Article type: Clinical Trial
Title: Sublingual tofacitinib for alopecia areata: a roll-over pilot clinical trial and analysis of pharmacokinetics
Authors: Vivien Wai Yun Lai, MBBS, BMedSc, Laita Bokhari, MPhil Med, Rodney Sinclair, MBBS, MD, FACD
Initials: V.W.Y. Lai, L. Bokhari, R. Sinclair
Affiliations:
1 Alfred Hospital, Melbourne VIC 3004
2 Sinclair Dermatology, East Melbourne VIC 3002
3 Dermatology, Melbourne University, Melbourne VIC 3010
Corresponding author:
Vivien Wai Yun Lai, MBBS, BMedSc
Alfred Hospital
55 Commercial Rd
Melbourne VIC 3004
https://orcid.org/0000-0003-4530-9864 (V.W.Y. Lai)
Email: lai.vvn@gmail.com
Funding sources: Australia Alopecia Areata Foundation
Conflicts of Interest: Vivien Lai and Laita Bokhari have no conflicts of interest to declare. Rodney Sinclair has acted as an Investigator for Pfizer and has patent on sublingual tofacitinib.
IRB approval status: Ethical approval for this study was received from Bellberry Human Research Ethics Committee (HREC No. 2018-08-607), Committee E (HREC Code: EC00450).
**Clinical trials Listing:** Australian New Zealand Clinical Trials Registry (ANZCTR)  
Registration No.: ACTRN12618001084279.

**Running Title:** Sublingual tofacitinib for alopecia areata

**Figures:** 1

**Tables:** 2

**Keywords:** JAK STAT Janus kinase inhibitors, hair loss, totalis, universalis, cyclosporine, Steroid-Sparing, Systemic Therapy

**Abstract**

**Background:** Tofacitinib is a JAK1/3 inhibitor used off-label to treat alopecia areata (AA). Oral tofacitinib undergoes extensive hepatic metabolism, has numerous drug interactions and a half-life of 3 hours necessitating twice daily dosing. Sublingual delivery bypasses hepatic first-pass metabolism which may provide pharmacokinetic benefits and reduce gastrointestinal side effects. We investigate sublingual tofacitinib as a novel form of administration in a cohort of treatment-resistant patients.

**Objective:** To assess the efficacy and pharmacokinetics of sublingual tofacitinib in moderate-to-severe AA patients.

**Methods:** An open-label, roll-over pilot clinical trial was conducted. Participants were recruited from a preceding trial. All responders (≥50% reduction in Severity of Alopecia Tool (SALT) score, SALT50) in the preceding trial continued on the same treatment (cyclosporine/placebo), while non-responders rolled-over to receive open-label sublingual tofacitinib 5mg twice daily for 12 weeks. Treatment response as reduction in SALT score after 12 weeks (low: 15-29%, medium: 30 – 49%, good: 50-75% and high grade: 75 – 100%) was measured. Pharmacokinetics was analysed using liquid chromatography tandem mass spectrometry.

**Results:** Eighteen participants completed the trial. Total treatment response to tofacitinib was 37.5%. SALT50 was achieved in 12.5%. The mean improvement in SALT score was 15.57%. Mean maximum plasma concentration was 43.18 ng/ml occurring after 1-hour. Elimination half-life is estimated to be up to 11 hours.

**Conclusion:** An estimated half-life of up to 11 hours may be achieved with sublingual tofacitinib, which is significantly longer than the oral form and may facilitate daily dosing. Larger clinical trials are required to further characterize its pharmacokinetics and efficacy.
Introduction

Alopecia areata (AA) is a common, relapsing and remitting, autoimmune hair loss condition (1). Disease spectrum varies from a single patch; to multifocal patches; to loss of entire scalp hair, known as alopecia totalis (AT); and to complete loss of all scalp and body hair, known as alopecia universalis (AU). There is an inverse association between disease duration and likelihood for spontaneous regrowth; patients with a patch of AA for greater than 12 months have a 45% risk of progressing to AT/AU (1). AA is associated with significant psychological morbidity. Estimated mean health utility, from a scale of 0 (death) to 1 (full health quality-adjusted life years) is calculated to be 0.748 for Australian AA patients (2).

