> infiltration.

Previous studies in AA have reported elevated NKG2D expression in circulating CD8 T cells and NK cells (Ito et al. 2008), supporting the feasibility of our approach. Here we will be able to correlate the numbers of CD8<sup>+</sup>NKG2D<sup>+</sup> cells in the blood with those infiltrating the target organ of patients with each other and as a function of disease status. The limited studies proposed here seeking an association of IFN- $\gamma$  and CD8<sup>+</sup>NKG2D<sup>+</sup> cells and clinical outcome will not, however, exhaust the “biobank” and all human materials [frozen biopsy tissue & RNA, serum and the remaining (90%) of PBMCs] will remain banked and available for future hypothesis driven questions. Subjects consent for retention and future use of blood and tissue will be requested in the informed consent.

#### Flow Cytometry Studies.

The presence of CD8<sup>+</sup>NKG2D<sup>+</sup> cells will be tracked in the blood and in the skin during treatment with Tofacitinib. By assessing CD8<sup>+</sup>NKG2D<sup>+</sup> involvement in longitudinally obtained skin biopsies at baseline and during treatment we can correlate disease severity and clinical response (progression or regression) with immunopathological evidence of resolution of total cellular infiltration (by H&E) or loss of specific CD8<sup>+</sup>NKG2D<sup>+</sup> cells in the skin (by IF) or in the blood (flow studies). CD8<sup>+</sup>NKG2D<sup>+</sup> frequency in the peripheral circulation will be quantified using freshly isolated PBMCs obtained from 60 mls of blood obtained upon entry into the trial and at 4 wks, and 24 wks during treatment. A portion of the PBMCs  $5 \times 10^6$  cells will be stained with anti-CD4, anti-CD8, anti-CD25, anti-CD28 and anti-NKG2D antibodies, stimulated with PMA/ionomycin in the presence of brefeldin and stained with anti-FOXP3 and anti-IFN- $\gamma$  Abs.

The remaining PBMCs will be used for RNA analysis (see below) and viably frozen for future ancillary studies. We will seek evidence of a comparable pathogenic T cell subset in the human that we observed in C3H mice, namely an activated CD25<sup>+</sup> IFN- $\gamma$ -producing CD8<sup>+</sup>NKG2D<sup>+</sup> T cell.

#### *Histology and Immunofluorescence Analysis.*

As shown in previous studies (Ito et al. 2008; Petukhova et al. 2010) in active AA NKG2D ligands are upregulated on hair follicles in association with peribulbar NKG2D-bearing CD8<sup>+</sup> T-cells and natural killer cells. We will seek evidence of resolution of total cellular infiltration (by H & E) and loss of specific CD8<sup>+</sup>NKG2D<sup>+</sup> cells (by IF) and NKG2D ligands by comparing baseline biopsies with biopsies obtained on treatment after 4 and 24 weeks. Frozen sections will be stained with anti-CD4, anti-CD8, anti-CD56, anti-NKG2D and anti-IFN- $\gamma$  antibodies to visualize the total number of leukocytes, cytotoxic T-cells and NK cells present in the baseline and drug treated skin, respectively. Immunostaining with anti-ULBP3, anti-MICA antibodies and recombinant NKG2D soluble receptor (NKG2D-Ig, detects all NKG2D ligands, R & D systems) will determine whether NKG2DL “danger/stress” signals are down modulated. Punch biopsies from patient skin before and after treatment will be embedded in OCT and 7-8 micron frozen sections will be cut. The sections will be fixed in 4% paraformaldehyde and stained overnight with primary antibodies for NKG2D ligands, receptor and immune markers. The following day, sections will be treated for 1 hr with fluorescence labeled antibody, counter-stained and mounted with DAPI containing media. Frozen sections will also be used to for H&E staining. The total number of immune cells, as well as NKG2D bearing cells, will be quantified by counting stained cells per magnification field using the NIH imaging software ImageJ and a student T-test will be carried out to determine significant changes in the number of infiltrating, NKG2D bearing immune cells to quantitatively correlate the simultaneous presence or absence of these cells in lesional skin and in circulation (by flow) during active disease or in remission.

