



PhD Thesis

Hair Regeneration

A Multi-Targeting Approach Using a Botanical-Vitamin-Mineral Compound in the Treatment of Hair Loss



**NORWICH
MEDICAL
SCHOOL**

Author:	Peter Rodney Feldman
Student #:	100422971/1
Email:	P.Feldman@uea.ac.uk
Course:	R2B992101 - PhD by Publication (Med)
Degree:	Doctor of Philosophy by Publication
Institution:	University of East Anglia
School:	Norwich Medical School
Address:	Norwich Research Park Norwich, NR4 7TJ United Kingdom
Word Count:	12,125 (Critical Analysis)
Date:	November 2023
Supervisor (Internal):	Prof. Nicole Horwood PhD
Supervisor (External):	Prof. Andrew Messenger MD (University of Sheffield, United Kingdom)
Examiner (Internal):	Dr. Rosemary Davidson PhD
Examiner (External):	Prof. Jeff Donovan MD, PhD (University of British Columbia, Canada)

This copy of the thesis has been supplied on condition that anyone who consults it is understood to recognise that its copyright rests with the author and that use of any information derived therefrom must be in accordance with current UK Copyright Law. In addition, any quotation or extract must include full attribution.

Abstract

Background: Interventions intending to prevent hair loss and restore the scalp hair phenotype have had limited effect, thus an unmet medical need remains. Hair follicle (HF) homeostasis and hair fibre production result from a finely tuned choreography of more than 1,000 gene expressions and at least 20 principal signalling pathways known as stem cell activators, cytokines, hormones, prostaglandins, and growth factors.

Objective: To evaluate the efficacy of a multi-targeting treatment for terminal hair regeneration in humans by aiming to modulate the primary signalling functions, including Wnt/ β -catenin, DHT, 5 α -Reductase, Prostaglandins, TNF- α , and TGF- β .

Materials and Methods: A botanical, vitamin and mineral (BVM) oral and topical treatment was administered to subjects with androgenetic alopecia in two randomised placebo-controlled trials and then compared to other therapies in a systematic review and Bayesian network meta-analysis (MA). Primary outcomes were terminal hair (TH) density changes over 24 weeks.

Results: At 24 weeks, the MA regrowth efficacies compared to placebo in TH/cm² and significance (*) in women were; BVM: 30.09*, Laser: 16.62*, Minoxidil 2%: 12.13*, Minoxidil 5%: 10.82*, and Nutrafol: 7.32*, and in men; BVM: 21.03*, Laser: 18.75*, Dutasteride: 18.37*, Viviscal: 13.23, Minoxidil 5%: 13.13*, Finasteride: 12.38, and Minoxidil 2%: 10.54. BVM regrowth rates were linear in men, accelerating in women. 99% of the BVM TH regrowth in women was derived from dormant HFs. In men, TH regrowth was 50/50 from inactive HFs / vellus-like hair. All other treatments found regrowth resistance after 12 weeks.

Findings: Consensus has formed that reactivating a dormant HF and maintaining hair growth requires upregulating the Wnt/ β -catenin pathway. Most expert opinions proposed multi-targeting or combination therapies to improve efficacy. The multi-targeting BVM treatment was found more effective than all other treatments. Additional studies are required to elucidate the mechanisms, optimise the BVM and confirm these findings.

Acknowledgements

First, I would like to acknowledge my wife, Deborah Cahan, who developed various persistent alopecic disorders. The poor efficacy of available treatments gave her the impetus to find a solution that needed to become available before the hope of restoration was lost. Deborah's belief and insistence that a solution could be found within naturally occurring substances led to this work. Some ten years after onset, her scalp hair was restored almost to that of her younger adult years. This discovery did not diminish my newfound obsession and fascination with stem cells, hair follicle biology, and hair formation. To my children, Amalya, Jacob, and Ori, who should all be A students in trichology, thank you for bearing with me on this journey. May your hair stay with you forever.

Thank you to all those in the sciences, medical sciences, and clinical fields of skin and hair who guided and supported me in this process over the past ten years - in particular: the late Dr Luciano Marra, who played a pivotal role in creating the multi-molecular targeting model before he suddenly passed away on July 4, 2018; David Salinger of the International Association of Trichologists, who provided an onramp into the world of trichology, supervised my clinical training and certification as a Clinical Trichologist in Sydney, Australia, in 2018; Dr Dennis Brown for his ongoing assistance and mind craft in patentable intellectual property development; Drs Charles Piwko, Klaus Fiebig, and Jaime Guevara, MD, whose experience in high-impact scientific publications helped craft the articles in Chapters 3 – 6; Dr Kurt Stenn, MD, who reviewed the multi-molecular targeting model and clinical trial results in detail and insisted they be published; Dr Claudia Schaefer, Editor-in-Chief of The Lancet's nascent eClinicalMedicine journal for publishing the articles in Chapters 3 and 4 and placing them in The Lancet's specialty collections on Regenerative Medicine and Dermatology; Drs Dominique Van Neste, MD, Hugh Rushton, Jeff Donovan, MD and Paradi Mirmirani, MD, who reached out to me after articles in Chapters 3 and 4 were published, and subsequently supporting my initiatives through the European and American Hair Research Societies.

I wish to acknowledge Prof John Bicheno, who instilled the art of experimental design to create real-world applications of technological innovations as part of my engineering degree in the 1980s. The nature of an engineer is tested when moving from a world of physics and generally definable matter, energy, and data models with controllable variables to those of biology and human behaviour, with unpredictable confounders and unknown influence on the investigated subject matter.

Finally, I must thank my PhD supervisors, Prof. Nicole Horwood, who enthusiastically accepted my studentship and provided support and guidance beyond all expectations, and Prof. Andrew Messenger, MD, whose lifetime dedication and expertise in dermatology and trichology ensured the prudence and relevance of this manuscript to the field.

Sincerely, Peter R Feldman

Access Condition and Agreement

Each deposit in UEA Digital Repository is protected by copyright and other intellectual property rights, and duplication or sale of all or part of any of the Data Collections is not permitted, except that material may be duplicated by you for your research use or for educational purposes in electronic or print form. You must obtain permission from the copyright holder, usually the author, for any other use. Exceptions only apply where a deposit may be explicitly provided under a stated licence, such as a Creative Commons licence or Open Government licence.

Electronic or print copies may not be offered, whether for sale or otherwise to anyone, unless explicitly stated under a Creative Commons or Open Government license. Unauthorised reproduction, editing or reformatting for resale purposes is explicitly prohibited (except where approved by the copyright holder themselves) and UEA reserves the right to take immediate 'take down' action on behalf of the copyright and/or rights holder if this Access condition of the UEA Digital Repository is breached. Any material in this database has been supplied on the understanding that it is copyright material and that no quotation from the material may be published without proper acknowledgement.

Conflicts of Interest

This body of work was initially developed to solve a complex case of alopecia in the PhD candidate's wife, which was successful. The candidate self-financed this thesis. The research published in Chapters 3 – 6 herein was funded by private sponsors and grants from the governments of Canada and Ontario. The author personally designed and conducted the research in these papers, except for the direct subject clinical investigations and resulting data generation of Chapters 3 – 5, that was conducted by independent dermatologist investigators and research organisations. The candidate of this thesis is a co-founder, director, employee and shareholder of Arbor Life Labs of Canada. A wholly owned subsidiary, Arbor Life Holdings, has filed patents on the multi-molecular targeting investigational interventions used in these studies, identified as ALRV5XR. The candidate and his wife wish to help as many people as possible who are afflicted by alopecic conditions and cannot find an effective solution.

Scientists discover the world that exists; engineers create the world that never was.

— Theodore von Kármán (1881-1963)

Table of Contents

<i>Abstract.....</i>	<i>2</i>
<i>Acknowledgements.....</i>	<i>3</i>
<i>Conflicts of Interest.....</i>	<i>4</i>
<i>Abbreviations.....</i>	<i>11</i>
<i>1 Introduction.....</i>	<i>12</i>
1.1 Human Scalp Hair.....	13
1.1.1 Hair types and structure	14
1.1.2 Hair diameter.....	16
1.2 Hair Follicle Anatomy.....	17
1.2.1 Normal hair growth cycle	18
1.2.2 Hair follicle anatomy	20
1.2.3 Follicular units	23
1.2.4 Terminal anagen hair	23
1.2.5 Terminal catagen hair.....	24
1.2.6 Terminal telogen hair	25
1.3 Hair Disorders, Frequency and Treatments.....	27
1.3.1 Introduction to hair loss and hair disorders.....	27
1.3.2 Non-scarring alopecia	28
1.3.3 Scarring or cicatricial alopecia.....	30
1.3.4 Other types of alopeciae.....	33
1.4 Biology of the Hair Follicle.....	34
1.4.1 The hair follicle and stem cells	34
1.4.2 Role of molecular pathways in hair growth and cycling disorders.....	37
1.5 Changes of Gene Expression in Hair Disorders	44

1.6	A Multi-Molecular Targeting Strategy for Hair Follicle Homeostasis	45
1.6.1	Hypothesis.....	46
1.6.2	Aims	46
2	<i>Materials and Methods</i>	47
2.1	Materials	47
2.1.1	Botanical formulation strategy	48
2.1.2	Vitamin, mineral and cofactor formulation strategy	49
2.1.3	Formulating ALRV5XR and methods of administration.....	49
2.2	Studies Design	50
2.2.1	Rationale for selecting subjects with androgenetic alopecia.....	50
2.2.2	Randomised placebo-controlled clinical trials	53
2.2.3	Sex differences study	54
2.2.4	Systematic review and Bayesian network meta-analysis.....	55
3	<i>Results – Paper 1: Safety and efficacy of ALRV5XR in women with androgenetic alopecia or telogen effluvium: A randomised, double-blinded, placebo-controlled clinical trial</i>	57
3.1	Introduction to Paper 1.....	57
3.2	Summary of Key Findings of Paper 1	74
4	<i>Results – Paper 2: Safety and efficacy of ALRV5XR in men with androgenetic alopecia: A randomised, double-blinded, placebo-controlled clinical trial</i>.....	75
4.1	Introduction to Paper 2.....	75
4.2	Summary of Key Findings of Paper 2	95
5	<i>Results – Paper 3: Sex differences in clinical trials of ALRV5XR treatment of androgenetic alopecia and telogen effluvium</i>.....	96
5.1	Introduction to Paper 3.....	96