Current therapies are suboptimal. Prednisolone, while effective, has significant side-effects and conjunctive use of steroid-sparing agents, including cyclosporine, methotrexate and azathioprine, only allow 35.9% of patients to wean-off prednisolone entirely at 12 months (3). Of the steroid-sparing agents, only one placebo-controlled randomized trial has investigated the efficacy of cyclosporine monotherapy in the treatment of AA, with an efficacy of up to 31.3%, however this did not reach statistical significance due to small sample size (4). Uncontrolled case series demonstrate the efficacy of monotherapy methotrexate and azathioprine to be between 38% to 43% (3). Ultimately, systematic reviews identify no reliable evidence-based treatments for chronic AA (5).

Many of the inflammatory responses involved in the pathogenesis of AA share common signalling transduction via the Janus Kinas – Signal Transducer and Activator of Transcription proteins (JAK-STAT) pathway, in which proinflammatory cytokines such as interferon gamma (IFN-γ) and interleukin-15 (IL-15) activate JAK kinase. Blockade of this pathway through JAK-inhibitors is a target of interest for new treatments.

Specifically, tofacitinib – a JAK1/3 inhibitor - has been investigated in multiple studies, with a meta-analysis of 6 clinical trials and 8 observational studies suggesting an overall 54.0% good/complete hair regrowth rate (6). Dose and treatment duration were important variables: 66.7% compared with 31.8% of participants achieved response on >5mg twice daily dosing versus ≤5 mg twice daily dosing respectively, and this result was the same for duration >6 months versus ≤6 months (6). Subgroup analyses demonstrated the good/complete response rate was lower in clinical trials, 34.5%, compared with observational studies, 56.6% (6).
Notably, oral tofacitinib undergoes extensive hepatic metabolism, has numerous drug interactions and an elimination half-life of 3 hours necessitating twice daily dosing. Adverse events include an increased risk of infections, lymphoma, thromboembolism, liver steatosis, gastrointestinal perforation, dyspepsia and diverticulitis (6-8). No studies have investigated sublingual tofacitinib, which may provide benefit as the sublingual form bypasses hepatic first-pass metabolism, circumvents gastrointestinal side-effects and may reduce drug interactions orchestrated through hepatic CYP450 enzyme metabolism. In this study, we report a pilot trial to evaluate the efficacy, pharmacokinetics and adverse events of sublingual tofacitinib in patients with moderate-to-severe, treatment-resistant AA.

Methods

We conducted an open-label, roll-over clinical trial. Adults aged 18 to 65 years with moderate-to-severe AA who completed an initial trial were recruited in person at the final visit of the initial trial to take part in this pilot study (9) (Figure 1). Responders (minimum 50% reduction in Severity of Alopecia Tool (SALT) score, i.e. SALT50) to treatment in the initial trial were allocated to continue a 12-week extension of the same blinded treatment (cyclosporine/placebo), while non-responders were allocated to receive open-label privately-compounded sublingual tofacitinib 5mg twice daily for 12 weeks after a 4-week washout period. Exclusion criteria included: pregnancy, lactation, non-adherence to prescribed contraception; use of hair-promoting treatments; tofacitinib use within 12 weeks; history of lymphoproliferative disorders, HIV, tuberculosis, hepatitis B or hepatitis C; active herpes simplex infection; and hypersensitivity to study medication.

Monthly assessments included: SALT score, eyelash and eyebrow scales (categorical rating from 0, none, to 3, normal), quality-of-life (QOL) questionnaires (Alopecia Areata Symptom Impact Scale, AASIS and Assessment of Quality of Life-8D, AQoL-8D) and adverse event monitoring. Adverse events were monitored through monthly blood biochemistry, together with patient self-reporting of symptoms and a thorough examination.

The SALT score is an internationally-recognized severity score representing total percentage hair loss through a weighted summation of left, right, superior and posterior scalp hair loss (10). The AASIS is a disease-specific instrument, used to characterize QOL impacted by AA (11), while the AQoL-8D is a generic instrument, which enables QOL comparison across diseases in Australia (12).

Blood samples were collected from tofacitinib-arm participants at baseline and after 12 weeks at 0, 0.5, 1, 3 and 24 hours. Samples were centrifuged and the resultant plasma
analysed using liquid chromatography tandem mass spectrometry method to determine tofacitinib plasma concentrations. The maximum observed plasma concentration ($C_{max}$) and time to $C_{max}$ ($t_{max}$) was calculated. Half-life was estimated through linear extrapolation.

Statistical analyses were performed using Stata 12 software. Descriptive statistics were summarized using means and standard deviations. Mann-Whitney U tests for non-normally distributed continuous data and chi-squared tests for categorical data were performed to compare groups. Statistical significance was defined as $p<0.05$. As this was a pilot study, the efficacy data presented may help calculate future sample sizes required for statistical significance.