#### *Analysis of Gene Expression by Quantitative RT-PCR*

We will analyze the **transcriptional expression signature of costimulatory and IFN-response genes in the skin and PMBC of drug-treated AA patients using qPCR** as an indirect measure of the efficacy of tofacitinib. Signature genes were selected based on their differential expression in affected vs. unaffected C3H mice (Carroll et al. 2002), and on published studies on human AA patients vs. controls (Subramanya et al. 2010) The genes in the list represent mediators and effectors of the IFN response, which we found to be instrumental in disease pathology. Briefly, biopsies and peripheral blood from baseline and tofacitinib treated patients will be collected at 0, 4, and 24 week time points and possibly additional timepoints if clinically indicated. RNA will be extracted from the tissues, and Sybr green-based qPCR assays will be performed. Differential gene expression resulting from inhibitor treatment will be normalized to the expression of a housekeeping gene. We expect many of the genes on this list to revert to a basal level of expression, as compared in unaffected skin of an AA patient, or skin from a healthy control.

#### *Serum inflammatory biomarkers*

Serum (5 ml) will be obtained at baseline and during treatment and assessed for resolution of elevated levels of IFN-inducible chemokines.

## 12 ADVERSE EVENTS

An **Adverse Event (AE)** 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 that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of investigational product, whether or not considered related to the investigational product.

### 12.1 Serious Adverse Events

A **Serious Adverse Event (SAE)** is any untoward medical occurrence that at any dose:

- results in death
- is life-threatening (defined as an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe)
- requires inpatient hospitalization or causes prolongation of existing hospitalization (see note below for exceptions)
- results in persistent or significant disability/incapacity
- is a congenital anomaly/birth defect

5) is an important medical event (defined as a medical event(s) that may not be immediately life-threatening or result in death or hospitalization but, based upon appropriate medical and scientific judgment, may jeopardize the subject or may require intervention [e.g., medical, surgical] to prevent one of the other serious outcomes listed in the definition above.) Examples of such events include, but are not limited to, intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization.) Potential drug induced liver injury (DILI) is also considered an important medical event. (See Section 6.6 for the definition of potential DILI.)

Suspected transmission of an infectious agent (e.g., pathogenic or nonpathogenic) via the study drug is an SAE.

Although pregnancy, overdose, cancer, and potential drug induced liver injury (DILI) are not always serious by regulatory definition, these events must be handled as SAEs and should also be reported to the IRB and appropriate regulatory bodies in an expedited manner.

**NOTE:** The following hospitalizations are not considered SAEs:

- a visit to the emergency room or other hospital department lasting less than 24 hours that does not result in admission (unless considered an “important medical event” or a life-threatening event)

- elective surgery planned before signing consent
- admissions as per protocol for a planned medical/surgical procedure
- routine health assessment requiring admission for baseline/trending of health status (e.g., routine colonoscopy)
- medical/surgical admission for purpose other than remedying ill health state that was planned before study entry. Appropriate documentation is required in these cases.
- admission encountered for another life circumstance that carries no bearing on health status and requires no medical/surgical intervention (e.g., lack of housing, economic inadequacy, caregiver respite, family circumstances, administrative).

## **12.2 Nonserious Adverse Events**

Nonserious adverse events are all adverse events that are not classified as SAEs.

## **12.3 Assignment of Adverse Event Intensity and Relationship to Tofacitinib**

All adverse events, including those that are serious, will be graded by the investigator as follows:

- Mild (Grade 1): awareness of event but easily tolerated
- Moderate (Grade 2): discomfort enough to cause some interference with usual activity
- Severe (Grade 3): inability to carry out usual activity
- Very Severe (Grade 4): debilitating; significantly incapacitates subject despite symptomatic therapy.

The following categories and definitions of causal relationship to investigational product as determined by a physician should be used:

- **Related:** There is a reasonable causal relationship to investigational product administration and the adverse event.
- **Not Related:** There is not a reasonable causal relationship to investigational product administration and the adverse event.

The expression “reasonable causal relationship” is meant to convey in general that there are facts (e.g., evidence such as de-challenge/re-challenge) or other arguments to suggest a positive causal relationship.

## **12.4 Collection and Reporting**

Adverse events can be spontaneously reported or elicited during open-ended questioning, examination, or evaluation of a subject. To prevent reporting bias, subjects should not be questioned regarding the specific occurrence of one or more adverse events.

If known, the diagnosis of the underlying illness or disorder should be recorded, rather than its individual symptoms. The following information should be captured for all AEs: onset, duration, intensity, seriousness, relationship to investigational product, action taken, and treatment required. If treatment for the event was administered, it should be recorded in the medical record. The investigator must supply the IRB and appropriate regulatory bodies with any additional information requested, notably for reported deaths of subjects.