5.2	Summary of Key Findings of Paper 3	110
6	<i>Results – Paper 4: Hair regrowth treatment efficacy and resistance in androgenetic alopecia: A systematic review and continuous Bayesian network meta-analysis.</i>	111
6.1	Introduction to Paper 4.....	111
6.2	Summary of Key Findings of Paper 4	180
7	<i>Critical Analysis.....</i>	182
7.1	Comparisons to Multi-Targeting Approaches to Hair Regeneration in the Literature..	184
7.1.1	Combination drug treatments.....	185
7.1.2	Mesotherapy	185
7.1.3	BVM formulations	186
7.1.4	Implications of comparisons of combination and multi-targeting hair loss treatments	188
7.2	Challenges in the Literature and the Interpretation of the Reported Clinical Outcomes Effects in Clinical Trials and SR/MAs	189
7.2.1	Comparisons to clinical trials and SR/MAs in the literature	190
7.2.2	Comparisons to long-term clinical trials in the literature.....	200
7.2.3	Implications for Outcomes Measures of Hair Loss Treatments.....	203
7.3	Expert Perspectives on Regenerating Hair.....	205
7.3.1	Implications of expert perspectives in regenerating hair	211
7.4	Novel Research that Might Lead to More Effective Therapeutics in Hair Regeneration	215
7.4.1	Wnt signalling is a major driver of hair follicle tissue homeostasis.	215
7.4.2	Novel opportunities and targets in hair follicle stem cells.....	216
7.5	Synthesis of Findings: A Multi-Targeting Approach to Hair Regeneration	218
7.5.1	Implications for the broader field of hair loss disorders	218
7.5.2	Implications for the wider field of skin disorders	219

7.5.3	Implications for other ageing and degenerative conditions	219
7.5.4	Limitations of the research.....	220
7.5.5	Proposed follow-up research.....	222
7.5.6	Considerations for multi-targeting formulation refinement.....	222
7.6	Conclusions.....	223
<i>References</i>		<i>224</i>
<i>Appendices.....</i>		<i>261</i>
Appendix 1: List of publications submitted for PhD by Publication		262
Appendix 2: Statement regarding published material submitted in this thesis and nature of contribution.....		263
Appendix 3: Copies of correspondence from co-authors confirming contribution.		267

Tables

Table 1.1: Glossary of hair follicle anatomy.....	22
Table 1.2: Non-scarring alopecia and characteristics	29
Table 1.3: Cicatricial alopecia characteristics.....	31
Table 1.4: Other types of alopecia and hair disorders, and their characteristics.....	33
Table 1.5: Molecular pathways in hair growth, cycling and disorders.	40
Table 2.1: Botanicals regulating molecular pathways in hair growth	48
Table 2.2: BVM investigational agent active ingredients.....	50
Table 7.1: Top-line efficacy comparisons to seminal SR/MAs	192
Table 7.2: Representative examples of non-comparable and confounding factors in SR/MAs	194
Table 7.3: Expert perspectives on regenerating hair.....	206

Figures

Figure 1.1 Schematic cross section of a hair fibre	14
Figure 1.2 Diameters of hair phenotypes	16
Figure 1.3 Cross section of layers of the skin	18
Figure 1.4 Schematic of hair follicle cycling	20
Figure 1.5 Histomorphology of the hair follicle	21
Figure 1.6 Follicular units	23
Figure 1.7 Anagen-catagen-telogen transition in the human hair follicle	25
Figure 1.8 Key stages of the hair cycle	26
Figure 7.1 Different mechanisms of action of botanicals and their active constituents for hair growth.	208
Figure 7.2 Photo-inducible labelling and tracing show upward lineage shifting of the matrix progenitors	216
Figure 7.3 Schematic model showing distinct types of stem cell divisions determine HF homeostasis and ageing	217

Abbreviations

Text Abbreviations	
AA	Alopecia Areata
AGA	Androgenetic Alopecia
BVM	Botanical, Vitamin and Mineral
FDA	Food and Drug Administration
FPHL	Female Pattern Hair Loss
HF	Hair Follicle
HFSC	Hair Follicle Stem Cell
HL	Hair Loss
MA	Meta-Analysis
MOA	Mechanism of Action
MPHL	Male Pattern Hair Loss
SA	Senescent Alopecia
SC	Stem Cell
SR	Systematic Review
TAHC	Target Area Hair Count
TE	Telogen Effluvium
TH	Terminal Hair
VH	Vellus Hair
Units of Measure	
CI/CrI	Confidence Interval/Credible Interval
cm	Centimetre
µm	Micrometre
mm	Millimetre
OR	Odds Ratio
%	Per cent
p	Statistical Probability – (Null Hypothesis)
Growth Factors	
EGF	Epidermal Growth Factor
FGF-7 and FGF-18	Fibroblast Growth Factor – 7, 18
HGF	Hepatocyte Growth Factor
IGF	Insulin Growth Factor
KGF	Keratinocyte Growth Factor
PDGF	platelet-derived growth factor
VEGF	Vascular Endothelial Growth Factor
Stem Cell stimulation	
BMP	Bone Morphogenetic Proteins
mTORC1	Mammalian Target of Rapamycin – 1
PKC	Protein Kinase C
PPARγ	Peroxisome Proliferator-Activated Receptor Gamma
Shh	Sonic Hedgehog
Wnt	Wingless-related integration site
β-catenin	Beta-catenin
Immunomodulation	
COX	Cyclooxygenase
IFN-α and IFN-γ	Interferons alpha and gamma
IL-1b, IL-6 and IL-12	Interleukins -1b, 6, 12
JAK	Janus Kinase
NF-κβ	Nuclear Factor Kappa Beta
PGE2 and PGD2	Prostaglandins E2 and D2
STAT	Signal Transducer and Activator of Transcription
TGF-β	Transforming Growth Factor beta
TNF-α	Tumour Necrosis Factor-alpha
Treg	Regulatory T cell
Hormones	
DHT	Dihydrotestosterone
Mediators and Transporters	
FPN	Ferroportin
5α-R	5 alpha Reductase
MDR	Multi-drug resistance
P-gp	P-glycoprotein
P450	Cytochrome P450

1 Introduction

Hair is thought to have evolved approximately 310 million years ago and emerged as the dominant skin appendage of mammals.^{1,2} Modern humans have several million hair follicles covering 95% of the body, and hair is most visibly pronounced on the scalp.²

In conjunction with body form, the appearance of scalp hair is a primary component of recognition and attraction. The quality of a person's hair may imply age, health, and fecundity. Hair in most cultures is at its zenith on the wedding day. All societies employ its styled form to express social status, cultural affiliation and confidence.²

Hair is a non-living appendage of the skin that grows inside a living and highly metabolically active mini-organ known as the hair follicle.³ Within the hair follicle, rapidly dividing cells transition into a structural material of keratin proteins that form the hair fibre and lose metabolic living function.⁴

Hair serves many functions, including protection, body temperature regulation, facilitation of perspiration, sensation, aesthetics, and psychosocial health.² Over time, the direct biological importance of hair for thermoregulation and protection has been largely lost in humans. Still, its effects on well-being remain and quality of life are significant, and hair loss disorders have been associated with ageing and various health and mortality risk factors.^{2,5-9}

A diverse range of hair loss treatments have been used throughout history as traditional medicines, with more recent characterisations of hair loss diseases being documented in *The Lancet* in 1843.^{10,11} Over the past 50 years, various hair loss treatments have been studied,

showing some efficacy, however complete or substantial endogenous scalp hair restoration remains an unmet need.¹²

This thesis describes human hair, scalp hair loss disorders and the biological mechanisms in the hair follicle required to maintain normal hair growth and induce the regrowth of dormant follicles. A model to support normal biological function and homeostasis in the hair follicle is proposed, hypothesising that a multi-targeting treatment made up of botanicals, vitamins and minerals (BVMs) can regenerate hair in subjects with androgenetic alopecia (AGA), the type of hair loss that affects approximately half of the adult human population. Results of randomised placebo-controlled clinical trials (RCTs) studying the multi-targeting treatment are reported, followed by a systematic review (SR) and network meta-analysis (MA) comparing the outcomes of the multi-targeting treatment to other treatments in the literature. These results are then critically analysed by comparing them to other multi-targeting treatments, seminal hair loss RCTs, SR/MAs and important expert opinions proposing novel ways of treating and reversing hair loss.

1.1 Human Scalp Hair

Once the body-wide intrauterine lanugo hairs are shed, visible follicular activity is limited to the scalp, with secondary sexual sites visible after puberty. Since the human head bears some 100,000–150,000 hair follicles, an adult with 30 months of continuous growth at 1 cm per month will carry some 30 kilometres or more hair. This hair mass is a significant investment in protein and energy consumption.^{2,4}

1.1.1 Hair types and structure

There are three essential types of hair in humans, which are dependent to some extent on the size of the follicle. **Lanugo** hair is fine and non-medullated hair that appears on the foetus and, with rare exceptions, is shed before or immediately after birth. **Vellus** hair is fine, short, and non- or lightly pigmented. It is the most numerous of human hairs. From the neonatal period onward, hair can be seen covering all surfaces other than the soles of the feet or palms of the hands. At puberty, some vellus hairs enlarge to become terminal hairs and develop sebaceous glands. Vellus hairs occur on the scalp but are far less numerous than terminal hairs. **Terminal** hair is thick, long, and pigmented.²

Terminal hairs are the dominant hairs on the scalp, eyebrows, lashes, axillae, and genital areas.