Ethical approval was received from Bellberry Human Research Ethics Committee E (EC00450).

**Results**

Twenty participants enrolled; 2 received continuation treatment (cyclosporine); 18 received tofacitinib. The majority of tofacitinib participants had AT/AU (72.22%), with 100% or some body hair loss (83.33%) (Table 1).

37.5% of participants showed some response to sublingual tofacitinib and 12.5% achieved SALT50 (Table 2). Mean reduction from baseline of SALT score was 15.57%.

37.5% of participants achieved at least a 1 grade improvement in eyelash assessment scale and 50% achieved at least a 1 grade improvement in eyebrow assessment scale. The two participants on cyclosporine did not achieve significant incremental improvement at 24 weeks compared the initial 12 weeks. There were no serious adverse events.

72 plasma samples were analysed. Mean $C_{max}$ was 43.18 ng/ml. $t_{max}$ was 1-hour. The approximate half-life of sublingual tofacitinib was 11 hours; albeit limited by a lack of time-point data between 3 and 24 hours.

**Discussion**

This is the first study to investigate sublingual tofacitinib. Only 12.5% of participants achieved SALT50, which is lower compared with meta-analysis data which suggests a 34.5% response rate to oral tofacitinib (6). The lower response rates seen in this clinical trial may represent either a lower efficacy of the sublingual tofacitinib compared with oral, or reflect difficulties treating a cohort of severe, treatment-resistant disease. The tofacitinib group had a mean duration of current episode of AA of 7.79 years, mean scalp hair loss at baseline of 86.01% and more than 70% of participants had AT/AU with more than 50% with duration...
greater than 2 years. The cohorts in current trials generally have a mean duration of current episode of AA of 5 years and 50% of participants with AT/AU (8, 13).

Optimal dosage of tofacitinib is important, given the toxicities associated with higher doses, particularly pulmonary embolism. In the two clinical trials reported for oral tofacitinib, one found a good response at 5mg twice daily in 32% of participants (8), while the other demonstrated limited response despite prolonged treatment and required dose-escalation to 10mg twice daily (13). Currently, due to safety concerns, a dosage of 10mg twice daily is not recommended for either rheumatoid arthritis or psoriatic arthritis (14). Our pharmacokinetics analysis suggests sublingual tofacitinib may have a substantially longer half-life of 11 hours, compared to a half-life of 3 hours for oral tofacitinib (15-17). Sublingual tofacitinib may enable once-daily, rather than twice-daily dosing. None of our participants received potent CYP3A4 inhibitors. Further pharmacokinetic studies are required to evaluate whether there remains substantial interaction with CYP3A4 drugs with sublingual administration. A further subgroup of interest would be those with hepatic impairment. In this 12-week clinical trial, we found no significant adverse events to sublingual tofacitinib. However, we note that a longer trial may be required to reveal these adverse events.

Tofacitinib is a selective inhibitor of JAK 1 and JAK3 and blocks several important cytokines (IL-2, -4, -7, -9, -15 and -21) involved in lymphocyte activation, proliferation and immune response. Further pharmacokinetic research with a greater number of time-points is required to determine full area under the plasma concentration time curve (AUC) required to elucidate any differences in metabolism, excretion and action that could explain lower sublingual response rates despite a similar maximum mean plasma concentration to oral tofacitinib.

Our results are limited by a small sample size. From these pilot study results, we may calculate that for a two-sided 5% significance level and power of 80%, a sample size of at least 100 participants per group is required, based on 12.5% achieving SALT50 in sublingual tofacitinib.

In conclusion, our study provides the relevant foundation for larger studies to confirm pharmacokinetics data and efficacy. We demonstrate potential efficacy of sublingual tofacitinib to treat moderate-to-severe AA as an alternative to oral administration. Further research is required to elucidate dose-dependent efficacy and safety of sublingual tofacitinib, characterize its drug interactions and determine differences in AUC compared to oral tofacitinib.
Acknowledgments: We would like to thank the Australia Alopecia Areata Foundation for funding this research project. We would also like to thank Monash Institute of Pharmaceutical Sciences for analysing our plasma samples.

