Systemic Treatments for Alopecia Areata: The Efficacy of Cyclosporin

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Abbreviations

Abbreviation	Name
AA	Alopecia Areata
AAAF	Australia Alopecia Areata Foundation
AASIS	Alopecia Areata Symptom Impact Scale
ALT	Alanine Aminotransferase
AMED	Allied and Complementary Medicine Database
ANA	Antinuclear antibody
Anti-Tg	Antithyroglobulin
ANZCTR	Australian New Zealand Clinical Trials Registry
ANZCTR	Australian New Zealand Clinical Trials Registry
AQoL-8D	Assessment of Quality of Life-8D
AST	Aspartate Aminotransferase
AT	Alopecia Totalis
AU	Alopecia Universalis
BMedSc(Hons)	Bachelor of Medical Science (Honours)
BMP-2/4	Bone morphogenetic protein-2/4
CD8	Cluster of differentiation 8
CENTRAL	Cochrane Central Register of Controlled Trials
CGT	Compound Glycyrrhizin Tablets
CMP	Calcium magnsium phosphate
Cr	Creatinine
DALYs	Disability-adjusted life years
DIRECT	Dermatology Investigational Research, Education and Clinical Trials
EBV	Epstein-Barr virus
ECG	Electrocardiogram
EOS	End of study
EOT	End of treatment
EUCTR	EU Clinical Trials register
FBE	Full blood examination
FGF5	Fibroblast growth factor 5
GGT	Gamma-glutamyl transferase
Hb	Haemoglobin
HIV	Human immunodeficiency virus
HLA	Human Leukocyte Antigen
HREC	Human Research Ethics Committee
HRQOL	Health-related quality of life
ICTRP	International Clinical Trials Registry Platform
IFN	Interferon
Intrauterine device	IUD
ITA	Intralesional triamcinolone acetonide

JAK	Janus kinase
K+	Potassium
LFT	Liver function tests
LILACS	Latin American and Caribbean Health Sciences Literature
Mg	Magnesium
MHC	Major histocompatibility complex
MSH	Melanocyte Stimulating Hormone
NTs	Neurotrophins
PBS	Pharmaceutical Benefits Scheme
PCA	Parietal Cell Antibody
PICF	Patient Information Consent Form
Plt	Platelets
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PT	Pulse Therapy
RCT	Randomised Controlled Trial
RNA	Ribonucleic acid
RS	Rodney Sinclair
SALT	Severity of Alopecia Tool
SALT100	100% reduction in SALT score
SALT30	At least 30% reduction in SALT score
SALT50	At least 50% reduction in SALT score
SALT75	At least 75% reduction in SALT score
SD	Standard deviation
SHH	Sonic hedgehog
SMA	Smooth muscle antibodies
STAT	Signal Transducer and Activator of Transcription
TGF-β	Transforming growth factor beta
TGPC	Total Glucosides of Paeony Capsule
Th1	Type 1 T helper
UEC	Urea electrolytes creatinine
USP	Unique Selling Proposition
UV	Ultraviolet
VL	Vivien Lai
WCC	White cell count
WNT	Wingless/Integrated

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Declarations

I declare that this thesis is my own original work. It contains no material previously published or written by another person, except where due reference has been made. The content of this thesis has not been previously submitted for the award of any other degree or diploma. Chapter 1 of this thesis comprises a shortened form of the systematic review previously submitted for the literature review component of the BMedSc(Hons) course. Chapter 1 has been updated to incorporate further background in addition to the systematic review, recent evidence, and feedback from examiners. This study was funded by the Australia Alopecia Areata Foundation. The study conception and design was developed by Prof. Rodney Sinclair, Dr. Gang Chen and myself. The ethics application for this study was written and submitted by myself, with the help of Laita Bokhari and Prof. Rodney Sinclair. I performed all recruitment, study visits and data acquisition for this study. I performed the data analysis and interpreted this with Prof. Rodney Sinclair and Dr. Gang Chen. I wrote this entire thesis, which was reviewed by Prof. Rodney Sinclair and Dr. Gang Chen. To the best of my knowledge, this thesis does not contain material previously published or written by another person, except with reference made in text.

Publications from BMedSc(Hons) Work Referenced

1. Lai VW, Gang C, Gin D, Sinclair R. Systemic Treatments for Alopecia Areata: A Systematic Review. Australas J Dermatol. 2018. DOI:10.1111/ajd.12913
2. Cranwell W, Lai VW, Photiou L, Meah N, Wall D, Sinclair R. Systemic treatment for alopecia areata: an expert consensus statement. Australas J Dermatol. 2018. DOI: 10.1111/ajd.12941

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Abstract

Background: Alopecia areata (AA) is a T-cell mediated autoimmune disease of the hair follicle resulting in acute or chronic patches of non-scarring hair loss, which may progress to loss of total scalp hair (alopecia totalis, AT), or universal loss of hair over the entire body (alopecia univeralis, AU). Systemic treatment for extensive disease has been poorly investigated. Despite widespread use of steroid-sparing agents, particularly cyclosporin, in the treatment of moderate to severe AA, there are no randomised, placebo-controlled trials evaluating its efficacy. Case series indicate the response rate to cyclosporin is in the range of 33% - 55%.

Aims: To evaluate the efficacy of cyclosporin compared to placebo at 3 months in patients aged 18 to 65 years with moderate to severe AA.

Methods: A double-blind, randomised, placebo-controlled trial was conducted. Adults aged 18 to 65 years of age with moderate to severe AA were randomised in a 1:1 ratio to receive 3 months of cyclosporin (4mg/kg/day) or matching placebo. The study was powered to detect a 50% reduction in SALT score in 50% of participants. Blinded assessments were conducted monthly and included: physical examination, blood biochemistry, photography, quality of life measurements and efficacy evaluation using Severity of Alopecia Tool (SALT) score, eyelash and eyebrow assessment scales. A per protocol interim analysis was performed for participants completing 3 months of treatment.

Results: 28 participants (cyclosporin: 13; placebo: 15) were analysed. At baseline, the mean SALT score was 79.4% and approximately half of participants in each group had AT or AU. The mean duration of current AA episode was 6.5 years. While the cyclosporin group had a greater mean change in SALT score (-10.3% versus -2.6%; p=0.59) and greater proportion of participants achieving at least a 50% reduction of SALT score (23.1% versus 6.7%; p=0.216) compared to placebo at 3 months, this did not achieve statistical significance. Only the proportion of participants achieving a 1 grade improvement in eyebrow assessment scale was significantly different between cyclosporin and placebo (23.1% versus 0.0%; p=0.049). Quality of life assessment did not show any statistically significant change for each group at the end of treatment compared to baseline.

Conclusion: This is the first randomised, placebo-controlled, prospective clinical trial investigating the effectiveness of 4mg/kg/day cyclosporin monotherapy in the treatment of

moderate to severe AA for 3 months. Interim results of 28 participants did not reveal a statistically significant difference between cyclosporin and placebo in reduction of scalp hair loss at 3 months of treatment. The trend for continued response over time suggests that trials employing a larger sample size and longer treatment duration may allow detection of lower response rates. These results suggest that any potential benefit associated with cyclosporin treatment is likely to be slower in onset than other inflammatory skin diseases, such as psoriasis and atopic dermatitis. These results may be interpreted for a cohort of patients with moderate to severe, long-standing AA and will guide clinicians in their choice of second-line agents for this patient cohort.

Abstract Word Count: 493

Chapter 1: Introduction and Literature Review

1.1 Introduction

Hair is a biological marker of health; associated with attractiveness and vitality. As a distinctive element of facial appearance, abnormal hair loss can result in substantial distress, particularly amidst a Western society where physical appearance and attractiveness are highly valued (1). Physiologically, hair also plays a role in protecting against ultraviolet damage of the scalp from the sun and more recently, has been implicated in immunological surveillance and regulation at the skin surface (2, 3).

Alopecia areata (AA) is the most common autoimmune disease in man (4) and the third most prevalent hair loss condition, following androgenetic and diffuse alopecia (5). It has a lifetime incidence of approximately 1.7% (6). It is a T-cell mediated autoimmune disease of the hair follicle that results in acute or chronic patches of non-scarring hair loss, often unpredictable, episodic and relapsing remitting in nature. Disease severity may range from a single solitary patch, to multiple focal patches, to complete loss of scalp hair (alopecia totalis, AT) or to complete loss of all hair on the scalp and body (alopecia universalis, AU). The complete aetiology remains unknown, though genetic, environmental and immune elements are involved.

Current management of AA is sub-optimal with uncertainties surrounding treatment choice, duration, indication and efficacy. Initial therapy often involves use of topical and intralesional corticosteroids. In extensive and refractory cases, systemic agents are trialled. However, the literature is deficient of high-quality studies evaluating systemic agents, so choice is largely dependent on clinician experience.

Specifically, the immunosuppressant medication, cyclosporin, is a popular second-line steroid-sparing agent used in clinical practice to arrest disease progression and induce hair regrowth. A number of uncontrolled single-arm studies, case series and retrospective reviews have suggested a relatively high and rapid response rate following use. However, the evidence from these studies may be critiqued for relatively small sample sizes, a lack of control, vague definitions of treatment success and in some instances, combination therapy with steroidal agents.

This dissertation aims to investigate the efficacy of systemic treatments for AA, specifically the efficacy of cyclosporin.

Chapter 1 reviews the relevant literature and includes a systematic review of systemic agents used for AA previously submitted for the BMedSc(Hons) degree. Chapter 2 outlines the materials and methodology for the main research project. Chapter 3 discusses the results of this project. Chapter 4 provides a discussion of the implications, limitations and future directions from this dissertation.

1.2 Hair Anatomy and Biology

Hair has both biological and social functions. Biologically, it protects the scalp, face and neck from ultraviolet radiation, aids in conserving heat otherwise lost through the scalp and provides tactile sensation (7). Socially, hair contributes to the aesthetic representation of beauty and attractiveness. A deviation from what is considered ‘normal’ hair often leads to significant psychological morbidity, whether it be excess or deficient hair, distribution, colour or quality of hair.

The hair follicle consists of the following regions: bulb, suprabulbar zone, isthmus and infundibulum (Figure 1) (8). The hair bulb is the lowest region of the hair follicle and contains the dermal papilla, an onion-shaped dense population of fibroblasts that controls hair growth (8). This is surrounded by the hair matrix, a collection of rapidly proliferating keratinocytes that differentiate into the hair shaft (9). The suprabulbar zone runs between the bulb and isthmus and contains the medulla and cortex of the hair shaft, as well as the inner and outer root sheath. The isthmus is the region between the attachment of the arrector pili muscle and opening of the sebaceous gland. Specifically, the arrector pili muscle attaches at the ‘bulge’, a segment of the outer root sheath, which contains epithelial stem cells. It has been suggested if the ‘bulge’ region and epithelial stem cells are damaged, the hair follicle is also destroyed (7). The infundibulum extends from the opening of the sebaceous gland to the epidermis of the skin.

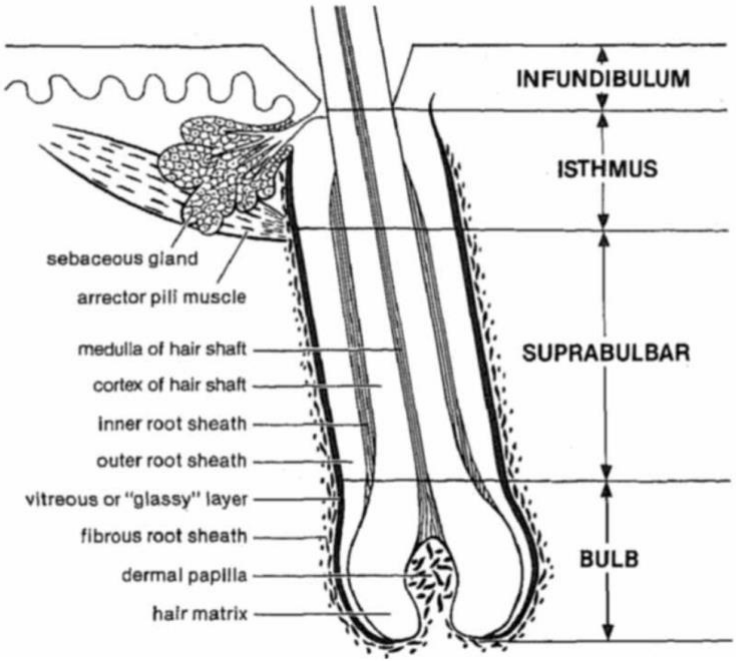

Figure 1. The anatomical structure of the hair follicle.
From Sperling LC. Hair anatomy for the clinician. J Am Acad Dermatol. 1991;25(1 Pt 1):1-17.

Hair continuously cycles through growth and degeneration throughout one's lifetime and is the only organ in the body to do so. The hair cycle consists of 3 stages: anagen (growth phase), catagen (regression phase) and telogen (rest phase).

Anagen is the longest phase of the hair cycle, lasting from 2 – 6 years depending on body site and age (9), during which epithelial cells in the hair matrix actively proliferate to result in increased hair length proportional to the length of anagen. During anagen, the hair matrix is particularly susceptible to damage from drugs, hormones, stress, immune injury and physical trauma. Approximately 85% – 99% of hairs are in the anagen phase (9).

Catagen follows anagen and is a phase of controlled keratinocyte apoptosis and suprabulb and bulb regression, during which proliferation of epithelial cells in the hair matrix ceases and there is a 50% reduction in the dermal papilla volume from loss of extracellular matrix and migration of cells to the dermal sheath (9). Only the lower two-thirds of the hair follicle undergoes involution during catagen; the infundibulum, isthmus and bulge region do not cycle. This phase lasts about 2 weeks. Less than 1% of total hairs at any time are in catagen. The exact signal responsible for initiating catagen from anagen is unknown, however morphogens implicated include FGF5, TGF- β, NTs and BMP-2/4 (Figure 2) (9).

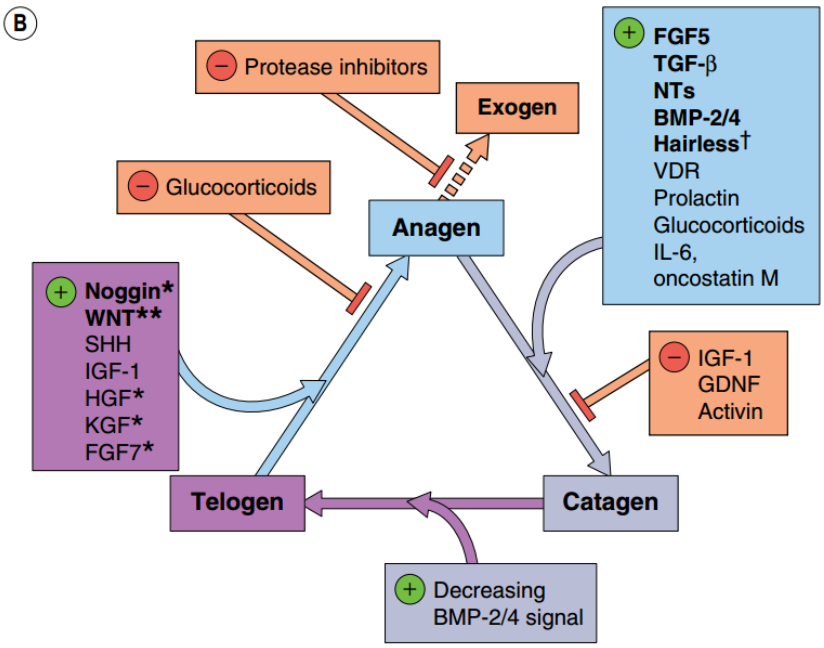

Figure 2. Regulation of hair follicle cycling.
From Wang E, de Berker, D., Christiano, A Biology of Hair and Nails. 4th ed. Bolognia J, Jorizzo, J. L., & Schaffer, J. V, editor. Philadelphia: Elsevier Saunders; 2018.

Telogen follows catagen and is a dormant phase of the hair cycle, lasting about 3 months, prior to the hair shedding at the end, known as exogen. About 10-15% of hairs are in the telogen phase (9).

The telogen-to-anagen transition is initiated by a number of factors, including WNT, SHH, noggin (Figure 2) and cessation of inhibitory FGF-18 (10-12), resulting in regenerative activity from the bulge region and hair matrix to begin anagen again.

1.3 Alopecia Areata

1.3.1 Presentation

The estimated lifetime incidence of AA is 1.7% (6), however solitary patches often remain undetected, without presentation to healthcare services, so this incidence is likely to be higher.

AA affects both sexes equally (6, 13) with no racial preponderance (14). While AA may develop at any age, it most commonly starts before the age of 40 (15), at an estimated mean of 30 years (13, 15, 16). Younger age groups are more likely to present for care than older age groups (15). Moreover, individuals less than 20 years of age presenting with AA are more likely to have severe disease (15-17).

There are various forms of AA. Patchy AA is the most common presentation where individuals develop one or more well-circumscribed ovoid areas of hair loss on the scalp. 58% of adults who first present with AA have patches affecting less than 50% of the scalp (15). The underlying scalp is smooth to touch and there is no scarring. The most frequently affected site on the scalp is the occipital region in about 35% of cases for males and females (18).

Other presentations include total loss of scalp hair (alopecia totalis, AT) or loss of entire scalp and body hair (alopecia universalis, AU). This represents approximately 7.3% of AA cases (15). Nail involvement, such as pitting, ridging, onycholysis or trachyonychia, occurs in approximately 64% of individuals with AA (19, 20) and is a poor prognostic factor (15). Pruritus and stinging of the scalp may also be experienced.

Characteristically, AA features peri-marginal hairs that are dystrophic, short and fractured, known as exclamation mark hairs. On dermoscopy, there may be hair breakage at the scalp (cadaverised hairs) or round yellow dots, markers of disease progression.

AA has a number of autoimmune and atopic disease associations, including thyroid disease, atopic dermatitis, asthma and vitiligo (21, 22). Concomitant thyroid disease is particularly more prevalent in females (23). Positive autoimmune serology, including ANA, SMA, Anti-Tg or PCA, is reported in 51.4% of AA patients (24).

It is difficult to determine how an individual's AA will fare. Some individuals may experience one patch of AA and have spontaneous remission, while others may develop multiple

patches over time. Poor prognostic factors include: extensive disease at presentation, oophiasis pattern (band-like involvement of the scalp), nail involvement, early age of onset and family history (15, 16, 25, 26). Ikeda et al.'s (27) study of 1987 individuals with AA showed that 91% of those who developed only 1 circular patch of AA were likely to experience spontaneous regrowth of hair within 6 months. However, this represents only 38% of individuals. Many are more likely to develop multifocal AA of which 65% recover within 12 months and 35% develop chronic disease. Those with disease beyond 12 months had a 50% chance of progressing to AT.

1.3.2 Burden of Disease

Globally, the disability-adjusted life years (DALYs) lost to AA is an estimated 1,332,800 (28, 29); a figure which has increased linearly from under 1 million in 1990. This is comparable to other dermatological conditions, including psoriasis (DALYs: 1,050,660). Disability weights, where 0 represents perfect health and 1 represents death, also show comparability of AA (disability weight = 0.035) to other skin diseases, including: urticaria (0.031), eczema (0.038) and cellulitis (0.035) (30).

1.3.3 Aetiology

AA is an organ-specific, CD8-driven, Th1-type, T-cell mediated autoimmune disease affecting the anagen phase of hair follicles (31). Histologically in AA, the lower hair follicle is surrounded by a lymphocytic infiltrate described as a “swarm of bees” pattern (32). The infiltrate is predominantly composed of CD4+ T-cells, while CD8+ T-cells are found within the follicular epithelium (33).

The exact aetiology is unknown, however genetic, environmental and immune associations have been implicated. The most recognised theory is the ‘immune privilege collapse model’ of AA. It has been found that the proximal hair follicle epithelium maintains an area of relative immune privilege during anagen, characterised by a low MHC class Ia antigen expression and production of immunosuppressive agents, α-MSH and TGF-β1 (34-36). This has been proposed to function as a mechanism of protection for the hair follicle against autoimmunity during the growth phase, sequestering anagen- and melanogenesis-associated autoantigens from attack by CD8+ T-cells (37). Damage to the protective hair follicle immune privilege, from insults such as infection, stress, skin trauma resulting in rises of inflammatory signals such as IFN-γ and abnormal expression of MHC class Ia antigen during anagen (38-40), thereby leads to the development of AA. Viruses, including Epstein-Barr virus, may be a triggering factor through mechanisms such as molecular mimicry (41,

42) and epitope spreading. Furthermore, melanogenesis-associated autoantigens are implicated in other autoimmune diseases such as vitiligo and halo naevi, and its involvement in AA may explain the tendency for non-pigmented hair to be spared and initial depigmented regrowth during recovery (43, 44). This theory is supported by Gilhar and Kalish's landmark study which showed that AA lesions were induced following transfer of MHC class I-restricted CD8+ T-cells only when anagen hair follicle antigens were present to stimulate T-cells or when melanogenesis-associated autoantigens were present (45).

This theory of immune privilege may be further contextualised by understanding genetic associations of AA as immunogenetically susceptible individuals may have hair follicles predisposed to autoimmune attack and immune privilege breakdown. AA is a complex polygenetic disorder, resulting in variable family history, with patients reporting figures between 0% to 8.6% (29). Genome-wide association studies have identified 14 susceptibility loci in AA (46). HLA serotypes DR11 and DQ7 are associated with AT and AU, and the general susceptibility gene, *HLA-DQB1*03* is found in up to 80% of AA patients (47).

1.3.4 Impact

Hair is a crucial component of facial identity for both men and women. In an image-oriented society, hair loss is psychologically devastating and more than 50% of patients with AA experience reduced health-related quality of life (HRQOL) (48). The prevalence of psychiatric disorders in patients with AA is between 66%-74% (49-51). Lifetime prevalence of depression is 39% and prevalence of generalised anxiety disorder is 40% - 60% (49-51).

Systematic reviews highlight the great impact of AA on HRQOL across a range of dimensions, most significantly role-emotional, mental health and vitality (29, 52). This occurs in both males and females alike (53)**,** however a greater burden is often associated with females due to a greater investment in physical appearance (54). Other risk factors for poor HRQOL are: 20-50 years of age, scalp hair loss greater than 25%, lightening of skin colour, family stress and occupational change (48).

The enigmatic occurrence of new patches in AA is also thought to compound anxiety associated with hair loss, due to the sporadic nature of relapses impairing the ability to cope and mentally prepare for change (55). Individuals also lose the ability to manipulate and improve appearance due to uncertainty (54).

There have been case reports of youth suicide in 4 adolescent Australian males (56) all of whom did not have pre-existing psychological disorders and presented with depressive symptoms following onset of AA.

1.4 Current Management

There is currently no curative treatment for AA. Treatment is tailored depending on disease severity, disease activity and psychosocial impact. Conservative management may be an option for patients with minimal disease, with up to 80% of cases self-resolving within 1 year (57).

Limited patchy disease is often treated with intralesional triamcinolone acetonide or topical agents, such as topical steroids and immunotherapy. Studies suggest that for solitary patches, intralesional triamcinolone acetonide (ITA) is more effective than topical agents, including betamethasone valerate (58, 59) or topical tacrolimus (59). ITA treatment is typically repeated every 4-6 weeks until remission of the patch. There is no difference in efficacy between lower concentrations of ITA (e.g. 2.5 mg/mL) and higher concentrations (e.g. 10 mg/mL) (60). Nevertheless, cumulative use of intralesional corticosteroids though, has been associated with a high toxicity profile, especially osteopenia and osteoporosis. Samroa et al. (61) showed that 50% of AA patients with a 20-month treatment period of intralesional corticosteroids had reduced bone mineral density on dual-energy x-ray absorptiometry.

Extensive disease, typically more than 30% loss of scalp hair, is generally treated with systemic agents. Commonly used systemic therapies include: corticosteroids or steroid-sparing agents including methotrexate, azathioprine and cyclosporin. Other tried systemic therapies include dapsone, mycophenolate, tacrolimus, sulfasalazine, for which evidence is weak (62).

The majority of these systemic agents have only been evaluated in case series, retrospective reviews and small uncontrolled trials. Systemic corticosteroids are effective in up to 89% of patients (63), however long-term use is limited by toxicity and not recommended. Alternatively, steroid-sparing agents are a viable treatment modality and are often used either in combination with prednisolone or as a monotherapy second-line agent (62).

1.5 Systematic Review of Systemic Treatments for AA, AT and AU

Only one previous systematic review published a decade ago has evaluated systemic therapy in patients with AA (64). This review found only 3 RCTs evaluating systemic therapy. There were no treatments found to be of long-term benefit for AA. Evidence for systemic treatment was poor.

Since that review, further trials have been conducted to evaluate systemic therapies. It is timely for an updated review on these trials. In a consensus between patients, carers, relatives and health professionals, quantifying the efficacy of systemic therapies, both immunosuppressant and biological, represented 2 of the top 3 research uncertainties to be prioritised (65).

We conducted a systematic review to identify studies that have investigated the use of a systemic agent for treatment of AA, AT or AU. To the best of our knowledge, this is the most updated systematic review since Delamere et al. (64) that will comprehensively assess systemic treatments being used.

The objectives of the review are:

- To identify RCTs of systemic treatments for AA, AT or AU
- To evaluate the efficacy of systemic treatments for the management of AA, AT or AU reported in RCTs
- To identify how efficacy is being measured in RCTs of systemic treatments for AA, AT or AU
- To assess the side effects of systemic treatments used in the management of AA, AT or AU reported in RCTs

1.5.1 Methods

This systematic review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (66) and registered on PROSPERO in advance (CRD42018088758). All RCTs that evaluated the effectiveness of systemic treatments for AA, AT or AU were included. We included all comparators of a systemic treatment to: placebo, other systemic treatment and non-systemic treatment.

Medline (1946 to present), Embase (1974 to present), Allied and Complementary Medicine Database (1985 to present), the Cochrane Central Register of Controlled Trials (1999 to present), PsychINFO (1806 to present) and Latin American and Caribbean Health Sciences

Literature (1987 to present) were searched through March 4, 2018 using a combination of free-text terms and medical subject headings (e.g. 'alopecia areata', 'randomised controlled trial') (Appendix 1).

Ongoing trials were searched through the following databases: Clinical-Trials.gov, metaRegister of Controlled Trials, the Australian New Zealand Clinical Trials Registry, the EU Clinical Trials register, and the World Health Organisation International Clinical Trials Registry Platform.

Both forward and backward hand-searching were employed to identify additional studies that satisfied the inclusion criteria. Abstracts and/or titles of every record retrieved were scanned and the full text of all potentially relevant articles were examined. Risk of bias in included studies was assessed using the guidelines of the Cochrane Handbook of Systematic Reviews of Interventions (66). Reporting bias was assessed through funnel plot analysis using Stata version 12 software for symmetry on visual inspection and Egger's test.

1.5.2 Results

A total of 2,830 articles were identified from the search strategy (Figure 3). After inclusion and exclusion criteria, 16 studies involving 768 randomised participants were included in the review (Table 1). All inclusion criteria entailed some form of AA, AT or AU, with variation across studies as to specificity of severity and duration. The age range of participants across all included studies was 2 – 66 years.

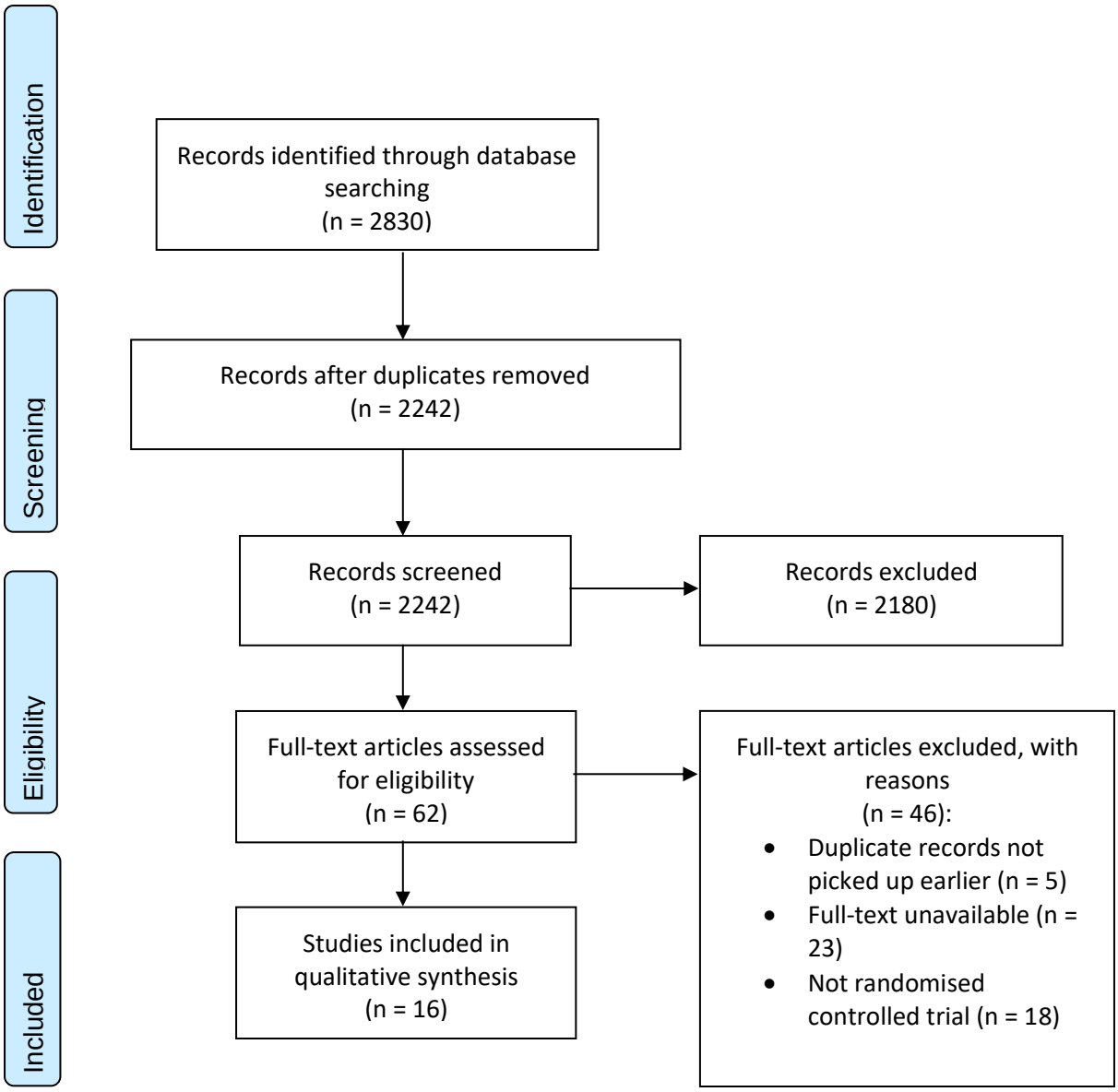

Figure 3. PRISMA flow diagram

Table 1. Characteristics of included studies.