² In men, terminal hairs are variably found on the trunk and legs. There is a tremendous regional difference in terminal body hairs.¹³ A cross-section of the terminal hair shaft reveals three major components:

- Cuticle—the outer protective layer
- Cortex—the core of the hair
- Medulla—a central soft protein core that is more common in thicker hair, particularly in white hairs. (See Figure 1.1)

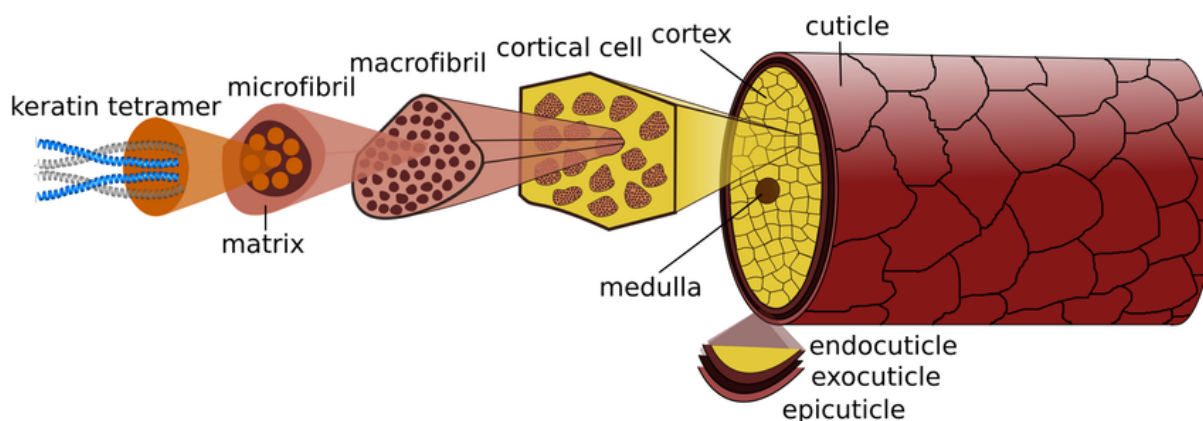


Figure 1.1 Schematic cross section of a hair fibre ¹⁴

The main constituents of these structures are sulphur-rich proteins, lipids, water, melanin, and trace elements. The **cuticle** comprises specialised keratins and six to eight layers of flattened overlapping cells with their free edges directed upward to the tip of the hair shaft. The cuticle may be damaged by any of four major “insults”— environmental, mechanical, chemical, and heat. The **cortex** consists of closely packed spindle-shaped cortical cells rich in keratin filaments oriented parallel to the longitudinal axis of the hair shaft and an amorphous matrix of high sulphur proteins. The disulfide bonds confer shape, stability, and resilience to the hair shaft, giving them high tensile strength, and can only be broken by external oxidative chemical agents such as occurs with perming or relaxing. Other weaker bonds link the keratin polypeptide chains, such as hydrogen bonds and salt links. These weaker bonds can be overcome with water, rendering curly hair temporarily straight. In addition, the cortex contains melanin granules that colour the fibre based on the number, distribution, and types of melanin granules. The **medulla** is a soft, proteinaceous core present in thicker and white hair. It has no known function in humans. The melanocytes determine hair colour in the follicle. Melanocytes transfer packages of melanin to the cortical cells during the active growth phase (anagen). Eumelanin is the dominant global pigment and confers black/brown hair. Pheomelanin, a mutation of eumelanin, is the predominant pigment in red hair. Blonde hair has a complex mixture of the two melanins but, in principle, is relatively devoid of pigment compared to black or brown hair. Greying of hair is a normal manifestation of ageing and illustrates a progressive reduction in melanocyte function.

1.1.2 Hair diameter

Human hair diameter ranges from 17 to 180 microns. Hair diameter $< 30 \mu\text{m}$ is regarded as vellus hair.^{2,15,16} Hair texture based on visible terminal hair (30 to 150 microns) is usually classified as follows:

- Fine less than 60 microns
- Medium 60 to 80 microns
- Thick 80 to 150 microns

Terminal hairs have traditionally been described as round, oval, or flat. People descending from subequatorial Africa have curved hair follicles. Although Sub-Saharan Africans are Earth's most genetically diverse group, their hair shafts are flattened and prone to tight curling. Asian or Eastern Asian hair is circular and of wide diameter, with a tendency to straightness and rigidity. This phenotype has a large diameter (up to 150 microns) and is primarily straight and black. Their hair diameters range from 80 to 150, with a South-to-North bias of increasing diameter. Indo-European hair is more oval and has variable characteristics — thin/straight to thicker or wavy/curly. The broad group of Indo-Europeans shows an East-to-West bias of darkly pigmented, straight hair to thinner, lighter hair. Those from the Indian subcontinent have classically straight hair. A range of straight, wavy, and curly hair occurs in Europe. The presence of pheomelanin pigment increases in the northwest of the Indo-European area. Only 1.8% of humans have naturally light/blond hair, with the highest prevalence in Estonia. (See Figure 1.2)

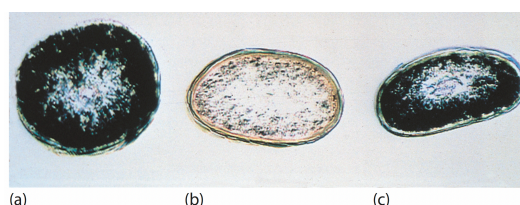


Figure 1.2 Diameters of hair phenotypes

(a) Asian - round, darkly pigmented. (b) Indo-European - oval hair of Indo-Europeans. (c) African - flattened hair of Sub-Saharan Africa.

Source: Gray J, *The World of Hair*, Macmillan, 1977

1.2 Hair Follicle Anatomy

There are approximately 5 million hair follicles on the body and around 100,000 to 150,000 scalp hairs present on a normal scalp.² The average scalp is 700 cm² and possesses a density of hair follicles ranging from 118 to 350 per square centimetre.^{16,17} Terminal hairs are rooted in subcutaneous tissue or the deep dermis, while vellus hairs are rooted in the upper dermis. A normal scalp averages seven terminal hairs per vellus hair. Vellus hairs are often less than 1 cm in length.¹⁸ True vellus hairs have thin, external root sheaths and short stelae in the upper dermis. Vellus-like miniaturised hairs have thicker external root sheaths and long stelae extending into the lower dermis or subcutaneous fat.¹⁸ Follicular stelae, in the upper dermis only, indicate vellus hairs. Follicular stelae in the lower dermis indicate terminal catagen or telogen hairs or miniaturised vellus-like hairs.

The skin appendages are epidermal and dermal-derived components. Each component has a unique structure, function, and histology. The skin appendages include eccrine and apocrine sweat glands, nails, and the pilosebaceous unit comprises the hair shaft, hair follicle, sebaceous gland, and arrector pili muscle — these appendages derive from a down growth of the epidermis.

Hair structure development starts after eight weeks of fetal life. The initial hair population is complete by 22 weeks. Fine lanugo hair develops in an advancing wave from the frontal to the occipital scalp, and it is shed by 36 weeks. A second coat of lanugo hair appears and is shed in a synchronised wave pattern at 3 to 4 months of life.² The bare occipital patch, often seen in infants, results from synchronised shedding of the final wave of lanugo telogen hairs before their replacement by normal scalp hairs.^{19,20} It is thought that the maximum number of scalp

hair follicles during the human life span is present at birth.² Thus hair follicle density is greatest in neonates and lessens progressively during childhood and adolescence due to the expansion of the cranium and scalp. (See Figure 1.3).

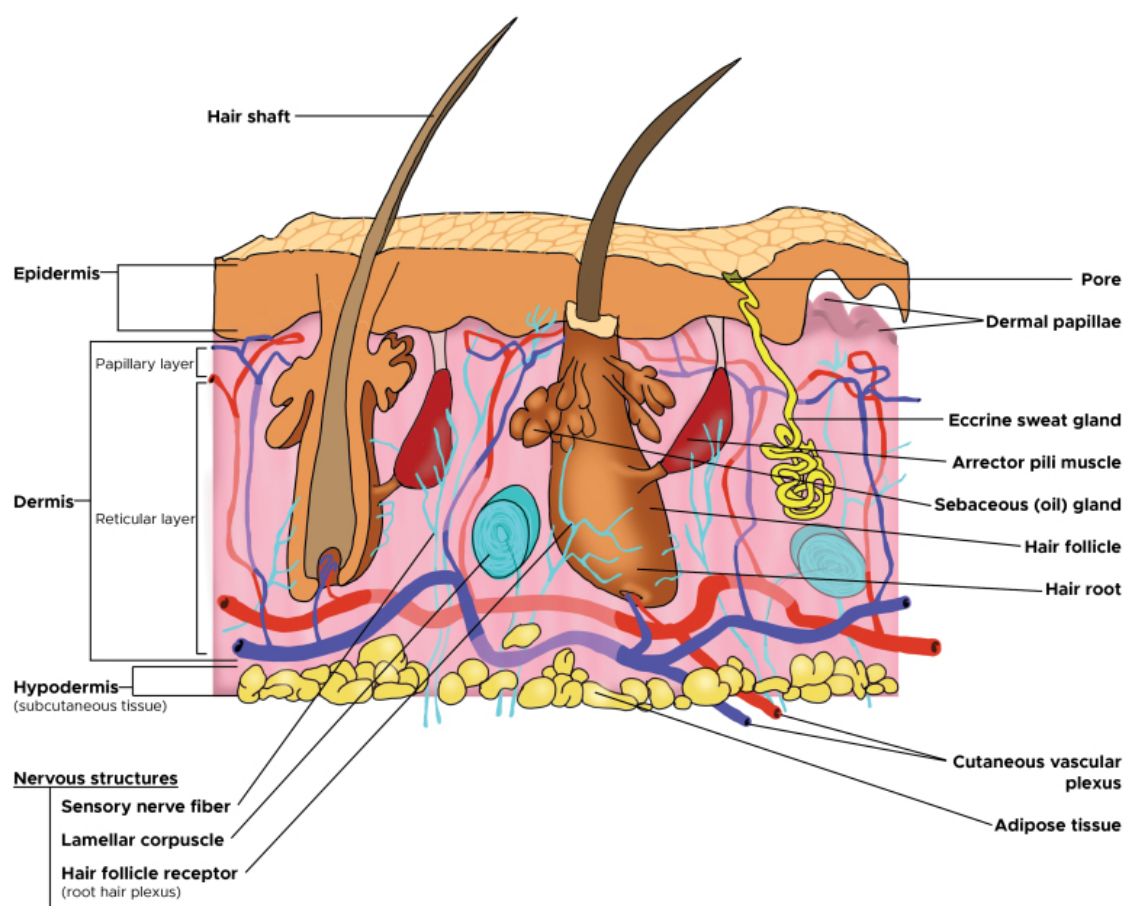


Figure 1.3 Cross section of layers of the skin

Hair follicles, hair roots and hair shafts, sweat glands, pores, epidermis, dermis, and hypodermis. Papillary and reticular layer. Eccrine sweat gland. Arrector pili muscles, sebaceous oil glands. Source: StatPearls Publishing; 2022 Jan.