References

Figure 1. Flow diagram of allocation, follow-up and analysis of participants

Total screened (n= 34)

Excluded (n= 14)
- Could not comply with visits (n= 5)
- Could not comply with contraception requirements (n= 1)
- Declined participation (n= 5)
- Met exclusion criteria (n= 3) e.g. TB, concomitant tofacitinib, active herpes zoster

This article is protected by copyright. All rights reserved
Table 1. Baseline demographic and clinical characteristics of all randomized participants.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All (n=20)</th>
<th>Continuation Arm (n=2)</th>
<th>Tofacitinib arm (n=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>44 (14.96)</td>
<td>34 (5)</td>
<td>45.11 (15.28)</td>
</tr>
<tr>
<td>Sex (female)</td>
<td>15 (75%)</td>
<td>1 (50%)</td>
<td>14 (77.78%)</td>
</tr>
<tr>
<td>Age at onset of first episode of AA (years)</td>
<td>26.75 (14.92)</td>
<td>18 (11)</td>
<td>27.72 (14.99)</td>
</tr>
<tr>
<td>Age at onset of current episode of AA (years)</td>
<td>37 (15.24)</td>
<td>32.5 (5.5)</td>
<td>37.50 (15.89)</td>
</tr>
<tr>
<td>Duration of current episode of AA (years)</td>
<td>7.13 (11.48)</td>
<td>1.25 (0.25)</td>
<td>7.79 (11.92)</td>
</tr>
<tr>
<td>Mean percentage scalp hair loss by SALT score at baseline (%)</td>
<td>81.59 (26.53)</td>
<td>41.75 (19.75)</td>
<td>86.01 (23.30)</td>
</tr>
<tr>
<td>Pattern of scalp hair loss, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AT</td>
<td>6 (30%)</td>
<td>0 (0%)</td>
<td>6 (33.33%)</td>
</tr>
<tr>
<td>AU</td>
<td>8 (40%)</td>
<td>1 (50%)</td>
<td>7 (38.89%)</td>
</tr>
<tr>
<td>Patchy</td>
<td>6 (30%)</td>
<td>1 (50%)</td>
<td>5 (27.78%)</td>
</tr>
<tr>
<td>Body hair loss, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100% loss</td>
<td>8 (40%)</td>
<td>1 (50%)</td>
<td>7 (38.89%)</td>
</tr>
<tr>
<td>No loss</td>
<td>3 (15%)</td>
<td>0 (0%)</td>
<td>3 (16.67%)</td>
</tr>
<tr>
<td>Some loss</td>
<td>9 (45%)</td>
<td>1 (50%)</td>
<td>8 (44.44%)</td>
</tr>
<tr>
<td>Nail involvement</td>
<td>10 (50%)</td>
<td>2 (100%)</td>
<td>8 (44.44%)</td>
</tr>
<tr>
<td>History of AT/AU at any time</td>
<td>16 (80%)</td>
<td>2 (100%)</td>
<td>14 (77.77%)</td>
</tr>
<tr>
<td>Duration of AT/AU, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤2 years</td>
<td>8 (50%)</td>
<td>2 (100%)</td>
<td>6 (42.89%)</td>
</tr>
<tr>
<td>&gt;2 years</td>
<td>8 (50%)</td>
<td>0 (0%)</td>
<td>8 (57.14%)</td>
</tr>
<tr>
<td>Medical history, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atopy</td>
<td>6 (30%)</td>
<td>0 (0%)</td>
<td>6 (33.33%)</td>
</tr>
<tr>
<td>Endocrine</td>
<td>2 (10%)</td>
<td>0 (0%)</td>
<td>2 (11.11%)</td>
</tr>
<tr>
<td>Psychological illness</td>
<td>2 (10%)</td>
<td>0 (0%)</td>
<td>2 (11.11%)</td>
</tr>
<tr>
<td>Family history of AA</td>
<td>2 (10%)</td>
<td>0 (0%)</td>
<td>2 (11.11%)</td>
</tr>
<tr>
<td>Score of 0 (no eyelashes) on eyelash assessment scale</td>
<td>10 (50%)</td>
<td>0 (0%)</td>
<td>10 (55.56%)</td>
</tr>
<tr>
<td>Score of 0 (no eyebrows) on</td>
<td>11 (55%)</td>
<td>0 (0%)</td>
<td>11 (61.11%)</td>
</tr>
</tbody>
</table>
Table 2. Summary of results for primary and secondary objectives