Completion of supplemental CRFs may be requested for AEs and/or laboratory abnormalities that are reported/identified during the course of the study.

## **12.5 Serious Adverse Events**

Following the subject's written consent to participate in the study, all SAEs must be collected, including those thought to be associated with protocol-specified procedures. Collection of all SAEs must continue for 30 days after the last administration of the investigational product. If applicable, SAEs must be collected that relate to any later protocol-specified procedure. The investigator should notify the IRB and FDA as appropriate of any SAE occurring after this time period that is believed to be related to the investigational product or protocol-specified procedure.

All SAEs, whether considered related or unrelated to tofacitinib, must be reported to the IRB and FDA (by the investigator or designee) within 24 hours of study personnel becoming aware of the event. If only limited information is initially available, follow-up reports are required. The original SAE form must be kept on file at the study site.

For studies conducted under an **Investigator IND**, any event that is both serious and unexpected must be reported to the Food and Drug Administration (FDA) as soon as possible **and no later than 7 days** (for a death or life-threatening event) **or 15 days** (for all other SAEs) **after the investigator's or institution's initial receipt of the information**. SAEs should be reported on MedWatch Form 3500A, which can be accessed at: <http://www.accessdata.fda.gov/scripts/medwatch/>.

**MedWatch SAE forms should be sent to the FDA at:**

**MEDWATCH**  
**5600 Fishers Lane**  
**Rockville, MD 20852-9787**  
**Fax: 1-800-FDA-0178 (1-800-332-0178)**  
[\*\*http://www.accessdata.fda.gov/scripts/medwatch/\*\*](http://www.accessdata.fda.gov/scripts/medwatch/)

## **12.6 Nonserious Adverse Events**

The investigator will begin collecting nonserious adverse event (NSAE) information once administration of the investigational product is initiated.

All identified NSAEs must be recorded and described in the medical record. If an ongoing NSAE worsens in its intensity, or if its relationship to the investigational product changes, a new NSAE entry for the event should be completed. NSAEs should be followed to resolution or stabilization, or reported as SAEs if they become serious. Follow-up is also required for NSAEs that cause interruption or discontinuation of investigational product, or those that are present at the end of study participation. Subjects with NSAEs at study completion should receive post-treatment follow-up as appropriate.

## **12.7      Laboratory Test Abnormalities**

All laboratory test results captured as part of the study should be recorded following institutional procedures. When reporting a test result that constitutes an adverse event, the clinical term should be used; for example, the event should be reported as “anemia” not “low hemoglobin.” Test results that constitute SAEs should be documented and reported as such.

## **12.8      Overdose**

An overdose is defined as the accidental or intentional ingestion or infusion of any dose of a product that is considered both excessive and medically important. All occurrences of overdose must be reported as an SAE.

## **12.9      Pregnancy**

Sexually active WOCBP must use an effective method of birth control during the course of the study, in such a manner that the risk of failure is minimized. (See Section 7.5 for the definition of WOCBP.)

Before study enrollment, WOCBP must be advised of the importance of avoiding pregnancy during study participation and of the potential risk factors for an unintentional pregnancy. The subject must sign an informed consent form documenting this discussion.

### **12.9.1      Requirements for Pregnancy Testing**

**All WOCBP MUST have a negative pregnancy test within 72 hours before receiving tofacitinib.** The minimum sensitivity of the pregnancy test must be 25 IU/L or equivalent units of HCG. If the pregnancy test is positive, the subject must not receive Tofacitinib and must not continue in the study.

**In addition, all WOCBP must be instructed to contact the investigator and/or other study personnel immediately if they suspect they might be pregnant (e.g., missed or late menstrual period) at any time during study participation.**

### **12.9.2      Reporting of Pregnancy**

If, following initiation of the investigational product, it is subsequently discovered that a study subject is pregnant or may have been pregnant at the time of investigational product exposure, including during at least 6 half-lives after administration, the investigational product will be permanently discontinued in an appropriate manner (e.g., dose tapering if necessary for subject safety).

Protocol-required procedures for study discontinuation and follow-up must be performed for the subject unless contraindicated by pregnancy (e.g., x-ray studies). Other appropriate pregnancy follow-up procedures should be considered if indicated. Information regarding the course of the pregnancy, including perinatal and neonatal outcome, must be reported to the IRB and/or FDA as appropriate. Infants should be followed for a minimum of 8 weeks.