Study Authors	Year	Study Design	Country	Frequency of follow up	Number Randomised	Age Range	Inclusion Criteria	Interventions
Berth-Jones et al	1990	RCT	UK	At 1, 2, 4 and 6 months	33	17-66 years	AT or AU of at least 12 months duration	1. Oral inosine pranobex 50 mg/kg/day in three doses for 6 months 2. Diphencyprone 1% solution to the right scalp weekly until sensitization, then applications of variable concentration, sufficient to maintain minimal irritation of the scalp weekly to the same side for 6 months. 3. Both oral inosine pranobex and diphencyprone for 6 months
Cipriani et al	2001	RCT	Italy	Every month	13	21-62 years	AA and psychiatric comorbidity	1. Oral paroxetine 20mg daily for 3 months 2. Placebo for 3 months
Dehghan et al	2013	RCT	Iran	-	40	-	AA with at least 30% involvement of scalp or more than 10 patches of alopecia in scalp and body	1. Oral prednisolone PT 200 mg in one dose every week for 3 months 2. Intravenous methylprednisolone PT 500 mg on 3 continuous days each month for 6 months
Ead et al	1981	RCT	UK	-	42	-	AA, AT or AU	1. Oral zinc sulphate, one capsule twice daily for 3 months 2. Placebo for 3 months
Galbraith et al	1986	Cross-over RCT	America	At 0, 2, 8, 14, 20, 22, 28, 34, and 40 weeks	34	-	AT of at least 1 year duration and documented evidence of cell-mediated immune dysfunction	1. Inosiplex for 20 weeks in total at 50 mg/kg/day up to 5 g/day from week 0 through week 2 and 9 through 20; 50 mg/kg 3 days a week from week 3 through week 8 2. Placebo for 20 weeks After 20 weeks, treatments were crossed-over for a further 20 weeks.
Georgala et al	2006	RCT	Greece	Every month	32	16-48 years	AA with at least 12 months duration and lesions refractory to at least one conventional therapy	1. Oral inosiplex 50 mg/kg/day given in the form of 500 mg tablets in five divided doses for 12 weeks 2. Placebo for 12 weeks
Kar et al	2005	RCT	India	Every month	43	-	AA with at least 40% loss of scalp hair or 10 patches scattered over the scalp and body	1. Oral prednisolone PT 200 mg once weekly for 3 months 2. Placebo for 3 months
Kurosawa et al	2005	Quasi-RCT	Japan	Every month	89	16-63 years	AA (single or multiple), AT or AU	1. Oral prednisolone PT 80 mg for 3 consecutive days once every 3 months for 12 months 2. Intramuscular triamcinolone 40 mg once a month for 6 months followed by 40 mg once every 1.5 months for 1 year 3. Oral dexamethasone 0.5 mg/day for 6 months
Mehta et al	2012	RCT	India	Every week	51	5-60 years	AA	1. Liquid phenol (20%) and oral betamethasone minipulse therapy. Group C (Regimen 3) applied liquid phenol (20%) weekly with oral Betamethasone minipulse therapy (1mg) 5 tab on sat/Sunday with milk for 3 months 2. Liquid phenol (20%) with topical minoxidil (2%). Group B (Regimen 2) applied liquid phenol (20%) weekly with topical minoxidil (2%) from second day twice daily (Regimen 2) for 3 months

								3. Liquid phenol (20%). Group A (Regimen 1) applied liquid phenol (20%) weekly for 3 months
Perini et al	1994	RCT	Italy	-	13	20-55 years	AT or AU with recent onset of symptoms (less than 6 months)	1. Imipramine 75mg daily for 6 months 2. Placebo for 6 months
Price et al	2008	RCT	America	-	62	18-59 years	AA with one of the following patterns for the previous 3 months: 50% to 95% of the scalp affected and a positive pull test; at least 95% of the scalp affected for up to 24 months; or AT for up to 12 months. Not responded adequately to topical, intralesional or systemic therapies and aged 18 to 70 years.	1. Subcutaneous efalizumab 1.0 mg/kg weekly for 12 weeks 2. Placebo weekly for 12 weeks
Saif et al	2012	RCT	Saudi Arabia	-	42	-	AT, AU or OA	Daily dose of 15mg/kg oral methylprednisolone PT in the following 3 regimens: 1. 3 consecutive days once every 2 weeks for 24 weeks. 2. 2 consecutive days every 3 weeks for 24 weeks. 3. 3 consecutive days every 3 weeks for 24 weeks..
Strober et al	2009	RCT	America	-	45	18-65 years	AA with at least a 50% to 95% patchy scalp hair loss of at least 6 months' duration aged 18 to 65 years	1. Alefacept 15mg weekly IM administration for 12 weeks 2. Placebo weekly IM administration for 12 weeks
Tosti et al	1991	RCT	Italy	Every month	26	16-48 years	AT or AU with no response from sensitizing therapy for at least 1 year	1. Intravenous thymopentin three times a week for 3 weeks, every 3 months, for 9 months. 2. Topical 10% cyclosporine in oily solution. 3. Photochemotherapy (PUVA) three times a week for 9 months. Squaric acid dibutylester 2% in acetone or diphencyprone 2% in acetone for sensitization in both groups.
Yang et al	2013	RCT	China	Every month	117	2-14 years	AA with severity ≥S3 (50% to 75% hair loss) aged 2-24 years	1. Oral TGPC 300mg, 3 times per day and Oral CGT 25mg, 3 times per day for 12 months 2. Oral CGT 25mg, 3 times per day for 12 months
Yang et al	2012	RCT	China	Every month	86	18-65 years	AA with severity less than S3 (50% to 75% hair loss) aged 18 to 65 years	1. Oral TGPC three times daily and 600 mg per time for 3 months 2. Oral CGT three times daily and 50 mg per time for 3 months Both groups had 10 mg vitamin B2 and tapped the bald patches with massage

-: not reported

Table 2. Outcome measures for treatment effect.

Study	Year	Numerical outcomes	Definition of numerical outcomes	Categorical outcomes	Definition of categorical outcomes
Berth-Jones et al, 1990	1990	-	-	Good, poor and no response	(1) Good response: a response of cosmetic value, between 20 and 100% terminal scalp hair regrowth, (2) Poor response: a response of no cosmetic value, less than 20% terminal scalp hair regrowth, (3) No response
Cipriani et al, 2001	2001	-	-	Complete response, partial response or no response	-
Dehghan et al, 2013	2013	-	-	Percentage categories of improvement in scalp hair	Percentage categories of improvement in scalp hair: (1) less than 30% improvement, (2) 30-60% improvement, (3) 60-99% improvement.
Ead et al, 1981	1981	-	-	Response or no response	-
Galbraith et al, 1986	1986	Mean length of scalp hair	-	Response or no response	-
Georgala et al, 2006	2006	-	-	Complete response, partial response or no response	(1) Complete response: total hair regrowth, (2) Partial response: at least 50% hair regrowth, (3) No response: less than 50% hair regrowth.
Kar et al, 2005	2005	-	-	Marked, moderate or poor regrowth Significant regrowth or no significant regrowth	(1) Marked: more than 60% regrowth, (2) Moderate: 31-60% regrowth, (3) Poor: regrowth less than 30%. Moderate to marked hair regrowth was significant regrowth.
Kurosawa et al, 2005	2005	-	-	Response or no response	Response defined as more than 40% regrowth of cosmetically acceptable terminal hair, or ability to abandon a wig or hat.
Mehta et al, 2012	2012	-	-	Grades of improvement 1-4	-
Perini et al, 1994	1994	-	-	Full regrowth, terminal hair, vellus hair or no regrowth	-
Price et al, 2008	2008	Percentage hair regrowth measured by SALT score Participant assessment of disease	Participant assessment of disease - Measured using a 100-mm visual analog scale. 0 represented no hair loss and 100 represented total hair loss.	Percentage categories of improvement in scalp hair Response or no response	Percentage categories of improvement in scalp hair: (1) at least 75% hair regrowth, (2) 50-74% hair regrowth, (3) 25-49% hair regrowth Response defined as at least 50% hair regrowth
Saif et al, 2012	2012	-	-	Adequate response, inadequate response or poor response	(1) Adequate response: >75% regrowth of terminal hair, (2) Inadequate response: 25-74% regrowth of terminal hair, (3) Poor response: <25% regrowth of terminal hair.

Strober et al, 2009	2009	Mean percentage of hair regrowth measured by SALT score		Response or no response Participant assessment of disease using 7-point qualitative scale: none, trace, mild, mild to moderate, moderate, moderate to severe, severe	Response defined as 50% or greater reduction in SALT score.
Tosti et al, 1991	1991	-	-	Cosmetic clinical improvement or no cosmetic clinical improvement Regrowth of terminal hair or no regrowth of terminal hair	-
Yang et al, 2012	2012	-	-	Cured, markedly effective, effective or failed	(1) Cured: hairs all grew out again, normal in density of distribution, color and luster, and negative in pulling hair test; (2) Markedly effective: 70% of hairs grew out again, almost normal in density of distribution, color and luster; (3) Effective: 30% of hairs grew out again, including fine hair and white hair, with no hair loss after treatment; (4) Failed: after a treatment of more than 3 months, new hairs grew out less than 30% or with continued hair loss
Yang et al, 2013	2013	Change in score of alopecia areata severity	Score of alopecia areata severity: S0 (no hair loss) was given 0 score; S1(<25% hair loss) was given 1 score; S2 (25%-49% hair loss) was given 2 scores; S3 (50%-74% hair loss) was given 3 scores; S4 (75%-99%) was given 4 scores; S5 was given 5 scores; S5B0 (AT) was given 6 scores; S5B1 (AT with partial body hair loss) was given 7 scores; S5B2 (AU) was given 8 scores.	Cured, markedly effective, effective or ineffective.	(1) Cured: all hairs grew out again, normal in density of distribution, color and luster, and negative in pulling hair test; (2) markedly effective: 50% of hairs grew out again, almost normal in density of distribution, color and luster, with many fine hair turning into hair, and negative in pulling hair test; (3) effective: 10% of hairs grew out again (including fine hair) but grew slowly, and negative or positive in pulling hair test; (4) ineffective: after a treatment of more than 3 months, no new hairs grew out or new hairs just less than 10% or continued with hair loss.

-: not reported

Table 3. Adverse events.

Study	Adverse events
Berth-Jones et al, 1990	No adverse effects from inosine pranobex. Diphencyprone produced occasional severe eczematous reactions and vitiligo in one patient. No adverse change in blood biochemistry; serum urates occasionally exceeded the normal range.
Cipriani et al, 2001	-
Dehghan et al, 2013	1. Oral prednisolone PT: 5 patients developed acne, 4 heartburn, 4 striae. 45% of patients developed side effects. 2. Intravenous methylprednisolone PT: 7 patients developed acne, 5 heartburn, 6 striae. 55% of patients developed side effects. The difference was not statistically significant
Ead et al, 1981	-
Galbraith et al, 1986	No clinically significant adverse reactions to inosiplex were encountered.
Georgala et al, 2006	Inosiplex was generally well tolerated.
Kar et al, 2005	11 (55%) patients developed side effects on oral prednisolone PT. General weakness for 1-2 days was the most common side effect. Other side effects: acneiform eruption, weight gain, gastrointestinal upset, facial mooning, and oligomenorrhea. All the side effects subsided in follow-up period.
Kurosawa et al, 2005	1. Oral prednisolone PT: side effects were noted in 3 or 29 patients (10%) - 2 developed dysmenorrhea, 1 complained of abdominal discomfort. 2. Intramuscular triamcinolone: 23 of 56 patients (41%) - 3 patients with abdominal discomfort, 1 patient with worsening acne, and 16 patients with dysmenorrhea. 3. Oral dexamethasone: 6 of 20 patients (30%) - 3 with weight gain, 2 with abdominal complaints, 1 with weakness, and 1 with mooning
Mehta et al, 2012	2 complications noted: secondary infection and hypopigmentation in 1 patient each. It was not noted in which treatment arms these patients were from.
Perini et al, 1994	Well tolerated.
Price et al, 2008	Efalizumab was well tolerated. Most frequent side effects: headache, fever, infection, nausea, rash, myalgia, and pharyngitis. With the exception of headache during the double-blind period, there were no statistically significant differences in frequency of AEs between the efalizumab and placebo treatment groups.
Saif et al, 2012	Relatively tolerated. Overall, 40 (95%) patients reported 186 adverse events. The most common side effect was fatigue (n=27, 64%) for 3 days after each pulse. Others include: weight gain (n=19, 45%), steroid induced acne (n=15, 35.7%), and sleep disturbances (n=14, 33%).
Strober et al, 2009	Adverse events of alefacept were similar to placebo group. Mostly mild and unrelated to treatment. Most frequent side effects in both groups: infections (upper respiratory in-fections, influenza), headaches, and nasal congestion.
Tosti et al, 1991	-
Yang et al, 2012	1. Oral TGPC: 6 cases of adverse reactions reported in the treatment group, mild and transient. 2. Oral CGT: 7 cases of adverse reactions reported in the control group, mild and transient. The incidence rates of both groups were similar without statistically significant difference (P=0.695)
Yang et al, 2013	1. Oral TGPC and CGT: 7 cases of adverse events, most frequently abdominal pain and loose stools 2. Oral CGT: 6 cases of adverse events, most frequently oedema, rash and weight gain All events were mild and transient. There was no statistically different in the incidence rate of adverse events between the two groups.

-: not reported

Table 4. Relapse rate.

Study	Length of time before relapse	Relapse Rate	Relapse definition
Berth-Jones et al, 1990	-	-	-
Cipriani et al, 2001	-	-	-
Dehghan et al, 2013	-	-	-
Ead et al, 1981	-	-	-
Galbraith et al, 1986	-	7 of the 11 (64%) patients had experienced loss of new hair growth within 2 to 11 months of inosiplex discontinuation.	-
Georgala et al, 2006	-	-	Reappearance of lesions
Kar et al, 2005	-	2 of 8 responders (25%) had relapse at the end of 3 months.	More than 20% hair loss compared with baseline during the follow-up period of 3 months
Kurosawa et al, 2005	Relapse was recognized 3 months or later after the discontinuation of steroid.	Relapse rate at 6 months after treatment was 33% in the prednisolone PT group (10/29), 46% in the intramuscular triamcinolone group (20/43), and 75% in the oral dexamethasone group (14/19). The relapse rate was significantly different only between the dexamethasone group and the prednisolone group. For patients with AT or AU, the relapse rate was 49% were in the prednisolone group, 71% in the intramuscular triamcinolone group, and 100% in the oral dexamethasone group. There was a significant difference in the relapse rate between the oral dexamethasone group and the prednisolone group.	Appearance of new bald patches or an abnormal increase of hair fall.
Mehta et al, 2012	-	-	-
Perini et al, 1994	-	-	-
Price et al, 2008	-	-	-
Saif et al, 2012	Variable and unpredictable. Some relapsed shortly after induction, others kept regrowth for 4 years off-treatment.	After 1-4 years off-treatment 13 of 34 (38.2%) patients relapsed, 5 (14.7%) patients developed moderate hair fall, 3 (8.8%) patients developed mild hair fall, 7 (20.1%) patients maintained their hair regrowth and 6 (17.6%) patients were lost to follow up.	Recurrence or worsening of alopecia from its original severity.
Strober et al, 2009	-	-	-
Tosti et al, 1991	-	-	-
Yang et al, 2012	-	-	-
Yang et al, 2013	-	-	-

-: not reported

Table 5. Risk of bias in included studies.

Study	Random sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data	Selective reporting	Other bias
Berth-Jones et al, 1990	Uncertain: Random sequence generation not specified.	Uncertain: No description of allocation concealment.	High: No description of blinding. Differing routes of administration make risk of bias high.	Uncertain: No description.	Uncertain: Intention to treat analysis not performed, though incomplete attrition reported.	Low: All outcomes reported.	Low
Cipriani et al, 2001	Uncertain: Random sequence generation not specified.	Uncertain: No description of allocation concealment.	Low: Identical placebo employed.	Uncertain: No description.	Uncertain: Attrition rate not reported.	Low: All outcomes reported.	Low
Dehghan et al, 2013	Uncertain: Random sequence generation not specified.	Uncertain: No description of allocation concealment.	High: No description of blinding. Differing routes of administration make risk of bias high.	Uncertain: No description.	Uncertain: Intention to treat analysis not performed, though incomplete attrition reported.	Low: All outcomes reported.	Low
Ead et al, 1981	Uncertain: Random sequence generation not specified.	Uncertain: No description of allocation concealment.	Low: Identical placebo employed.	Uncertain: No description.	Uncertain: Intention to treat analysis not performed, though incomplete attrition reported.	Uncertain: Potential for selective reporting of 'response' given this was not defined.	Low
Galbraith et al, 1986	Low: Random sequence used.	Low: Allocation concealed by sponsor	Low: Identical placebo employed.	Low: Likely low given allocation concealment, double-blinding and identifical placebo tablets being employed.	Uncertain: Intention to treat analysis not performed, though incomplete attrition reported.	Uncertain: Potential for selective reporting of 'response' given this was not defined.	Low
Georgala et al, 2006	Low: Random number table used.	Uncertain: No description of allocation concealment.	Low: Identical placebo employed.	Low: Blinded investigator	Low: Intention to treat analysis performed.	Low: All outcomes reported.	Low
Kar et al, 2005	Low: Random number table used.	Uncertain: No description of allocation concealment.	Low: Identical placebo employed.	Uncertain: No description.	Uncertain: Intention to treat analysis not performed, though incomplete attrition reported.	Low: All outcomes reported.	Low
Kurosawa et al, 2005	High: Quasi-RCT.	High: Likely to know allocation based on initial visit	High: No description of blinding. Differing routes of administration make risk of bias high.	Uncertain: No description.	Uncertain: Inconsistent reporting of numbers randomised to treatments: Sum of AA/multiplex patients in Table 1 is 53, whereas 51 reported to have been recruited in total.	Low: All outcomes reported.	Low
Mehta et al, 2012	Uncertain: Random sequence generation not specified.	Uncertain: No description of allocation concealment.	High: No description of blinding. Differing routes of administration make risk of bias high.	Low: Blinded investigator	Uncertain: Attrition rate not reported.	Uncertain: Potential for selective reporting of 'grades' and 'clinical improvement' given this was not defined.	Low

Perini et al, 1994	Uncertain: Random sequence generation not specified.	Uncertain: No description of allocation concealment.	Low: Identical placebo employed.	Low: Blinded investigator	Low: All recruited participants included in outcome assessment.	Low: All outcomes reported.	Low
Price et al, 2008	Low: Random sequence used.	Low: Allocation concealed by blinded randomisation manager	Low: Identical placebo employed.	Low: Likely low given allocation concealement, double-blinding and placebo use	Low: Intention to treat analysis performed.	Low: All outcomes reported.	Low
Saif et al, 2012	Uncertain: Random sequence generation not specified.	Uncertain: No description of allocation concealment.	High: No description of blinding. Differing routes of administration make risk of bias high.	Low: Blinded investigator	Uncertain: Intention to treat analysis not performed, though incomplete attrition reported.	Low: All outcomes reported.	High
Strober et al, 2009	Low: Random sequence used.	Low: Allocation concealed by study coordinator/pharmacist	Low: Identical placebo employed.	Low: Blinded investigator	Low: Intention to treat analysis performed	Low: All outcomes reported.	Low
Tosti et al, 1991	Uncertain: Random sequence generation not specified.	Uncertain: No description of allocation concealment.	High: No description of blinding. Differing routes of administration make risk of bias high.	Uncertain: No description.	Low: All recruited participants included in outcome assessment.	Uncertain: Potential for selective reporting of 'cosmetic improvement' given this was not defined.	Low
Yang et al, 2012	Low: Random number table used.	Uncertain: No description of allocation concealment.	Uncertain: Similarity of tablets not specified.	Uncertain: No description.	Low: All recruited participants included in outcome assessment.	Low: All outcomes reported.	Low
Yang et al, 2013	Low: Random number table used.	Uncertain: No description of allocation concealment.	High: No description of blinding. Differing routes of administration make risk of bias high.	Uncertain: No description.	Low: All recruited participants included in outcome assessment.	Low: All outcomes reported.	Low

Table 6. Response rate.

Study	Intervention 1	Intervention 2	Intervention 3
Berth-Jones et al, 1990	Oral inosine pranobex: 0/10 (0%)	Diphencyprone 1% solution: 1/11 (9%)	Oral inosine pranobex and diphencyprone: 1/11 (9%)
Cipriani et al, 2001	Oral paroxetine: 2/8 (25%)	Placebo: 1/5 (20%)	N/A
Dehghan et al, 2013[1]	Oral prednisolone PT: 5/18 (27.8%)	Intravenous methylprednisolone PT: 13/17 (76%)	N/A
Ead et al, 1981	Oral zinc sulphate: -	Placebo: -	N/A
Galbraith et al, 1986	Oral inosiplex: 8/17 (47%)	Placebo: -	N/A
Georgala et al, 2006[2]	Oral inosiplex: 5/15 (33.3%)	Placebo: 0/14 (0%)	N/A
Kar et al, 2005	Oral prednisolone PT: 8/20 (40%)	Placebo: 0/16 (0%)	N/A
Kurosawa et al, 2005	Oral prednisolone PT: 19/29 (66%)	Intramuscular triamcinolone: 32/43 (74%)	Oral dexamethasone: 7/19 (37%)
Mehta et al, 2012[3]	Liquid phenol (20%) and oral betamethasone minipulse therapy: 15/17 (88.23%)	Liquid phenol (20%) with topical minoxidil (2%): 9/17 (52.94%)	Liquid phenol (20%): 8/17 (47.06%)
Perini et al, 1994[4]	Imipramine: 1/7 (14%)	Placebo: 0/6 (0%)	N/A
Price et al, 2008	Subcutaneous efalizumab: 2/37 (5%)	Placebo: 0/25 (0%)	N/A
Saif et al, 2012	Oral methylprednisolone PT, 3 consecutive days once every 2 weeks for 24 weeks: 3/6 (50%)	Oral methylprednisolone PT, 2 consecutive daily pulses every 3 weeks for 24 weeks: 3/9 (33%)	Oral methylprednisolone PT, 3 consecutive daily pulses every 3 weeks for 24 weeks: 6/27 (22%)
Strober et al, 2009	Oral alefacept: 2/23 (9%)	Placebo: 2/22 (9%)	N/A
Tosti et al, 1991	Intravenous thymopentin: 0/10 (0%)	Topical 10% cyclosporine in oily solution: 0/8 (0%)	Photochemotherapy: 0/8 (0%)
Yang et al, 2012[5]	Oral TGPC: 30/44 (68%)	Oral CGT: 30/42 (71%)	N/A
Yang et al, 2013[5]	Oral TGPC and Oral CGT: 49/60 (82%)	Oral CGT: 31/57 (54%)	N/A

Given the range of outcome measures we present the summary data as response rate, with 'response' defined by the individual RCT i.e. number who respond / total number of participants of that arm.

[1] For categorical outcomes where 'response' was stratified into multiple percentage categories, we used at least 60% hair regrowth as a cut-off to combine data in the same binary format.

[2] For categorical outcomes defined as ‘complete’, ‘partial’ or ‘no’ response, ‘complete’ response has been reported.

[3] Grade 4 improvement has been reported.

[4] Full response reported.

[5] ‘Cured’ and ‘markedly effective’.

-: not reported

A total of 15 different systemic interventions were examined across the 16 included studies. The most frequently examined intervention was oral prednisolone pulse therapy (PT) (67-69) and oral inosiplex (70-72). Eight studies were placebo-controlled RCTs, 3 studies compared 2 different systemic treatments and 5 studies compared 3 different treatments.

All studies included a categorical endpoint for efficacy, while only 4 studies included a numerical endpoint (70, 73-75). There was large variation in the definition of treatment 'response' (Table 2) with little consistency between studies.

Thirteen studies reported adverse events (Table 3). Relapse rate following cessation of treatment was reported in 4 studies (Table 4) which was considerable across these studies (69, 70, 76, 77). Only 3 studies performed scalp biopsies to determine histological changes from treatment (70, 75, 78).

The most robust studies were Strober et al. (74) and Price et al. (75) which scored low across all domains on risk of bias assessment (Table 5). Many studies had significant bias in blinding of participants and personnel and uncertainties in allocation concealment and blinding of outcome assessment.

To explore risk of publication bias, funnel plot analysis was performed (Figure 4) which demonstrated symmetry, indicating that risk of publication bias was low.

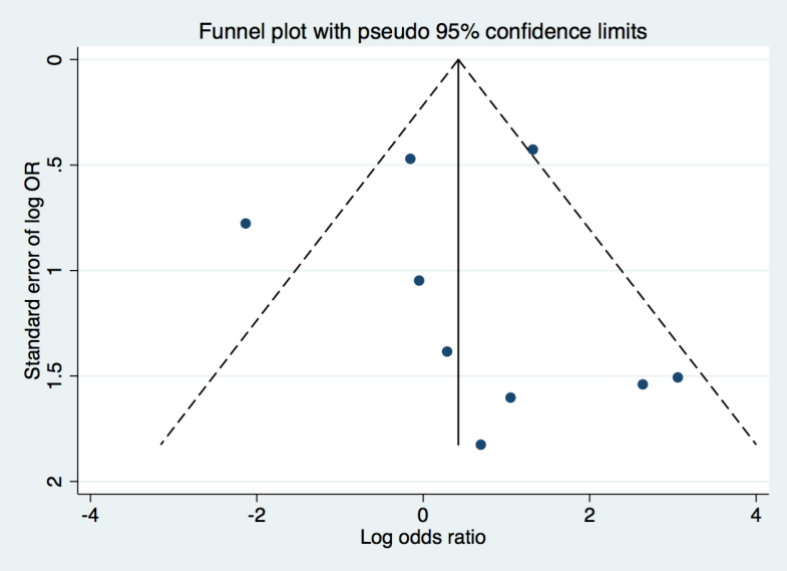

Figure 4. Funnel plot analysis.
For construction of the funnel plot we could only include double-arm trials, studies with complete data and studies reporting outcomes translatable to odds ratios. This resulted in analysis of 9 studies (68, 69, 71, 73-75, 79-81).

We identified 4 ongoing RCTs from trial registries (Appendix 2). Systemic therapies being evaluated by these trials are: apremilast, tralokinumab, 'PF-06651600' and 'PF-06700841', 'CTP-543' and 'TS-133'.

There were no studies sufficiently similar to support pooling of data in a meta-analysis. We present a summarised discussion of the evidence on systemic interventions in the included studies below. A summarised table of response rates is in Table 6.

1.5.2.1 Evidence for Systemic Glucocorticoids

Five trials evaluated systemic glucocorticoids: 1 placebo-controlled RCT (69) and 4 comparative trials (68, 76, 77, 82). Results from the placebo-controlled trial suggest a 40% (8/20) response rate after treatment with oral prednisolone PT (69). In comparative studies, there were significantly greater response rates with intramuscular triamcinolone, intravenous methylprednisolonse and oral betamethasone minipulse therapy with liquid phenol, when compared to oral dexamethasone (76), oral prednisolone pulse therapy (PT) (68) and liquid phenol with and without topical minoxidil respectively (82).

1.5.2.2 Evidence for Immunomodulator Agents

Two immunomodulator agents have been studied in RCTs: oral inosiplex (isoprinosine, inosine pranobex), a synthetic immunomodulator with anti-viral effects, and intravenous thymopentin, a synthetic immunostimulant studied in acquired immune deficiency syndrome (AIDS)

Three studies evaluated oral inosiplex, including 2 placebo-controlled trials. One trial found complete hair regrowth in 33.3% (5/15) of patients in the oral inosiplex group (71); the other trial did not report placebo response rates, so an accurate difference could not be evaluated. Oral inosiplex was also compared to topical diphencyprone or both for 6 months by Berth-Jones et al. (72), with poor response for all 3 treatment arms. No participants on intravenous thymopentin achieved any regrowth in a comparative trial to topical 10% cyclosporin or photochemotherapy.

1.5.2.3 Evidence for Biologics

Two biologics have been investigated in separate trials: intramuscular alefacept, an immunosuppressive biological agent that inhibits T-cell activation, and subcutaneous efalizumab, a humanised monoclonal antibody and T-cell blocker. Both were well-

conducted, double-blind placebo-controlled trials of moderate size. Both concluded no difference between treatment and placebo.

1.5.2.4 Evidence for Antidepressants

Two antidepressants have been studied in placebo-controlled trials: oral imipramine and oral paroxetine. Oral imipramine induced a regrowth rate of 71% (5/7), however the small sample size of 13 randomised participants limits these results. Similarly, a complete regrowth was found in 25% (2/8) of participants on oral paroxetine, however this study was too small to achieve statistical significance.

1.5.2.5 Evidence for Complementary and Alternative Medicine

Ead et al. (83) evaluated oral zinc sulphate in 42 participants with AA, AT or AU and found no difference compared to placebo.

Yang et al. (81) investigated oral total glucosides of paeony capsule (TGPC) and oral compound glycyrrhizin tablets (CGT), plant extracts of glycosides with proposed immunoregulatory functions. In his comparative study, they were deemed of similar efficacy, with a 70% response rate each. He subsequently compared oral TGPC plus oral CGT with oral CGT alone in children aged 2 to 14 years old (73). He found that combination therapy was significantly more effective than oral CGT alone, 82% versus 54% response rate respectively. There was no placebo for either of these studies.

1.5.3 Discussion

1.5.3.1 Summary of Evidence

Overall, we identified 16 RCTs, cross-over RCTs or quasi-RCTs evaluating systemic therapy for AA, AT or AU. There was no systemic therapy that clearly had a robust body of high-quality clinical trials to support its efficacy. Overall, the evidence was not vigorous to conclude percentage or comparative efficacy for most treatments. This was particularly due to small sample sizes. Apart from trials investigating systemic corticosteroids, most other systemic treatments were well tolerated with few side effects (Table 3).

1.5.3.2 Quality of the Evidence

We found large variation in the preciseness of endpoints (Table 2). Many studies lacked well-defined quantitative endpoints (70, 78-80, 82, 83). This is a significant barrier to attaining precise and comparable data on efficacy. Numerical outcomes provide more exact quantification of response, however were only used in 4 studies (70, 73-75). Efficacy may

be recorded through percentage change in the Severity of Alopecia Tool (SALT) score, a visual quantification of hair loss through summation of percentage hair loss from 4 views of the scalp (84). A $SALT_{50}$, i.e. 50% improvement, is an acceptable definition of 'response' to use as an endpoint in clinical trials evaluating systemic therapy for participants with extensive AA (84). Only Strober et al. (74) and Price et al. (75) employed $SALT_{50}$ as an endpoint. More consistent measures would allow meta-analyses in the future.

In conjunction with hair regrowth, an important measurement of efficacy is an improvement in quantitative measurements of quality of life (29, 52). No studies completely evaluated quality of life. Two studies investigated anti-depressants (79, 80) and used psychometric measures traditionally for depression and anxiety. A third study, Price et al. (75), did not use the complete Dermatology Quality of Life Scales tool, employing only the first 17 questions. The validity of this tool may therefore be impaired. None of these measures are currently validated in the AA population.

Very few studies scored low across all domains on risk of bias assessment. Blinding of participants and personnel was the most concerning domain to cause a high risk of bias in a large number of studies.

1.5.3.3 Relationship with Previous Literature

Delamere et al. (64) performed a Cochrane review of all interventions for AA in 2008 and found only 3 trials investigating systemic therapy (69, 70, 80). Seven RCTs have been published since 2008 included in our review. These trials conclude that intramuscular alefacept and subcutaneous efalizumab are ineffective. Comparable findings include: no RCTs evaluating steroid-sparing agents, few studies conducted with a large sample size, few studies evaluating interventional impact on quality of life and few studies with rigorous methodology limiting bias.

1.5.3.4 Study Implications

Current choice of systemic agent is based on clinician experience and preference. Unfortunately, there is still insufficient evidence from RCTs supporting a particular systemic therapy for AA. A management plan developed from an understanding of potential, but not certain success is necessary. There remains a need for high quality RCTs to be conducted involving systemic treatments for AA to define efficacy, guide treatment in an evidence-based fashion and compare treatments currently used in practice. For future meta-analyses, trials should employ standardised outcome measures. This has been addressed by Olsen et

al. (84), who suggests a >50% reduction in SALT score is an appropriate endpoint for moderate to severe AA. Quality of life instruments, both disease-specific and generic, should be utilised to capture impact of pharmacotherapy on quality of life and allow economic evaluation. This may enable evidence from RCTs to guide decision makers on allocation of healthcare resources, which is pertinent in a disease where there is currently no systemic treatment subsidised on the Pharmaceutical Benefits Scheme in Australia.

1.5.3.6 Strengths and Limitations

We used a systematic search strategy that was unlikely to introduce bias. A large number of databases were searched, and our search terms were broad to allow detection of all relevant studies (Appendix 1). To reduce publication bias we did not limit publications based on date published or language. Additionally, we searched registries for ongoing trials (Appendix 2). Our protocol was created and registered on PROSPERO prior to commencement to reduce bias during conduct of the systematic review. We were comprehensive in our inclusion of RCTs by considering all outcome measures and comparators.

However, this review has several limitations. The heterogeneity of studies, particularly with outcome measurement, meant we were unable to perform a meta-analysis. This was an unavoidable limitation due to the studies themselves, rather than our inclusion criteria as we analysed that even a small meta-analysis with a subgroup of relatively similar studies would contain significance differences affecting a pooled result.

We only considered RCTs for inclusion in this systematic review. Evidence from non-RCTs may be relevant, particularly for other systemic treatments. However, in view of performing a systematic review to answer questions regarding efficacy of systemic treatments, we chose to limit studies to RCTs designed to answer such questions.

1.5.3.7 Conclusions

There remains no systemic therapy that is supported by robust evidence from high quality RCTs. To further define efficacy of systemic treatments in AA, there is a need for higher quality RCTs with clearly defined endpoints to be conducted in the future.

1.6 Steroid-sparing agents, azathioprine, methotrexate, cyclosporin and sulfasalazine, as second-line therapies

Many other anti-inflammatory drugs used with corticosteroids are collectively known as steroid-sparing agents. Our systematic review found no RCTs evaluating these agents despite common use in clinical practice as second-line therapies to systemic corticosteroids. As there is a lack of high-quality evidence, there are no specific guidelines over the choice of agent; this currently depends on clinical judgement, consideration of patient comorbidities, adverse events, disease severity, lifestyle and psychosocial impact.

Evidence from smaller, uncontrolled studies have estimated response rates of these agents (Table 7). Briefly the most robust studies for each agent were by Jang et al. (85) for cyclosporin, Rashidi et al. (86) for sulfasalazine, Royer et al. (87) for methotrexate and, Vano-Galva et al. (88) for azathioprine.

Jang et al. (85) conducted the largest study on cyclosporin and retrospectively compared 88 patients treated for at least 3 months with either oral cyclosporine (n=51) or betamethasone minipulse (n=37). A 55% response rate in the cyclosporin arm was found. Rashidi et al. (86) performed a single-arm uncontrolled trial investigating 3g sulfasalazine for 6 months in 39 patients with recalcitrant AA and reported a ‘good’ response in only 25% of patients. A retrospective study of 14 paediatric AA patients treated with methotrexate at a mean dose of 18.9 mg weekly and mean duration of treatment 14.2 months summarised a 38% response rate, defined as more than 50% regrowth of hair (87). Azathioprine 2.5mg/kg/day was evaluated in a prospective uncontrolled study enrolling 14 patients, with 43% attaining therapeutic response (88). Table 7 provides a summary of these studies. Overall, the current evidence most extensively evaluates and favourably suggests that cyclosporin is the most effective monotherapy, second-line agent.

To further assess the effectiveness of these agents, we briefly evaluated the number of patients in a single large Melbourne hair loss clinic who continued taking each agent for 12 months (Table 8). Those who continued were classed as responders; those who commenced treatment but stopped prior to 12 months were considered non-responders. For retrospective analysis of efficacy, the continuation rate of medication functions as a crude proxy for response, predicated on the basis that only patients who tolerate, are satisfied and respond to treatment continue buying and taking the medication. Patients who attained complete remission and therefore ceased treatment were also considered responders.