1.2.1 Normal hair growth cycle

Each hair follicle performs a repetitive cycle of growth (anagen), regression (catagen), and rest (telogen).^{2,21} Each hair follicle grows and cycles independently of other follicles in an asynchronous manner. Postnatally until puberty, 90% or more of scalp hair is in anagen.²² In adults, anagen rates vary with observations of seasonal variations showing a peak anagen rate

slightly over 90% on the scalp during the late winter, then falling steadily to about 80% by the end of the summer, which corresponds with the least shedding over the winter and greatest shedding rate at the end of the summer.²³ These observations have been similarly reported in populations in northern latitudes of the United Kingdom, China and central Europe.²³⁻²⁵ This late summer shedding is consistent with the annual moult cycle found in mammals and is thought to be associated with changes in nocturnal melatonin secretion in the pineal gland and its pituitary cascade regulating prolactin secretions.²⁶

The variation in hair length is proportional to the duration of the anagen, depending on the body site. The anagen growth phase of scalp hair lasts 1 to 7 years. Scalp hair grows an average of 1 cm per month. Therefore, uncut scalp hairs can grow 12 to 84 cm in length.² In contrast, eyebrow hairs remain in anagen for 8 to 12 weeks, leg hairs for 19 to 26 weeks, arm hairs for 6 to 12 weeks, and upper lip hairs for 4 to 14 weeks, and produce short hairs. Studies on hair growth parameters indicated that rates of hair growth on the Asian scalp are highest, followed by European and then African hair.^{27,28} However, the rate of hair growth is variable by many factors such as ethnic group, sex, genetic influences, body site, climate, age, nutrition, hormonal, and other factors.^{2,13,28-31}

At the end of the anagen phase, the follicle enters the intermediate or catagen phase, marked by programmed cell death, or apoptosis, and lasts approximately two weeks. In catagen, the hair shaft and inner root sheath retreat upward while the outer root sheath undergoes cell death, and the hyaline membrane thickens and folds as it compacts upward. As a result, the lower follicle disappears, leaving an angiofibrotic strand or streamer (stela) indicating the former position of the anagen root.^{2,16,18}

The telogen phase lasts an average of 3 months before a new anagen hair develops. In telogen, the resting club root is situated at the “bulge” level, where the arrector pili muscle attaches to the hair follicle.³² The telogen bulb lacks pigment and inner root sheath.

Telogen hair is shed during washing and grooming, called the “exogen phase.” Exogen occurs in either late telogen or early anagen. It is unclear whether this event requires molecular signalling or mechanical stimulus to dislodge the telogen club hair.³² As many as 100 hairs per day may be lost by exogen. After exogen occurs in a telogen follicle, the period during which the empty follicle remains in is known as the “kenogen phase.”³³ A follicle can remain in kenogen for several weeks or months until the follicle experiences neogen, or the initiation of next anagen cycle, whereafter the cycle is repeated. (See Figure 1.4).^{2,21,34,35}

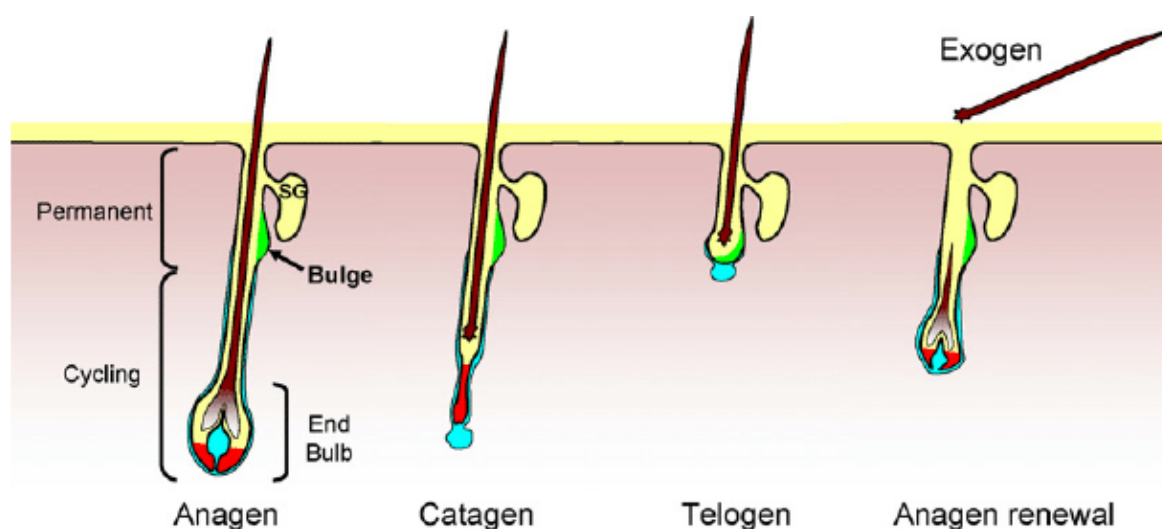


Figure 1.4 Schematic of hair follicle cycling

Source: Waters, J et al. Seminars in cell & developmental biology, 2007.

1.2.2 Hair follicle anatomy

Hair follicles can be viewed microscopically from vertical and horizontal planes of biopsied sections. Terminal hair follicles penetrate deep into the dermis, extending into the subcutaneous tissue. In the vertical plane of the section, the terminal hair follicle has a permanent upper

segment of follicular infundibulum and isthmus and an impermanent lower segment of the hair follicle consisting of the lower follicle and root (bulb). A glossary of the hair follicle anatomy can be found in Table 1.1, and a cross-sectional histomorphology view of the hair follicle is in Figure 1.5.

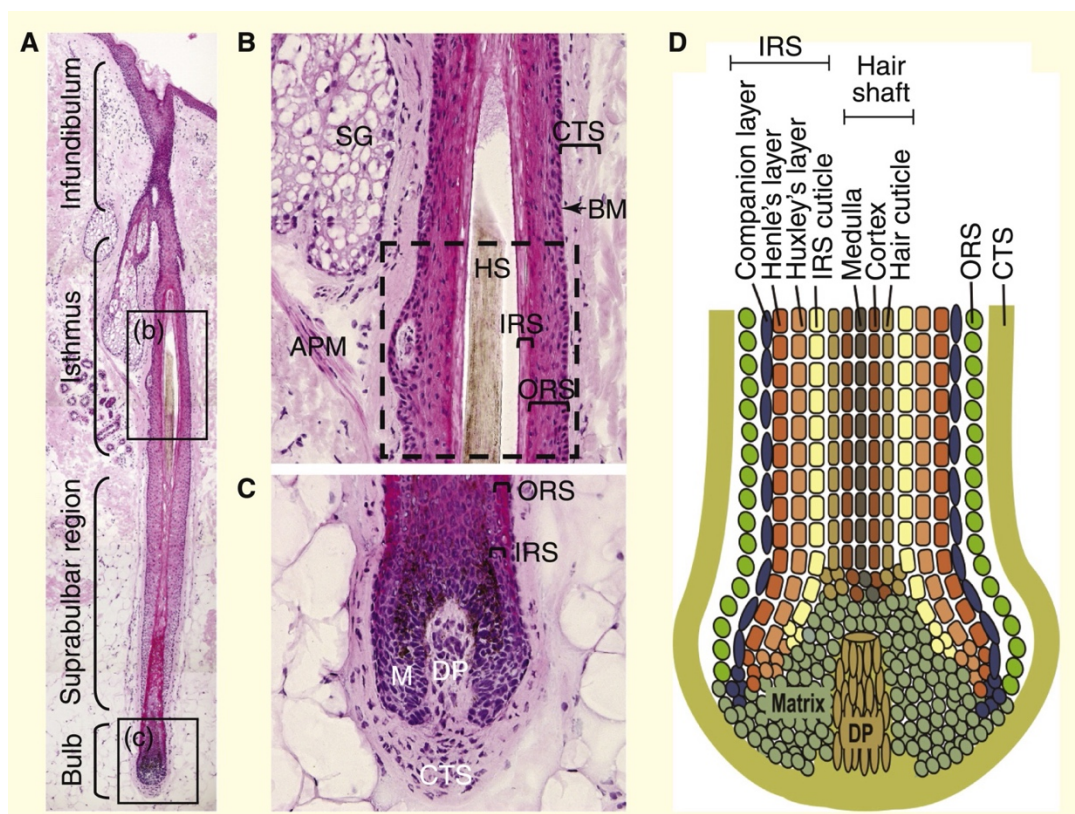


Figure 1.5 Histomorphology of the hair follicle³

(A) Sagittal section through a human scalp hair follicle (anagen VI) showing the permanent (infundibulum, isthmus) and anagen-associated (suprabulbar and bulbar area) components of the hair follicle. (B) High-magnification image of the isthmus. The dashed square indicates the approximate location of the bulge. (C) High-magnification image of the bulb. (D) Schematic drawing illustrating the concentric layers of the outer root sheath (ORS), inner root sheath (IRS) and shaft in the bulb. The inner root sheath is composed of four layers: Companion layer (CL), Henle's layer, Huxley's layer, and the inner root sheath cuticle. The companion layer cells are tightly bound to Henle's layer but not to the outer root sheath, thus allowing the companion layer to function as a slippage plane between the stationary outer root sheath and the upwards-moving inner root sheath. Further inwards, the inner root sheath cuticle is composed of scales that interlock with the scales of the hair shaft cuticle, anchoring the shaft in the follicle and enabling both layers to move jointly during hair follicle growth. The hair shaft is wrapped by a protective layer of overlapping scales and in mice, but not in humans, shows in its centre regular arrows of air spaces believed to play a role in thermal insulation (BM: basal membrane; APM: arrector pili muscle; CTS: connective tissue sheath; DP: dermal papilla; M: matrix; HS: hair shaft, IRS: inner root sheath; ORS: outer root sheath; SG: sebaceous gland).