## **12.10 Other Safety Considerations**

Any significant worsening noted during interim or final physical examinations, electrocardiograms, x-rays, and any other potential safety assessments, whether or not these procedures are required by the protocol, should also be recorded in the medical record.

# **13 STATISTICAL CONSIDERATIONS**

## **13.1 Sample Size Determination**

Based on two recently completed randomized trials (Price et al, 2008; Strober et al, 2009) in similar patient populations (moderate to severe AA) we expect placebo response rates to be between 6% (>50% improvement in SALT score) and 12% (>25% improvement). We have chosen our primary endpoint conservatively (proportion of patients with >50% improvement in SALT index) as a relatively strict criteria that should minimize placebo response rates to 10% or less (0-1 of 10 subjects). This allows a higher degree of confidence that appreciable response rates are attributable to drug rather than spontaneous remission.

With our sample of n=15, we will have greater than 80% power to reject the null hypothesis that the true underlying remission rate is 10% if we observe a rate in our sample of 48%, holding type I error to 5%. If we assume that the underlying remission rate is 30%, then we have greater than 80% power to reject that null hypothesis if the observed rate is 73% or above, again holding type I error to 5%.

## **13.2 Populations for Analyses**

The Dermatology clinic and private practice at CUMC currently sees 600 AA patients yearly, of which we anticipate 20-30% would be eligible (>30% hair loss). Accrual will be greatly facilitated by targeted recruitment through our existing NAAF registry, which includes 500 AA patients in the New York area, the majority of which have patchy type disease. Thus, complete accrual of 60 subjects should be feasible within 1.0 years from a pool of >100 eligible subjects each year.

### 13.3 Endpoint Definitions

1. The study's *primary efficacy endpoint* will be the proportion of responders after 6 to 18 months of treatment, with response defined as 50% or greater hair re-growth from baseline as assessed by SALT score at week 24 to 72.

#### Secondary Endpoints

1. Percent hair regrowth from baseline determined by SALT measurements following end of treatment (week 24 to 72) and during the observational phase at 8, 16, and 24 weeks after end of treatment.
2. Comparison of the proportion of responders in the experimental group with the historically known placebo response rate (<10%), with response defined as 50% change in SALT score (50% regrowth) from baseline, at end of treatment (week 24 to 72), and during the observational phase at 8, 16, and 24 weeks after end of treatment.
3. Comparison of the proportion of subjects in each group attaining global overall improvement SALT score of A5 (100% coverage) supported by  $\leq$  SALT 25% at the end of treatment (week 24 to 72), and during the observational phase at 8, 16, and 24 weeks after end of treatment.
4. Change in PGA (Physician Global Assessment) based on live evaluations and evaluation of standardized photographs between baseline, and selected subsequent visits during the treatment period and the follow up period.
5. Change in patient global assessment between baseline, and subsequent selected visits during the treatment period and the follow up period.
6. Change in patient quality of life assessment from baseline to and each subsequent visit during the treatment period and the follow up period.
7. Frequency of occurrence and timing of relapse (as defined above) in responders followed for 6 months off therapy.
8. Safety will be evaluated as a secondary endpoint using descriptive statistics to summarize the cumulative incidence and types of AEs.
9. The proportion of patients who discontinued treatment will be summarized.

10.

## **13.4 Analyses**

### **13.4.1 Demographics and Baseline Characteristics**

Demographics and baseline characteristics will be summarized via descriptive statistics.

### **13.4.2 Safety Analyses**

To assess safety, we will summarize the incidence of adverse events for the study group. Analysis of safety is included as a secondary endpoint. All subjects will receive tofacitinib and will be evaluated for safety. Safety outcomes include adverse events, clinically significant changes in vital signs, laboratory test abnormalities, and clinical tolerability of the drug. The investigator will determine the severity of each adverse event as mild, moderate, severe, or very severe. Laboratory findings that the investigator feels are clinically relevant should be recorded as adverse events. In addition, the investigator will determine the relationship of the adverse event to the administration of the study drug. Any occurrence of a SAE from time of consent forward, up to and including follow-up visits will be reported. See Section 12 for the SAE reporting procedures.