55% of patients on cyclosporine, 45% of patients on methotrexate and 65% of patients on azathioprine continued treatment for 12 months. The higher rate of continuation on azathioprine compared to cyclosporin, may reflect a difference in adverse event profile and long-term tolerability, considering hypertension and renal impairment are associated with cyclosporin and limit long-term therapy. There is often a continuation of concurrent prednisolone with each second-line agent. A larger review of clinic patients may more accurately reflect the use of these second-line agents; this evidence provides a snapshot of how common these agents are utilised in clinical practice and continuation rates for AA.

Table 7. Studies investigating steroid-sparing agents: azathioprine, cyclosporin, methotrexate and sulfasalazine.

Drug	Study Authors	Study Design	Sample Size	Mean duration of AA (years)	Mean dose	Mean Duration of Treatment (months)	Response Rate
Monotherapy							
Azathioprine	Farshi et al.[1]	Uncontrolled single-arm interventional study	20	2	2 mg/kg/day	6	-
	Vano-Galvan et al.	Uncontrolled single-arm interventional study	14	2	2.5 mg/kg/day	9.8	6/14 (43%)
Cyclosporin	Acikgoz et al.	Case series	22	8.3	4.54 mg/kg/day	4.14	10/22 (45%
	Ferrando et al.	Uncontrolled single-arm interventional study	15	9.8	5 mg/kg/day	9	5/15 (33%)
	Gupta et al.	Uncontrolled single-arm interventional study	6	8	6 mg/kg/day	3	3/6 (50%)
	Jang et al.	Case series	51	2	3 mg/kg/day	13.2	28/51 (55%)
	Paquet et al.	Case report	1	2	6 mg/kg/day	3	0/1 (0%)
Methotrexate	Royer et al.	Retrospective review	14	5.7	18.9 mg weekly	14.2	5/13 (38%)
	Hammerschmidt et al.	Retrospective review	9	4	17.5 mg weekly	-	4/9 (44.4%)
	Joly et al.	Retrospective review	6	11	21.5 mg weekly	-	3/6 (50%)
Sulfasalazine	Rashidi et al.	Uncontrolled single-arm interventional study	39	4	3 g/day	6	10/39 (25%)
	Aghaei et al.	Uncontrolled single-arm interventional study	26		1 g/day for 1 month, 2g/day for 1 month, then 3g/day for 4 months	6	6/22 (27.3%)
	Misery et al.	Case report	1	7	1 g/day for 1 month, 2g/day for 1 month, the 3g/day	9	1/1 (100%)
	Ellis et al.	Retrospective review	39	-	-	-	7/39 (18%)
Combination therapy							
Cyclosporin and prednisolone	Gensure et al.	Case report	1	-	4 mg/kg/day cyclosporin; 50mg/day tapering dose of prednisolone	3	1/1 (100%)
	Kim et al.	Uncontrolled single-arm interventional study	43	5	6 mg/kg/day cyclosporin; 30mg/day tapering dose of prednisolone	3	38/43 (88.4%)
	Lee et al.	Uncontrolled single-arm interventional study	34	4	3.75 mg/kg/day cyclosporin; 30mg/day tapering dose of prednisolone	6	24/34 (77%)
	Shapiro et al.	Uncontrolled single-arm interventional study	8	7.5	5 mg/kg/day cyclosporin; 5mg/day prednisolone	6	2/8 (25%)

	Teshima et al.	Uncontrolled single-arm interventional study	6	5.8	2.5 mg/kg/day cyclosporin; 5mg/day prednisolone	5	6/6 (100%)
Cyclosporin and pulse IV methylprednisolone	Shaheedi-Dadras et al.	Uncontrolled single-arm interventional study	18	6.3	2.5 mg/kg/day cyclosporin; monthly pulses of 500mg IV methylprednisolone	6.5	6/18 (33%)
Methotrexate and prednisolone	Anuset et al.	Retrospective review	26	6	10 mg methotrexate weekly; 6mg prednisolone daily	-	15/26 (57%)
	Hammersch midt et al.	Retrospective review	22	4	17.5 mg methotrexate weekly; 25 mg prednisolone daily	6	17/22 (77.3%)
	Joly et al.	Retrospective review	16	11	21.5 mg methotrexate weekly; 19.4mg prednisolone daily	-	11/16 (69%)
	Landis et al.	Case series	14	-	7.3 mg methotrexate weekly; 33mg tapering dose of prednisolone	-	8/14 (57%)
Methotrexate and pulse IV methylprednisolone	Droitcourt et al.	Retrospective review	20	2	18.75 mg methotrexate weekly; 500mg/day methylprednisolone for 3 days each month for 3 months	12	11/20 (55%)
Sulfasalazine and methylprednisolone	Bakar et al.	6 case reports	6	9	3g sulfasalazine daily; 1mg/kg/day oral methylprednisolone	5	6/6 (100%)

-: not reported

[1]Response rate not reported, however mean regrowth percentage was 52.3%.

Table 8. Systemic therapy continuation rates at 12 months.

Agent	Non-Responders[1]	Responders[2]	Proportion of responders also using concurrent prednisolone	Average daily dose of concurrent prednisolone (mg)
Azathioprine	7/20 (35%)	13/20 (65%)	5/13 (38%)	5.6
Cyclosporin	9/20 (45%)	11/20 (55%)	7/11 (64%)	6.4
Methotrexate	11/20 (55%)	9/20 (45%)	5/9 (56%)	4.8

Data are proportions (percentage)
[1]Non-responders are defined as patients who stopped the medication prior to 12 months either due to side-effects or lack of efficacy (including relapse while on treatment).
[2]Responders are defined as patients who have either continued to take the medication for 12 months or longer or who have stopped the medication due to complete remission.

1.6.1 Cyclosporin

As suggested from the currently available evidence, cyclosporin is a popular first-line alternative to prednisolone used commonly for the treatment of AA (89). It is viewed to be highly efficacious (89) and to have a rapid onset, as seen in other dermatological conditions such as psoriasis.

Cyclosporin is a calcineurin inhibitor that works through binding to cyclophilin of T-cells, inhibiting calcineurin and transcription of interleukin 2 to reduce effector T-cell function, which is pathogenic in AA (90). Scalp biopsies from patients treated with oral cyclosporin showed a decrease in T-cell lymphocytic infiltration of the hair follicle and perifollicular matrix (91). The decrease in the mean number of CD4 helper T-cells per hair follicle was significantly associated with the degree of terminal hair regrowth during therapy (91). Patients with better clinical response had fewer CD4 cell counts per hair follicle (91).

Cyclosporin has only been evaluated as monotherapy in a total of 5 studies so far (Table 7), the largest of which is Jang et al. (85) as described earlier, with a response rate of 55%. Studies in favour suggest a response rate between 33% to 55%. However, a case report by Paquet et al. (92) suggested contrary, unfavourable results. A female with extensive AA was treated with cyclosporin for 3 months with no improvement. There are a number of uncontrolled studies investigating cyclosporin in combination with glucocorticoids; response varies from 25% to 100% (Table 7).

The adverse event profile of cyclosporin is well described. It is associated with hypertrichosis, hypertension, renal impairment, gastrointestinal symptoms, headaches, gum hypertrophy and electrolyte disturbances. Cyclosporin is a pregnancy category C drug and may be associated with harmful effects to the foetus, though not malformations. Jang et al. (85) describes the adverse event profile of cyclosporin at a mean daily dose of 180.9 mg in patients with AA. 56.9% of patients experienced an adverse event, the most common of which was transient gastrointestinal discomfort (19/51, 37.3%). Nephrotoxicity was only found in 3 patients. This was reversible.

Currently, cyclosporin is used for a number of other dermatological conditions, including eczema and psoriasis. Australian guidelines suggest a maximum dose of 5mg/kg/day for treatment of both these conditions (93). A response should be seen within 6 weeks at a dose of 5mg/kg/day, otherwise treatment should be ceased (93). While generally titrated up from an initial dose of 2.5mg/kg/day, induction doses of 5mg/kg/day are recommended for rapid improvement (93).

Doses of up to 6mg/kg/day have been studied in patients with AA. Studies suggest that higher doses are more effective than lower doses, with potentially the most optimal risk-benefit ratio achieved at 4mg/kg/day. Doses higher than 4mg/kg/day show similar efficacy (94, 95). American guidelines report maximum efficacy should be observed at 6 weeks on 4mg/kg/day for psoriasis and rheumatoid arthritis (96).

1.7 Research Question and Aims

It has been difficult to estimate the effectiveness of systemic agents used for AA, AT and AU due to a lack of randomised-controlled trials. Without a suitable control, the underlying relapsing remitting nature of AA may hide or enhance a treatment's effect. Furthermore, in practice, agents are commonly used together, so while combination therapy may be evaluated, monotherapy effectiveness is harder to isolate.

Current literature supports the relatively high efficacy of cyclosporin for treatment of AA, however this has not been studied in a randomised, placebo-controlled trial. This dissertation aims to evaluate the following research question: How effective is cyclosporin treatment in patients with moderate to severe alopecia areata?

This will be evaluated through a prospective, randomised, placebo-controlled trial, with the objectives and endpoints displayed in Table 9.

Table 9. Study objectives and endpoints

Primary Objective:	**Primary Endpoint:**
• To evaluate the efficacy of cyclosporin compared to placebo at Week 12 in patients aged 18 to 65 years with moderate to severe alopecia areata	• Change from baseline of Severity of Alopecia Tool (SALT) score at Week 12
Secondary Objectives:	**Secondary Endpoints:**
• To evaluate the efficacy of cyclosporin on additional efficacy endpoints in patients aged 18 to 65 years with moderate to severe alopecia areata	• Proportion of participants achieving a 30%, 50%, 75% and 100% reduction in SALT (i.e. SALT30, SALT50, SALT75, SALT100) at Week 12 • Change from baseline in non-vellus hair counts by macrophotography at Week 12 • Proportion of participants achieving at least 1 grade improvement in eyelash assessment scale at Week 12 • Proportion of participants achieving at least 1 grade improvement in eyebrow assessment scale at Week 12
• To evaluate the effect of cyclosporin compared to placebo on quality of life at Week 12 in patients aged 18 to 65 years	• Change from baseline in Assessment of Quality of Life-8D (AQoL-8D) score at Week 12 • Change from baseline in Alopecia Areata Symptom Impact Scale (AASIS) score at Week 12
• To evaluate the safety and tolerability of cyclosporin over time in patients aged 18 to 65 years with moderate to severe alopecia areata	• Incidence of treatment-emergent adverse events (AEs) • Incidence of specific clinical laboratory abnormalities including but not limited to anaemia, neutropenia, thrombocytopenia, lymphopenia, changes in lipid profile, and liver function tests (LFTs)

Chapter 2: Materials and Methodology

2.1 Ethical approval

The protocol for this study (2017-11-824-A-2) received ethics approval on 9th May 2018 from Bellberry Human Research Ethics Committee (HREC), Committee E (HREC Code: EC00450). This study was also registered with Monash University HREC on 10th May 2018 (Project Number: 13116).

The project was funded by the Australia Alopecia Areata Foundation (AAAF). The funders had no role in the study design, data collection and analysis, or preparation of this thesis.

2.2 Trial design

This was a single-centre, double-blind, randomised, placebo-controlled, phase 4, parallel-group study conducted in Melbourne, Australia. This study was registered with the Australian New Zealand Clinical Trials Registry (ANZCTR) prior to enrolment of first patient (Registration No.: ACTRN12618001084279).

Briefly, participants with moderate to severe AA were randomised in a 1:1 ratio to receive 3 months of either: cyclosporin (4mg/kg/day) or matching placebo (Figure 5).

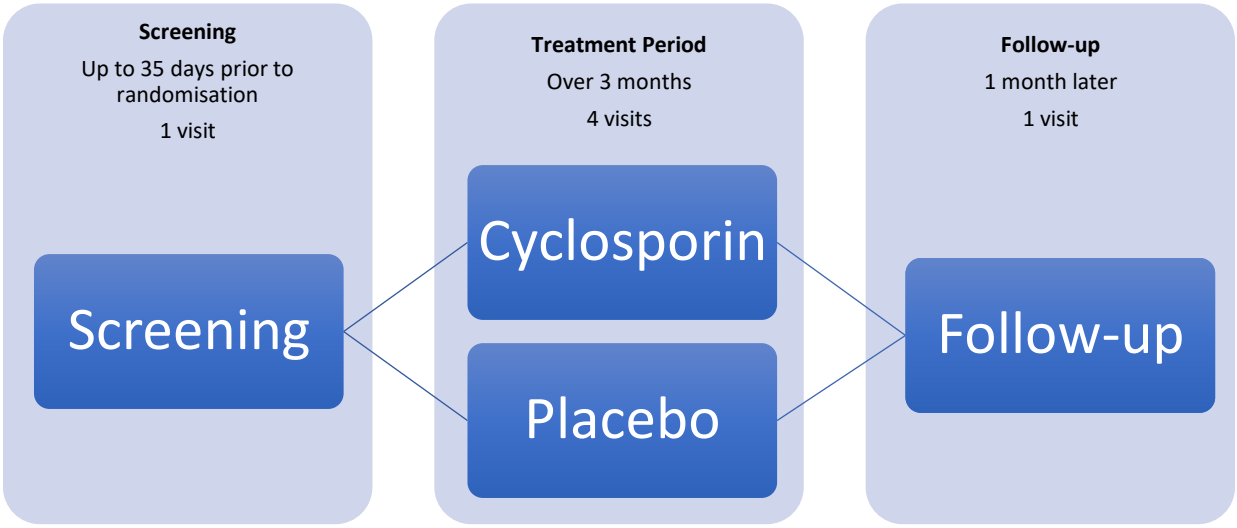

Figure 5. Study design schematic

2.3 Participants

2.3.1 Eligibility criteria for participants

Eligible participants were all adults aged 18 to 65 years of age with moderate to severe AA.

Exclusion criteria were pregnancy and lactation, history of any lymphoproliferative disorder, history of human immunodeficiency virus (HIV), history of hepatitis B or hepatitis C, hypersensitivity to any ingredient of the study medication, use of any hair regrowth treatments prior to the study without an adequate washout period (generally 5 half-lives), inability to adhere to study procedures and visits and any acute or chronic medical or laboratory abnormality that may increase the risk of study participation.

2.3.2 Lifestyle requirements of participants

All females of childbearing potential were required to have 2 negative pregnancy tests before receiving study medication – 1 negative test at screening and another prior to randomisation. Females of childbearing potential were required to use highly effective contraception during the study e.g. intrauterine device, combined oral contraceptive pill, sterilised male partner or abstinence.

Participants were required to refrain from shaving their scalp hair to less than 0.5cm in length during the study for accurate photography of hair growth.

Concomitant medications were reviewed for any interactions with cyclosporin, and participants were monitored accordingly to risk.

2.3.3 Settings

The study took place at Sinclair Dermatology Investigational Research, Education and Clinical Trials (DIRECT) in Melbourne, Australia from May 2018 and is ongoing.

2.3.4 Participant recruitment and screening

Participants were recruited from May 2018 to July 2018 through:

- Online advertisement on clinic website
- Poster advertisements placed within the clinic
- Sponsor advertisement in newsletter to AA patients
- Sponsor advertisement on social media platform
- Referral of interested AA patients from dermatologists

Participants expressed interest by completing an online form or contacting the study researcher. These participants were provided a Patient Information Sheet/Consent Form (Appendix 3) via e-mail and also had the study verbally explained to them via telephone call in terms of purpose, procedures and potential side effects. They were then invited to attend a screening appointment to confirm eligibility for the study.

At the screening appointment, inclusion and exclusion criteria were revisited and written informed consent for the study was obtained. A copy of the signed consent form was provided to the participants. Patient demographics, AA disease history, relevant medical history and prior medication were noted. A complete physical examination and 12-lead electrocardiogram (ECG) was conducted. Photography of the scalp was taken to record hair loss. Blood biochemistry (Appendix 3) was taken. All female participants of child-bearing potential completed a urine pregnancy test. The assessment was reviewed to confirm eligibility prior to randomisation at the next visit.

2.4 Interventions

2.4.1 Trial medication

Participants were randomised in a 1:1 ratio to receive 3 months of either: cyclosporin (4mg/kg/day) or matching placebo. The trial medication was in capsule form, supplied in bottles for twice daily oral administration and prepared by PharmacySmart Compounding Pharmacy for each participant's weight. Trial medication had capsules made of gelatin and contained either 2mg/kg 100% cyclosporine A USP or 2mg/kg Flocel PH-102. There were 66 capsules per bottle.

A cyclosporin dose of 4mg/kg/day was determined as optimal to investigate efficacy and provide a significant difference between the intervention and control group at 3 months. Previously published studies show that a dose of 4mg/kg/day attains similar efficacy to higher doses (94, 95). For psoriasis and rheumatoid arthritis, American guidelines report that maximum efficacy should be observed at 6 weeks on 4mg/kg/day (96).

Participants received the study medication as outpatients. The study researcher (VL) communicated with participants how to take the capsules at home: twice daily, ideally 12 hours apart, swallowed whole with water, with or without food. If a dose was missed and the interval to the next dose was less than 6 hours, the missed dose was not to be taken. On visit days, participants were instructed to withhold their morning dose of medication until cyclosporin trough levels were taken.

2.4.2 Randomisation

Independent pharmacists randomly assigned and dispensed the trial medication for each participant according to a computer-generated randomisation list. This was performed in a 1:1 ratio for cyclosporin or placebo group.

2.4.3 Blinding

PharmacySmart Compounding Pharmacy prepared all study medication, including both active medication (cyclosporin) and control (placebo). The placebo was identical to the active medication in shape (oblong, bisect caplet), size (capsule size #1), colour (white) and taste (gelatin).

Participants, study researchers and all outcome assessors were kept blinded to the allocation sequence through the pharmacy who performed the randomisation and had no clinical involvement in the trial. Participants had no contact with the pharmacy.

2.4.4 Study assessments

Participants attended Sinclair DIRECT for all study assessments. For each participant, the study took place over a maximum of 21 weeks with 6 visits to the clinic over the following periods:

1. Screening period – 1 visit prior to commencing treatment to determine eligibility.
2. Treatment period – 4 visits over 3 months, each visit 1 month apart.
3. Follow-up period – 1 visit one month after the end of the treatment period.

During the screening period, participants attended a screening appointment (Visit 1) as previously described. Upon confirming eligibility, participants entered the treatment period and were randomised (Visit 2) and then attended monthly clinical reviews (Visit 3 to 5). Following the end-of-treatment (EOT) clinical review (Visit 5), there was one follow-up visit one month later (Visit 6) which was the end-of-study (EOS) visit. Each study visit was 1 to 1.5 hours in duration. Participants were given a $\pm$3 day window for study visits based on Visit 1. They were given the respective pills to cover for this window period.

Each study visit consisted a number of assessments to review health and safety of participants and record efficacy of treatment. These included: physical examination, blood biochemistry, urine pregnancy testing for females of child-bearing potential, adverse events, SALT score, eyelash and eyebrow assessment, photography and questionnaires (Figure 6).

The detailed description of the procedures conducted at each study visit is found in (Appendix 4). Results of all assessments were recorded on a proforma for each visit.

2.4.4.1 Physical examination

At each study visit, a physical examination was conducted by the study investigator (VL), which included vital signs (blood pressure, heart rate, respiratory rate and temperature) and weight.

Vital signs were performed before laboratory blood collection. Blood pressure was measured manually using a standard calibrated blood pressure device. Pulse and respiratory rate were measured for a minimum of 30 seconds each after blood pressure was taken. Temperature was measured with a tympanic thermometer. Weight was measured using standard calibrated electronic scales.

A complete physical examination comprised an assessment of general appearance; skin; ears, nose and throat; heart; lungs; abdomen; neurologic function and lymph nodes. A targeted physical examination comprised an assessment of the skin, heart, lungs, abdomen and other relevant systems as signified by symptom complaints from the participant.

A single 12-lead ECG was performed at the screening and end-of-study visit.

2.4.4.2 Pregnancy testing

All female participants of child-bearing potential completed a urine pregnancy test at each study visit, and a serum pregnancy test at screening and end-of-study visits. All pregnancy tests had a sensitivity of at least 25 mIU/mL. The serum pregnancy test was performed by Dorovitch Pathology.

Protocol Activity	Screening	Treatment Period				Follow Up
Week	**-5 to 0**	**0**	**4**	**8**	**12**	**16**
Day	**-35 to 0**	**1**	**28**	**56**	**84**	**112**
Visit	**1**	**2**	**3**	**4**	**5**	**6**
Visit window (days)	**N/A**	**±3 based on Day 1 visit**				
Average time in clinic (hrs)	**2.0**	**1.5**	**1.5**	**1.5**	**1.5**	**1.5**
Study Preparation						
Obtain written informed consent	x					
Review eligibility	x	x				
Demographics	x					
AA disease history and medical history	x					
Prior and current concomitant medications	x	x	x	x	x	x
Randomisation (cyclosporin/placebo)		x				
Dispense medication[1]		x	x	x		
Check medication			x	x	x	
Safety Assessments						
Vital signs and Physical examination[2]	x#	x#	x*	x*	x#	x#
ECG	x					x
Height	x					
Weight	x	x				x
Safety blood testing[3]	x*#	x	x*	x	x	x
Blood drug levels[4]		x	x	x	x	x
Pregnancy testing (if applicable)[5]	x*	x	x	x	x	x*
Adverse events and Investigator Safety Assessment	x	x	x	x	x	x
Contraception check	x	x	x	x	x	x
Efficacy Assessments						
Photography[6]	x*	x	x	x	x	x
SALT score	x	x	x	x	x	x
Eyelash and Eyebrow Assessment Scale	x	x	x	x	x	x
Patient Reported Outcomes						
Participant questionnaire (AQol-8D and AASIS)		x	x	x	x	x

Figure 6. Study visits schedule

[1]Dispense medication. Medication will be dispensed by PharmacySmart Compounding Pharmacy.
[2]Vital signs and physical examination will be performed at all visits marked 'x'. #Indicates a visit with a complete physical examination. *Indicates a visit with a targeted physical examination only.
[3]Safety blood testing. Includes full blood examination (FBE), electrolytes (UEC and CMP), and liver function tests (LFT) for all visits marked 'x'. #Indicates a visit with additional HIV, Hepatitis B Serology, Hepatitis C Serology testing. *Indicates a visit with additional Fasting Lipid testing for which patients are required to fast for 6-8 hours prior to the blood test.
[4]Blood drug levels. Patients will take study medication at the clinic after this test.
[5]Pregnancy testing will be performed with urine pregnancy test at each visit marked 'x'. *Indicates a visit with additional serum pregnancy testing. Pregnancy tests will be performed for all female participants except those at least 2 years post-menopausal or surgically sterile.
[6]Photography will be performed at all visits marked 'x'. This includes full scalp photography, close-up photography of the scalp and a normal front-on photo of your face. *Indicates a visit with full scalp photography only.

2.4.4.3 Blood biochemistry

Blood tests were performed at each study visit (Table 10 and Figure 6). Samples were collected by a certified phlebotomist of Dorovitch Pathology and analysed at the laboratory of Dorovitch Pathology. The results of the blood biochemistry were reviewed by the study investigator (VL) prior to the participant's next appointment. In addition, unscheduled blood biochemistry could be obtained at any time due to any perceived safety concerns at the investigators' discretion (VL, RS).

Table 10. Blood biochemistry.

Haematology	Serum Chemistry	Infection Screening	Other
FBE[1]	UEC[1] CMP[1] LFT[1] Fasting lipid profile panel[3]	HIV[2] Hepatitis B Serology[2] Hepatitis C[2] Serology[2]	Serum pregnancy test[4] Cyclosporin level[5]

[1]Performed for all study visits
[2]Performed at the screening visit only
[3]Performed at the screening visit and at Visit 3
[4]Performed at the screening visit and at Visit 6 (end-of-study)
[5]Performed at Visit 2 through to Visit 6. Sample was collected at least 12 hours post last dose of study medication.
FBE: Full Blood Examination; UEC: Urea electrolytes creatinine; CMP: Calcium magnesium phosphate; LFT: Liver function tests; HIV: Human immunodeficiency virus

Following commencement of trial medication, any abnormal findings with a clinically significant change from baseline were recorded as adverse events. These were monitored to resolution or stabilisation. Any changes posing an immediate harm to the participant were reported to the principal investigator (RS).

2.4.4.4 Review of concomitant medications

All concomitant medications were documented. This included drug name, dose and indication. At each visit, participants were asked about any new concomitant medications and changes in already documented medication.

2.4.4.5 Adverse events

An adverse event was defined as any untoward medical occurrence in a participant on study medication; this need not have a causal relationship. A serious adverse event was defined as an important medical event, that either: resulted in death, was life-threatening, required inpatient hospitalisation, resulted in persistent or significant incapacity.

Participants were prompted to elicit any adverse events at each visit. The physical examinations and blood biochemistry were evaluated for any adverse events by the study investigators (VL, RS). All observed or participant-reported adverse events were documented to allow medical assessment.

2.4.4.6 Review of medication compliance

Medication compliance was checked at each study visit by the study investigator (VL) who physically sighted and counted the remaining capsules in the bottle. Percentage compliance was calculated and recorded. Participants who had <80% compliance were counselled in regard to strategies to improve compliance.

2.4.4.7 SALT Score

A SALT score was determined for each participant at every study visit (Figure 7). The SALT score is a summation of the weighted percentage hair loss across 4 views of the scalp (left, right, back and superior). This was calculated on visual inspection of the 4 views (left, right, back and superior) in person by the study investigator (VL) during the visit for all participants.

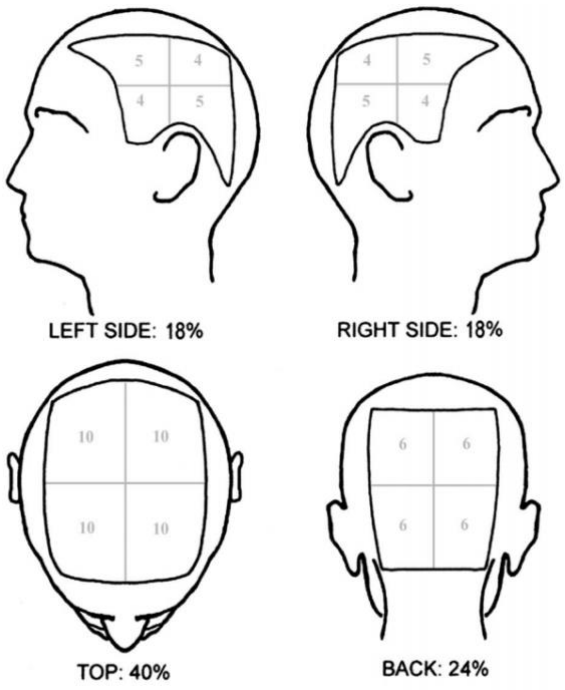

Figure 7. SALT Score
From Olsen EA, Hordinsky MK, Price VH, Roberts JL, Shapiro J, Canfield D, et al. Alopecia areata investigational assessment guidelines--Part II. National Alopecia Areata Foundation. J Am Acad Dermatol. 2004;51(3):440-7.

2.4.4.8 Eyelash and Eyebrow Assessment

Quantity of eyelash and eyebrow was rated categorically from 0 (none) to 3 (normal) for each participant at each visit by the study investigator (VL) (Figure 8). For the eyelash assessment, participants were asked to close their eyes to enable accurate visualisation of the eyelashes. For the eyebrow assessment, quantity of non-vellus hairs along the eyebrow line was inspected and given a categorical rating.

Eyelash Assessment Scale
The numeric rating scale ranges from 0 (none) to 3 (normal) for eyelash assessment.

Score	Description
0	None eyelash
1	Minimal eyelash
2	Moderate eyelash
3	Normal eyelash

Eyebrow Assessment Scale
The numeric rating scale ranges from 0 (none) to 3 (normal) for eyebrow assessment.

Score	Description
0	None eyebrow
1	Minimal eyebrow
2	Moderate eyebrow
3	Normal eyebrow

Figure 8. Eyelash and eyebrow assessment scales

2.4.4.9 Photography

A series of photographs were taken at each study visit according to Appendix 4. A Nikon D7500 camera was used for photographs of each participant's front, left side, right side and back view of scalp. Photographs were taken with the camera propped on a photography stand at height of 140cm**,** and distance of 200cm from the participant**.** Participants had hair pinned back to reveal patches of alopecia. Superior views were taken using a tripod. VECTRA®WB360 3D photography was performed at baseline (Visit 2), end-of-treatment (Visit 5), and follow-up (Visit 6). A patch of alopecia was selected by the study investigator (VL), its location recorded on the VECTRA®WB360 3D photograph, and macrophotography taken using a Canon EOS 1300D camera at Visit 2, 5 and 6. The study investigator (VL) counted all non-vellus hairs on macrophotography.

2.4.4.10 Patient reported outcomes

Participants self-completed 2 questionnaires, the Alopecia Areata Symptom Impact Scale (AASIS) and the Assessment of Quality of Life-8D (AQoL-8D) at each study visit from Visit 2 to 6. Any queries were clarified by the study investigator (VL) without promoting an answer.

2.5 Outcomes

2.5.1 Data Collection

Data was collected prospectively during the study visits and recorded on a hardcopy proforma for each participant. All data collected were kept confidential.

2.5.2 Statistical Analysis

All statistical analyses were performed using Stata 12. A per protocol interim analysis was performed for 28 participants at 3 months. An intention-to-treat analysis will be performed when the trial finishes.

Sample size was calculated from estimated proportions attaining response in each group, defined as a 50% reduction in SALT score at 3 months compared to baseline. Studies suggest the proportion of participants on cyclosporin that respond is 55% (85), and the proportion on placebo is 5% (97). For a two-sided 5% significance level and a power of 80%, a sample size of 16 participants per group was required.

Descriptive statistics of participant demographics and outcome measures were summarised using means and standard deviations as there were no significant outliers. All data were checked for normality on histogram and accordingly either independent t-tests for normally distributed continuous data or Mann-Whitney U tests for non-normally distributed continuous data were performed to compare groups. Chi-squared tests were performed for categorical data.

Potential covariates identified from baseline demographics were analysed for any confounding effect on the primary and secondary endpoints by examining correlations. Where a significant correlation was found this was adjusted for.

We performed subgroup analyses excluding those with AT or AU and those with current episode of AA greater than 5 years to examine for any difference in results on the remaining cohort.

The primary endpoint, change from baseline in SALT score, was analysed using a linear mixed-effect repeated measures model with fixed effects for treatment, time and baseline SALT score, and a random effect for subject.

The key secondary efficacy endpoints, proportion of participants achieving SALT30, SALT50, SALT75 and SALT100 were analysed with a logistic regression model, adjusted for potential covariates.

Statistical significance was defined as $p<0.05$.

Chapter 3: Results

3.1 Participant recruitment

Participants were recruited from May 2018 to July 2018. In total 110 expressions of interest were received across 5 main sources: study posters, clinic website, sponsor advertisement, dermatologist referral and the clinical trials interest form (Figure 9). Most participants were recruited through the sponsor advertisement.

A total of 42 patients were screened and of these, 36 met inclusion criteria and were randomly assigned treatment (Figure 10).

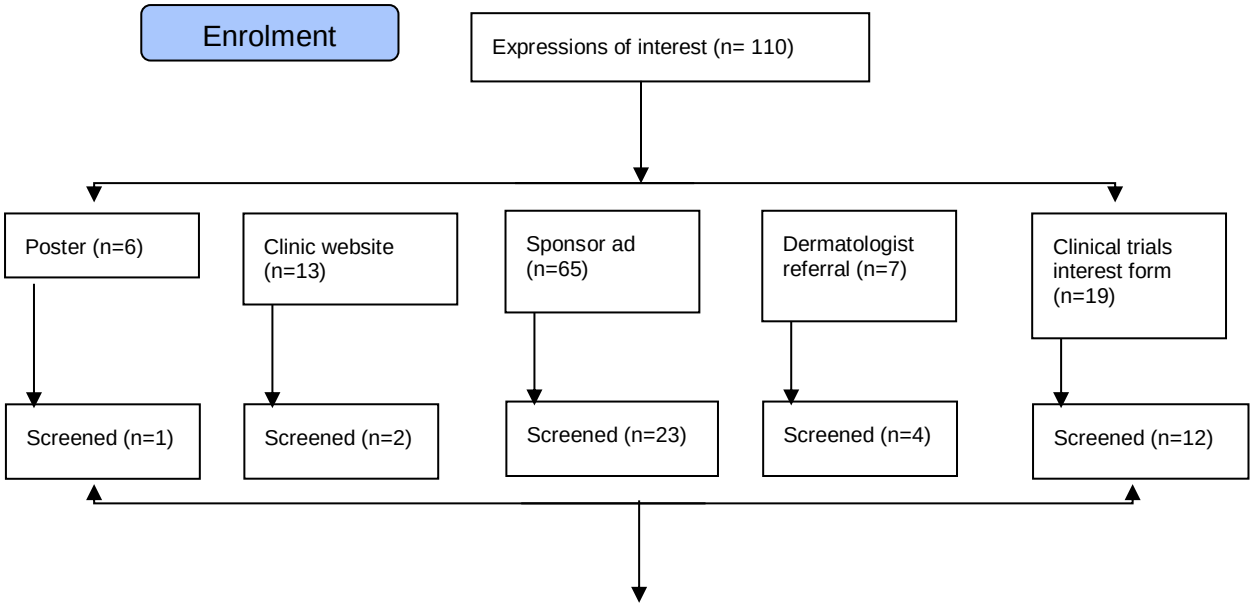

Figure 9. Expressions of interest and sources for recruitment

3.2 Participant follow-up

This clinical trial is ongoing, with the last participant scheduled for completion in November. Of the 36 randomised participants, 30 have completed their 3-month follow-up visit; the primary timepoint of this study (Figure 10).