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Tofacitinib Group (n=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary Objective – Efficacy of sublingual tofacitinib</strong></td>
<td></td>
</tr>
<tr>
<td>Mean reduction from baseline of SALT score after 12 weeks</td>
<td>15.57 (23.41)</td>
</tr>
<tr>
<td>Treatment Response (Reduction in SALT score after 12 weeks)</td>
<td></td>
</tr>
<tr>
<td>Low grade respondents (15 – 29%)</td>
<td>3/16 (18.75%)</td>
</tr>
<tr>
<td>Medium grade respondents (30 – 49%)</td>
<td>1/16 (6.25%)</td>
</tr>
<tr>
<td>Good grade respondents (50 – 75%)</td>
<td>1/16 (6.25%)</td>
</tr>
<tr>
<td>High grade respondents (75 – 100%)</td>
<td>1/16 (6.25%)</td>
</tr>
<tr>
<td>Total participants showing some response</td>
<td>6/16 (37.5%)</td>
</tr>
<tr>
<td>Proportion of participants achieving at least 1 grade improvement in eyelash assessment scale after 12 weeks</td>
<td>6/16 (37.5%)</td>
</tr>
<tr>
<td>Proportion of participants achieving at least 1 grade improvement in eyebrow assessment scale after 12 weeks</td>
<td>8/16 (50%)</td>
</tr>
<tr>
<td><strong>Secondary Objective – Quality of life measurements</strong></td>
<td></td>
</tr>
<tr>
<td>Mean change from baseline in Assessment of Quality of Life-8D(^1) (AQoL-8D) score after 12 weeks (n=13)</td>
<td>-0.0148 (0.0515)</td>
</tr>
<tr>
<td>Mean change from baseline in Alopecia Areata Symptom Impact Scale (AASIS) score after 12 weeks – Global Symptom Impact Score(^2) (n=14)</td>
<td>-0.1306 (0.1252)</td>
</tr>
<tr>
<td>Mean change from baseline in Alopecia Areata Symptom Impact Scale (AASIS) score after 12 weeks – Scalp Hair Loss Score(^3) (n=14)</td>
<td>-2.2142 (2.3916)</td>
</tr>
<tr>
<td><strong>Secondary Objective – Pharmacokinetics</strong></td>
<td></td>
</tr>
<tr>
<td>Mean maximum plasma concentration (C(_{\text{max}}))</td>
<td>43.18 ng/ml</td>
</tr>
<tr>
<td>Time to mean maximum plasma concentration (t(_{\text{max}}))</td>
<td>1 hour</td>
</tr>
</tbody>
</table>

Data are mean (standard deviation) or proportion (percentage).

\(^1\)The AQoL-8D scale measures QOL on a scale from 0 (death) to 1 (full health), positive values reflect an improvement in QOL. \(^2\)The Global Symptom Impact Score measures all AA.
symptom impact on a scale of 0 (all symptoms not present) to 1 (all symptoms as bad as you can imagine), negative values reflect an improvement in symptom impact. \(^3\) The Scalp Hair Loss Score measures AA scalp hair loss from 0 (not present) to 10 (as bad as you can imagine), negative values reflect an improvement in scalp hair loss.
Total screened (n=34)

Excluded (n=14)
- Could not comply with visits (n=5)
- Could not comply with contraception requirements (n=1)
- Declined participation (n=5)
- Met exclusion criteria (n=3) e.g. TB, concomitant tofacitinib, active herpes zoster

Recruited (n=20)

Allocation

Responder (≥50% improvement in SALT score) – continuation of cyclosporine/placebo (n=2)
- Cyclosporine (n=2)
- Placebo (n=0)

Lost to follow-up before 16 weeks (n=0)

Completed 16 weeks (n=2)

Lost to follow-up before 20 weeks (n=0)

Completed 20 weeks (n=2)

Lost to follow-up before 24 weeks (n=0)

Completed 24 weeks (n=2)

Analysis

Per protocol analysis (n=2)

Non-responders (<50% improvement in SALT score) – rolled-over to open-label tofacitinib (n=18)
- Non-responder to cyclosporine (n=8)
- Non-responder to placebo (n=10)

Lost to follow-up before 4 weeks (n=0)

Completed 4 weeks (n=18)

Lost to follow-up before 8 weeks (n=2):
- Logistic reasons (n=2)

Completed 8 weeks (n=16)

Lost to follow-up before 12 weeks (n=0)

Completed 12 weeks (n=16)

Per protocol analysis (n=16)
Reasons for exclusions:
- Lost to follow-up (n=2)
Author/s:
Lai, VWY; Bokhari, L; Sinclair, R

Title:
Sublingual tofacitinib for alopecia areata: a roll-over pilot clinical trial and analysis of pharmacokinetics

Date:
2021-05-18

Citation:

Persistent Link:
http://hdl.handle.net/11343/278519