### **13.4.3 Efficacy Analyses**

Since this is a small open label proof of concept study, we recognize that it will likely lack adequate power to conclusively demonstrate small efficacy signals (particularly if the assumed detectable difference of 38% is not met). Yet, descriptive summaries of all the primary and secondary efficacy outcomes (proportions for binary outcomes, means for continuous outcomes) will provide a preliminary indication of the effect size (even if smaller than 38%) that will aid design of subsequent efficacy trials.

## **14 ADMINISTRATIVE SECTION**

### **14.1 Compliance with the Protocol**

The study must be conducted as described in the final IRB-approved protocol. No protocol amendments will be implemented until written approval has been given by the IRB, except when necessary to eliminate an immediate hazard to study subjects.

If a protocol amendment mandates a revision to the informed consent, the revised consent must be used to obtain consent from subjects currently enrolled in the study if it affects them (e.g., if it contains new information regarding safety), and the revised consent must be used to obtain consent from new subjects before enrollment.

## **14.2 Records Retention**

The investigator will retain, in a confidential manner, all data pertinent to the study for all treated subjects as well as those entered as control subjects. The investigator will retain source documents and accurate case histories that record all observations and other data pertinent to the investigation (e.g., the medical record) for the maximum period required by applicable regulations and guidelines or following institutional procedures.

The Columbia University Research Pharmacy or responsible research pharmacy will ensure that a current record of disposition of investigational product is maintained at each study site where the investigational product is inventoried and disposed. Records or logs must comply with applicable regulations and guidelines and should include:

- amount received and placed in storage area
- amount currently in storage area
- label identification number or batch number and use date or expiry date
- dates and initials of person responsible for each inventory entry/movement
- amount dispensed to and returned by each subject, including unique subject identifiers
- amount transferred to another area/site for dispensing or storage
- non-study disposition (e.g, lost, wasted, broken), and
- amount destroyed at study site.

## **14.3 Destruction of Investigational Product**

All study drug dispensation, monitoring and disposal will be managed by the Columbia University Research Pharmacy or responsible research pharmacy according to applicable regulations, guidelines, and institutional procedures. Appropriate records of the disposal will be maintained.

## 15 GLOSSARY OF TERMS

Term	Definition
Adverse Reaction	An adverse event that is considered by either the investigator or the sponsor to be related to the investigational product
Expedited Safety Report	Rapid notification to investigators of all SAEs that are suspected (related to the investigational product) and unexpected (i.e., not previously described in the Investigator Brochure), or that could be associated with the study procedures.
SUSAR	Suspected, Unexpected, Serious Adverse Reaction as termed by the European Clinical Trial Directive (2001/20/EC).
Unexpected Adverse Reaction	An adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g., Investigator Brochure for an unapproved investigational product)

## 16 LIST OF ABBREVIATIONS

AA	Alopecia areata
AE	Adverse event
ALT	Alanine Transaminase
AST	Aspartate Transaminase
BCG	Bacillus Calmette-Guérin
CBC	Complete Blood Count
CDC-ACIP	Centers for Disease Control and Prevention Advisory Committee on Immunization Practices
CFR	Code of Federal Regulations
CMV	Cytomegalovirus
CRF	Case Report Forms
CTLA	Cytotoxic T-Lymphocyte Associated
DMARD	Disease-Modifying Anti-Rheumatic Drug
DNA	Deoxyribonucleic Acid
FDA	Food and Drug Administration
FSH	Follicle-Stimulating Hormone
GCP	Good Clinical Practice
GM-CSF	Granulocyte Macrophage Colony-Stimulating Factor
HCG	Human Chorionic Gonadotropin
HIV	Human Immunodeficiency Virus
HLA	Histocompatibility Leukocyte Antigen
HRT	Hormone Replacement Therapy
IB	Investigator Brochure
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
IL	Interleukin
IRB	Independent Review Board
IU	International Unit
IV	Intravenous
MHC	Major Histocompatibility Complex
NSAE	Non-Serious Adverse Event

NSAID	Non-Steroidal Anti-inflammatory Drug
PCR	Polymerase Chain Reaction
PPD	Purified Protein Derivative
RA	Rheumatoid Arthritis
SAE	Serious Adverse Event
SALT	Severity of alopecia tool
Se	Sensitivity
SmPC	Summary of Product Characteristics
Sp	Specificity
SUSAR	Suspected Unexpected Serious Adverse Reaction
TB	Tuberculosis
TNF	Tumor Necrosis Factor
ULN	Upper Level of Normal
WBC	White Blood Cell
WOCBP	Women of Childbearing Potential

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