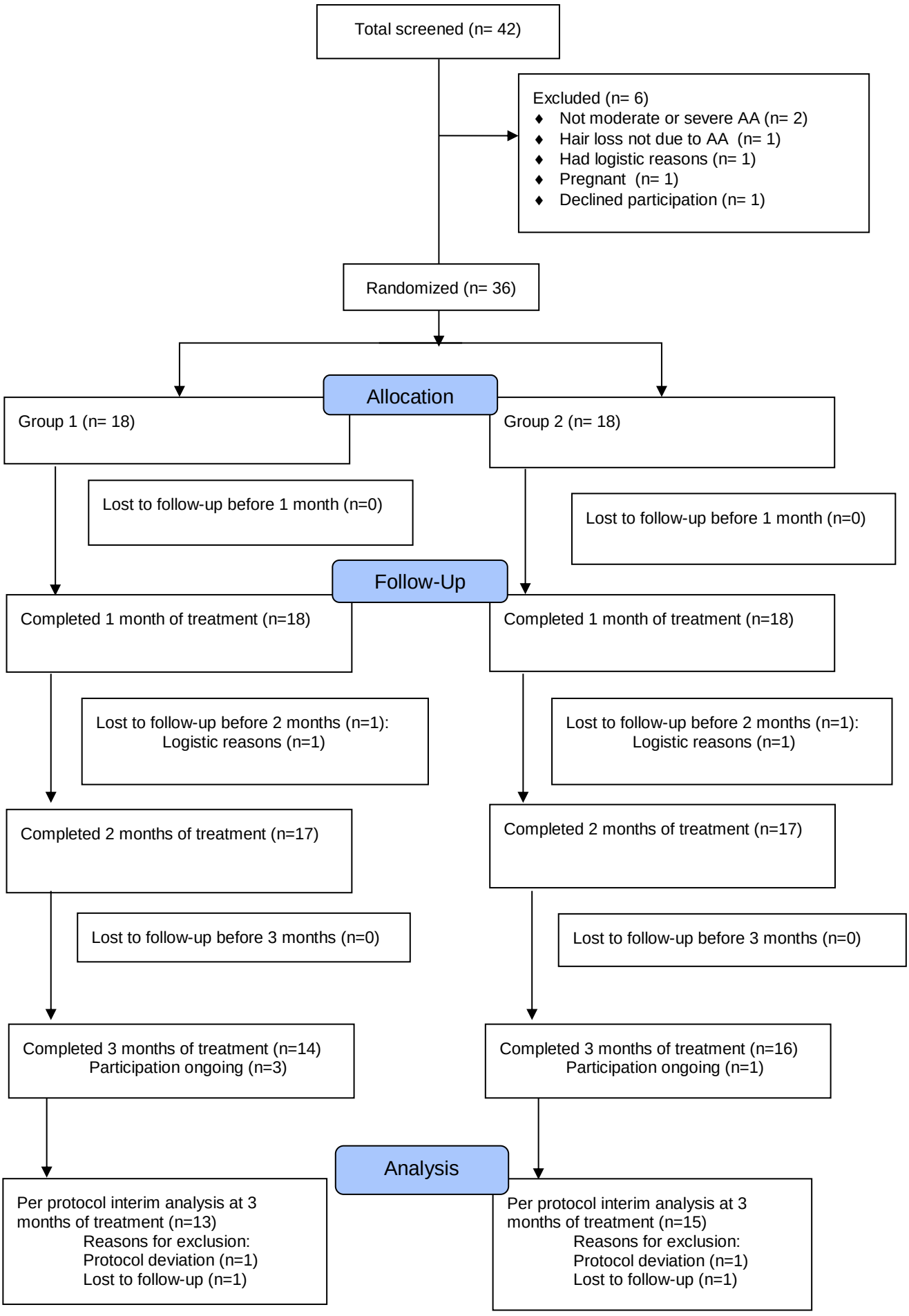

Figure 10. Flow diagram of allocation, follow-up and analysis of participants

3.3 Participant demographics

Participants were mostly similar across both groups (Table 11). The mean age was 41.0 years and mean age at onset of first episode of AA was 24.5 years. The cohort consisted 80.6% females. The duration of current episode of AA was on average 6.5 years, and this was slightly longer for the cyclosporin group (mean 7.4) than placebo group (mean 5.7) (p=0.75). The mean percentage scalp hair loss by SALT score at baseline was 79.4%. This was less for the cyclosporin group (mean 77.8%) than placebo group (81.1%) (p=0.56). About half of the participants in each group had AT or AU (cyclosporin: 55.5%; placebo: 61.1%; p=0.92). The rates of 100% body hair loss were similar for both groups (cyclosporin: 38.9%; placebo: 33.3%; p=0.31). Nail involvement was more common in the cyclosporin group (50.0%), than placebo group (38.9%) (p=0.45). 72.2% of all participants had a history of AT or AU at any time, for which the duration of AT or AU was greater than 2 years in more participants of the placebo group (69.2%) compared with the cyclosporin group (46.2%) (p=0.23). Having another autoimmune disease was reported in 8.3% of participants and having a family history of AA was reported in 4% of participants. Around half of the participants had no eyelashes (50.0%) or eyebrows (52.8%) at baseline.

Table 11. Baseline demographic and clinical characteristics of all randomised participants.

	All (n=36)	Cyclosporin (n=18)	Placebo (n=18)	P value[1]
Age (years)	41 (14.5)	36.4 (11.3)	45.7 (16.2)	0.12
Sex (female)	29 (80.6%)	13 (72.2%)	16 (88.9%)	0.21
Age at onset of first episode of AA	24.5 (13.9)	19.7 (10.6)	29.3 (15.4)	0.06
Age at onset of current episode of AA	34.5 (14.3)	28.9 (10.8)	40.1 (15.3)	0.04
Duration of current episode of AA (years)	6.5 (9.7)	7.4 (11.6)	5.7 (7.5)	0.75
Percentage scalp hair loss by SALT score at baseline	79.4 (28.3)	77.8 (31.0)	81.1 (26.1)	0.56
Pattern of scalp hair loss:				0.92*
AT	9 (25.0%)	4 (22.2%)	5 (27.8%)	
AU	12 (33.3%)	6 (33.3%)	6 (33.3%)	
Patchy	15 (41.7%)	8 (44.4%)	7 (38.9%)	
Body hair loss:				0.31*
100% loss	13 (36.1%)	7 (38.9%)	6 (33.3%)	
No loss	7 (19.4%)	5 (27.8%)	2 (11.1%)	
Some loss	16 (44.4%)	6 (33.3%)	10 (55.6%)	
Nail involvement	16 (44.4%)	9 (50.0%)	7 (38.9%)	0.50*
History of AT or AU at any time	26 (72.2%)	13 (72.2%)	13 (72.2%)	0.70*
Duration of AT or AU:				0.23*
≤2 years	11 (42.3%)	7 (53.9%)	4 (30.8%)	
>2 years	15 (57.7%)	6 (46.2%)	9 (69.2%)	
Medical history:				0.24*
Atopy	12 (33.3%)	4 (22.2%)	8 (44.4%)	
Other autoimmune disease	3 (8.3%)	2 (11.1%)	1 (5.6%)	
Endocrine	2 (5.6%)	1 (5.6%)	1 (5.6%)	
Psychological illness	3 (8.3%)	3 (16.7%)	0	
Family history of AA	4 (11.1%)	3 (16.7%)	1 (5.6%)	
Score of 0 (no eyelashes) on eyelash assessment scale	18 (50.0%)	9 (50.0%)	9 (50.0%)	0.57*
Score of 0 (no eyebrows) on eyebrow assessment scale	19 (52.8%)	9 (47.4%)	10 (52.6%)	0.88*

Data are means (SD) or numbers (%)
AA, alopecia areata; AT, alopecia totalis; AU, alopecia universalis
[1]Mann-Whitney U test used for all continuous data; *chi-squared test used

3.4 Outcomes

A per protocol interim analysis of 28 participants (cyclosporin: 13; placebo: 15) was performed at 3 months (Table 12). Both ‘Age at onset of first episode of AA’ (p=0.06) and ‘Age at onset of current episode of AA’ (p=0.04) were controlled, with results remaining insignificant and no significant confounding effect (p values = 0.57, 0.54 respectively). Results for each endpoint are presented below.

Table 12. Summary of results for primary and secondary endpoints at 3 months.

Endpoint	Cyclosporin Group (n=13)	Placebo Group (n=15)	p value[1]
Primary Endpoint			
Mean change from baseline of SALT score at 3 months	-10.3 (23.7)	-2.6 (7.7)	0.593
Secondary Endpoints			
Proportion of participants achieving at least a 30% reduction in SALT score (SALT30) at 3 months	3/13 (23.1)	1/15 (6.7)	0.216*
Proportion of participants achieving at least a 50% reduction in SALT score (SALT50) at 3 months	3/13 (23.1)	1/15 (6.7)	0.216*
Proportion of participants achieving at least a 75% reduction in SALT score (SALT75) at 3 months	1/13 (7.7)	0/15 (0.0)	0.274*
Proportion of participants achieving a 100% reduction in SALT score (SALT100) at 3 months	1/13 (7.7)	0/15 (0.0)	0.274*
Change from baseline in non-vellus hair counts by macrophotography at 3 months	19.9 (40.5)	1.9 (26.0)	0.133
Proportion of participants achieving at least 1 grade improvement in eyelash assessment scale at 3 months	1/13 (7.7)	0/15 (0.0)	0.274*
Proportion of participants achieving at least 1 grade improvement in eyebrow assessment scale at 3 months	3/13 (23.1)	0/15 (0.0)	0.049*
Change from baseline in Assessment of Quality of Life-8D (AQoL-8D) score at 3 months	0.067 (0.091)	0.046 (0.098)	0.629
Change from baseline in Alopecia Areata Symptom Impact Scale (AASIS) score at 3 months – Global Symptom Impact Score	-0.045 (0.131)	0.024 (0.143)	0.344
Change from baseline in Alopecia Areata Symptom Impact Scale (AASIS) score at 3 months – Scalp Hair Loss Score	0.077 (1.553)	0.267 (1.580)	0.824

Data are mean (standard deviation) or proportion (percentage).
[1]Mann-Whitney U test used for all continuous data; *chi-squared test used

3.4.1 Primary endpoint: Change from baseline of SALT score at 3 months

There was no statistically significant difference between cyclosporin and placebo groups in change from baseline of SALT score at 3 months (Table 12).

Mean SALT score decreased over time for both cyclosporin and placebo groups (Figure 11). Participants in the cyclosporin group had on average a greater decrease in SALT score over time, than participants in the placebo group (-10.3% versus -2.6%; p=0.59) (Figure 12). The mean SALT score at the end of 3 months was 65.8% in the cyclosporin group, compared to 84.2% in the placebo group.

The mean change in SALT score during the first month was almost equivalent between the cyclosporin and placebo groups (Figure 12), following which the mean change in SALT score decreased at a greater extent for the cyclosporin group during the second and third month.

Both the greatest decrease (-69.5%) and increase (15.0%) in SALT score occurred in the cyclosporin group. The greatest decrease in SALT score for the placebo group was -29.0%, while the greatest increase was 2.0%. As represented by the standard deviations (Figure 12), changes in SALT scores varied further from the mean in the cyclosporin group compared to the placebo group.

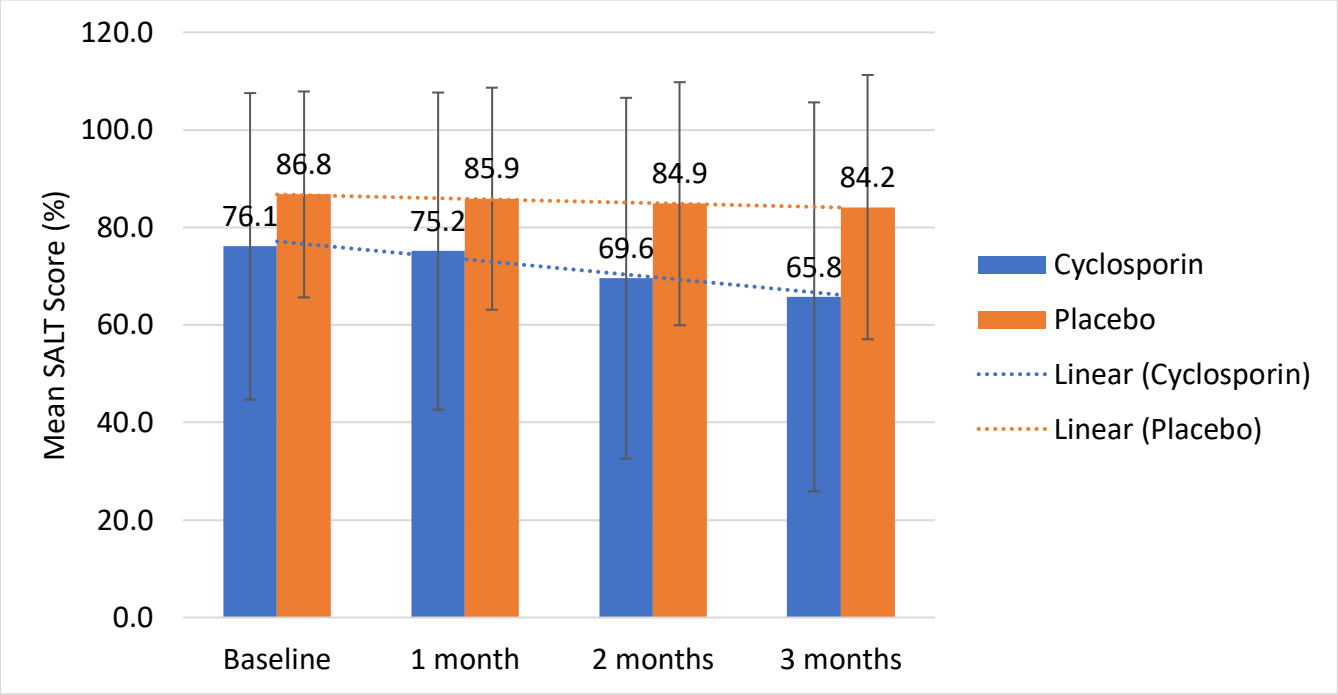

Figure 11. Mean SALT score over time

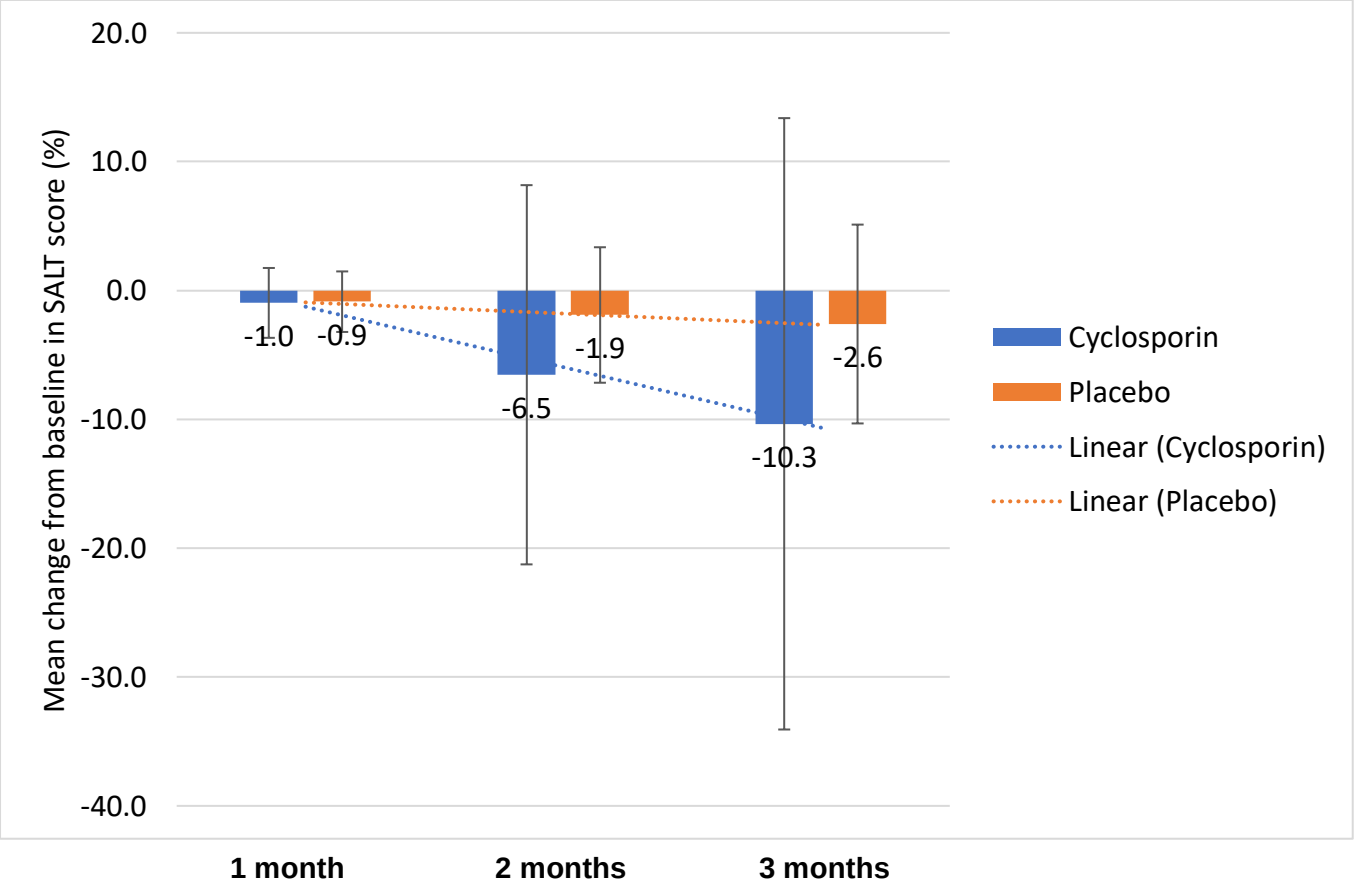

Figure 12. Mean change from baseline of SALT score over time

3.4.2 Secondary Endpoint 1: Proportion of participants achieving a 30%, 50%, 75% and 100% reduction in SALT score at 3 months

There was no statistically significant difference between cyclosporin and placebo groups in proportion of participants achieving at least a 30%, 50%, 75% and 100% reduction in SALT score at 3 months (Table 12).

Overall, 3 participants (23.1%) in the cyclosporin group achieved at least a 50% improvement at the end of 3 months, compared to 1 participant (6.7%) in the placebo group (p=0.216). 1 participant (7.7%) in the cyclosporin group achieved SALT100 at 3 months, whereas none achieved this in the placebo group (p=0.274).

The proportions of participants achieving SALT30, SALT50, SALT75 and SALT100 over time in the cyclosporin group is displayed in Figure 13.

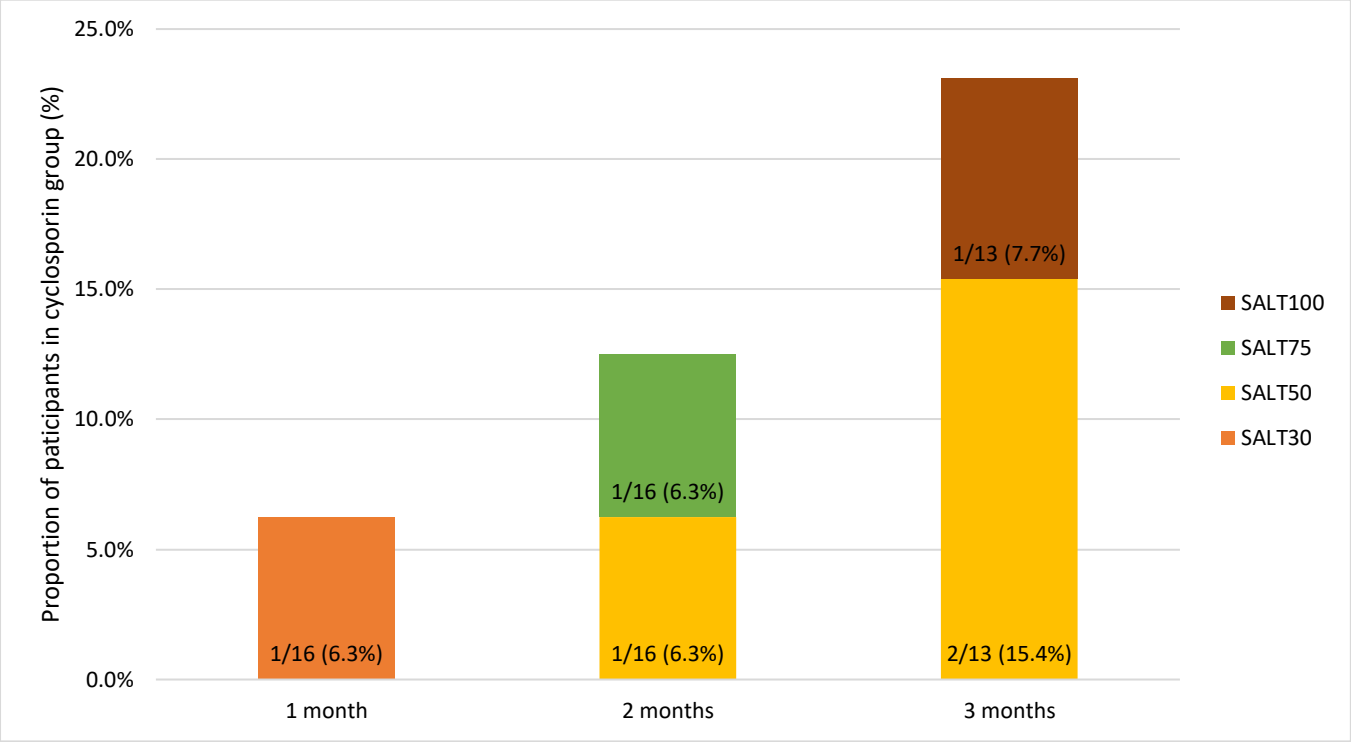

Figure 13. Proportion of participants achieving SALT30, SALT50, SALT75, SALT100 over time in cyclosporin group

3.4.3 Secondary Endpoint 2: Change from baseline in non-vellus hair counts by macrophotography at 3 months

There was no statistically significant difference between cyclosporin and placebo groups in change from baseline in non-vellus hair counts by macrophotography at 3 months (p=0.133) (Table 12).

On average, the hair count increased more for the cyclosporin group, than the placebo group after 3 months (19.9 versus 1.9; p=0.133) (Figure 14). In the cyclosporin group, the maximum change from baseline in hair count was 115, while the minimum was -21. In the placebo group, the maximum change from baseline in hair count was 62, while the minimum was -49.

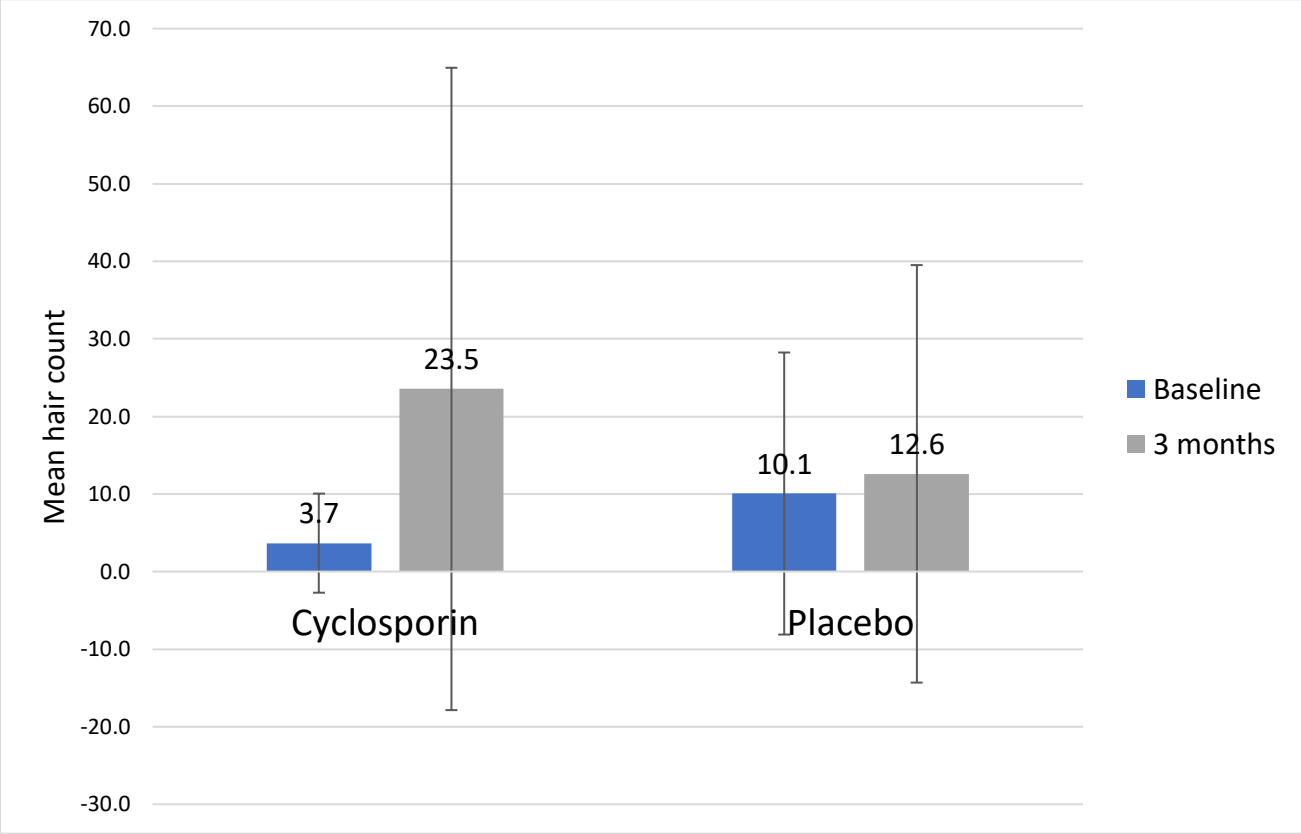

Figure 14. Hair count in cyclosporin and placebo groups at baseline and 3 months

3.4.4 Secondary Endpoint 3: Proportion of participants achieving at least 1 grade improvement in eyelash assessment scale at 3 months

There was no statistically significant difference between cyclosporin and placebo groups in proportion of participants achieving at least 1 grade improvement in eyelash assessment scale at 3 months (7.7% versus 0.0%; p=0.274) (Table 12). 1 participant in the cyclosporin group achieved a 1 grade improvement in eyelash assessment scale, whereas no participants on placebo achieved this at 3 months.

Figure 15 depicts the proportions of participants achieving at least 1 grade improvement in eyelash assessment scale over time in the cyclosporin group. The second participant achieving a 1 grade improvement at 2 months has not completed the 3-month visit yet. No participants in the placebo group attained a 1 grade improvement at any time point.

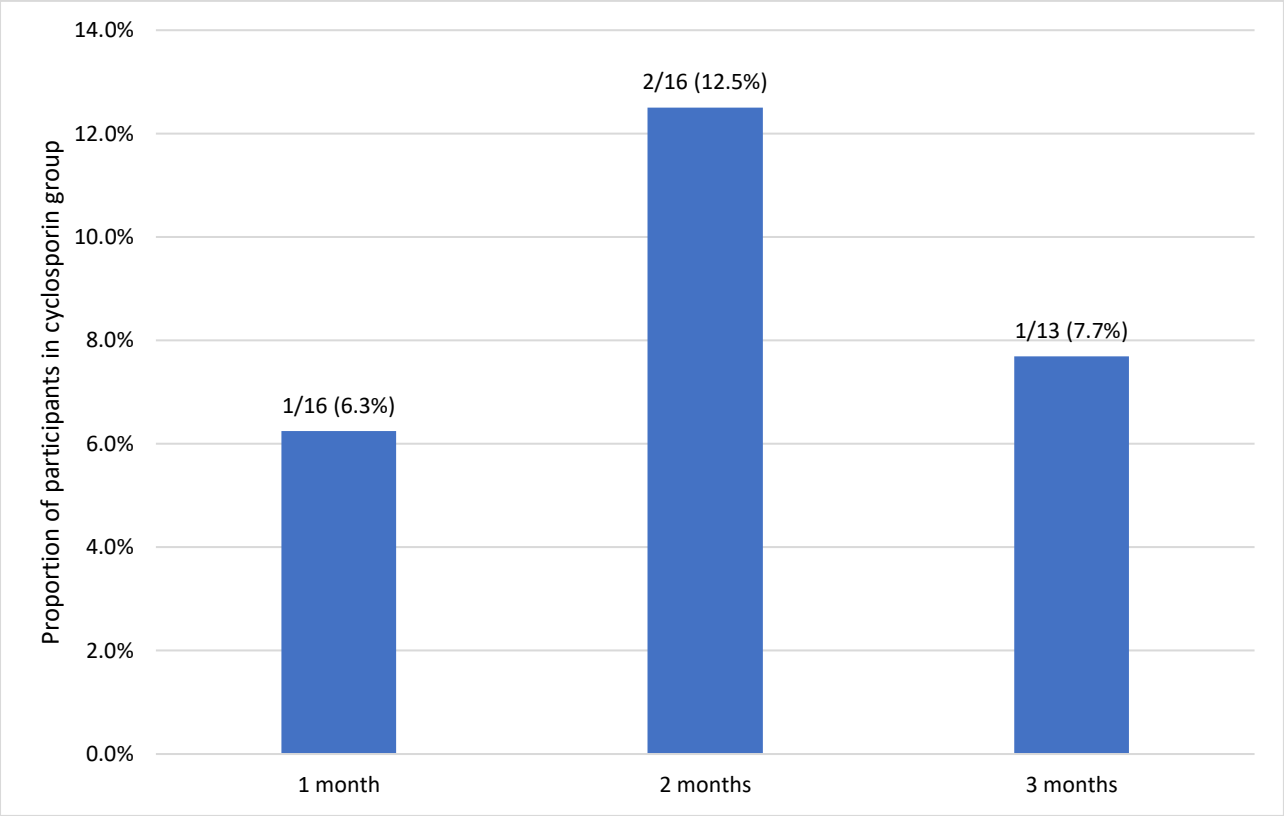

Figure 15. Proportion of participants achieving a 1 grade improvement in eyelash assessment scale over time in the cyclosporin group

3.4.5 Secondary Endpoint 4: Proportion of participants achieving at least 1 grade improvement in eyebrow assessment scale at 3 months

There was a statistically significant difference between cyclosporin and placebo groups in proportion of participants achieving at least 1 grade improvement in eyebrow assessment scale at 3 months (23.1% versus 0.0%; p=0.049) (Table 12). 3 participants in the cyclosporin group achieved a 1 grade improvement in eyebrow assessment scale, whereas no participants in the placebo group achieved this at 3 months.

Figure 16 depicts the proportions of participants achieving at least a 1 grade improvement in eyebrow assessment scale over time in the cyclosporin group. This was achieved from the second month onwards. No participants in the placebo group attained a 1 grade improvement at any time point.

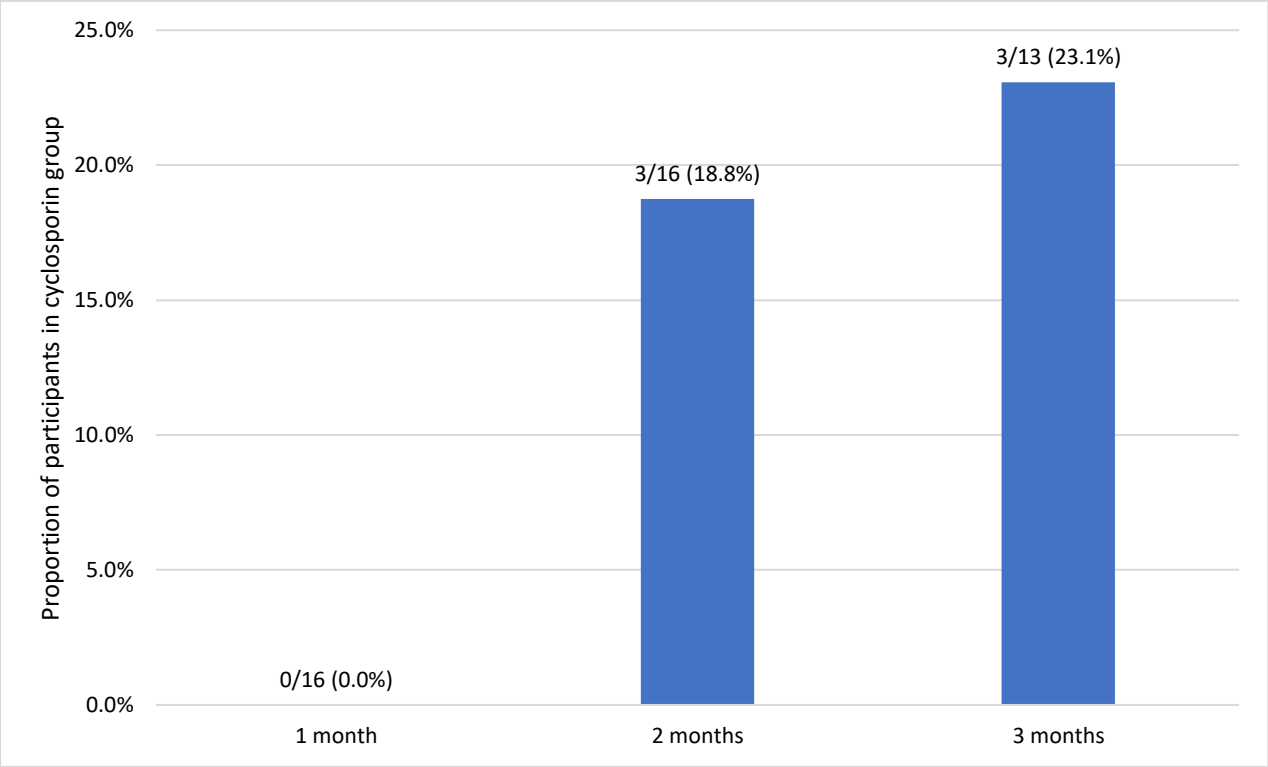

Figure 16. Proportion of participants achieving a 1 grade improvement in eyebrow assessment scale over time in the cyclosporin group

3.4.6 Secondary Endpoint 5: Change from baseline in AQoL-8D score at 3 months

There was no statistically significant difference between cyclosporin and placebo groups in change from baseline in AQoL-8D score at 3 months (0.067 versus 0.046; p=0.629) (Table 12). The AQoL-8D score lies on a 0 – 1, death – full health quality-adjusted life years (QALYs) scale.