Table 1.1: Glossary of hair follicle anatomy

This table describes the main anatomical elements of a hair follicle.³

Arrector pili muscle	Tiny smooth muscle that connects the hair follicle with the dermis. When contracted, the arrector pili causes the ‘raising’ of the hair.
Bulb	Thickening of the proximal end of the hair follicle. Contains rapidly proliferating, rather undifferentiated matrix cells (transient amplifying cells), melanocytes and outer root sheath cells.
Bulge	Convex protrusion of the outer root sheath in the most distal permanent portion of the hair follicle, just below the sebaceous gland and at the insertion site of the muscle arrector pili. Contains the hair follicle stem cells.
Club hair	Fully keratinised, dead hair (telogen follicle) formed during catagen and telogen.
Dermal papilla	Mesodermal signalling centre of the hair follicle consisting of closely packed specialised mesenchymal fibroblasts. It is framed by the enlarged bulb matrix in anagen.
Hair canal	Tubular connection between the epidermal surface and the most distal part of the inner root sheath. Contains the hair shaft.
Hair germ	Also called ‘hair placode’ depending on the developmental stage. Bud-like thickening in the fetal epidermis consisting of elongated keratinocytes, which at the distal end are in touch with numerous aggregated specialised dermal fibroblasts, the dermal condensate.
Hair peg	Column of keratinocytes growing into the dermis during embryonic hair follicle development (developmental stages 3-5). The concave proximal end starts to encase the dermal condensate, the future dermal papilla.
Hair shaft and cuticle	The hair, per se, is composed of trichocytes, which are terminally differentiated hair follicle keratinocytes. It is composed of the medulla, the central part with loosely connected keratinised cells and large air spaces, and the cortex, which is the bulk of the hair shaft, consisting of keratinised cells, keratin filaments, and melanin granules in pigmented hairs. The cuticle comprises specialised keratins and six to eight layers of flattened overlapping cells.
Infundibulum	Most proximal part of the hair follicle relative to the epidermis, extending from the sebaceous duct to the epidermal surface. Includes the hair canal and the distal Outer root sheath.
Inner root sheath	A multi-layered, rigid tube composed of terminally differentiated hair follicle keratinocytes surrounded by the outer root sheath.
Isthmus	Middle part of the hair follicle extending from the sebaceous duct to the bulge.
Outer root sheath	The outermost layer of the hair follicle. Merges proximally with the basal layer of the interfollicular epidermis and distally with the hair bulb.
Sebaceous gland	Acinar gland composed of lipid-filled sebocytes, localised close to the insertion of the arrector pili muscle. Secretes sebum to the epidermal surface via a holocrine mechanism. Sebum helps to make hair and skin waterproof. Together with the hair follicle and the arrector pili muscle, it forms the pilosebaceous unit.

1.2.3 Follicular units

A follicular unit in the adult human scalp usually consists of two to four terminal follicles, and one, or rarely, two vellus follicles, the associated sebaceous lobules with glands and ducts, and the insertions of the arrector pili muscles of the terminal follicles.¹⁸ Each follicular unit is invested with a single arrector muscle which divides distally to envelop individual hair follicles within the unit. Follicular units are roughly hexagonal in shape and are surrounded by a loose network of collagen (see Figure 1.6).¹⁸ In adults, the mean area of a follicular unit is 1 mm²; thus, 12 to 14 follicular units are usually found in a horizontal section of a 4-mm punch biopsy, which has an actual area of 12.57 mm².

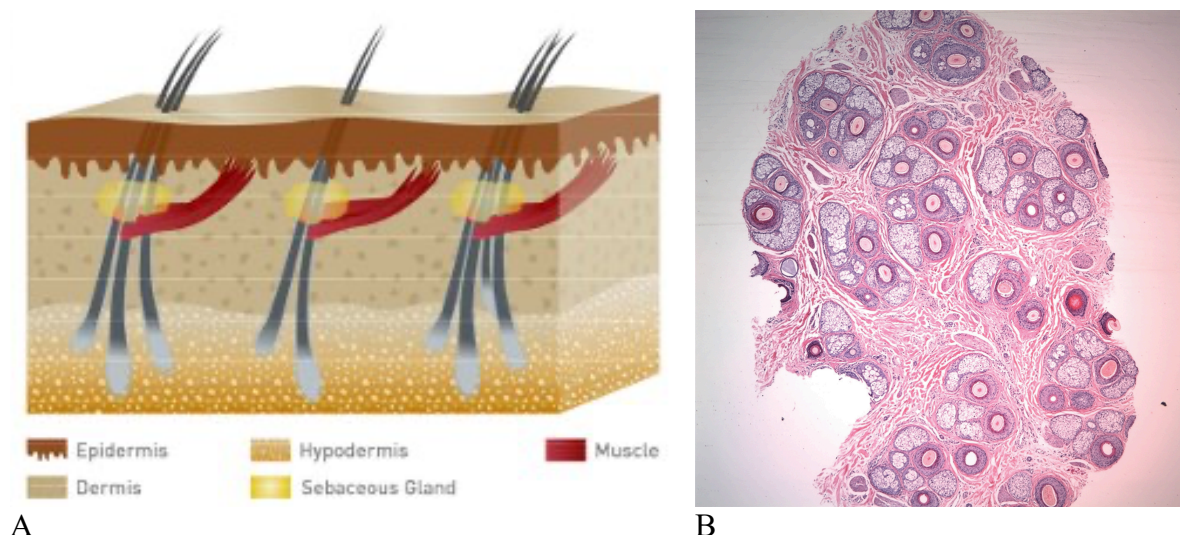


Figure 1.6 Follicular units^{2,18}

(A) Schematic of two, three and four hair follicular units. (Source *The International Society of Hair Restoration Surgery*). (B) Follicular units shown in a horizontal biopsy section at upper isthmus level using hematoxylin and eosin stains.

1.2.4 Terminal anagen hair

Anagen involves the complete regrowth or regeneration of the lower cycling portion of the follicle. Anagen hair formation begins with activating a cluster of epithelial cells present at the outer root sheath keratinocytes of a telogen hair follicle called secondary hair germ (telogen germinal unit), that gives rise to the matrical cells. An exchange of molecular signals causes

the secondary germ and the dermal papilla to migrate downward into the dermis. The dermal papilla signals the matrical cells to actively proliferate and grow upward, differentiating into the internal root sheath and hair shaft. The volume of the dermal papilla dictates the size of the hair shaft.³⁶ The terminal anagen hair follicle penetrates deep into the dermis, extending into the subcutaneous tissue. (See Key stages of the hair cycle in Figures 1.7 and Anagen-catagen-telogen transition in the human hair follicle in Figure 1.8).

1.2.5 Terminal catagen hair

When anagen ends, hair goes into catagen, the intermediate transition stage between growth and rest, for 10 to 14 days. The catagen stage heralds the involution of the hair follicle. This process involves regression of the lower cycling portion of the hair follicle via apoptosis. The catagen stage begins with the lower hair follicle and the dermal papilla retracting upward, leaving behind a collapsed perifollicular sheath forming an angiofibrotic streamer or stela, linking the follicle to the site of the prior anagen bulb. The hair shaft and inner root sheath slide upward together inside the outer root sheath, leaving an elongated mass of trichilemmal outer root sheath below. Apoptosis of trichilemmal cells produces a marked shrinkage of the outer root sheath. Thickening and wrinkling of the surrounding hyaline layer occur with this shrinkage of the trichilemma. As the hair shaft withdraws further upward, its base becomes club-shaped. It is surrounded by a pocket of trichilemmal keratin, with the dermal papilla beneath.¹⁸ In horizontal sections, the catagen hair is generally round or oval. A thickened hyaline membrane surrounds it, often concertinaed by compression from the ascending hair bulb. The catagen hair usually contains apoptotic cells, which stain reddish with hematoxylin and eosin. (See Figures 1.7 and 1.8).

1.2.6 Terminal telogen hair

After catagen, the hair follicle enters telogen, where the hair follicle matures into a club hair. The hair follicle retracts to the level of the “bulge” at the site of insertion of the arrector pili muscle into the follicle. Here the resting hair comprises a telogen germinal unit situated below the telogen club. The telogen germinal unit consists of trichilemma, which is somewhat convoluted and surrounded by palisading basaloid cells. The telogen germinal unit has a characteristic appearance and shows no obvious apoptosis.² A telogen club comprises a central mass of trichilemmal keratin, star-shaped in a horizontal section, surrounded by trichilemmal and fibrous sheaths, connecting telogen germinal units and hair shafts. The recognition of terminal, anagen, catagen and telogen hairs is only possible from the examination of the lower follicle below the “bulge” level for the presence of inner root sheath, apoptosis, or trichilemmal club, respectively.²

After 2 to 4 months of telogen, the telogen germinal cell envelops the dermal papilla. Then, it elongates the existing follicular tract or stela to form an anagen hair. Subsequent hair cycling will continue throughout life for as long as the hair follicle is viable. (See Figures 1.7 and 1.8).