On average, AQol-8D scores increased for both groups over time (Figure 17). Participants in the cyclosporin group, had on average lower AQoL-8D scores than participants on placebo at baseline. At the end of 3 months, participants in the cyclosporin group had on average a marginally higher AQoL-8D score than participants on placebo.

Figure 18 and 19 depicts the scores of the 8 dimensions measured by AQoL-8D over time in the cyclosporin and placebo groups respectively. There were no statistically significant changes in any of the 8 dimensions in either group over time. In both groups, mental health and relationship domains were the two most significantly affected. In both groups, the dimensions that improved over time were: independent living, happiness, mental health, coping, relationships, self-worth and sensation. Only pain did not improve over time. For both groups, mental health improved the most over time (0.050 versus 0.065).

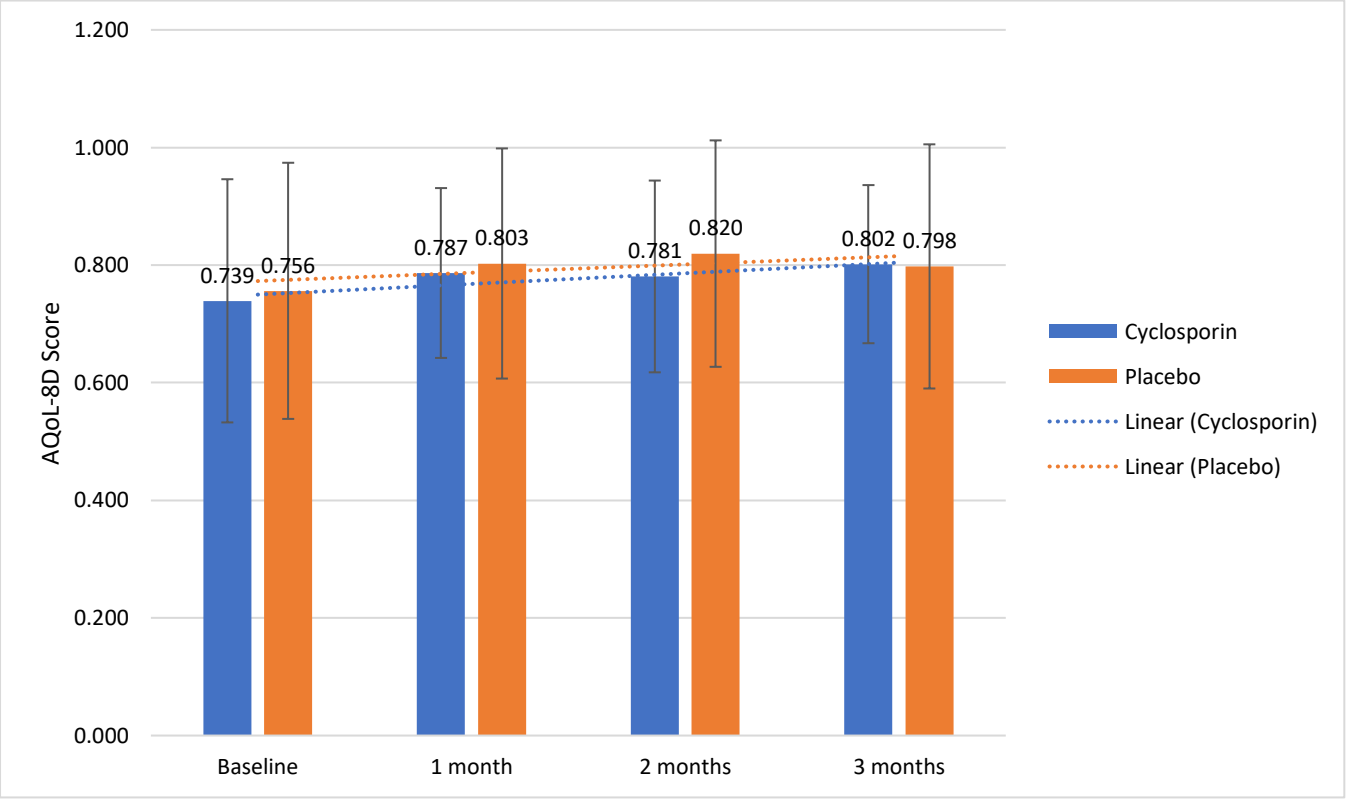

Figure 17. AQoL-8D scores over time

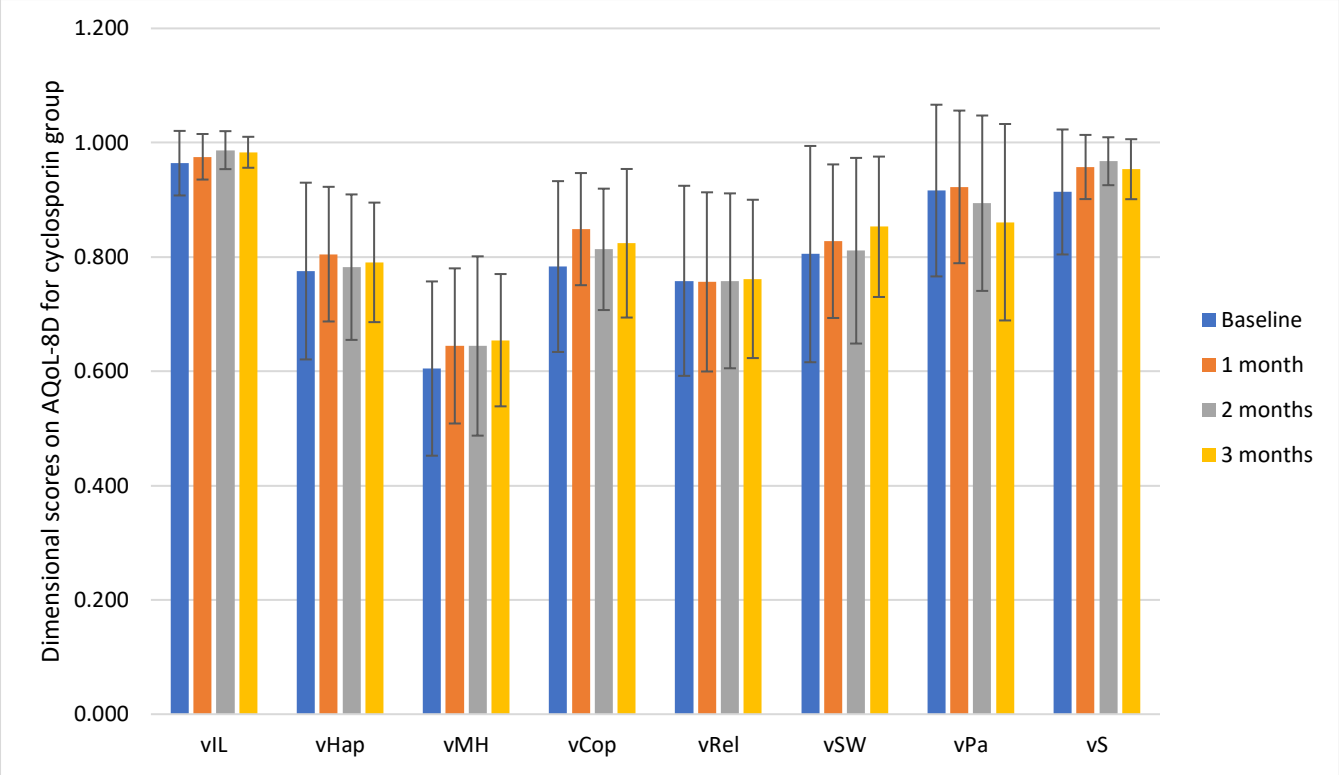

Figure 18. Dimension scores of AQoL-8D in cyclosporin group over time
vIL: independent living; vHap: happiness; vMH: mental health; vCop: coping; vRel: relationship; vSW: self-worth; vPa: pain; vS: sensation

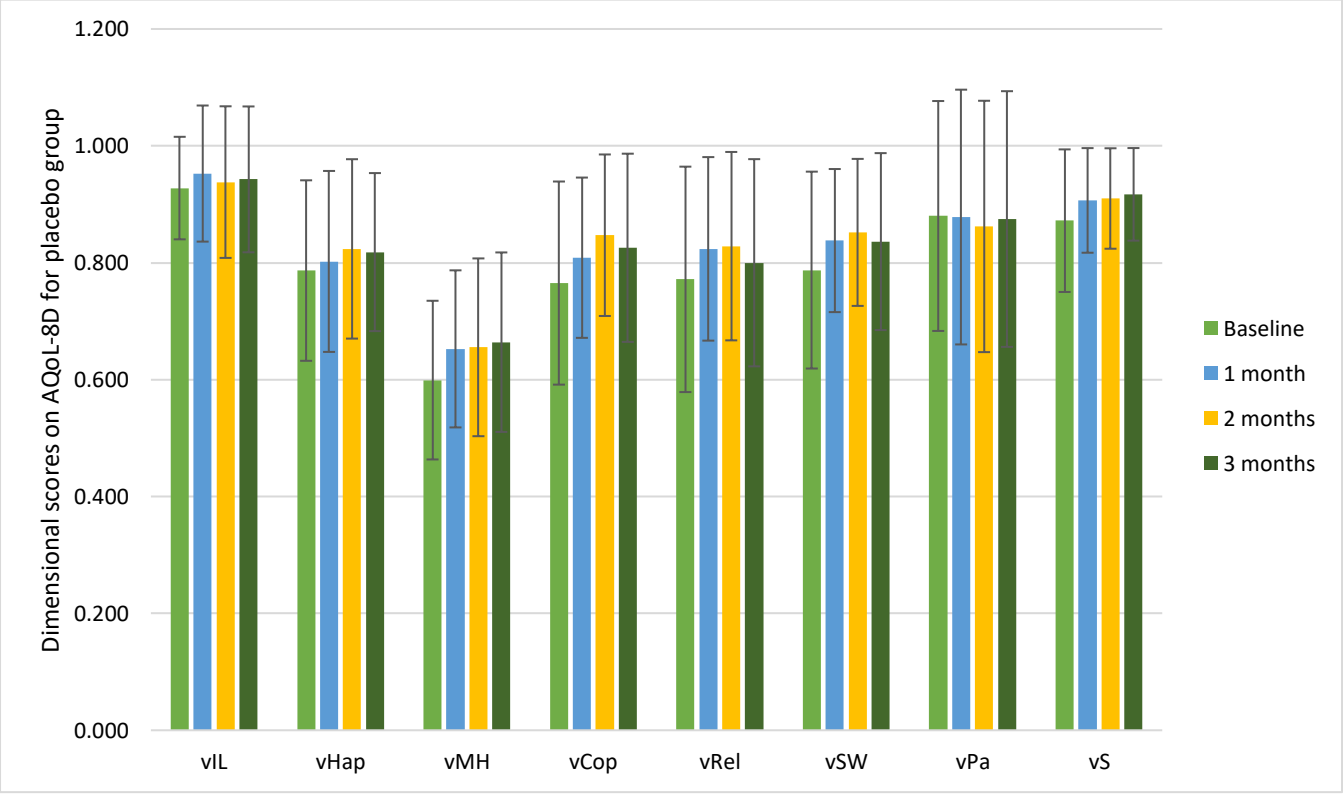

Figure 19. Dimension scores of AQoL-8D in placebo group over time
vIL: independent living; vHap: happiness; vMH: mental health; vCop: coping; vRel: relationship; vSW: self-worth; vPa: pain; vS: sensation

3.4.7 Secondary Endpoint 6: Change from baseline in AASIS score at 3 months

There was no statistically significant difference between cyclosporin and placebo groups in change from baseline in Global Symptom Impact score and Scalp Hair Loss score on the AASIS at 3 months (Table 12). The Global Symptom Impact score is an unweighted average of 7 symptom impact scores rated from 0 (not present) to 10 (as bad as you can imagine); for which the Scalp Hair Loss score is one comprising symptom. On average, the Global Symptom Impact score decreased over time for the cyclosporin group whereas increased over time for the placebo group (-0.045 versus 0.024; p=0.344). While the Scalp Hair Loss score stayed relatively stable for the cyclosporin group, it increased slightly over time for the placebo group (0.077 versus 0.267; p=0.824).

Figure 20 depicts the Global Symptom Impact score for cyclosporin and placebo groups over time. On average, the Global Symptom Impact Score had the greatest decrease within the first month for both groups. Scores then continued to increase for the placebo group, whereas fluctuated for the cyclosporin group during the second and third month.

Figure 21 depicts the Scalp Hair Loss Score in the cyclosporin and placebo groups over time. This showed the greatest decrease within the first month for both groups. Scores then continued to rise for the placebo group, whereas fluctuated for the cylcosporin group in the second and third months.

Each symptom score over time is shown in Figure 22 for cyclosporin and Figure 23 for placebo. There were no statistically significant changes in either group over time. In the cyclosporin group, scalp hair loss, body or eyelash hair loss, itchy or painful skin, irritated skin, feeling anxious or worried and feeling sad on average decreased over time as rated by the participants. Tingling or numbness of the scalp though on average increased over time. In the placebo group, tingling or numbness of the scalp, itchy or painful skin and feeling anxious or worried on average decreased over time as rated by the participants. In contrast, scalp hair loss, body or eyelash hair loss, irritated skin and feeling sad on average increased over time.

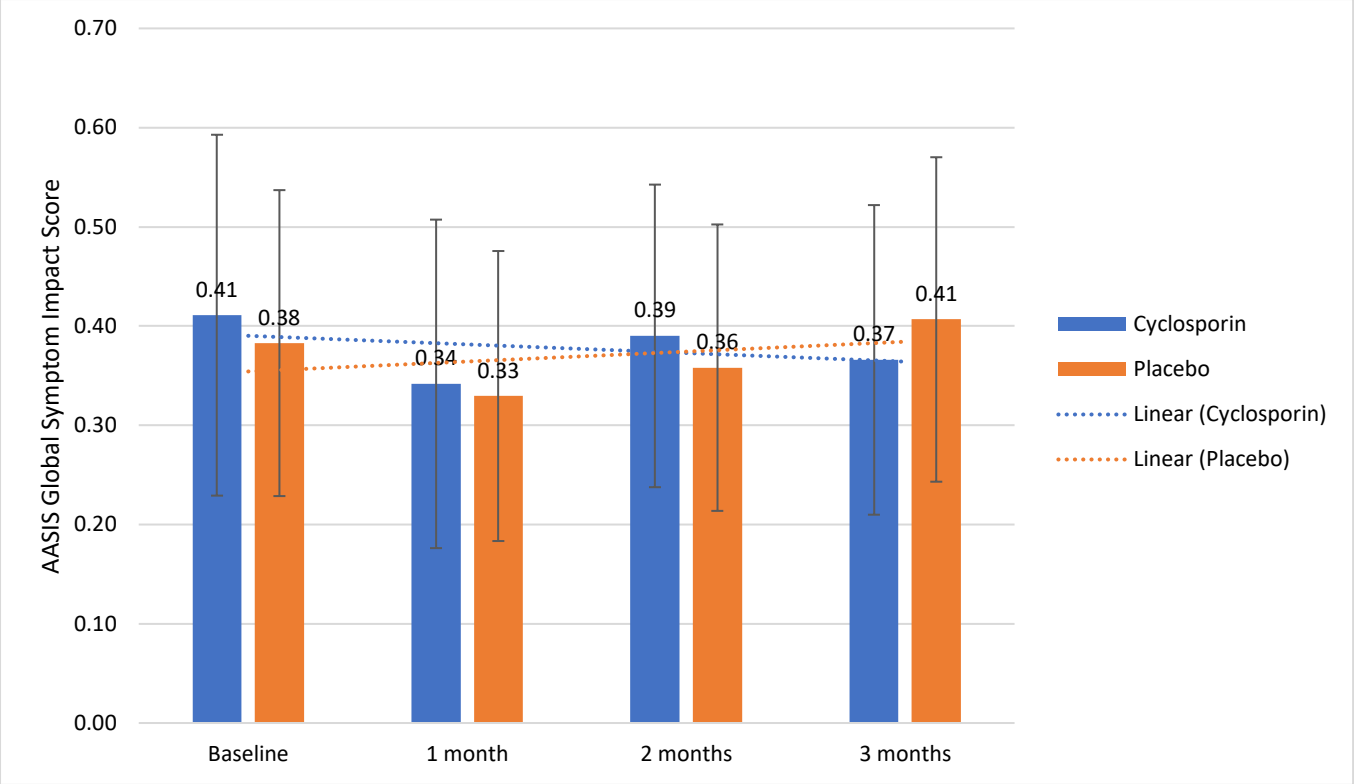

Figure 20. Global Symptom Impact Score over time

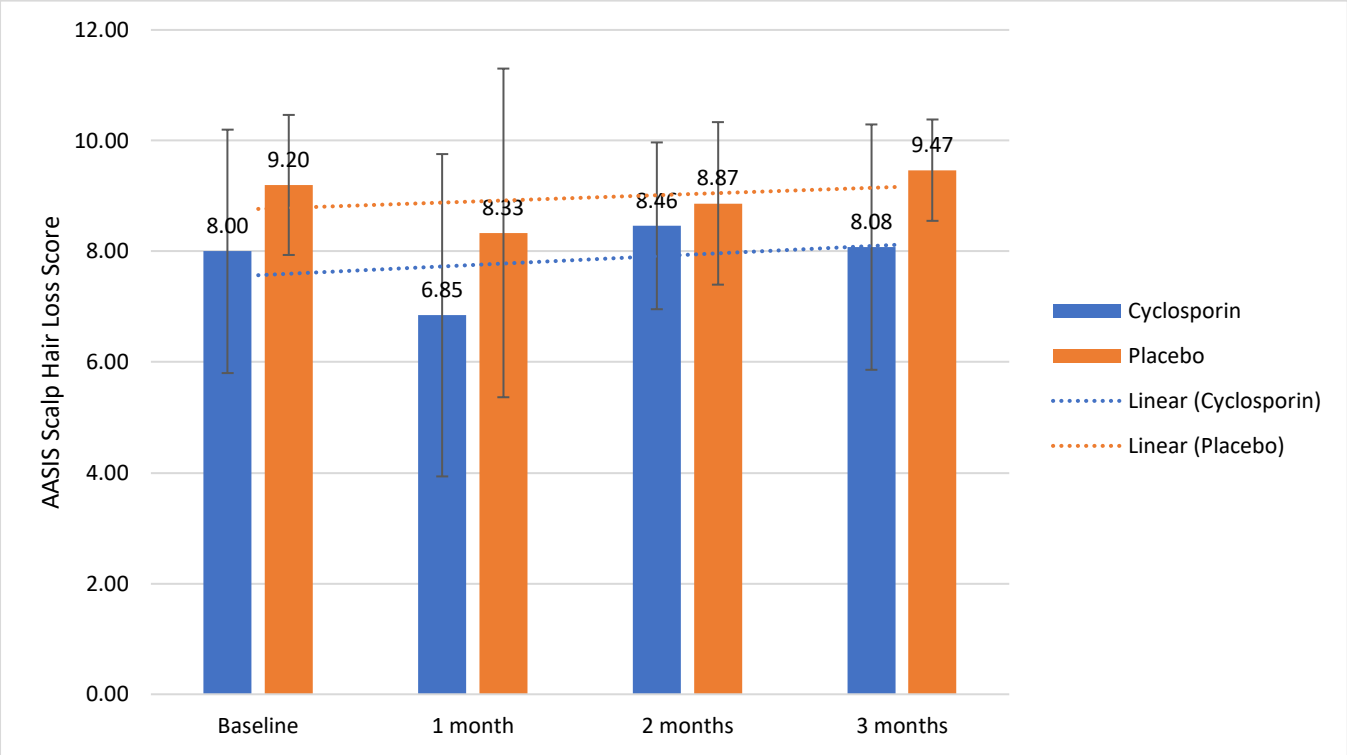

Figure 21. Scalp Hair Loss Score over time

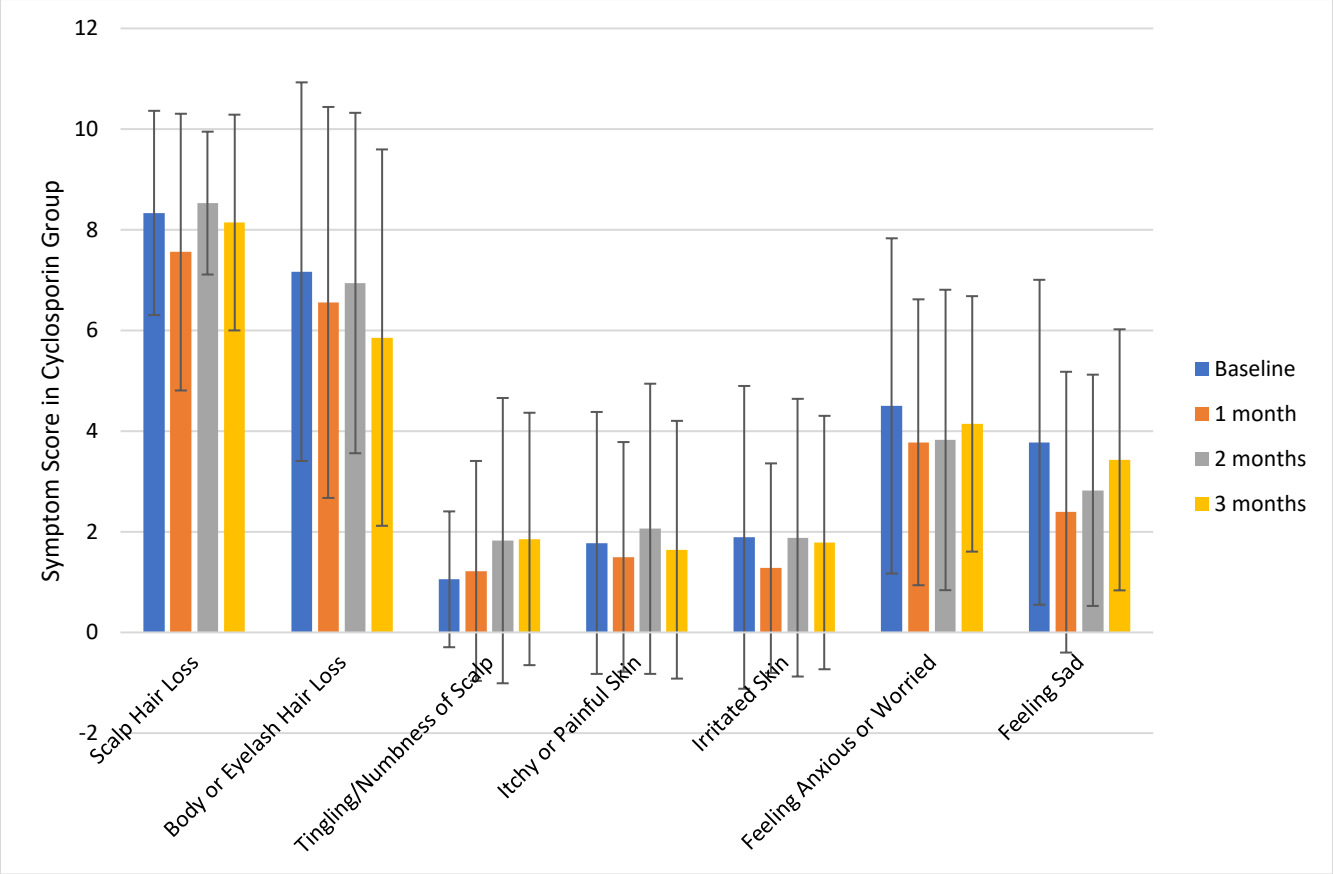

Figure 22. Symptom scores over time for cyclosporin group

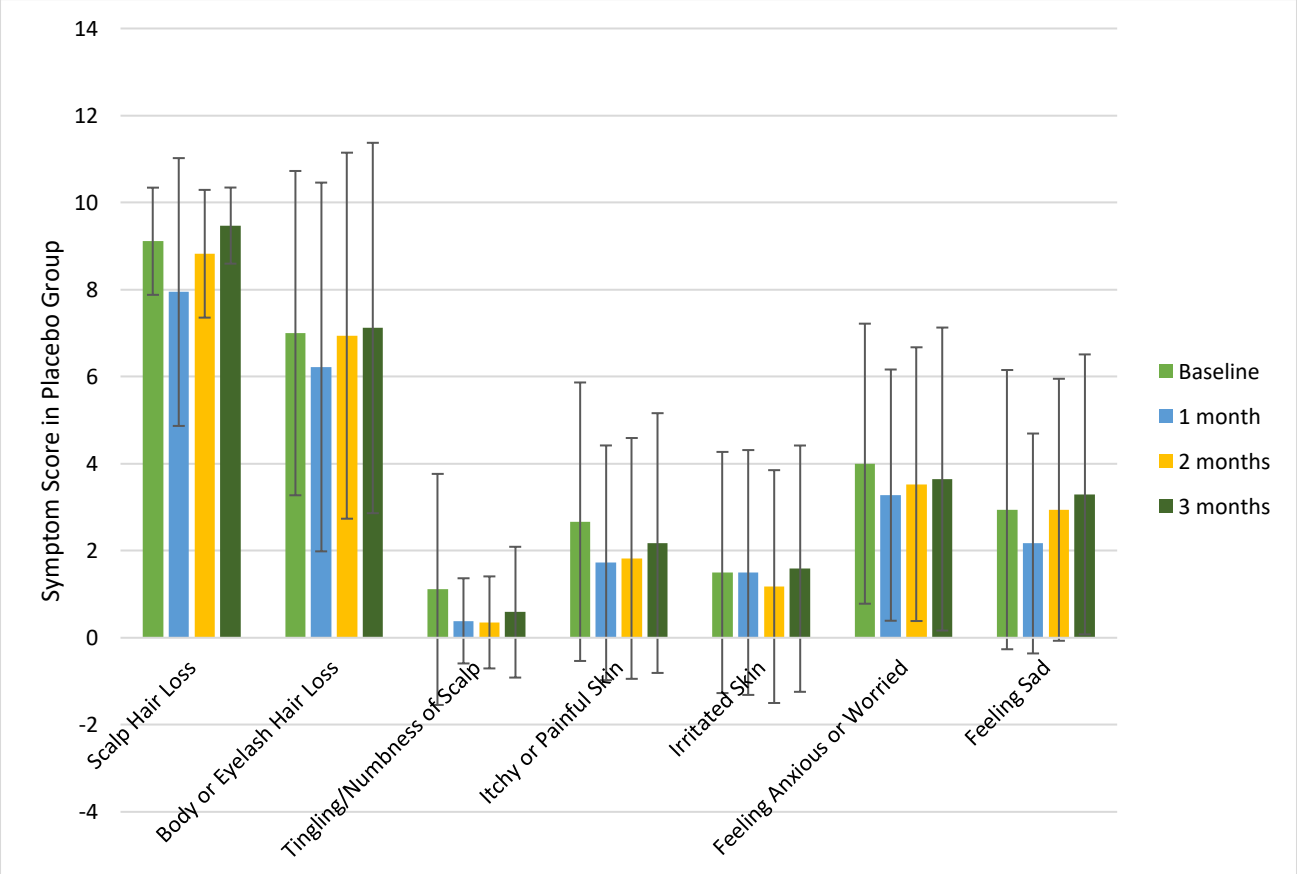

Figure 23. Symptom scores over time for placebo group

3.4.8 Secondary Endpoint 7: Incidence of adverse events

There was no statistically significant difference between cyclosporin and placebo groups in incidence of adverse events across 3 months (Table 13).

Overall, 83% of participants reported a total of 47 adverse events during the trial. The incidence of adverse events was similar between cyclosporin and placebo (25 versus 22). Adverse events spanned a range of systems, with the most frequent complaints being headaches (n=11) and hirsutism (n=9). The cumulative incidence of headache over 3 months was greater in the placebo group than the cyclosporin group (7 versus 4) and these complaints were frequently made by the same participants (4 versus 4). 4 participants in the placebo group complained of ‘hirsutism’ during the trial, compared to 5 participants in the cylcosporin group.

The only adverse event that approached a statistically significant difference were respiratory disorders (p=0.07). The incidence was greater for the cyclosporin group than the placebo group (4 versus 0). Respiratory disorders included upper respiratory tract infections reported by 3 participants; additionally, 1 participant complained of increased asthmatic symptoms during the trial.

More adverse events were reported during the first month than the second and third month for both groups. There were no serious adverse events during the trial.

Table 13. Incidence of adverse events.

Complaint	Cyclosporin (n=18)	Placebo (n=18)	Total (n=36)	p value
Participants with AEs	15 (83%), 25	15 (83%), 22	30 (83%), 47	1.00
Nervous system disorders				
Headaches	4 (22.2%), 4	4 (22.2%), 7	8 (22.2%), 11	1.00
Paraesthesia	1 (5.6%), 1	2 (11.1%), 3	3 (8.3%), 4	0.55
Gastrointestinal disorders				
Abdominal pain	2 (11.1%), 3	2 (11.1%), 2	4 (11.1%), 5	1.00
Nausea	0 (0%), 0	2 (11.1%), 2	2 (5.6%), 2	0.15
Increased appetite	1 (5.6%), 1	0 (0%), 0	1 (2.8%), 1	0.31
Infections				
Urinary Tract Infection	1 (5.6%), 1	0 (0%), 0	1 (2.8%), 1	0.31
MSK	3 (16.7%), 3	2 (11.1%), 3	5 (13.9%), 6	0.63
Respiratory disorders	3 (16.7%), 4	0 (0%), 0	3 (2.8%), 4	0.07
Dermatological disorders				
Pruritus	1 (5.6%), 2	1 (5.6%), 1	2 (5.6%), 3	1.00
Hirsutism	5 (27.8%), 5	4 (22.2%), 4	9 (25%), 9	0.70
Ophthalmological disorders	1 (5.6%), 1	0 (0%), 0	1 (2.8%), 1	0.31

Data are number of participants (%), cumulative incidence of adverse events from 1 month to 3 months of treatment. P values are reported for number of participants (%) in each group.

3.4.9 Secondary Endpoint 8: Incidence of specific clinical laboratory abnormalities

The majority of changes in blood biochemistry between cyclosporin and placebo groups were statistically insignificant at 3 months (Table 14).

On average and for each individual patient, there were no clinically important changes in blood biochemistry during the trial. Only the mean change in ALP at 3 months was statistically significant between the two groups (p=0.03). While the cyclosporin group's ALP changes were greater than the placebo group on average (9.1 versus -7.5; p=0.03), this difference was not clinically significant. Mean change in total cholesterol levels, measured at baseline and 1 month afterwards, approached statistical significance between the cyclosporin and placebo groups (0.4 versus -0.1; p=0.06), however this change was also not clinically significant. Both the greater increase in ALP and total cholesterol found in the cyclosporin group are known clinical laboratory abnormalities associated with cyclosporin.

Overall, the mean haemoglobin levels of the cyclosporin group decreased more than the placebo group during the trial (-2.9 versus 0; p=0.38). There was no difference in the change in mean white cell counts between the two groups after 3 months (-0.1 versus -0.1; p=0.78). The mean creatinine level increased marginally for the cyclosporin group compared to the placebo group (0.2 versus -2.6; p=0.42). These trends were not clinically significant.

Table 14. Mean change in blood biochemistry at 3 months.

	Cyclosporin (n=13)	Placebo (n=15)	Total	p value
Change in Hb	-2.9 (9.6)	0 (10.3)	-1.3 (9.8)	0.38
Change in WCC	-0.1 (2.3)	-0.1 (1.6)	-0.1 (0.9)	0.78
Change in Plt	6.9 (38.9)	4.5 (54.9)	5.6 (47.6)	0.95
Change in K+	0.1 (0.3)	0.1 (0.2)	0.1 (0.3)	0.69
Change in Cr	0.2 (8.5)	-2.6 (8.5)	-1.4 (8.5)	0.42
Change in Total bilirubin	-0.4 (6.8)	1 (3.7)	0.4 (5.1)	0.47
Change in ALT	3.7 (21.3)	-1.8 (6.2)	0.6 (14.7)	0.93
Change in AST	4.1 (14.7)	-1.2 (4.2)	1.1 (10.2)	0.32
Change in ALP	9.1 (12.3)	-7.5 (25.7)	-0.3 (22.2)	0.03
Change in GGT	-0.7 (4.1)	6.9 (15.4)	3.6 (12.3)	0.10
Change in Mg	-0.1 (0.1)	0 (0.1)	0.0 (0.1)	0.26
Change in Total cholesterol	0.4 (0.7)	-0.1 (0.5)	0.1 (0.6)	0.06
Change in Triglycerides	0.3 (0.4)	-0.03 (0.3)	0.1 (0.4)	0.11

Data are mean (SD). Hb: haemoglobin; WCC: white cell count; Plt: platelets; K+: potassium; Cr: creatinine; ALT: alanine transaminase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; GGT: gamma-glutamyl transferase; Mg: magnesium

Chapter 4: Discussion and Conclusion

4.1 Key findings

This randomised, double-blind, placebo-controlled, parallel group prospective clinical trial was designed to investigate the efficacy of cyclosporin in participants with moderate to severe AA. The efficacy of monotherapy cyclosporin has been difficult to estimate thus far with literature only consistent of case series, retrospective reviews and small uncontrolled trials.

This interim analysis of 28 participants suggests that there is no difference between cyclosporin treatment at 4mg/kg/day for 3 months compared to placebo in patients with moderate to severe AA.

There was no statistically significant difference in a number of efficacy endpoints, including the primary endpoint, change from baseline of SALT score at 3 months, as well as secondary efficacy endpoints, proportion of participants achieving a 30%, 50%, 75% and 100% reduction in SALT score at 3 months, change from baseline in non-vellus hair counts and proportion of participants achieving at least 1 grade improvement in eyelash assessment scale at 3 months. The response rate (at least 50% reduction in SALT score) in the cyclosporin group was 23.1% at the end of 3 months. The only secondary efficacy endpoint that was statistically significant was the proportion of participants achieving at least 1 grade improvement in eyebrow assessment scale at 3 months.

Furthermore, the interim analysis of quality of life measurements highlighted that active pharmacotherapy with 4mg/kg/day cyclosporin did not yield results that produced an improvement in participants' quality of life on both the generic quality of life instrument, AQoL-8D, and the disease-specific instrument, AASIS in all dimensions. There was no significant difference in participants' ratings of scalp hair loss, as measured by the Scalp Hair Loss Score on AASIS, at the end of 3 months.

Overall, cyclosporin 4mg/kg/day was well-tolerated over the 3-month period in a cohort of participants with a mean age of 41 years, ranging from 18 to 64 years. The incidence of adverse events did not differ between cyclosporin and placebo groups. There were no serious adverse events. There were no clinical laboratory abnormalities that were clinically significant.

There are 4 ongoing participants yet to complete end-of-treatment visit at 3 months. The following is a discussion of the interim results.

4.2 Interpretation of findings

In this interim analysis of 28 participants, 23.1% of participants on cyclosporin achieved a response, defined as 50% reduction in SALT score at the end of 3 months. To interpret this result, we revisit hair follicle morphogenesis, AA pathogenesis and the mechanism of action of cyclosporin in the treatment of AA.

Cyclosporin is a hydrophobic endecapeptide that selectively inhibits T-cell activation by disturbing interleukin-2 gene expression through inhibiting calcineurin, a calmodulin-dependent phosphatase. While cyclosporin's mechanisms for promoting hair growth are not fully elucidated, both dose-dependent hypertrichosis (98), and T-cell inhibition are suggested additive pathways for promoting regrowth in AA. The hypertrichotic effect of cyclosporin causes increased keratinisation, density and diameter of the hair (99, 100), recently found to result from SFRP1 inhibition through WAY-316606 (101). Importantly, the role of androgens is not implicated in cyclosporin's therapeutic effect, from studies of cyclosporin-induced hypertrichotic patients that show a normal circulating plasma androgen, no virilisation and hair growth in non-androgen associated sites (102, 103). The T-cell inhibitory effect on hair regrowth was investigated by Gupta et al. (91) in patients with AA treated with 3 months of oral cyclosporin 6mg/kg/day. Clinical response correlated with decreases to the perifollicular infiltrate that is characteristic of AA histology, with decreases in helper and cytotoxic T-cells correlating with degree of terminal hair regrowth (91).

While we did not perform scalp biopsies on participants in the clinical trial, potential reasons for the low response rate may be explored through examining updates in aetiological models of AA. According to the prevailing immune privilege collapse model, it is the loss of immune privilege of the hair follicle epithelium during anagen that results in susceptibility to autoimmune attack and generation of AA. Specifically, the increase in IFN-γ secretion most potently upregulates MHC class Ia expression in the proximal hair follicle epithelium that was otherwise MHC class I-negative (39). It has therefore been suggested that treatment should be targeted at downregulating IFN-γ-induced ectopic MHC class I expression to restore immune privilege of the hair follicle. Immunomodulators, such as α-MSH, TGF-β1, and IGF-1, produced by the anagen hair bulb, have been shown to do so and suggested as new therapeutic modalities for immune privilege restoration (104-106). Cyclosporin, however, works by inhibiting secondary autoimmune sequelae, i.e. the attack from autoreactive CD8+

T-cells, and so may therefore be less effective when the primary cause of AA, i.e. loss of immune privilege, is still left unaddressed. This may also explain the high rates of relapse associated with treatment cessation in studies of steroid-sparing agents. Nevertheless, the secondary autoimmune sequelae may be impairing the hair follicle's ability to recover from autoimmune attack and restore immune privilege, allowing some individuals to recover when this immune cell infiltrate is decreased by cyclosporin.

In addition to the immune privilege collapse model, Paus et al. suggested that cyclosporin induces hair regrowth by inhibiting catagen demonstrated through the inhibition of dexamethasone-induced catagen changes after intraperitoneal cyclosporin on murine models (107). Recently, an experiment on human hair follicles stimulated with cyclosporin for 6 days, showed that cyclosporin reduces the migration of fibroblasts from the dermal papilla, a marker of catagen onset, therefore delaying catagen (108). This mechanism for cyclosporin to inhibit catagen onset supports the clinical use of cyclosporin as a maintenance agent. Relatively low response rates to monotherapy cyclosporin were seen in this trial despite induction doses of 4mg/kg/day, perhaps suggesting that an alternative agent is required to initiate anagen or regrowth. As found in the retrospective review of our clinic patients (Table 8), 64% of cyclosporin 'responders' were using concurrent prednisolone. Additionally, this figure does not account for individuals who were treated with prednisolone initially. Despite Paus et al. suggesting that cyclosporin also induces anagen in telogen hair follicles (109, 110), these studies are limited to murine models and no human studies have been performed to investigate this. Further studies are required to elucidate the mechanism of action of cyclosporin on hair regrowth in humans.

It is known that patients with more severe and long-standing disease, including AT and AU, are more resistant to treatment than those with limited, patchy disease of short duration of onset (111). Many participants in our trial had treatment-resistant, long-standing, extensive disease. On average, the mean duration of current episode of AA was 6.5 years and mean percentage scalp hair loss at baseline was 79.4%. Longstanding lesions are associated with miniaturisation of the anagen hair follicles by the surrounding lymphocytic infiltrate of the hair bulb (112), resulting in difficult hair regrowth. There may also be a decline in follicle density over time, suggested to result from perifollicular fibrosis (113). However, as the infiltrate surrounds the hair bulb and not the 'bulge' zone of the isthmus where follicular stem cells are located, theoretically the hair follicles retain regenerative ability despite many years of disease. A 10 year retrospective longitudinal study of 70 patients with AT/AU showed that 17.1% (12/70) of patients achieved a complete recovery with active treatment (114). 1 of 3 participants who achieved SALT50 at 3 months in our trial had AT/AU. We performed a

subgroup analysis excluding those with AT/AU and found no statistically significant differences in treatment effect. Nevertheless, the severity of disease in this cohort of participants should be considered in the interpretation of low response rates.

There is a paucity of literature on the biology of eyelash and eyebrow hair follicles, however it is known that the ultrastructure of the lashes and eyebrows are different (115). 2 participants achieved a 1 grade improvement in eyelash assessment scale at 2 months in the cyclosporin group, compared to none in the placebo group. In general, eyelashes may require a longer treatment period to regrow than scalp hair. A retrospective review found a mean time of 28 months for complete regrowth of the lashes with patients on a number of topical and systemic treatments (116). Therefore, it is possible that longer treatment with cyclosporin may have resulted in favourable results. We found a statistically significant difference in participants achieving a 1 grade improvement in eyebrow assessment scale compared to placebo. Further studies are required to investigate the differences in eyelash, eyebrow and scalp hair follicle biology as well as the impact of cyclosporin treatment on each in AA.

Overall, we found that quality of life did not significantly change with cyclosporin intervention, despite some participants experiencing improvement. This occurred for both quality of life measured using the generic instrument, AQoL-8D, and the disease-specific instrument, AASIS. This is likely to be the result of both a low response rate and a sample size inadequately powered to detect small changes in quality of life data. Even in our participant who achieved a 100% reduction in SALT score, her final scalp hair loss rating was 8. While there is an element of variation between individuals in the value one places on hair and appearance, many participants voiced that partial regrowth of a patch would still lead them to shave the hair off, that is, regrowth in patches is unwearable and still deemed abnormal. In this sense, quality of life measurements are likely only to substantially improve if an individual feels that their hair is ‘normal’; an outcome that even a 100% reduction in SALT score may not capture due to its scoring mechanism. An alternative explanation is a delayed psychological adjustment for increased hair growth. The unpredictable nature of AA is greatly responsible for the psychological morbidity (55). Participants in this relatively short trial of 3 months may require a greater adjustment time to believe that hair will persist following treatment, instead of shedding again, thereby causing stagnant ratings of scalp hair loss within this short duration. Further studies examining impact of pharmacotherapy intervention on quality of life may allow greater quantification of this interaction.

Our clinical trial did not detect a statistically significant difference between treatment and placebo, and while biological mechanisms for the response rate have been discussed, the small-moderate sample size of this study may be insufficiently powered to detect lower responses. Furthermore, we cannot rule out that a longer treatment period would not have yielded positive results. These limitations are discussed below.

4.3 Relationship with similar literature

There are no other published randomised, placebo-controlled, double-blind clinical trials investigating cyclosporin for the treatment of AA. Case series and uncontrolled single-arm studies have suggested response rates to monotherapy cyclosporin ranging from 33% to 55% (Table 7). Compared to published literature, there are 3 main treatment reasons why our study potentially yielded a lower response rate: a participant cohort with severe disease, a lower dose of cyclosporin in comparison to these studies, and a shorter treatment period compared to the mean treatment period of these studies.

Jang et al. (85) reports the highest response rate of these published studies – 55% of participants treated with an average dose of 3mg/kg/day cyclosporin attained a response, defined as at least 50% terminal regrowth in alopecic areas. The higher response may have resulted from overall milder disease seen in Jang et al.'s study in comparison to those investigated in this trial. His study cohort of 51 AA patients comprised 78% (40/51) patchy AA and 22% (11/51) AT/AU. These figures are compared to the 56% (10/18) of participants in this trial that had AT/AU. Furthermore, the mean duration of AA was 2 years in Jang et al.'s study, whereas the mean duration of AA in this study was 16.5 years, with a mean duration of current episode, 6.5 years. The comparably more severe disease of our participant cohort may result in greater treatment resistance. The average treatment duration of 13.2 months in Jang et al.'s study, compared to 3 months in our study, is also another crucial difference to explain response rate.

Ferrando et al. (89) similarly had participants with a shorter duration of AA, 9.8 years, and examined a longer duration of treatment, mean 9 months, as well as a higher dose of cyclosporin, 5mg/kg/day.

Both Acikgoz et al. (117) and Gupta et al. (91) had similar characteristics in their study cohorts to ours. Acikgoz et al. examined a cohort of 25 participants, 13 (52%) of which had AT/AU. This was a retrospective case series, with mean dose of 4.5 mg/kg/day and mean duration of treatment 4 months. A 45% (10/22) response rate was found, defined as

‘significant hair growth’, including 5 patients with AT/AU. Gupta et al. investigated 3 months of cyclosporin 6mg/kg/day treatment in 6 patients, finding a 50% response rate. 4 of the 6 patients had AT/AU. Both studies reported higher treatment doses and Acikgoz et al. had a longer treatment duration. Additionally, it may be difficult to compare ‘response’ between all studies due to qualitative outcome measures.

There are also a number of intrinsic study design considerations that may influence results and therefore lead to the difference in response rates seen. Compared to the published literature, our study employed: a control arm (placebo), blinded outcome measurements, blinded treatment, prospective evaluation and standardised treatment regimens and doses.

In the studies by Acikgoz et al. and Jang et al. patients treated in a clinic were reviewed. Given the high rates of combination therapy used in clinical practice, it may be difficult to verify that patients treated solely with cyclosporin were analysed, as clinicians may supplement ongoing cyclosporin treatment with occasional topical therapy and intralesional steroid injections. Importantly, there was no information on whether cyclosporin was used as an induction agent to initiate remission or whether it was used as a maintenance agent following an alternative induction agent which was weaned off. In the latter situation, both the difference in functional use of cyclosporin as well as the potential for delayed therapeutic action from the induction agent may result in a higher response rate. Comparatively, in our study we ensured that participants had an adequate ‘washout’ period from their previous treatments prior to enrolment and cyclosporin monotherapy was investigated with no other hair growth-promoting agents used during the trial. The ability to increase or decrease doses according to clinical response is another privilege in clinic not afforded in clinical trials. This patient-tailored approach to treatment may have also increased response rates.

Lack of blinding in these other studies may also lead to outcome assessment and performance bias. All measures used to determine treatment response in AA are currently subjective to some degree; there is no computerised test to evaluate regrowth. For studies which employed numerical gradings, it was not reported whether the same outcome assessor was employed for all ratings of a particular patient; variation in numerical outcomes between assessors has been reported (84).

Additionally, this is the first study that employs a control arm of participants with similar baseline disease to investigate the treatment effect of cyclosporin. None of the previous studies had suitable controls by which to compare treatment response. Particularly in patchy AA, it may be challenging to distinguish the effect of a treatment from spontaneous

remission of a patch and this may be particularly pertinent in Jang et al. with the majority of the cohort with multifocal AA.

No studies have reported the impact of any steroid-sparing agent on the growth of eyelash or eyebrow hair. In comparison to studies investigating agents used solely to treat eyelash or eyebrow alopecia (116, 118), our study had a shorter treatment duration.

This is the only clinical trial employing both disease-specific and generic quality of life instruments to evaluate impact of pharmacotherapy on quality of life as a secondary efficacy endpoint. The generic quality of life instrument allowed comparability between AA quality of life information with the general Australian population; while the specific quality of life instrument provided greater sensitivity to detect changes in AA symptoms following pharmacotherapy intervention. These measures have previously been overlooked in clinical trials of systemic agents for AA; our systematic review (119) identified only 3 studies that incorporated this, two of which employed only depression and anxiety scales (79, 80). Price et al. (75) used a dermatology-specific quality of life instrument, the Dermatology Quality of Life Scales tool. In her study, subcutaneous efalizumab was found to be ineffective for treating AA and reflectively, there was no significant difference in quality of life data between placebo and efalizumab at the end of 3 months. We similarly did not find a significant difference in quality of life measurements at the end of 3 months in our trial, which may be related to sample size, treatment effect or delayed psychological adjustment as discussed previously. This is the first study in which the AQoL-8D has been used for a population of AA patients. It has previously been employed in diseases such as arthritis, asthma, cancer, depression, heart disease, hearing loss and diabetes. Similarly, to our knowledge, this is the first time the AASIS has been used in a study of AA patients, having previously been validated in 1400 patients with clinician review (120).

In examining data from other RCTs of systemic agents, oral prednisolone PT had a response rate of 40% within 3 months (119). Furthermore, a recent trial of JAK inhibitors, PF-06651600 and PF-06700841, reported a 30% and 42% mean reduction of SALT score respectively at 3 months and 48% and 60% of participants respectively achieved SALT30 at 6 months (121). When we compare these figures to the 10% mean reduction in SALT score and 23% achievement of SALT30 for cyclosporin in this trial, cyclosporin is likely an inferior agent to such established and emerging treatments.

4.4 Study strengths and limitations

Our study has a number of strengths and limitations. To answer our research question in regard to the efficacy of cyclosporin treatment in patients with moderate to severe AA, we employed a study design most suitable to deriving this outcome: a double-blind, randomised, placebo-controlled, parallel, prospective clinical trial. This study design resolves some key barriers to estimating a true efficacy rate found in the current literature: lack of control, combination with other therapies, changing doses and selection bias. We achieved a double-blind through the use of an identical placebo and a third-party pharmacy who performed all randomisation and concealed the allocation sequence. Outcome assessments for all participants were graded by the same investigator to ensure consistency. We selected a therapeutic dose and maintained this dose throughout the study, with near perfect compliance from trial participants (mean: 96%). Outcome was assessed through both well-defined numerical and categorical outcomes - mean change in SALT score and proportions attaining 30%, 50%, 75% and 100% reduction in SALT – enabling our results to be compared with other trials using this standardised measurement, such as for future meta-analyses. This is also the only clinical trial for AA to employ both disease-specific and generic quality of life instruments to measure efficacy of pharmacotherapy from a psychosocial perspective.

However, our study also has a number of limitations. Our sample size was powered for a prediction that approximately 50% of those treated with 4mg/kg/day cyclosporin would achieve a treatment response, i.e. 50% reduction in SALT score, at the end of 3 months. This figure was based off previous literature as summarised in Chapter 1. Therefore, if the true response rate is less than the predicted 50% of participants, then our sample size is insufficiently powered to detect this response. For example, if only 20% of participants achieve a treatment response with cyclosporin 4mg/kg/day at the end of 3 months, then the required sample size to detect this would be 176 participants, with a power of 80% and an alpha of 0.05. Our final results may be able to conclude that the response rate of cyclosporin 4mg/kg/day for 3 months is not the hypothesised 50%, however we would not be able to significantly conclude a lower percentage efficacy.

We chose a treatment period of 3 months. Previous studies investigating cyclosporin monotherapy (Table 7) had a mean duration of treatment ranging from 3 months to 13.2 months and investigational guidelines suggest that the active treatment period should be at a minimum 3 months for AA (84). It may be possible that a longer treatment duration would detect a greater response, given the delay in onset of action with all agents for treatment of

AA and the trend of continued improvement seen in this trial. However, cyclosporin is often considered relatively rapid-onset and efficacy does not depend on cumulative dose, therefore the lower response at 3 months suggests that response may be low for durations longer than 3 months as well. This trend has previously been reported, with low yield for longer treatment durations in patients unresponsive at 4 months of treatment (89).

The SALT score was chosen as our numerical measurement for efficacy. It is the only standardised method of assessment for AA and is used in commercials trials for AA as recommended by investigational guidelines (122). However, this scoring system has limitations. SALT scores are derived from a *visual* estimation of the percentage scalp hair loss, making the scoring outcome assessor dependent. Differences in the method outcome assessors estimate percentage scalp hair loss, may result in variation in the final score. These differences may arise from over- or under- estimation of percentage scalp surface area involved, difficulties with distinguishing vellus from non-vellus hairs (particularly depigmented non-vellus hairs), and not visualising patches covered by longer hairs, particularly when scores are performed retrospectively on photography. This has previously been described, with differences in evaluators as much as 20% (84). While the sensitivity of the score has not been defined in the literature, it is likely to have a degree of imprecision. We attempted to ameliorate this limitation by maintaining the same outcome assessor for all participants in the study, attaining SALT scores in person at the visit, as well as through extensive photography both 2D and 3D which could retrospectively be viewed to compare SALT scores across time points for each participant. The primary efficacy endpoint, mean change, also reduces error associated with determining the exact score, as only the change is of primary interest.

Furthermore, while the SALT score may be a useful efficacy measurement to standardise outcome assessment in clinical trials, the score themselves may not optimally reflect severity and impact of disease from a patient's perspective. Only completely alopecic areas on the scalp are counted; areas of partial regrowth and patches with sparse terminal hair of lower than normal density, are not counted. However, these areas of partial regrowth and sparseness are often still considered 'abnormal' by the patient. One may therefore attain 100% reduction in SALT score, with partial regrowth in all alopecic areas, however this may still be clinically suboptimal. To negotiate differences in a successful response as defined by SALT or a successful response as defined by patient satisfaction, we employed quality of life measurements as a secondary efficacy endpoint. The AQoL-8D is particularly suited to this cohort as opposed to other generic quality of life instruments, as it has greater emphasis on psychosocial dimensions. We used the AASIS as a disease-specific measure to more

sensitively capture symptoms of AA and any changes during the trial. This measurement has previously demonstrated exceptional internal consistency and content validity (52). We did not find any statistically significant changes in quality of life at the end of 3 months treatment compared to baseline. While this may be related to the efficacy of cyclosporin found in this trial, our sample size may also be insufficiently powered to detect a change in the quality of life measurements.

The generalisability of these results is limited to a cohort of moderate to severe patients with AA. On average, participants had a long duration of current episode of AA, 6.5 years, and 55.5% of the cyclosporin group had AT/AU. 72.2% of the cohort had a history of AT/AU at any time. Patients with AT/AU and longstanding patches respond poorer to treatment than patchy disease of shorter duration. We chose a cohort of moderate to severe patients as those with limited disease are typically treated with intralesional steroids or topical treatments, however the relative severity of this cohort, particularly in regard to length of current AA episode, should be considered in interpretation of these results.

4.5 Clinical implications

Clinical insights from this study may be gained by interpreting the results with consideration for the cohort of relatively severe disease, the treatment regimen of *monotherapy* cyclosporin and the relatively short treatment period of 3 months.

The interim analysis suggests that there is no difference between 4mg/kg/day cyclosporin and placebo in the treatment of moderate to severe AA for 3 months. This trial is powered to detect response rates of at least 50% at 3 months. While lower response rates may not be detected, the results from this trial indicate that in a cohort of moderate to severe patients with AA, 4mg/kg/day cyclosporin monotherapy is unlikely to yield a 50% response rate when used for 3 months. This new data suggests that what was previously thought to be a relatively efficacious, rapid-onset agent for the treatment of AA has only mild treatment effect.

Clinically, this new efficacy data will aid clinicians in deciding a second-line treatment. This is pertinent in patients who fail to respond to systemic corticosteroids or are corticosteroid-responsive but corticosteroid-dependent and must wean corticosteroid treatment due to cumulative side effects. Evaluating the risk-benefit ratio of second-line agents in AA is important as many are associated with adverse events, require long-term treatment, may require ongoing blood biochemistry monitoring and have variable efficacy. The use

cyclosporin is a balancing act between the risk of adverse events, particularly nephrotoxicity, hypertension and hyperlipidaemia, and its efficacy rates. Many clinicians would be hesitant in treating chronic AA with doses higher than 4mg/kg/day for prolonged periods, as cumulative toxicity would outweigh potential benefits. Therefore, the response rates shown in this study at 4mg/kg/day may prompt clinicians to consider other second-line agents with fewer side effects or superior efficacy as therapeutic options.

Additionally, this study may suggest that cyclosporin *monotherapy* is relatively weak at inducing remission at doses of 4mg/kg/day. Therefore, when used in clinical practice, its function may be primarily to maintain remission once an alternative agent has initiated this. While we did not examine its effect when combined with glucocorticoids, previous literature indicates favourable results (Table 7) and we cannot exclude that cyclosporin does not have a synergistic effect when used in combination therapies, based on the results of this trial.

4.6 Future directions

This clinical trial is due for completion in November, after which the final results may be analysed.

The inferior response rates of this clinical trial for cyclosporin highlight the need for further research into the biological mechanisms of AA pathogenesis. In an AA priority setting partnership between patients, carers, relatives and healthcare professionals, the greatest uncertainty in AA management and treatment surrounded understanding the aetiology of AA (65). This includes investigation into both the acute pathogenesis of AA and also the chronic impacts of AA on the hair follicle, given that clinical treatment is often poor in long-standing, severe disease, as is seen in this clinical trial. The mechanism of action of cyclosporin in the treatment of AA - how it induces hair regrowth and why response rates vary between individuals - is another area requiring investigation.

The effectiveness of cyclosporin may be further studied through a larger clinical trial powered to detect lower response rates. The response rate for cyclosporin was 23.1% in this trial. Therefore, future trials should employ a sample size of approximately 176 participants for a power of 80% and alpha of 0.05 to detect a significant result based off the response rates of this trial. In order to determine what clinical response is deemed worthwhile for active intervention though, further studies quantifying the impact of pharmacotherapy on quality of life in patients with AA may be necessary.

While the SALT score is the current investigational standard in trials for AA, future clinical trials may also find the reporting of scalp surface area affected a useful marker of disease response that may better align with patient perspectives on disease. This may therefore make information from clinical trials more translatable to clinical practice. Scalp surface area affected includes all areas deemed ‘less than normal’, rather than only areas completely devoid of terminal hair as measured by SALT.

It has been suggested that future treatment be focussed on alternate pathways and targets involved in AA pathogenesis, such as targeting immunomodulators that restore ‘immune privilege’ of the hair follicle. Of note, the activation of the JAK/STAT cytokine pathway in AA has been on great interest lately through the potential to target this through JAK inhibitors, such as tofacitinib. Promising large-scale trials investigating these treatments are underway by pharmaceutical companies.

4.7 Conclusion

This is the first randomised, placebo-controlled, prospective clinical trial investigating the effectiveness of 4mg/kg/day cyclosporin monotherapy in the treatment of moderate to severe AA for 3 months. Interim results of 28 participants suggests there is no statistically significant difference between cyclosporin and placebo in reduction of scalp hair loss at the end of 3 months of treatment. Based on these interim results, it is unlikely for the treatment response of cyclosporin to be 50% at 3 months. Trials employing a larger sample size and longer treatment duration may detect response rates lower than 50%. These results may be interpreted for a cohort of patients with moderate to severe, long-standing AA and will guide clinicians in their choice of second-line agents for this patient cohort.

References

1. Gilbert ST, J. . Body shame in childhood and adolescence. Miles GJ, editor. New York: Hove: Brunner-Routledge; 2002.
2. Adachi T, Kobayashi T, Sugihara E, Yamada T, Ikuta K, Pittaluga S, et al. Hair follicle-derived IL-7 and IL-15 mediate skin-resident memory T cell homeostasis and lymphoma. Nat Med. 2015;21(11):1272-9.
3. Nagao K, Kobayashi T, Moro K, Ohyama M, Adachi T, Kitashima DY, et al. Stress-induced production of chemokines by hair follicles regulates the trafficking of dendritic cells in skin. Nature immunology. 2012;13(8):744-52.
4. Cooper GS, Bynum MLK, Somers EC. Recent Insights in the Epidemiology of Autoimmune Diseases: Improved Prevalence Estimates and Understanding of Clustering of Diseases. Journal of autoimmunity. 2009;33(3-4):197-207.
5. Le Cleach L, Chosidow O. Clinical practice. Lichen planus. N Engl J Med. 2012;366(8):723-32.
6. Safavi KH MS, Suman VJ, Moshell AN, Melton LJ, 3rd. Incidence of alopecia areata in Olmsted County, Minnesota, 1975 through 1989. Mayo Clinic proceedings Mayo Clinic. 1995;70(7):628-33.
7. Cotsarelis G, Botchkarev, V. Biology of Hair Follicles. 7th ed. Wolff K GL, Katz SI, Gilchrest BA, Paller AS, Leffell DJ, editor. New York: McGraw-Hill; 2008.
8. Sperling LC. Hair anatomy for the clinician. J Am Acad Dermatol. 1991;25(1 Pt 1):1-17.
9. Wang E, de Berker, D., Christiano, A Biology of Hair and Nails. 4th ed. Bolognia J, Jorizzo, J. L., & Schaffer, J. V, editor. Philadelphia: Elsevier Saunders; 2018.
10. Plikus MV, Mayer JA, de la Cruz D, Baker RE, Maini PK, Maxson R, et al. Cyclic dermal BMP signalling regulates stem cell activation during hair regeneration. Nature. 2008;451(7176):340-4.
11. Greco V, Chen T, Rendl M, Schober M, Pasolli HA, Stokes N, et al. A two-step mechanism for stem cell activation during hair regeneration. Cell stem cell. 2009;4(2):155-69.
12. Mardaryev AN, Ahmed MI, Vlahov NV, Fessing MY, Gill JH, Sharov AA, et al. Micro-RNA-31 controls hair cycle-associated changes in gene expression programs of the skin and hair follicle. FASEB journal : official publication of the Federation of American Societies for Experimental Biology. 2010;24(10):3869-81.
13. Mirzoyev SA, Schrum AG, Davis MDP, Torgerson RR. Lifetime incidence risk of alopecia areata estimated at 2.1% by Rochester Epidemiology Project, 1990-2009. J Invest Dermatol. 2014;134(4):1141-2.
14. Goh C, Finkel M, Christos PJ, Sinha AA. Profile of 513 patients with alopecia areata: associations of disease subtypes with atopy, autoimmune disease and positive family history. J Eur Acad Dermatol Venereol. 2006;20(9):1055-60.
15. Tan E, Tay YK, Goh CL, Chin Giam Y. The pattern and profile of alopecia areata in Singapore--a study of 219 Asians. Int J Dermatol. 2002;41(11):748-53.
16. Yang S, Yang J, Liu JB, Wang HY, Yang Q, Gao M, et al. The genetic epidemiology of alopecia areata in China. Br J Dermatol. 2004;151(1):16-23.
17. Sharma VK, Dawn G, Kumar B. Profile of alopecia areata in Northern India. Int J Dermatol. 1996;35(1):22-7.

18. Tak WJ CY, Ro BI. A clinical study on alopecia areata (1996–2000). Korean J Dermatol 2002;40(7):791-800.
19. Roest YBM, van Middendorp HT, Evers AWM, van de Kerkhof PCM, Pasch MC. Nail Involvement in Alopecia Areata: A Questionnaire-based Survey on Clinical Signs, Impact on Quality of Life and Review of the Literature. Acta Derm Venereol. 2018;98(2):212-7.
20. Gandhi V, Baruah MC, Bhattacharaya SN. Nail changes in alopecia areata: incidence and pattern. Indian J Dermatol Venereol Leprol. 2003;69(2):114-5.
21. Huang KP, Mullangi S, Guo Y, Qureshi AA. Autoimmune, atopic, and mental health comorbid conditions associated with alopecia areata in the united states. JAMA Dermatology. 2013;149(7):789-94.
22. Thomas EA, Kadyan RS. ALOPECIA AREATA AND AUTOIMMUNITY: A CLINICAL STUDY. Indian Journal of Dermatology. 2008;53(2):70-4.
23. Lundin M, Chawa S, Sachdev A, Bhanusali D, Seiffert-Sinha K, Sinha AA. Gender differences in alopecia areata. Journal of drugs in dermatology : JDD. 2014;13(4):409-13.
24. Seyrafi H, Akhiani M, Abbasi H, Mirpour S, Gholamrezanezhad A. Evaluation of the profile of alopecia areata and the prevalence of thyroid function test abnormalities and serum autoantibodies in Iranian patients. BMC dermatology. 2005;5:11.
25. Tosti A BS, Iorizzo M. Alopecia areata: a long term followup study of 191 patients. J Am Acad Dermatol. 2006;55(1):438-41.
26. De Waard-van der Spek FB, Oranje AP, De Raeymaecker DM, Peereboom-Wynia JD. Juvenile versus maturity-onset alopecia areata--a comparative retrospective clinical study. Clin Exp Dermatol. 1989;14(6):429-33.
27. Ikeda T. A New Classification of Alopecia Areata. Dermatology. 1965;131(6):421-45.
28. Evaluation IfHMa. Search GBD Data [webpage on the Internet] Seattle, WA2016 [13th September 2018]. Available from: Available from: http://www.healthdata.org/search-gbd-data?s=Alopecia%20areata.
29. Villasante Fricke AC, Miteva M. Epidemiology and burden of alopecia areata: a systematic review. Clin Cosmet Investig Dermatol. 2015;8:397-403.
30. Karimkhani C, Boyers LN, Prescott L, Welch V, Delamere FM, Nasser M, et al. Global burden of skin disease as reflected in Cochrane Database of Systematic Reviews. JAMA Dermatol. 2014;150(9):945-51.
31. King LE, Jr., McElwee KJ, Sundberg JP. Alopecia areata. Current directions in autoimmunity. 2008;10:280-312.
32. Sperling LC, Lupton GP. Histopathology of non-scarring alopecia. J Cutan Pathol. 1995;22(2):97-114.
33. Todes-Taylor N, Turner R, Wood GS, Stratte PT, Morhenn VB. T cell subpopulations in alopecia areata. J Am Acad Dermatol. 1984;11(2 Pt 1):216-23.
34. Paus R, Cotsarelis G. The biology of hair follicles. N Engl J Med. 1999;341(7):491-7.
35. Cotsarelis G, Millar SE. Towards a molecular understanding of hair loss and its treatment. Trends in molecular medicine. 2001;7(7):293-301.
36. Head JR, Billingham RE. Immunologically privileged sites in transplantation immunology and oncology. Perspectives in biology and medicine. 1985;29(1):115-31.
37. Paus R, Ito N, Takigawa M, Ito T. The hair follicle and immune privilege. J Investig Dermatol Symp Proc. 2003;8(2):188-94.
38. Gilhar A, Paus R, Kalish RS. Lymphocytes, neuropeptides, and genes involved in alopecia areata. The Journal of clinical investigation. 2007;117(8):2019-27.