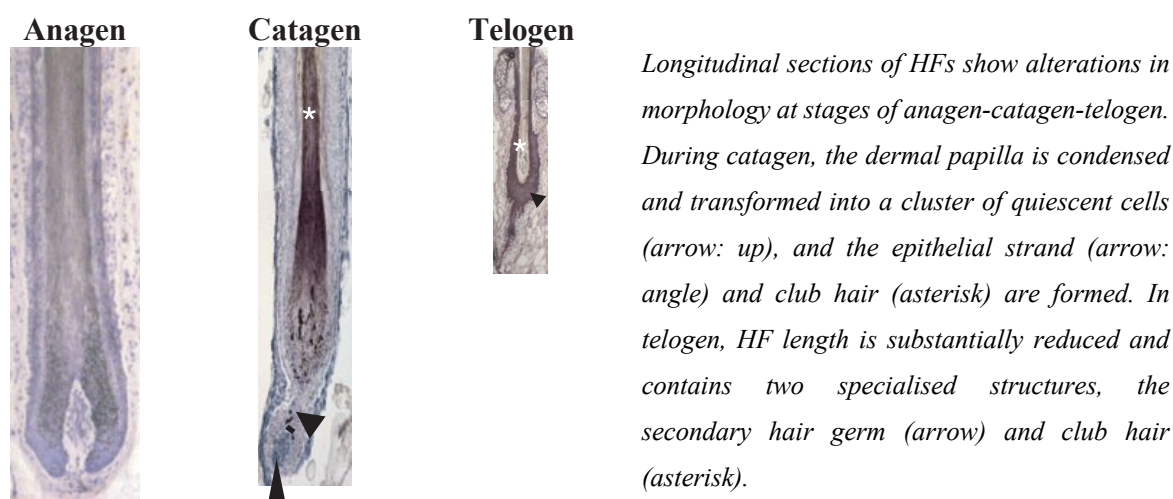


Figure 1.7 Anagen-catagen-telogen transition in the human hair follicle³⁷

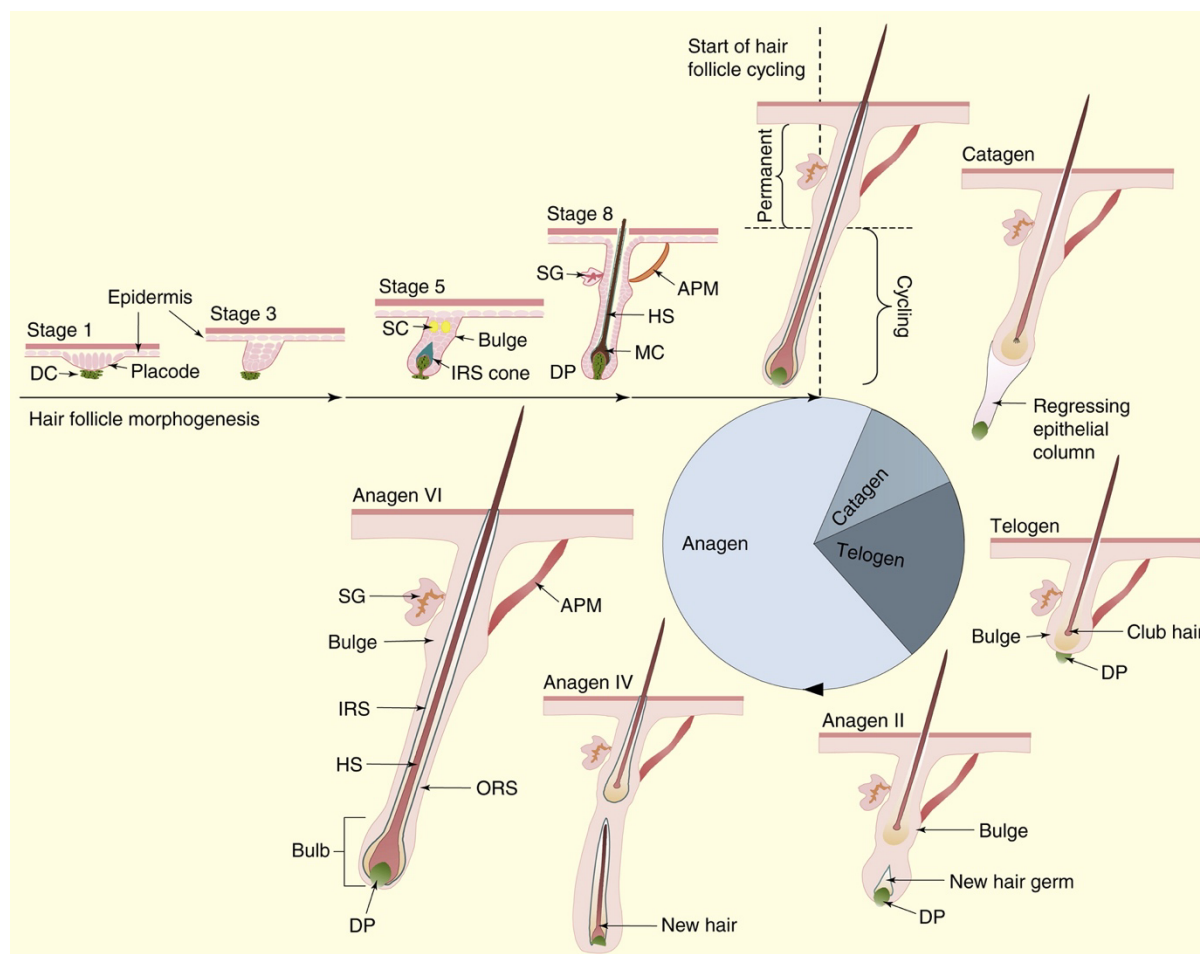


Figure 1.8 Key stages of the hair cycle³

The hair cycle is divided into three phases: Anagen (growth phase), catagen (regression phase) and telogen (resting phase). Postnatal hair morphogenesis leads to elongation of the follicle and production of the hair fibre, which emerges from the skin. Once the hair follicle has matured, it enters the regression phase, during which the lower cycling portion of the hair follicle is degraded. This process brings the dermal papilla into close proximity to the bulge, where the hair stem cells (HSCs) reside. The molecular interaction between the HSCs and the dermal papilla is essential to form a new hair follicle. The proximity between the bulge and dermal papilla is maintained throughout telogen, the resting phase. Only when a critical concentration of hair growth activating signals is reached, anagen phase is entered, and a new hair is regrown. The first postnatal hair cycle is initiated and passed by all hair follicles at the same time point, while subsequent cycles are no longer synchronised. Stages 1–8 of embryonic hair development are depicted (upper left), demonstrating the continuous transition between hair follicle development and the first postnatal hair cycle. APM: arrector pili muscle; DC, dermal condensate (green); DP: dermal papilla (green); HS: hair shaft (brown); IRS: inner root sheath (blue); MC: melanocytes; ORS: outer root sheath; SC: sebocytes (yellow); SG: sebaceous gland.

1.3 Hair Disorders, Frequency and Treatments

The primary function of each hair follicle is to produce the shaft. The hair shaft can vary in size, shape, curl, and colour. A certain proportion of the hair follicles undergo growth, regression, and rest continuously and independently. This process involves the orchestration of a complex yet delicate interplay of molecular signals. Disruption of the signalling process can lead to follicle and hair disorders and, ultimately, hair loss. A range of hair and follicle disorders have been documented and characterised initially by clinical observation and subsequently microscopically using scalp biopsy and non-invasive trichoscopy imaging methods.^{2,31,38}

1.3.1 Introduction to hair loss and hair disorders

Hair disorders are characterised by scarring vs non-scarring; however, non-scarring disorders can also lead to permanent hair loss. Auto-immune disorders can be short-term and can also lead to long-term or permanent hair loss; however, follicles are not necessarily scarred.

Most men and women will experience hair loss of one or more types of disorders over their lifetime. Men, in particular, tend to view their patterned form of hair loss as hereditary, and women often use hair styling, wigs and other prosthetic or fashion features to mask their hair loss. Epidemiology studies in hair loss disorders have not characterised incidence or prevalence rates very well. There are few studies reporting estimates from small sample surveys, closed or insured populations and hair loss clinic incident reporting rates. Prevalence of each hair loss disorder tends to vary by age, sex, race and geographic region.

In general, the prevalence of scarring vs non-scarring alopecia is thought to account for ~1% vs ~99%, although these estimates are not well supported. Most people affected tend not to seek medical treatment; however, those with rarer types of hair loss, particularly with

disfiguring signs and scarring alopecia, seek treatment disproportionately more often than those with more common types of hair loss. A recent study of hair loss clinics found that 67.1% of patients presented with non-scarring alopecia, 27.3% with scarring alopeciae and 5.3% with pulling out their hair or very rare and genetic types of hair dysmorphia.³⁹ However, since patients seek treatment more frequently for alopecia areata and scarring alopecia than those with AGA or TE, these incident rates tend to be disproportionately skewed compared to the presumed prevalence rates.³⁹

1.3.2 Non-scarring alopecia

Non-scarring alopeciae are unrelated types of hair loss that typically do not lead to the destruction of the hair follicle. (See Table 1.2) However, the follicle's productivity is permanently or temporarily impaired and presents as localised patches, patterns, or diffuse thinning. The most prevalent form of all alopecia is androgenetic alopecia (AGA), which is also known as male pattern hair loss (MPHL) or female pattern hair loss (FPHL). AGA and telogen effluvium (TE) are believed to account for at least 95% of all alopeciae. Differential clinical diagnosis between AGA and senescent alopecia (SA) is challenging due to similar hair miniaturisation features. AGA and TE are frequently present simultaneously in women.^{2,40,41}

A recent prevalence and incidence study in a USA employer-sponsored insured population of 13,455 people, reported that in 2019, prevalence and incidence of alopecia areata (AA) was 0.222% and 92 per 100,000 person years, and alopecia totalis (AT) and alopecia universalis (AU) combined was 0.019% and 8.92 per 100,000 person-years. Prevalence and incidence of AA and AT/AU were higher among female vs male individuals, adults vs children and adolescents, and in the Northeast vs other regions.⁴²

Table 1.2: Non-scarring alopecia and characteristics

This table identifies various types of non-scarring alopeciae and the characteristics that make them identifiable.^{2,39,41,43,44}

Type of Non-Scarring Alopecia	Characteristics
Androgenetic Alopecia (AGA)	AGA is the most common form of alopecia, affecting up to 80% of men and 50% of women in the course of their life. The progressive reduction in the hair's diameter, length and pigmentation characterises AGA. It is understood to be primarily induced by the testosterone metabolite dihydrotestosterone (DHT) on androgen-sensitive hair follicles. It has been understood that multiple factors play a role in AGA. AGA thinning is generally in a sex-related pattern. It is also called patterned hair loss.
Telogen Effluvium (TE)	TE is a temporary hair loss caused by a stressor or a change to the body, including medication. It is the most common cause of diffuse hair loss on the scalp. TE is more common in women. It is non-scarring and occurs around three months after a triggering event. It is usually self-limiting, lasting about six months, but can remain chronic if the underlying cause remains unresolved. Chronic TE can also be considered idiopathic chronic TE when an underlying cause is unknown. It is usually readily recognisable by its clinical features and the clinical history, although a strongly positive hair pull test throughout the scalp, a trichogram exhibiting >25% telogen hairs or an increased proportion of telogen hairs (>15%) on scalp biopsy can support diagnosis. ^{45,46}
Anagen Effluvium (AE)	AE is a form of alopecia commonly associated with chemotherapy. Affected anagen (growing) hairs suffer a toxic or inflammatory insult, fracturing the hair shaft. Shedding usually takes place within 14 days of administration of the offending drug; however, in many instances, it is reversible, with hair regrowth occurring after discontinuation of the offending agent. It can also be caused by the toxicity of heavy metals.
Senescent Alopecia (SA)	SA is age-related hair thinning that affects men and women in their later decades with no familial history or evidence of pattern balding. It usually starts around age 50, with thinning all over the scalp. It shares similar features to AGA. The cause is not known.
Alopecia Areata (AA)	AA is an auto-immune disease that attacks the body's hair follicles, causing hair loss. It is a non-scarring alopecic patch or multiple patches. Its clinical course is unpredictable, although approximately 67% of affected tend to self-resolve.
Totalis (AT) Universalis (AU)	AA in a small percentage of patients may extend to the entire scalp (AT). AA can also lead to total body hair loss (AU) with a poor prognosis.