39. Ruckert R, Hofmann U, van der Veen C, Bulfone-Paus S, Paus R. MHC class I expression in murine skin: developmentally controlled and strikingly restricted intraepithelial expression during hair follicle morphogenesis and cycling, and response to cytokine treatment in vivo. J Invest Dermatol. 1998;111(1):25-30.
40. Yazdan P. Update on the genetics of androgenetic alopecia, female pattern hair loss, and alopecia areata: implications for molecular diagnostic testing. Seminars in cutaneous medicine and surgery. 2012;31(4):258-66.
41. Koyama S, Ishii KJ, Coban C, Akira S. Innate immune response to viral infection. Cytokine. 2008;43(3):336-41.
42. Ercolini AM, Miller SD. The role of infections in autoimmune disease. Clinical and experimental immunology. 2009;155(1):1-15.
43. McElwee KJ, Gilhar A, Tobin DJ, Ramot Y, Sundberg JP, Nakamura M, et al. What causes alopecia areata? Exp Dermatol. 2013;22(9):609-26.
44. Paus R, Slominski A, Czarnetzki BM. Is alopecia areata an autoimmune-response against melanogenesis-related proteins, exposed by abnormal MHC class I expression in the anagen hair bulb? The Yale Journal of Biology and Medicine. 1993;66(6):541-54.
45. Gilhar A, Ullmann Y, Berkutzki T, Assy B, Kalish RS. Autoimmune hair loss (alopecia areata) transferred by T lymphocytes to human scalp explants on SCID mice. The Journal of clinical investigation. 1998;101(1):62-7.
46. Petukhova L, Duvic M, Hordinsky M, Norris D, Price V, Shimomura Y, et al. Genome-wide association study in alopecia areata implicates both innate and adaptive immunity. Nature. 2010;466:113.
47. Colombe BW, Lou CD, Price VH. The genetic basis of alopecia areata: HLA associations with patchy alopecia areata versus alopecia totalis and alopecia universalis. J Investig Dermatol Symp Proc. 1999;4(3):216-9.
48. Shi Q, Duvic M, Osei JS, Hordinsky MK, Norris DA, Price VH, et al. Health-Related Quality of Life (HRQoL) in alopecia areata patients-a secondary analysis of the National Alopecia Areata Registry Data. J Investig Dermatol Symp Proc. 2013;16(1):S49-50.
49. Ruiz-Doblado S CA, García-Hernández MJ. Alopecia areata: psychiatric comorbidity and adjustment to illness. International journal of dermatology. 2003;42(6):434-7.
50. Sellami R, Masmoudi J, Ouali U, Mnif L, Amouri M, Turki H, et al. The Relationship Between Alopecia Areata and Alexithymia, Anxiety and Depression: A Case-Control Study. Indian Journal of Dermatology. 2014;59(4):421-.
51. Colon EA, Popkin MK, Callies AL, Dessert NJ, Hordinsky MK. Lifetime prevalence of psychiatric disorders in patients with alopecia areata. Comprehensive psychiatry. 1991;32(3):245-51.
52. Rencz F, Gulacsi L, Pentek M, Wikonkal N, Baji P, Brodszky V. Alopecia areata and health-related quality of life: a systematic review and meta-analysis. Br J Dermatol. 2016;175(3):561-71.
53. Zhang M, Zhang N. Quality of life assessment in patients with alopecia areata and androgenetic alopecia in the People's Republic of China. Patient preference and adherence. 2017;11:151-5.
54. Cash TF, Price VH, Savin RC. Psychological effects of androgenetic alopecia on women: comparisons with balding men and with female control subjects. J Am Acad Dermatol. 1993;29(4):568-75.
55. Papadopolous L, & Bor, R. Psychological Approaches to Dermatology. Leicester: BPS Books; 1999.

56. Sinclair RD. Alopecia areata and suicide of children. Med J Aust. 2014;200(3):145.
57. Ito T. Advances in the management of alopecia areata. J Dermatol. 2012;39(1):11-7.
58. Devi M, Rashid A, Ghafoor R. Intralesional Triamcinolone Acetonide Versus Topical Betamethasone Valearate in the Management of Localized Alopecia Areata. Journal of the college of physicians and surgeons--pakistan : JCPSP [Internet]. 2015; 25(12):[860-2 pp.]. Available from: http://onlinelibrary.wiley.com/o/cochrane/clcentral/articles/181/CN-01125181/frame.html.
59. Kuldeep C, Singhal H, Khare AK, Mittal A, Gupta LK, Garg A. Randomized comparison of topical betamethasone valerate foam, intralesional triamcinolone acetonide and tacrolimus ointment in management of localized alopecia areata. International journal of trichology. 2011;3(1):20-4.
60. Chu TW, AlJasser M, Alharbi A, Abahussein O, McElwee K, Shapiro J. Benefit of different concentrations of intralesional triamcinolone acetonide in alopecia areata: An intrasubject pilot study. Journal of the American Academy of Dermatology. 2015;73(2):338-40.
61. Samrao A, Fu JM, Harris ST, Price VH. Bone mineral density in patients with alopecia areata treated with long-term intralesional corticosteroids. Journal of drugs in dermatology : JDD. 2013;12(2):e36-40.
62. Cranwell W, Lai, V., Photiou, L., Meah, N., Wall, D., Rathnayake, D., Joseph, S., Chitreddy, V., Gunatheesan, S., Sindhu, K., Sharma, P., Green, J., Eisman, S., Yip, L., Jones, L., Sinclair, R. Treatment of alopecia areata: An Australian expert consensus statement. Australas J Dermatol. Forthcoming 2018.
63. Fiedler VC. Alopecia areata. A review of therapy, efficacy, safety, and mechanism. Arch Dermatol. 1992;128(11):1519-29.
64. Delamere FM, Sladden MJ, Dobbins HM, Leonardi-Bee J. Interventions for alopecia areata. Cochrane Database of Systematic Reviews [Internet]. 2008; (2). Available from: http://onlinelibrary.wiley.com/doi/10.1002/14651858.CD004413.pub2/abstract.
65. Macbeth AE, Tomlinson J, Messenger AG, Moore-Millar K, Michaelides C, Shipman AR, et al. Establishing and prioritizing research questions for the treatment of alopecia areata: the Alopecia Areata Priority Setting Partnership. Br J Dermatol. 2017;176(5):1316-20.
66. Higgins JPT, Altman DG, Gøtzsche PC, Jüni P, Moher D, Oxman AD, et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. The BMJ. 2011;343:d5928.
67. Kurosawa M, Nakagawa S, Mizuashi M, Sasaki Y, Kawamura M, Saito M, et al. A comparison of the efficacy, relapse rate and side effects among three modalities of systemic corticosteroid therapy for alopecia areata. Dermatology (basel, switzerland) [Internet]. 2006; 212(4):[361-5 pp.]. Available from: http://onlinelibrary.wiley.com/o/cochrane/clcentral/articles/443/CN-00565443/frame.html.
68. Dehghan M, Alborzi A, Shahini N. Comparison of oral prednisolone pulse therapy with intravenous methylprednisolone pulse therapy in severe alopecia areata. Journal of Pakistan Association of Dermatologists. 2013;23.
69. Kar B, Handa S, Dogra S, Kumar B. Placebo-controlled oral pulse prednisolone therapy in alopecia areata. Journal of the american academy of dermatology [Internet]. 2005; 52(2):[287-90 pp.]. Available from: http://onlinelibrary.wiley.com/o/cochrane/clcentral/articles/985/CN-00520985/frame.html.

70. Galbraith GMP, Thiers BH, Jensen J, Hoehler F. A randomized double-blind study of inosiplex (Isoprinosine) therapy in patients with alopecia totalis. Journal of the American Academy of Dermatology. 1987;16(5, Part 1):977-83.
71. Georgala S, Katoulis A, Befon A, Georgala K, Stavropoulos P. Inosiplex for treatment of alopecia areata: a randomized placebo-controlled study. Acta dermato-venereologica [Internet]. 2006; 86(5):[422-4 pp.]. Available from: http://onlinelibrary.wiley.com/o/cochrane/clcentral/articles/864/CN-00567864/frame.html.
72. Berth-Jones J, Hutchinson PE. Treatment of alopecia totalis with a combination of inosine pranobex and diphencyprone compared to each treatment alone. Clinical and Experimental Dermatology. 1991;16(3):172-5.
73. Yang D, Zheng J, Zhang Y, Jin Y, Gan C, Bai Y. Total glucosides of paeony capsule plus compound glycyrrhizin tablets for the treatment of severe alopecia areata in children: a randomized controlled trial. Evidence-based complementary and alternative medicine [Internet]. 2013. Available from: http://onlinelibrary.wiley.com/o/cochrane/clcentral/articles/655/CN-00913655/frame.html.
74. Strober BE, Menon K, McMichael A, et al. Alefacept for severe alopecia areata: A randomized, double-blind, placebo-controlled study. Archives of Dermatology. 2009;145(11):1262-6.
75. Price VH, Hordinsky MK, Olsen EA, Roberts JL, Siegfried EC, Rafal ES, et al. Subcutaneous efalizumab is not effective in the treatment of alopecia areata. Journal of the American Academy of Dermatology. 2008;58(3):395-402.
76. Kurosawa M, Nakagawa S, Mizuashi M, Sasaki Y, Kawamura M, Saito M, et al. A Comparison of the Efficacy, Relapse Rate and Side Effects among Three Modalities of Systemic Corticosteroid Therapy for Alopecia Areata. Dermatology. 2006;212(4):361-5.
77. Saif G, Al-Khawajah M, Al-Otaibi H, Al-Roujayee A, Alzolibani A, Kalantan H, et al. Efficacy and safety of oral mega pulse methylprednisolone for severe therapy resistant Alopecia areata. Saudi medical journal [Internet]. 2012; 33(3):[284-91 pp.]. Available from: http://onlinelibrary.wiley.com/o/cochrane/clcentral/articles/243/CN-01018243/frame.html.
78. Tosti A, Bardazzi F, Guerra L. Alopecia totalis: is treating nonresponder patients useful? Journal of the american academy of dermatology [Internet]. 1991; 24(3):[455-6 pp.]. Available from: http://onlinelibrary.wiley.com/o/cochrane/clcentral/articles/423/CN-00076423/frame.html.
79. Cipriani R, Perini G, Rampinelli S. Paroxetine in alopecia areata. International journal of dermatology [Internet]. 2001; 40(9):[600-1 pp.]. Available from: http://onlinelibrary.wiley.com/o/cochrane/clcentral/articles/122/CN-00376122/frame.html.
80. Perini G, Zara M, Cipriani R, Carraro C, Preti A, Gava F, et al. Imipramine in alopecia areata. A double-blind, placebo-controlled study. Psychotherapy and psychosomatics [Internet]. 1994; 61(3-4):[195-8 pp.]. Available from: http://onlinelibrary.wiley.com/o/cochrane/clcentral/articles/806/CN-00103806/frame.html.
81. Yang D, You L, Song P, Zhang L, Bai Y. A randomized controlled trial comparing total glucosides of paeony capsule and compound glycyrrhizin tablet for alopecia areata. Chinese journal of integrative medicine [Internet]. 2012; 18(8):[621-5 pp.]. Available from: http://onlinelibrary.wiley.com/o/cochrane/clcentral/articles/389/CN-00879389/frame.html.
82. Mehta B, Raka A, Bharmbhatt V. Comparative study of various regimens in Alopecia areata. Internet journal of dermatology [Internet]. 2011; 9(1). Available from: http://onlinelibrary.wiley.com/o/cochrane/clcentral/articles/541/CN-00897541/frame.html.

83. Ead R. Oral zinc sulphate in alopacia areata-a double blind trial. British journal of dermatology [Internet]. 1981; 104(4):[483-4 pp.]. Available from: http://onlinelibrary.wiley.com/o/cochrane/clcentral/articles/960/CN-00568960/frame.html.
84. Olsen EA, Hordinsky MK, Price VH, Roberts JL, Shapiro J, Canfield D, et al. Alopecia areata investigational assessment guidelines--Part II. National Alopecia Areata Foundation. J Am Acad Dermatol. 2004;51(3):440-7.
85. Jang YH, Kim SL, Lee KC, Kim MJ, Park KH, Lee WJ, et al. A Comparative Study of Oral Cyclosporine and Betamethasone Minipulse Therapy in the Treatment of Alopecia Areata. Ann Dermatol. 2016;28(5):569-74.
86. Rashidi T MA. Treatment of persistent alopecia areata with sulfasalazine. Int J Dermatol. 2008;47(8):850-2.
87. Royer M, Bodemer C, Vabres P, Pajot C, Barbarot S, Paul C, et al. Efficacy and tolerability of methotrexate in severe childhood alopecia areata. Br J Dermatol. 2011;165(2):407-10.
88. Vano-Galvan S, Hermosa-Gelbard A, Sanchez-Neila N, Miguel-Gomez L, Saceda-Corralo D, Rodrigues-Barata R, et al. Treatment of recalcitrant adult alopecia areata universalis with oral azathioprine. J Am Acad Dermatol. 2016;74(5):1007-8.
89. Ferrando J, Grimalt R. Partial response of severe alopecia areata to cyclosporine A. Dermatology. 1999;199(1):67-9.
90. Matsuda S, Koyasu S. Mechanisms of action of cyclosporine. Immunopharmacology. 2000;47(2-3):119-25.
91. Gupta AK, Ellis CN, Cooper KD, Nickoloff BJ, Ho VC, Chan LS, et al. Oral cyclosporine for the treatment of alopecia areata. A clinical and immunohistochemical analysis. J Am Acad Dermatol. 1990;22(2 Pt 1):242-50.
92. Paquet P, Arrese Estrada J, Pierard GE. Oral cyclosporin and alopecia areata. Dermatology. 1992;185(4):314-5.
93. Ciclosporin (dermatology): Australian Medicines Handbook Pty Ltd; 2017 [updated July 2017. Available from: https://amhonline-amh-net-au/chapters/chap-08/psoriasis-drugs-for/immunosuppressants-psoriasis/cyclosporin-01.
94. Lee D OD, Kim JW, Park SW, Oh MK, Sung HS, Hwang SW. Treatment of Severe Alopecia Areata: Combination Therapy Using Systemic Cyclosporine A with Low Dose Corticosteroids. Ann Dermatol. 2008;20(4):172-8.
95. Fradin MS, Ellis CN, Voorhees JJ. Efficacy of cyclosporin A in psoriasis: a summary of the United States' experience. Br J Dermatol. 1990;122 Suppl 36:21-5.
96. Reference.medscape.com. Neoral, Sandimmune (cyclosporine) dosing, indications, interactions, adverse effects and more 2018 [19 April 2018]. Available from: https://reference.medscape.com/drug/neoral-sandimmune-cyclosporine-343196.
97. Price V. Double-blind, placebo-controlled evaluation of topical minoxidil in extensive alopecia areata. Journal of the american academy of dermatology [Internet]. 1987; 16(3 Pt 2):[730-6 pp.]. Available from: http://onlinelibrary.wiley.com/o/cochrane/clcentral/articles/193/CN-00047193/frame.html.
98. Lutz G. Effects of cyclosporin A on hair. Skin pharmacology : the official journal of the Skin Pharmacology Society. 1994;7(1-2):101-4.
99. Gilhar A, Krueger GG. Hair growth in scalp grafts from patients with alopecia areata and alopecia universalis grafted onto nude mice. Arch Dermatol. 1987;123(1):44-50.
100. Yamamoto S, Kato R. Hair growth-stimulating effects of cyclosporin A and FK506, potent immunosuppressants. Journal of Dermatological Science. 1994;7:S47-S54.

101. Hawkshaw NJ, Hardman JA, Haslam IS, Shahmalak A, Gilhar A, Lim X, et al. Identifying novel strategies for treating human hair loss disorders: Cyclosporine A suppresses the Wnt inhibitor, SFRP1, in the dermal papilla of human scalp hair follicles. PLOS Biology. 2018;16(5):e2003705.
102. Schmidt JB, Gebhart W, Kopsa H, Spona J. Does cyclosporin A influence sex hormone level? Experimental and clinical endocrinology. 1986;88(2):207-11.
103. Wysocki GP, Daley TD. Hypertrichosis in patients receiving cyclosporine therapy. Clin Exp Dermatol. 1987;12(3):191-6.
104. Slominski A, Paus R. Melanogenesis is coupled to murine anagen: toward new concepts for the role of melanocytes and the regulation of melanogenesis in hair growth. J Invest Dermatol. 1993;101(1 Suppl):90s-7s.
105. Botchkareva NV, Botchkarev VA, Chen LH, Lindner G, Paus R. A role for p75 neurotrophin receptor in the control of hair follicle morphogenesis. Developmental biology. 1999;216(1):135-53.
106. Slominski A, Wortsman J, Luger T, Paus R, Solomon S. Corticotropin releasing hormone and proopiomelanocortin involvement in the cutaneous response to stress. Physiological reviews. 2000;80(3):979-1020.
107. Paus R, Handjiski B, Czarnetzki BM, Eichmuller S. A murine model for inducing and manipulating hair follicle regression (catagen): effects of dexamethasone and cyclosporin A. J Invest Dermatol. 1994;103(2):143-7.
108. Hawkshaw NJ, Haslam IS, Ansell DM, Shamalak A, Paus R. Re-Evaluating Cyclosporine A as a Hair Growth–Promoting Agent in Human Scalp Hair Follicles. Journal of Investigative Dermatology. 2015;135(8):2129-32.
109. Paus R, A Böttge J, M. Henz B, Maurer M. Hair growth control by immunosuppression1996. 408-10 p.
110. Paus R, Stenn KS, Link RE. The induction of anagen hair growth in telogen mouse skin by cyclosporine A administration. Laboratory investigation; a journal of technical methods and pathology. 1989;60(3):365-9.
111. Chiang KS, Mesinkovska NA, Piliang MP, Bergfeld WF. Clinical Efficacy of Diphenylcyclopropenone in Alopecia Areata: Retrospective Data Analysis of 50 Patients. J Investig Dermatol Symp Proc. 2015;17(2):50-5.
112. McDonagh AJ, Messenger AG. The pathogenesis of alopecia areata. Dermatol Clin. 1996;14(4):661-70.
113. Whiting DA. Diagnostic and predictive value of horizontal sections of scalp biopsy specimens in male pattern androgenetic alopecia. J Am Acad Dermatol. 1993;28(5 Pt 1):755-63.
114. Jang YH, Hong NS, Moon SY, Eun DH, Lee WK, Chi SG, et al. Long-Term Prognosis of Alopecia Totalis and Alopecia Universalis: A Longitudinal Study with More than 10 Years of Follow-Up: Better than Reported. Dermatology. 2017;233(2-3):250-6.
115. Liotet S, Riera M, Nguyen H. [The lashes. Physiology, structure, pathology (author's transl)]. Archives d'ophtalmologie. 1977;37(11):697-708.
116. Modjtahedi BS, Kishan AU, Schwab IR, Jackson WB, Maibach HI. Eyelash alopecia areata: case series and literature review. Canadian Journal of Ophthalmology / Journal Canadien d'Ophtalmologie. 2012;47(4):333-8.
117. Acikgoz G, Caliskan E, Tunca M, Yeniay Y, Akar A. The effect of oral cyclosporine in the treatment of severe alopecia areata. Cutan Ocul Toxicol. 2014;33(3):247-52.

118. Vila TO, Camacho Martinez FM. Bimatoprost in the Treatment of Eyelash Universalis Alopecia Areata. International journal of trichology. 2010;2(2):86-8.
119. Lai VW CG, Gin D, Sinclair R. Systemic Treatments for Alopecia Areata: A Systematic Review. Australas J Dermatol. 2018;Article accepted on 14 July, 2018.
120. Mendoza TR, Osei JS, Shi Q, Duvic M. Development of the alopecia areata symptom impact scale. J Investig Dermatol Symp Proc. 2013;16(1):S51-2.
121. A Phase 2a Randomized, Placebo-controlled Study to Evaluate Efficacy and Safety of Janus Kinase Inhibitors PF-06651600 and PF-06700841 in Alopecia Areata: 24-Week Results (Abstract) [press release]. Abstract presented at EADV 2018: EADV2018.
122. Olsen EA, Roberts J, Sperling L, Tosti A, Shapiro J, McMichael A, et al. Objective Outcome Measures: Collecting Meaningful Data on Alopecia Areata. Journal of the American Academy of Dermatology. 2017.

Appendices

Appendix 1: Search terms

MEDLINE (OVID) search strategy

(i) Search strategy to locate RCTs

Search terms 1-29, as given in the Cochrane Handbook (Higgins 2005), Appendix 5b.2

(ii) Search strategy to locate alopecia areata.

30. exp Alopecia Areata/

31. alopecia areata.mp. [mp=title, original title, abstract, name of substance word, subject heading word]

32. (alopecia adj totalis).mp. [mp=title, original title, abstract, name of substance word, subject heading word]

33. (alopecia adj universalis).mp. [mp=title, original title, abstract, name of substance word, subject heading word]

34. (alopecia adj celsi).mp. [mp=title, original title, abstract, name of substance word, subject heading word]

35. ophia$.mp. [mp=title, original title, abstract, name of substance word, subject heading word]

36. (nonscarring adj hair adj loss).mp. [mp=title, original title, abstract, name of substance word, subject heading word]

37. 30 or 31 or 32 or 33 or 34 or 35 or 36

38. 29 and 37

The results of searches (i) and (ii) were combined with the Boolean operator AND.

EMBASE (OVID) search strategy

1. random$.mp.

2. crossover procedure/or double blind procedure/or single blind procedure/

3. comparative study/ or controlled study/ or clinical trial/

4. factorial$.mp.

5. PLACEBO/ or placebo$.mp.

6. versus.mp.

7. (single or double or treble or triple).mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer name]

8. (blind or mask).mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer name]

9. Alopecia Areata/ or alopecia areata.mp.
10. Alopecia Areata/th, dt [Therapy, Drug Therapy]
11. alopecia totalis.mp.
12. alopecia universalis.mp.
13. alopecia celsi.mp.
14. ophiasis.mp.
15. nonscarring hair loss.mp.
16. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8
17. 9 or 10 or 11 or 12 or 13 or 14 or 15
18. 16 and 17

AMED search strategy

1. randomised controlled trial$/
2. random allocation/
3. double blind method/
4. single blind method.mp.
5. exp Clinical trials/
6. (clin$ adj25 trial$).mp. [mp=abstract, heading words, title]
7. ((singl$ or doubl$ or trebl$ or tripl$) adj25 (blind$ or mask$ or dummy)).mp. [mp=abstract, heading words, title]
8. (placebo$ or random$).mp. [mp=abstract, heading words, title]
9. research design/ or clinical trials/ or comparative study/ or double blind method/ or random allocation/
10. prospective studies.mp.
11. cross over studies.mp.
12. Follow up studies/
13. control$.mp.
14. (multicent$ or multi-cent$).mp. [mp=abstract, heading words, title]
15. ((stud or design$) adj25 (factorial or prospective or intervention or crossver or cross-over or quasi-experiment$)).mp. [mp=abstract,
heading words, title]
16. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15
17. alopecia areata.mp.
18. alopecia universalis.mp.
19. ophia$.mp.
20. (nonscarring ajd hair adj loss).mp. [mp=abstract, heading words, title]
21. alopecia celsi.mp.

22. 17 or 18 or 18 or 19 or 20 or 21

23. 16 and 22

Cochrane Controlled Trials Search Strategy

#1 alopecia areata in All Fields in all products

#2 MeSH descriptor Alopecia Areata, this term only in MeSH products

#3 alopecia totalis in All Fields in all products

#4 alopecia universalis in All Fields in all products

#5 alopecia celsi in All Fields in all products

#6 ophia$ in All Fields in all products

#7 nonscarr* NEAR hair NEAR loss in All Fields in all products

#8 (#1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7)

PsychINFO search strategy

1. alopecia areata.mp.

2. exp ALOPECIA/

3. exp Clinical Trials/

4. 1 or 2

5. 3 and 4

LILACS Search Strategy

((Pt randomised controlled trial OR Pt controlled clinical trial OR Mh randomised controlled trials OR Mh random allocation OR Mh
double-blind method OR Mh single-blind method) AND NOT (Ct animal AND NOT (Ct human and Ct animal)) OR (Pt clinical
trial OR Ex E05.318.760.535$ OR (Tw clin$ AND (Tw trial$ OR Tw ensa$ OR Tw estud$ OR Tw experim$ OR Tw investiga$))
OR ((Tw singl$ OR Tw simple$ OR Tw doubl$ OR Tw doble$ OR Tw duplo$ OR Tw trebl$ OR Tw trip$) AND (Tw blind$ OR
Tw cego$ OR Tw ciego$ OR Tw mask$ OR Tw mascar$)) OR Mh placebos OR Tw placebo$ OR (Tw random$ OR Tw randon$ OR
Tw casual$ OR Tw acaso$ OR Tw azar OR Tw aleator$) OR Mh research design) AND NOT (Ct animal AND NOT (Ct human and
Ct animal)) OR (Ct comparative study OR Ex E05.337$ OR Mh follow-up studies OR Mh prospective studies OR Tw control$ OR
Tw prospectiv$ OR Tw volunt$ OR Tw volunteer$) AND NOT (Ct animal AND NOT (Ct human and Ct animal))) [Words] and

alopecia and (areata or universalis or totalis or celsi) or (nonscarring and hair and loss)

[Words]

Appendix 2: Details of ongoing trials

Trial name	Pilot Study of the Safety and Efficacy of Apremilast in Subjects With Moderate to Severe Alopecia Areata
ID	NCT02684123
Brief summary	Alopecia areata is a medical condition, in which the hair falls out in patches. The hair can fall out on the scalp or elsewhere on the face and body. Alopecia areata is an autoimmune skin disease, which means that the immune system is recognizing the hair follicles as foreign and attacking them, causing round patches of hair loss. It can progress to total scalp hair loss (alopecia totalis) or complete body hair loss (alopecia universalis). The scalp is the most commonly affected area, but the beard or any hair-bearing site can be affected alone or together with the scalp. Alopecia areata occurs in males and females of all ages, and is a highly unpredictable condition that tends to recur. Alopecia areata can cause significant distress to both patients and their families. In this study, the aim to assess the effects of a new treatment called apremilast in patients with alopecia areata. A total of 30 patients will be included in the study, which will run for a total of 52 weeks.
Methods	This is a randomized, double-blind, placebo-controlled pilot study consisting of two phases. A total of 30 subjects with moderate to severe alopecia areata (including universalis and totalis) involving 50-100% of the scalp will be enrolled. A possible maximum of 15 patients (approximately 7 patients each) with current episodes of AA totalis / universalis may be included in this study. In Phase 1, subjects will be randomized (2:1) to either receive apremilast or placebo for 24 weeks. In Phase 2, eligible subjects will receive apremilast from Week 24 through Week 48. The following subjects will be eligible to enter into Phase 2: 1. Subjects who received placebo in Phase 1 of the study 2. Subjects who received apremilast in Phase 1 of the study, and who achieved a minimum of 50% regrowth (SALT50) at Week 24, compared to Baseline.
Participants	Age: 18 years and older Subject with a diagnosis of patchy scalp alopecia areata present for at least 6 months, and up to a maximum of 10 years. Patients with ≥50% and <95% total scalp hair loss at Baseline as measured using the SALT score to qualify as moderate to severe AA; and 95%-100% scalp hair loss to qualify as AA totalis/universalis.
Interventions	1. Apremilast 30mg twice daily

	2. Placebo pills twice daily
Outcomes	Primary Outcome Measures: SALT50 [Time Frame: Baseline and Week 24]
Starting date	February 2016
Contact information	https://clinicaltrials.gov/ct2/show/NCT02684123 Principal Investigator: Emma Guttman, MD, PhD ISMMS

Trial name	A Pilot Study of Tralokinumab in Subjects With Moderate to Severe Alopecia Areata
ID	NCT02684097
Brief summary	The purpose of this study is to assess whether tralokinumab can be a helpful treatment for alopecia areata.
Methods	This is a randomized, double-blind, placebo-controlled pilot study of a total of 30 subjects with moderate to severe alopecia areata involving 30-100% of the scalp. Expected is 50% of these subjects to have concomitant alopecia areata (AA) and atopic dermatitis (AD). Subjects with AA alone (15 subjects) will be randomized (2:1) to either receive tralokinumab or placebo via subcutaneous injection every 2 weeks for 24 weeks. Subjects with concomitant alopecia areata and atopic dermatitis (15 subjects) will be randomized separately in a 2:1 ratio to receive tralokinumab or placebo via subcutaneous injection every 2 weeks for 24 weeks.
Participants	Age: 18 to 75 years Subject has a history of alopecia areata for at least 3 months. Subject has extensive patchy alopecia areata (at least 30% scalp hair loss).
Interventions	1. Tralokinumab subcutaneous injection every two weeks for 24 weeks 2. Placebo: Saline subcutaneous injection every two weeks for 24 weeks.
Outcomes	Primary Outcome Measures: • Gene expression changes in Th2/IL-13, "T22"/IL-22, S100A7 and S100A8, Th1/IFN-gamma, and Th17/IL-17A jointly correlated [Time Frame: Week 24] • Change from baseline in cellular, and molecular markers in skin biopsies after treatment
Starting date	January 2016
Contact information	https://clinicaltrials.gov/ct2/show/NCT02684097 Emma.Guttman, Associate Professor, Icahn School of Medicine at Mount Sinai

Trial name	Study To Evaluate The Efficacy And Safety Profile Of PF-06651600 And PF-06700841 In Subjects With Alopecia Areata
ID	NCT02974868
Brief summary	This is a Phase 2a, randomized, double blind, parallel group, multicenter study with an extension period.
Methods	The study will have a maximum duration of approximately 85 weeks. This includes an up to 5 weeks screening period, a 24 week treatment period, a 4 week drug holiday, an up to 12 month extension period, and a 4 week follow up period. The study will enroll a total of approximately 132 subjects. The study will be conducted at approximately 30 to 40 sites.
Participants	Age: 18 to 75 years Must have moderate to severe alopecia areata
Interventions	1. Drug: PF-06651600: 200 mg QD during induction and 50 mg QD during Maintenance 2. Drug: PF-06700841: 60 mg QD during induction and 30 mg QD during maintenance 3. Placebo
Outcomes	Primary Outcome Measures: 1. Change from baseline of Severity of Alopecia Tool (SALT) score at Week 24. [Time Frame: baseline, 24 weeks] assessment of the overall severity of alopecia areata 2. Incidence of treatment emergent adverse events (AEs) during the Extension Period [Time Frame: Baseline of extension period (Week 28) up to 12 months] 3. Incidence of specific clinical laboratory abnormalities including but not limited to anemia, neutropenia, thrombocytopenia, lymphopenia, changes in lipid profile, and liver function tests (LFTs) during the Extension Period [Time Frame: Baseline of extension period (Week 28) up to 12 months]
Starting date	December 15, 2016
Contact information	https://clinicaltrials.gov/ct2/show/NCT02974868 Pfizer

Trial name	Study to Evaluate the Safety and Efficacy of CTP-543 in Adult Patients With Moderate to Severe Alopecia Areata
ID	
Brief summary	This study will evaluate the safety and efficacy of CTP-543 in adult patients with chronic, moderate to severe alopecia areata.
Methods	This is a double-blind, randomized, placebo-controlled multi-center study consisting of 2 cohorts to assess the safety and efficacy of CTP-543. Each Cohort will be initiated sequentially in ascending dose order. Subjects will be randomized to either an active dose of CTP-543 or placebo for a 24-week treatment period.
Participants	Age: 18 to 65 years Definitive diagnosis of alopecia areata with a current episode lasting at least 6 months and not exceeding 10 years at the time of Screening. Total disease duration greater than 10 years is permitted. At least 50% scalp hair loss, as defined by a SALT score ≥50, at Screening and Baseline.

Interventions	1. CTP-543, 4 mg Oral tablet, dosed twice-daily 2. CTP-543, 8 mg Oral tablet, dosed twice-daily 3. Placebo, Oral tablet, dosed twice-daily
Outcomes	Primary Outcome Measures: 1. Effect of CTP-543 on treating hair loss as measured by the Severity of Alopecia Tool (SALT) [Time Frame: 24 weeks] 2. Number of Participants with Adverse Events as a Measure of Safety [Time Frame: 24 weeks]
Starting date	August 9, 2017
Contact information	https://clinicaltrials.gov/ct2/show/NCT03137381 Concert Pharmaceuticals

Appendix 3: Patient information consent form

Participant Information Sheet/Consent Form

Interventional Study - *Adult providing own consent*

Sinclair Dermatology

Title	*A randomised, double-blind, placebo-controlled study to evaluate the efficacy of cyclosporin in subjects with moderate to severe alopecia areata*
Protocol Number	*CYC001*
Project Sponsor	*Samson Clinical*
Coordinating Principal Investigator/ Principal Investigator	*Prof. Rodney Sinclair*
Associate Investigator(s) *(if required by institution)*	*Dr. Vivien Lai* *Laita Bokhari* *Dr. Gang Chen*
Location *(where CPI/PI will recruit)*	*Sinclair Dermatology*

Part 1 What does my participation involve?

1 Introduction

You are invited to take part in a clinical research trial because you have been diagnosed with a hair loss condition called Alopecia Areata. The clinical research trial is testing a potential new treatment for Alopecia Areata. The potential new treatment is cyclosporin.