Treatment of AGA has had limited success in restoring the person's cardinal scalp phenotype, and approximately 50% of users do not respond to treatments.^{47,48} These treatments can include Dutasteride, Finasteride, Minoxidil, low-level laser therapy, micro-needling and platelet-rich

plasma (PRP). Alopecia areata (AA) treatments include topical, intralesional, and systemic steroids; often, localised irritants are also used. Recently a class of treatments known as JAK inhibitors have also been used for alopecia areata (AA). There is no specific treatment for TE, however diagnosed underlying conditions are treated accordingly, and topical minoxidil is commonly used. It is also very common to find consumer products for hair loss, such as dietary supplements, cosmetic sera and traditional botanical medicines, although evidence is limited.^{12,49}

1.3.3 Scarring or cicatricial alopecia

Cicatricial Alopecia (CA) comprises a group of disorders in which inflammation leads to the destruction of the upper permanent part of the follicle including the stem-cell region and replacement by fibrous tissue that prevents hair regrowth⁵⁰ (See Table 1.3). Generally thought to be 3 – 5% of all alopeciae, one recent multicenter study found that patients presenting to specialist hair clinics with scarring alopeciae comprised approximately 26.8% of total alopeciae cases. The most common was frontal fibrosing alopecia (FFA) with an incidence of 40.1% of cicatricial alopecias³⁹. Treatment of scarring alopecia is challenging; at best, they can prevent further scarring progression. They can include oral Finasteride, Dutasteride, topical, intralesional, systemic steroids, Minoxidil, topical tacrolimus, oral hydroxychloroquine, platelet-rich plasma (PRP) and Vitamin D.^{2,51,52}

In a recent prevalence and incidence study in a New York City health system population of 1,189,507 people treated between 2015 and 2018, crude prevalence of FFA was 0.015% and Lichen Planopilaris (LPP) was 0.017%. Although both were similar, they were also more common in White and Asian female patients aged between 40 and 70 years.⁵³ This study was limited by the common diagnostic coding for FFA and LPP, which required chart reviews of biopsies for accurate diagnosis.

In March of 2023, the Centers for Disease Control and Prevention in the USA, proposed new ICD-10-CM diagnostic codes for Central Centrifugal Cicatricial Alopecia (CCCA) with prevalence estimated from 2.7 – 5.6%, occurring predominantly in black women aged 30 to 55 years. Similarly new codes were proposed for FFA, classic Lichen Planopilaris (LPP), and Graham–Little Piccardi-Lasseur Syndrome (GLPLS), each having distinct clinical and pathological features, which had been grouped under LPP. Implementing these codes will facilitate improved disease characterisation, potentially identify unknown comorbidities and provide quality data for epidemiologic studies at the population level.⁵⁴

Table 1.3: Cicatricial alopecia characteristics

This table identifies various types of scarring alopecia and the characteristics that make them identifiable.^{2,39,52,55}

Type of Cicatricial Alopecia	Characteristics
Lymphocytic	
Central centrifugal cicatricial alopecia (CCCA)	CCCA is a unique form of scarring alopecia that presents as patches of permanent hair loss on the vertex or crown of the scalp, and it spreads centrifugally. In addition, CCCA is associated with inflammation. It is a common condition that usually affects women of African descent and runs in families.
Discoid lupus erythematosus (DLE)	DLE is a form of cutaneous lupus erythematosus (CLE). CLE is a family of lupus that affects the skin. People with discoid lupus get round sores, usually on their face or scalp. Another name for discoid lupus is chronic cutaneous lupus.
Lichen planopilaris (LPP)	LPP is a primary lymphocytic cicatricial alopecia, which starts as a sudden, irregular, patchy hair loss in the vertex area and is also associated with redness, scaling and pruritus. LPP has a worse prognosis than AGA, leading to follicular dropout and scarring. Lichen planopilaris is the follicular type of lichen planus. Besides classic LPP, there are three variants: GLPLS and FFA. (See below)
Frontal fibrosing alopecia (FFA)	FFA is a form of lichen planopilaris characterised by slowly progressive hair loss and scarring on the scalp near the forehead. The eyebrows, eyelashes or other parts of the body may be involved, as well. It has been suggested that FFA may be due to hormonal changes or an auto-immune response, and recently it has been associated with the long-term use of sun protection compounds in cosmetics. However, the exact cause of this condition is not yet known.
Graham–Little–Piccardi-Lasseur syndrome (GLPLS)	GLPLS is a type of LPP. The onset of symptoms in GLPLS can occur in any order. However, the condition often begins with the development of hyperkeratotic papules on the trunk and extremities before the development of alopecia.

Pseudopelade of Brocq (PPB)	PPB is a rare, idiopathic self-limiting hair disorder resulting in progressive cicatricial alopecia. It is frequently described as discrete, asymmetrical, slightly depressed alopecic patches primarily involving the parietal scalp and vertex.
Follicular mucinosis (FM)	FM is a rare cutaneous mucinosis characterised by the accumulation of mucin at the external root sheath and sebaceous glands. It manifests clinically as follicular papules distributed on the trunk, proximal limbs, scalp, and face; it may also exist as indurated plaques. FM is most commonly a manifestation of cutaneous T-cell lymphoma. It has also been described broadly as a primary benign condition, running an acute or chronic course, or as a secondary disorder to various other benign and malignant conditions.
Keratosis follicularis spinulosa decalvans (KFSD)	KFSD is a hereditary disorder of the hair follicle which presents with scarring alopecia and follicular papules affecting the scalp and other areas of the body.
Neutrophilic	
Folliculitis decalvans (FD)	FD is a rare, chronic cicatricial (scarring) alopecia that occurs in adults and classically presents as an expanding patch of alopecia with peripheral pustules on the scalp. Patients may experience associated pruritus or pain.
Dissecting cellulitis of the scalp (DCS)	DCS , also known as perifolliculitis capitis abscedens et suffodiens or Hoffman disease, is a chronic inflammatory disorder of the scalp characterised by boggy, suppurative nodules that are often associated with patchy hair loss.
Mixed	
Acne keloidalis nuchae (AKN)	AKN is an inflammatory skin disorder of the hair follicles on the neck and back of the scalp. It presents as red patches on the skin, which can develop into papules, plaques, pustules, infections, scarring and hair loss. AKN is most common among men with curly hair.
Acne necrotica (AN)	AN is a disorder of adults of obscure aetiology, featuring repeated cropping of inflammatory papulonodules, which rapidly necrotise, leaving varying degrees of superficial scarring with pathological features in early lesions of a necrotising lymphocytic folliculitis.
Erosive pustular dermatosis of the scalp (EPDS)	EPDS is characterised by an erythematous skin erosion with thick crusts and pustules with alopecia. Over time, recurrent erosions, crusts, and pustules result in scarring and subsequent alopecia, telangiectasia, and atrophy of the skin. However, the hyperkeratotic lesions are not painful, and pruritus is rare.

1.3.4 Other types of alopecia

Other types of alopecia and hair disorders can be seen in Table 1.4.

Table 1.4: Other types of alopecia and hair disorders, and their characteristics

This table identifies other types of alopecia and hair disorders, and describes their identifiable characteristics.^{2,56}

Other Types of Alopecia	Characteristics
Genetic Hair Shaft Disorders	
Trichothiodystrophy	A rare, genetic, syndromic hair shaft abnormality disorder characterised by short, dry, sulphur-deficient, brittle hair. A more subtle feature of this syndrome is a “tiger tail” banding pattern in hair shafts when examined microscopically under polarised light.
Menkes (kinky hair) syndrome	A disorder that affects copper levels in the body. It is characterised by sparse, kinky hair.
Monilethrix	A rare inherited disorder characterised by sparse, dry, and often brittle hair that can break before reaching more than a few inches in length. The hair may lack lustre, and there may be patchy areas of hair loss (alopecia). Another common sign is the appearance of elevated spots (papules) surrounding the hair follicles that may be covered with grey or brown crusts or scales (perifollicular hyperkeratosis). Under a microscope, the hair shaft resembles a string of evenly spaced beads.
Uncombable hair syndrome	A rare genetic condition that causes a child’s hair to grow in multiple directions. It makes hair rough, frizzy and dry. As a result, combing or brushing hair is difficult to impossible. No treatment is available for this condition, and Tends to improve during adolescence.
Pili annulati (PA)	PA, also known as ringed or Morse alphabet hair, is a very rare benign disorder characterised by a unique alternating light and dark speckled and banded appearance of the hair. The hair shaft develops with periodic abnormal air-filled cavities. PA is thought to be caused by a protein metabolic disorder.
Self Induced	
Trichotillomania	Also known as a hair-pulling disorder. Hair is pulled from anywhere on the body repeatedly, appearing as hair loss but is caused by the patient’s action.
Traction alopecia	A disorder caused by hairstyles that produce a continuous pulling force on the hair roots. It is most common in hairstyles involving tight braids or buns. It is preventable and reversible if addressed early but can become permanent.
Trichorrhexis nodosa	A common disorder characterised by hair breakage due to weak points in the hair caused by physical or chemical trauma to the hair as part of hair care practices. Typical causes are heat, chemical straightening or permanent waving, permanent hair colour, and drying hair products.
Other	
Premature Canities	A premature hair greying disorder before age 20 in European and before 30 years in African populations. It also presents in AA, but hair is remelanised when AA resolves.

Tinea Capitis (TC)	TC , also known as ringworm or herpes tonsurans infection, is a fungal infection of the scalp hair. It is caused primarily by the dermatophyte species microsporum and trichophyton. The fungi can penetrate the hair follicle's outer root sheath and may invade the hair shaft. Commonly, the infection may look like severe dandruff that appears in various places on the scalp. Some infections cause patches of hair loss. The inflammatory type (kerion) is associated with pus discharge and might lead to permanent hair loss. TC is treatable with systemic antifungal medication.
Loose Anagen Syndrome (LA)	LA is generally a self-resolving condition where anagen hairs are easily and painlessly pulled from the follicle. It is a disorder of abnormal anagen hair anchorage in the follicle.