Cyclosporin is an immunosuppressive agent. These medicines help to control your body's immune system. They can be used to treat conditions thought to be caused by a problem with the immune system. Cyclosporin is used to treat a number of conditions, including a kidney disease called nephrotic syndrome, severe cases of rheumatoid arthritis, severe cases of psoriasis and eczema.

This Participant Information Sheet/Consent Form tells you about the research project. It explains the tests and treatments involved. Knowing what is involved will help you decide if you want to take part in the research.

Please read this information carefully. Ask questions about anything that you don't understand or want to know more about. Before deciding whether or not to take part, you might want to talk about it with a relative, friend or your local doctor.

Participation in this research is voluntary. If you don't wish to take part, you don't have to. You will receive the best possible care whether or not you take part.

If you decide you want to take part in the research project, you will be asked to sign the consent section. By signing it you are telling us that you:
• Understand what you have read
• Consent to take part in the research project
• Consent to have the tests and treatments that are described
• Consent to the use of your personal and health information as described.

You will be given a copy of this Participant Information and Consent Form to keep.

2 What is the purpose of this research?

Alopecia Areata is a difficult disease to treat. So far, there are very limited treatments available for this condition. This research aims to evaluate the effectiveness of cyclosporin versus placebo in treating moderate to severe Alopecia Areata.

So far there have not been any studies that evaluate the effectiveness of cyclosporin in comparison to a placebo for the treatment of Alopecia Areata. Studies that examine cyclosporin for the treatment of Alopecia Areata so far have shown promising results.

Cyclosporin is approved in Australia to treat psoriasis and eczema. However it is not approved to treat alopecia areata. Therefore, it is an experimental treatment for alopecia areata. This means that it must be tested to see if it is an effective treatment for alopecia areata.

This study aims to provide information about the effectiveness of cyclosporin. It is possible that this information may be used to determine whether this drug could be made available to alopecia areata patients on the Pharmaceutical Benefits Scheme (PBS) in the future.

The research has been initiated by Professor Rodney Sinclair and is a self-funded project. It will be sponsored in Australia by Sinclair Dermatology.

The results of this research may contribute towards Dr. Vivien Lai being awarded a Bachelor of Medical Science (Hons) degree.

3 What does participation in this research involve?

Study Summary

You will be participating in a randomised controlled research project. Sometimes we do not know which treatment is best for treating a condition. To find out we need to compare different treatments. We put people into groups and give each group a different treatment. The results are compared to see if one is better. To try to make sure the groups are the same, each participant is put into a group by chance (random).

You will be participating in a double-blind study. This means that neither you nor your study doctor will know which treatment you are receiving. However, in certain circumstances your study doctor can find out which treatment you are receiving.

In the initial treatment period, one group will receive the investigational product, cyclosporin. The other group will receive a placebo. You will be randomly allocated to either group. A

placebo is a medication with no active ingredients. It looks like the real thing but is not. You have a 50% chance of receiving the investigational product, cyclosporin.

We aim to recruit a maximum of 38 participants in total into the study.

This research project has been designed to make sure the researchers interpret the results in a fair and appropriate way and avoids study doctors or participants jumping to conclusions.

There are no additional costs associated with participating in this research project, nor will you be paid. All medication, tests and medical care required as part of the research project will be provided to you free of charge.

The principal investigator will advise your local doctor of your decision to participate in this research project.

Duration of Study
The study will take place over a maximum of 21 weeks. There will be 6 visits to the clinic over the following periods:
1. Screening period – 1 visit prior to commencing the treatment period
2. Treatment period – 4 visits over 12 weeks
3. Follow-up period – 1 visit over 4 weeks

Study Procedures
All study procedures will take place in the clinic at Sinclair Dermatology.

You will first be screened for eligibility of this trial. This will include reviewing your medical history, completing a physical examination, having a blood test and assessing your hair. The blood test will include tests for Hepatitis B, Hepatitis C and Human Immunodeficiency Virus (HIV). You will be informed of the results and what the results mean for you. Any positive results will be subject to mandatory reporting to the Victorian Department of Health. If you have any questions regarding these tests, you may discuss this with your study doctor.

A consent form must be signed prior to any study assessments being performed.

You will be randomised and allocated to receive either cyclosporin (2mg/kg twice daily) or placebo.

PharmacySmart Compounding Pharmacy will dispense to you the medication in bottles. You will be taking 1 capsule twice daily.

Visits to the clinic every 4 weeks will be conducted to assess your response and check your progress with the treatment. Each visit will take approximately 1.5 hours. In the follow-up visit, we will again monitor your response and check for any side effects.

What will be done during the study?
A number of check-ups will be performed at each study visit, including physical examination, review of any concomitant medications, safety blood testing, pregnancy testing (if applicable), side effect checking, questionnaires to monitor progress and photography.

After you start the medication both you and your doctor will rate how effective you think the medication is. Your study doctor will assess you for any side effects at each visit.

The study visit schedule is attached below to outline what will be done and when.

Study Visit Schedule

Protocol Activity	**Screening**	**Treatment Period**				**Follow Up**
Week	**-5 to 0**	**0**	**4**	**8**	**12**	**16**
Day	**-35 to 0**	**1**	**28**	**56**	**84**	**112**
Visit	**1**	**2**	**3**	**4**	**5**	**6**
Visit window (days)	**N/A**	**±3 based on Day 1 visit**				
Average time in clinic (hrs)	**2.0**	**1.5**	**1.5**	**1.5**	**1.5**	**1.5**
Study Preparation						
Obtain written informed consent	x					
Review eligibility	x	x				
Demographics	x					
AA disease history and medical history	x					
Prior and current concomitant medications	x	x	x	x	x	x
Randomisation (cyclosporin/placebo)		x				
Dispense medication[1]		x	x	x		
Check medication			x	x	x	
Safety Assessments						
Vital signs and Physical examination[2]	x#	x#	x*	x*	x#	x#
ECG	x					x
Height	x					
Weight	x	x				x
Safety blood testing[3]	x*#	x	x*	x	x	x
Blood drug levels[4]		x	x	x	x	x
Pregnancy testing (if applicable)[5]	x*	x	x	x	x	x*
Adverse events and Investigator Safety Assessment	x	x	x	x	x	x
Contraception check	x	x	x	x	x	x
Efficacy Assessments						
Photography[6]	x*	x	x	x	x	x
SALT score	x	x	x	x	x	x
Eyelash and Eyebrow Assessment Scale	x	x	x	x	x	x
Patient Reported Outcomes						
Participant questionnaire (AQol-8D and AASIS)		x	x	x	x	x

[1]Dispense medication. Medication will be dispensed by PharmacySmart Compounding Pharmacy.
[2]Vital signs and physical examination will be performed at all visits marked 'x'. #Indicates a visit with a complete physical examination. *Indicates a visit with a targeted physical examination only.
[3]Safety blood testing. Includes full blood examination (FBE), electrolytes (UEC and CMP), and liver function tests (LFT) for all visits marked 'x'. #Indicates a visit with additional HIV, Hepatitis B Serology, Hepatitis C Serology testing. *Indicates a visit with additional Fasting Lipid testing for which patients are required to fast for 6-8 hours prior to the blood test.
[4]Blood drug levels. Patients will take study medication at the clinic after this test.
[5]Pregnancy testing will be performed with urine pregnancy test at each visit marked 'x'. *Indicates a visit with additional serum pregnancy testing. Pregnancy tests will be performed for all female participants except those at least 2 years post-menopausal or surgically sterile.
[6]Photography will be performed at all visits marked 'x'. This includes full scalp photography, close-up photography of the scalp and a normal front-on photo of your face. *Indicates a visit with full scalp photography only.

4 What do I have to do?

At your screening visit your doctor will ask you what medications you are taking, both topically and orally. If you are taking certain medications you will be excluded from the trial. These include: oral JAK inhibitors, products that promote scalp hair growth (e.g. finasteride or minoxidil), spironolactone, flutamide, cyproterone acetate, cimetidine.

A full list of medications that should be avoided is discussed in Page 8 ('12 Can I have other treatments during this research project?') of this information sheet. Please discuss your medications with your study doctor and they will advise as to how best to manage these medications.

Participants should not drink grapefruit juice during the course of the study as this affects how the study drug is absorbed.

If you need to commence any new medications while the trial is being conducted please let the study doctors know.

This study will not be recruiting any women that are pregnant or breastfeeding. In the event that a female subject becomes pregnant after Visit 1 through to the final visit the participant will be discontinued from the study, and the outcome of the pregnancy will be collected.

Females of childbearing potential must use highly effective contraception during the study.

Examples of acceptable forms of highly effective contraception include:

1. Placement of an intrauterine device (IUD) or intrauterine system (IUS).
2. Sterilised male partner (with the appropriate post-vasectomy documentation of the absence of sperm in the ejaculate).
3. True abstinence: When this is in line with your preferred and usual lifestyle

Examples of non-acceptable methods of contraception include:

1. Double barrier
2. Periodic abstinence (e.g. calendar, ovulation, sympthothermal, post ovulation)
3. Withdrawal
4. Spermicide (as it is not approved as a method of contraception in Australia)

Sexually active male subjects must also prevent potential transfer to and exposure of partner(s) to drug through ejaculate by using a condom consistently and correctly, beginning with the first dose of study medication and continuing for 30 days after the last dose of study medication.

If you are uncertain of what form of contraception is acceptable for use during the study, then please ask your study doctor.

If you are participating in the trial you will not be able to donate blood during the period of the study.

There will be some visits that require you to fast for a blood test prior to attending the clinic. These visits are noted in the Study Visit Schedule. Fasting is when you consume no food or drinks, with the exception of water, for 6 - 8 hours prior to your test. Fasting is necessary for certain tests because the nutrients in recently consumed foods and beverages can impact your test results.

Blood drug levels, also known as pharmacokinetic testing, will be done at each clinic visit. A small amount of blood sample will be taken, together with the safety blood testing. Blood drug levels testing shows us how the body absorbs, breaks down and removes the study drug from your body. On these clinic visits, you are required to take the study medication after this test at the clinic. You will be informed by the site staff when you can take it.

5 Do I have to take part in this research project?

Participation in any research project is voluntary. If you do not wish to take part, you do not have to. If you decide to take part and later change your mind, you are free to withdraw from the project at any stage.

If you do decide to take part, you will be given this Participant Information and Consent Form to sign and you will be given a copy to keep.

Your decision whether to take part or not to take part, or to take part and then withdraw, will not affect your routine treatment, your relationship with those treating you or your relationship with Sinclair Dermatology.

6 What are the alternatives to participation?

You do not have to take part in this research project to receive treatment at this clinic. Other options are available; these may include no treatment, oral steroids, topical steroids or injecting steroids into your scalp. Your study doctor will discuss these options with you before you decide whether or not to take part in this research project. You can also discuss the options with your local doctor.

7 What are the possible benefits of taking part?

We cannot guarantee or promise that you will receive any benefits from this research; however, possible benefits may include hair regrowth.

8 What are the possible risks and disadvantages of taking part?

Medical treatments often cause side effects. You may have none, some or all of the effects listed below, and they may be mild, moderate or severe. If you have any of these side effects, or are worried about them, talk with your study doctor. Your study doctor will also be looking out for side effects.

There may be side effects that the researchers do not expect or do not know about and that may be serious. Tell your study doctor immediately about any new or unusual symptoms that you get.

Many side effects go away shortly after treatment ends. However, sometimes side effects can be serious, long lasting or permanent. If a severe side effect or reaction occurs, your study doctor may need to stop your treatment. Your study doctor will discuss the best way of managing any side effects with you.

Potential side effects of cyclosporin include:

- Tiredness, lack of energy
- Burning feeling in hands and feet, usually during the first week of treatment
- Excessive growth of body and facial hair
- Overgrown, thickened, swollen or bleeding gums
- Stomach upset, including nausea (feeling sick), vomiting, loss of appetite, diarrhoea, stomach cramps, stomach ulcer
- Tremor (shaking)
- Headache, including migraine
- Sensitivity to light
- Weight loss or gain

- Feeling depressed (sad)
- Flushing of face, acne, darkening of skin
- Painful menstrual periods or lack of periods
- Increase in size of breasts in males and females
- Muscle cramps, tenderness or weakness
- Blocked or stuffy nose
- Pain of lower extremities

The above side effects are moderately common (1% to 10%).

Tell your doctor immediately if you notice any of the following:

- Fever (temperature above 37°C)
- Constant 'flu-like' symptoms such as chills, sore throat, aching joints, swollen glands, or any other signs of infection
- Unusual bleeding or bruising
- Signs of allergy such as rash, itching or hives on the skin; swelling of the face, lips, tongue or other part of the body; shortness of breath, wheezing or troubled breathing
- New lumps or moles, or changes to existing moles, anywhere on the body
- Swelling of the eyelids, hands, or feet due to excess fluid
- A change in the amount of urine passed or in the number of times you urinate, pain on urinating, bloody or smelly urine
- Yellowing of the skin and/or eyes (jaundice) often accompanied by generally feeling unwell (for example, tiredness, lack of energy, loss of appetite, nausea and vomiting, pain in the abdomen)
- Severe pain or tenderness in the stomach or abdomen
- Vomiting blood or material that looks like coffee grounds; black sticky bowel motions or bloody diarrhoea
- Unusual tiredness or weakness, which may be accompanied by dizziness, spots before the eyes, shortness of breath and pale skin
- Numbness or "pins and needles" in the hands and feet
- A disturbance in brain function which may cause a variety of symptoms, including personality changes, confusion, disorientation, agitation, inability to sleep, decreased responsiveness, weakness and loss of coordination in arms and legs with or without abnormal speech or eye movements, seizures (fits), clumsiness, memory loss, difficulty speaking or understanding what others say, visual hallucinations (seeing things that are not there) or other problems with vision, coma, paralysis of part or all of the body, stiff neck
- Buzzing or ringing in the ears, difficulty hearing

The above are serious side effects that need medical attention.

Some side effects may not give you any symptoms and can only be found when tests are done. Safety blood tests will be performed to monitor for these side effects throughout the study as per the study schedule. Some of these side effects include:

- Changes in kidney or liver function, or liver injury (with or without yellow eyes or skin)
- Raised blood pressure
- Increase in the amount of potassium or cholesterol in the blood
- Decrease in the amount of magnesium in the blood
- Increase in the amount of uric acid in the blood, which can lead to gout
- Low white blood cell count
- Low levels of red blood cells
- Low levels of platelets in the blood

The effects of cyclosporin on the unborn child and on the newborn baby are not known. Because of this, it is important that research project participants are not pregnant or breast-

feeding and do not become pregnant during the course of the research project. You must not participate in the research if you are pregnant or trying to become pregnant, or breast-feeding. If you are female and child-bearing is a possibility, you will be required to undergo pregnancy tests during the study. If you are male, you should not father a child or donate sperm for at least 30 days after the last dose of study medication.

Both male and female participants must agree to use highly effective contraception during the course of the research and for 30 days after completion of the research project. You should discuss methods of highly effective contraception with your study doctor.

For female participants, if you do become pregnant whilst participating in the research project, you should advise your study doctor immediately. Your study doctor will withdraw you from the research project and advise on further medical attention should this be necessary. You must not continue in the research if you become pregnant.

For male participants, you should advise your study doctor if you father a child while participating in the research project. Your study doctor will advise on medical attention for your partner should this be necessary.

11 What if new information arises during this research project?

Sometimes during the course of a research project, new information becomes available about the treatment that is being studied. If this happens, your study doctor will tell you about it and discuss with you whether you want to continue in the research project. If you decide to withdraw, your study doctor will make arrangements for your regular health care to continue. If you decide to continue in the research project you will be asked to sign an updated consent form.

Also, on receiving new information, your study doctor might consider it to be in your best interests to withdraw you from the research project. If this happens, he/ she will explain the reasons and arrange for your regular health care to continue.

12 Can I have other treatments during this research project?

Whilst you are participating in this research project, you may not be able to take some or all of the medications or treatments you have been taking for your condition or for other reasons. It is important to tell your study doctor and the study staff about any treatments or medications you may be taking, including over-the-counter medications, vitamins or herbal remedies, acupuncture or other alternative treatments. You should also tell your study doctor about any changes to these during your participation in the research project. Your study doctor should also explain to you which treatments or medications need to be stopped for the time you are involved in the research project.

Many other medicines may be affected by cyclosporin or they may affect how well cyclosporin works. This includes:

- St John's wort
- Grapefruit juice
- Methotrexate
- Certain antibiotics including gentamicin, tobramycin, ciprofloxacin, macrolides, and trimethoprim
- Certain antifungal agents containing amphotericin B and the azole type (e.g. fluconazole)
- Cytostatics containing melphalan
- Some pain medications including non-steroid anti-inflammatory drugs e.g. diclofenac
- Acid secretion inhibitors of the H2-receptor antagonist type e.g. cimetidine, ranitidine

- Other drugs which may affect the kidneys
- Oral contraceptives (Levonorgestrel and Norethisterone)
- Protease inhibitors, used to treat or prevent infections caused by viruses
- Certain cancer treatments including imatinib, doxorubicin, etoposide,
- Certain blood pressure reducing agents of the calcium antagonist type (e.g. nifidipine), the endothelin receptor antagonist type (e.g. bosentan) or aliskiren
- Certain anticonvulsives, used to prevent fits or seizures (e.g. carbamazepine, phenytoin)
- Digoxin
- Colchicine
- Certain medications used to treat high cholesterol, including statins of fibric acid derivatives
- Prednisolone
- Repaglinide
- Potassium sparing drugs or potassium containing drugs
- Triclopidine
- Octreotide
- Orlistat
- Danazol
- Allopurinol
- Metoclopramide
- Cholic acid and derivatives
- Tacrolimus, everolimus or sirolimus
- Dabigatran

You may need to take different amounts of your medicines or you may need to take different medicines. Your study doctor will advise on this depending on your medication and the reason for taking it.

It may also be appropriate or necessary for you to take medication during or after the research project to address side effects or symptoms that you may have. You may need to pay for these medications and so it is important that you ask your doctor about this possibility.

13 What if I withdraw from this research project?

If you decide to withdraw from the project, please notify a member of the research team before you withdraw. This notice will allow that person or the research supervisor to discuss any health risks or special requirements linked to withdrawing.

If you do withdraw your consent during the research project, the study doctor and relevant study staff will not collect additional personal information from you, although personal information already collected will be retained to ensure that the results of the research project can be measured properly and to comply with law. You should be aware that data collected by the study team up to the time you withdraw will form part of the research project results. If you do not want them to do this, you must tell them before you join the research project.

14 Could this research project be stopped unexpectedly?

This research project may be stopped unexpectedly for a variety of reasons. These may include reasons such as:
• Unacceptable side effects
• The drug/treatment/device being shown not to be effective
• The drug/treatment/device being shown to work and not need further testing

• Decisions made in the commercial interests of the sponsor or by local regulatory/health authorities.

15 What happens when the research project ends?

When the research project ends you can still continue to have your regular medical follow up visits with your usual dermatologist. There are other treatment options available to treat alopecia areata and these treatments will be made available to you. Note that once the study ends, the medications received in the study and any other treatment options will not be subsidised and you will need to pay for them yourself.

Part 2 How is the research project being conducted?

16 What will happen to information about me?

By signing the consent form you consent to the study doctor and relevant research staff collecting and using personal information about you for the research project. Any information obtained in connection with this research project that can identify you will remain confidential. The information will be kept on a password-secured computer and your name will be coded. Any data stored on hard copies will be kept under lock and key. Your information will only be used for the purpose of this research project and it will only be disclosed with your permission, except as required by law.

Study records must be kept for a minimum of 10 years after completion or discontinuation of the study or for longer if required by law.

Information about you may be obtained from your health records held at this and other health services for the purpose of this research. By signing the consent form you agree to the study team accessing health records if they are relevant to your participation in this research project.

Your health records and any information obtained during the research project are subject to inspection (for the purpose of verifying the procedures and the data) by the relevant authorities, the institution relevant to this Participant Information Sheet, or as required by law. By signing the Consent Form, you authorise release of, or access to, this confidential information to the relevant study personnel and regulatory authorities as noted above.

It is anticipated that the results of this research project will be published and/or presented in a variety of forums. In any publication and/or presentation, information will be provided in such a way that you cannot be identified, except with your permission.

Information about your participation in this research project may be recorded in your health records.

In accordance with relevant Australian and Victorian privacy and other relevant laws, you have the right to request access to your information collected and stored by the research team. You also have the right to request that any information with which you disagree be corrected. Please contact the study team member named at the end of this document if you would like to access your information.

Any information obtained for the purpose of this research project that can identify you will be treated as confidential and securely stored. It will be disclosed only with your permission, or as required by law.

17 Complaints and compensation

If you are injured as a result of your participation in this trial you may be entitled to compensation.

Sponsors of clinical trials in Australia have agreed that the guidelines developed by their industry body, Medicines Australia, will govern the way in which compensation claims from injured participants are managed by sponsors.

However, as guidelines, they do NOT in any way dictate the pathway you should follow to seek compensation. The sponsor is obliged to follow these guidelines.

These guidelines are available for your inspection on the Medicines Australia Website (www.medicinesaustralia.com.au) under Policy – Clinical Trials – Indemnity & Compensation Guidelines. Alternatively, your study doctor can provide you with a hard-copy of the guidelines.

It is the recommendation of the independent ethics committee responsible for the review of this trial that you seek independent legal advice before taking any steps towards compensation for injury.

18 Who is organising and funding the research?

This research project is being conducted and funded by Samson Clinical. Professor Sinclair is the sole Director and Shareholder of Samson Clinical and owner and director of Dr. Rodney Sinclair Pty Ltd, trading as Sinclair Dermatology.

You will not benefit financially from your involvement in this research project. No member of the research team will receive a personal financial benefit from your involvement in this research project (other than their ordinary wages).

19 Who has reviewed the research project?

All research in Australia involving humans is reviewed by an independent group of people called a Human Research Ethics Committee (HREC). The ethical aspects of this research project have been approved by the HREC of Bellberry.

This project will be carried out according to the *National Statement on Ethical Conduct in Human Research (2007)*. This statement has been developed to protect the interests of people who agree to participate in human research studies.

20 Further information and who to contact

The person you may need to contact will depend on the nature of your query.

If you want any further information concerning this project or if you have any medical problems which may be related to your involvement in the project (for example, any side effects), you can contact the principal study doctor on (03) 9654 2426 or any of the following people:

Clinical contact person
Name: Dr. Vivien Lai
Position: Associate Investigator
Telephone: (03) 9654 2426
E-mail: Vivien@sinclairdermatology.com.au

Name: Laita Bokhari
Position: Associate Investigator
Telephone: (03) 9654 2426
E-mail: laita.bokhari@sinclairdermatology.com.au

If you have any complaints about any aspect of the project, the way it is being conducted or any questions about being a research participant in general, then you may contact:

Bellberry HREC
Position: Operations Manager
Telephone: 08 8361 3222
Fax: 08 8361 3322
Email: bellberry@bellberry.com.au
Address: 129 Glen Osmond Road Eastwood South Australia 5063

Consent Form - *Adult providing own consent*

Title	A randomised, double-blind, placebo-controlled study to evaluate the efficacy of cyclosporin in subjects with moderate to severe alopecia areata
Protocol Number	*CYC001*
Project Sponsor	Samson Clinical
Coordinating Principal Investigator/ Principal Investigator	Prof. Rodney Sinclair
Associate Investigator(s) *(if required by institution)*	*Dr. Vivien Lai* *Laita Bokhari* *Dr. Gang Chen*
Location *(where CPI/PI will recruit)*	Sinclair Dermatology

Declaration by Participant

I am aged 18 years or older.

I have read the Participant Information Sheet or someone has read it to me in a language that I understand.

I understand the purposes, procedures and risks of the research described in the project.

I give permission for my doctors, other health professionals, hospitals or laboratories outside this hospital to release information to Sinclair Dermatology concerning my disease and treatment for the purposes of this project. I understand that such information will remain confidential.

I give permission for the principal investigator to communicate my decision to participate in this research project to my local doctor.

I understand that, if I decide to discontinue the study treatment, I may be asked to attend follow-up visits to allow collection of information regarding my health status. Alternatively, a member of the research team may request my permission to obtain access to my medical records for collection of follow-up information for the purposes of research and analysis.

I have had an opportunity to ask questions and I am satisfied with the answers I have received.

I freely agree to participate in this research project as described and understand that I am free to withdraw at any time during the study without affecting my future health care.

I understand that I will be given a signed copy of this document to keep.

Name of Participant (please print) _____

Signature _____ Date _____

Under certain circumstances (see Note for Guidance on Good Clinical Practice CPMP/ICH/135/95 at 4.8.9*) a witness* to informed consent is required.*

Name of Witness* to Participant's Signature (please print) _____

Signature _____ Date _____

* Witness is not to be the investigator, a member of the study team or their delegate. In the event that an interpreter is used, the interpreter may not act as a witness to the consent process. Witness must be 18 years or older.

Declaration by Study Doctor/Senior Researcher†

I have given a verbal explanation of the research project, its procedures and risks and I believe that the participant has understood that explanation.

Name of Study Doctor/
Senior Researcher† (please print) _____

Signature _____ Date _____

† A senior member of the research team must provide the explanation of, and information concerning, the research project.

Note: All parties signing the consent section must date their own signature.

Form for Withdrawal of Participation - *Adult providing own consent*

Title	A randomised, double-blind, placebo-controlled study to evaluate the efficacy of cyclosporin in subjects with moderate to severe alopecia areata
Protocol Number	*CYC001*
Project Sponsor	Samson Clinical
Coordinating Principal Investigator/ Principal Investigator	Prof. Rodney Sinclair
Associate Investigator(s) *(if required by institution)*	*Dr. Vivien Lai* *Laita Bokhari* *Dr. Gang Chen*
Location *(where CPI/PI will recruit)*	Sinclair Dermatology

Declaration by Participant

I wish to withdraw from participation in the above research project and understand that such withdrawal will not affect my routine treatment, my relationship with those treating me or my relationship with Sinclair Dermatology.

Name of Participant (please print) _____

Signature _____ Date _____

Declaration by Study Doctor/Senior Researcher†

I have given a verbal explanation of the implications of withdrawal from the research project and I believe that the participant has understood that explanation.

Name of Study Doctor/
Senior Researcher† (please print) _____

Signature _____ Date _____

† A senior member of the research team must provide the explanation of and information concerning withdrawal from the research project.

Note: All parties signing the consent section must date their own signature.

Appendix 4: Study procedures

1.1 Screening

Subjects will have up to 35 days of a screening period to confirm that they meet the subject selection criteria for the study.

Screening laboratory tests with abnormal results may be repeated **once** to confirm abnormal results (with the same screening number); the last value will be used to determine eligibility. If results return to normal within the screening period, the subject may enter the study.

The site will be permitted to re-screen subjects (with a new screening number) who initially do not meet eligibility criteria **once**.

The following procedures will be completed:

- Obtain written informed consent.
- Review Inclusion and Exclusion criteria.
- Participant demographics, includes date of birth, sex, ethnicity and occupation
- AA disease history, includes collection of details of AA at Screening: AA background, AA history, AA diagnosis, pattern of scalp hair loss, body hair loss, nail involvement, the use of topical treatments, systemic treatments and other treatments for AA.
- Relevant medical history, in addition to AA history.
- Prior medication history, including all prescription or nonprescription drugs, and dietary and herbal supplements taken within 35 days prior to the Screening Visit and all previous drug treatments for AA (including use of topical, intralesional, systemic and other treatments) in the past 2 years.
- Conduct complete physical examination including dermatological full body examination
- Obtain vital signs including pulse rate, blood pressure, respiratory rate and oral or tympanic temperature.
- Perform single 12-lead electrocardiogram (ECG).
- Obtain weight and height.
- Obtain **fasting** samples for fasting lipid panel: For cyclosporin it is advisable to perform lipid determinations before treatment and after the first month of therapy.
- Obtain safety blood testing: FBE, UEC, CMP, LFT, HIV, Hepatitis B Serology, Hepatitis C Serology
- Obtain pregnancy testing (female subjects of childbearing potential): serum pregnancy test, urine pregnancy test.
- Photographs to verify eligibility (Global photography).
- Conduct clinical evaluations including SALT, Eyelash Assessment Scale, Eyebrow Assessment Scale.
- Agree to use proper contraceptive methods.
- Conduct Investigator Safety Assessment and Assess for occurrence of Adverse Events: The reporting period starts with the signing of the informed consent.

1.2 Study Period

1.2.1 Visit 2, Day 1, Week 0

- Review of Inclusion/Exclusion Criteria.
- If subject meets all Inclusion/Exclusion criteria, officially randomise subject into the study.
- Review any changes in the subject's prior and concomitant treatment information.
- Obtain vital signs including pulse rate, blood pressure, respiratory rate and oral or tympanic temperature.
- Obtain weight.

- Conduct complete physical examination.
- Obtain safety blood testing: FBE, UEC, CMP, LFT.
- Obtain PK sampling.
- Obtain pregnancy testing (female subjects of childbearing potential): urine pregnancy test.
- Photography – Global, Macro- and Frontal
- Conduct clinical evaluations using SALT, Eyelash Assessment Scale, Eyebrow Assessment Scale
- Administer Patient Reported Outcomes: AQol-8D, AASIS
- Confirm proper contraception is being used.
- Administer first dose of study drug to subject.
- Dispense study drug supply to the subject.
- Conduct Investigator Safety Assessment and Assess for occurrence of Adverse Events by spontaneous reporting of adverse events and by asking the subjects to respond to a non-leading question such as “How do you feel?”.

1.2.2 Visit 3, Day 28, Week 4 (±3 day)

- Conduct targeted physical examination.
- Obtain vital signs including pulse rate, blood pressure, respiratory rate and oral or tympanic temperature.
- Obtain **fasting** samples for fasting lipid panel: For cyclosporin it is advisable to perform lipid determinations before treatment and after the first month of therapy.
- Obtain safety blood testing: FBE, UEC, CMP, LFT.
- Obtain PK sampling.
- Obtain pregnancy testing (female subjects of childbearing potential): urine pregnancy test.
- Photography – Global, Macro- and Frontal
- Conduct clinical evaluations using SALT, Eyelash Assessment Scale, Eyebrow Assessment Scale
- Administer Patient Reported Outcomes: AQol-8D, AASIS
- Confirm proper contraception is being used.
- Review any changes in the subject’s concomitant treatments information.
- Perform drug accountability procedures.
- Administer study drug to the subject.
- Dispense study drug to the subject.
- Conduct Investigator Safety Assessment and Assess for occurrence of Adverse Events by spontaneous reporting of adverse events and by asking the subjects to respond to a non-leading question such as “How do you feel?”.

1.2.3 Visit 4, Day 56, Week 8 (±3 day)

- Conduct targeted physical examination.
- Obtain vital signs including pulse rate, blood pressure, respiratory rate and oral or tympanic temperature.
- Obtain safety blood testing: FBE, UEC, CMP, LFT.
- Obtain PK sampling.
- Obtain pregnancy testing (female subjects of childbearing potential): urine pregnancy test.
- Photography – Global, Macro- and Frontal
- Conduct clinical evaluations using SALT, Eyelash Assessment Scale, Eyebrow Assessment Scale
- Administer Patient Reported Outcomes: AQol-8D, AASIS
- Confirm proper contraception is being used.

- Review any changes in the subject's concomitant treatments information.
- Perform drug accountability procedures.
- Administer study drug to the subject.
- Dispense study drug to the subject.
- Conduct Investigator Safety Assessment and Assess for occurrence of Adverse Events by spontaneous reporting of adverse events and by asking the subjects to respond to a non-leading question such as "How do you feel?".

1.2.4 Visit 5, Day 84, Week 12 (±3 day)/End of Treatment (EOT)

- Conduct complete physical examination.
- Obtain vital signs including pulse rate, blood pressure, respiratory rate and oral or tympanic temperature.
- Obtain safety blood testing: FBE, UEC, CMP, LFT.
- Obtain PK sampling.
- Obtain pregnancy testing (female subjects of childbearing potential): urine pregnancy test.
- Photography – Global, Macro- and Frontal
- Conduct clinical evaluations using SALT, Eyelash Assessment Scale, Eyebrow Assessment Scale
- Administer Patient Reported Outcomes: AQol-8D, AASIS
- Confirm proper contraception is being used.
- Review any changes in the subject's concomitant treatments information.
- Perform drug accountability procedures.
- Conduct Investigator Safety Assessment and Assess for occurrence of Adverse Events by spontaneous reporting of adverse events and by asking the subjects to respond to a non-leading question such as "How do you feel?".

1.3 Follow-up Visits

1.3.1 Visit 6, Day 112, Week 16 (±3 day)/End of Study (EOS)

- Conduct complete physical examination.
- Obtain vital signs including pulse rate, blood pressure, respiratory rate and oral or tympanic temperature.
- Perform single 12-lead electrocardiogram (ECG).
- Obtain safety blood testing: FBE, UEC, CMP, LFT.
- Obtain PK sampling.
- Obtain pregnancy testing (female subjects of childbearing potential): serum pregnancy test, urine pregnancy test.
- Obtain weight.
- Photography – Global, Macro- and Frontal
- Conduct clinical evaluations using SALT, Eyelash Assessment Scale, Eyebrow Assessment Scale
- Administer Patient Reported Outcomes: AQol-8D, AASIS
- Confirm proper contraception is being used.
- Review any changes in the subject's concomitant treatments information.
- Conduct Investigator Safety Assessment and Assess for occurrence of Adverse Events by spontaneous reporting of adverse events and by asking the subjects to respond to a non-leading question such as "How do you feel?".