1.4 Biology of the Hair Follicle

HFs are epithelial skin mini-organs that modulate and sustain hair regrowth over repeated hair cycles. Each hair follicle has its own stem cells, nerves, blood vessels, muscle, glands, and immune privilege. It functions over time with its own anagen-catagen-telogen cycle – independent of its immediate neighbouring hair follicles and even those sharing the same follicular or pilosebaceous unit.^{3,57}

Cell signalling is part of cells' communication process to govern basic activities and actions. The ability of cells to perceive and correctly respond to their environment is important for normal development, tissue repair, and tissue maintenance in a normal state. By understanding and being able to favourably influence cell signalling, normal hair growth may be more effectively attained.^{3,21}

1.4.1 The hair follicle and stem cells

Stem cells are mainly characterised by self-renewal properties and the potency to differentiate into diverse cell types.⁵⁸ Studies have demonstrated that different growth factors, including members of the Wnt/ β -catenin protein family, regulate these properties.⁵⁹ In telogen hair follicles, stem cells are “quiescent,” meaning they are normally inactive, but they activate during a new anagen hair cycle to produce daughter cells that migrate down the outer root

sheath to enter the hair bulb matrix driving new hair growth. Hair follicle stem cells remain dormant during telogen before proliferating during anagen.⁶⁰ Therefore, the stem cells give the follicle its regenerative capacity, allowing it to cycle through its growth phases. Many factors regulate the quiescence of hair follicle stem cells. In certain cases, they fail to activate, which is what causes hair loss. Wnt proteins are secreted glycoproteins that can activate different intracellular signalling pathways.⁶¹ Recent studies have suggested that hair loss occurs when the supply or the activity of hair follicle stem cells is exhausted.⁶² How they retain their stemness or ability to self-renew and differentiate appears to be intimately related to Wnt/ β -catenin activity.⁶³

In mammals, Wnt/ β -catenin signalling features prominently in stem cells. The interactions between Wnt ligands and their receptors activate various intracellular signalling cascades that can be cross-connected or act independently. Depending on the pathway activated, Wnt signalling can regulate a variety of diverse processes, including cell proliferation, differentiation, migration, polarity and asymmetric cell division.⁶⁴ Not surprisingly, the disruption of Wnt signalling has been linked to a number of human diseases and also hair loss.⁶⁵ Biological processes associated with abnormal inactivation of Wnt/ β -catenin signalling include fate determination of hair follicle stem cells (HFSCs), regeneration of adult hair follicles, regulation of hair shaft growth, and regeneration of melanocytes.⁶⁶

Wnt pathways fall into two general categories: canonical and non-canonical Wnt signalling.⁶¹ Canonical Wnt signalling is often referred to as the Wnt/ β -catenin pathway, as it happens when Wnt-stimulated signals trigger β -catenin-dependent transcriptional activation.⁶¹ In contrast, the non-canonical is referred to as the β -catenin-independent pathway.⁶¹ In the absence of Wnt, β -catenin is targeted by a destructive complex of proteins.⁶¹ Binding of Wnt to receptors

activates cytosolic proteins, leading to the inhibition of the destructive protein complex and the stabilisation of β -catenin.⁶¹ Accumulation of stabilised β -catenin in the presence of transcription factors results in their translocation into the nucleus to activate Wnt-responsive genes.⁶⁷ Canonical Wnt/ β -catenin signalling in the hair follicle has been shown to play a major role in differentiating stem cells into each of the seven tissue types of the hair follicle, however the rate of these signals can vary depending other signals and the stage in the cycle of the follicle.^{3,68,69} (See Figures 1.9 and 1.10).

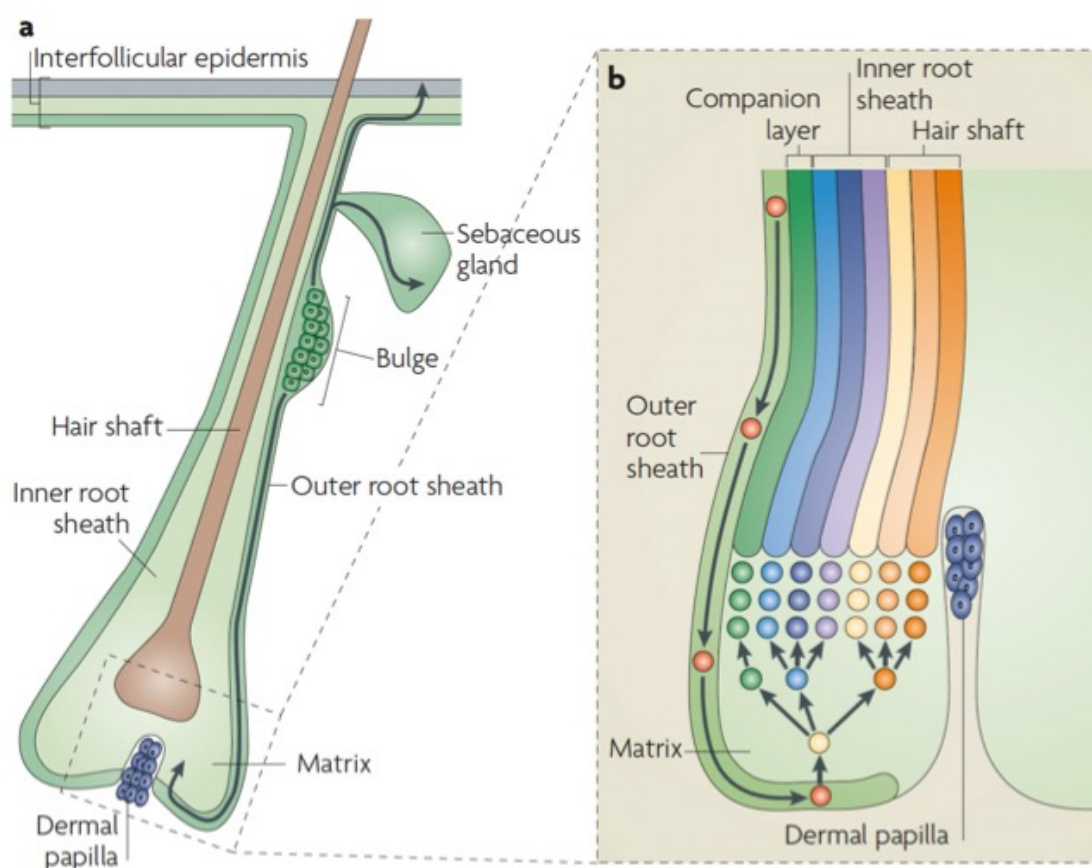


Figure 1.9: Stem cells from the bulge migrate to the matrix and differentiate into one of seven hair lineages⁵⁸

During normal homeostasis, bulge stem cells (SCs) periodically activated to migrate along the outer root sheath (ORS) towards the matrix at the base of the hair follicle (HF) to regenerate and sustain anagen growth of the HF (see panel a). As bulge SC progeny move down the ORS to the base of the follicle, they subsequently become matrix cells, which, after proliferation, differentiate along one of seven hair lineages (see panel b) to sustain the differentiation and production of the cells that form the hair shaft and its channel, the inner root sheath. Under conditions of injury in which the resident interfollicular epidermal or sebaceous gland SCs are damaged, bulge SCs become mobilised and migrate to and differentiate along these lineages (see panels a and b).

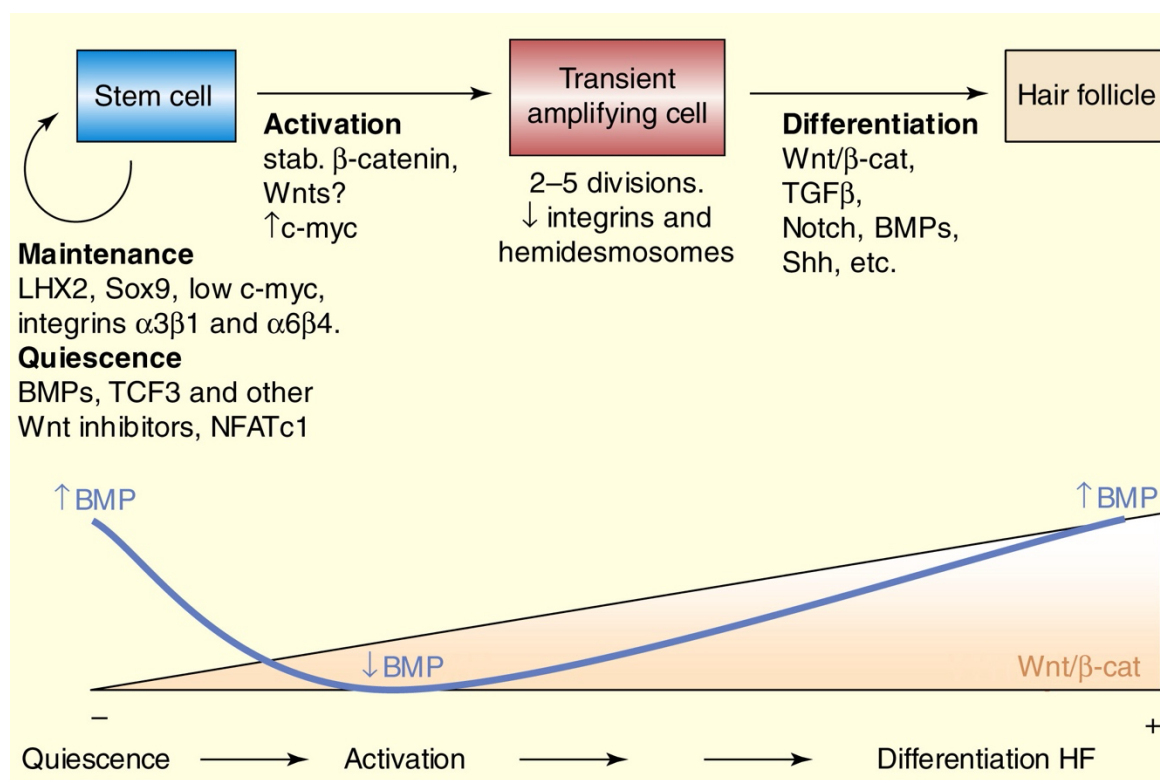


Figure 1.10 Schematic presentation of the temporal interplay of the molecular players in hair stem cell activation ³

Upper panel: List of molecules required for stem cell maintenance/quiescence, activation and differentiation.

Lower panel: Comparison between the relative amounts of BMP (blue) and Wnt/ β -catenin (orange) activity needed for quiescence, activation and differentiation of hair follicle stem cells.

1.4.2 Role of molecular pathways in hair growth and cycling disorders.

Molecular pathways are a series of actions among molecules in a cell that leads to a certain endpoint or cell function. They can be intracellular or extracellular, and they can facilitate communication between cells. They have evolved to maintain homeostasis and survival of the organism. In the hair follicle, similar mechanisms regulate morphogenesis and homeostasis (See Figure 1.11). ⁵⁸ Unfortunately, biological pathways do not always work correctly. When something goes wrong in a pathway, the result can lead to a temporary, chronic or catastrophic disorder or disease. ^{70,71}

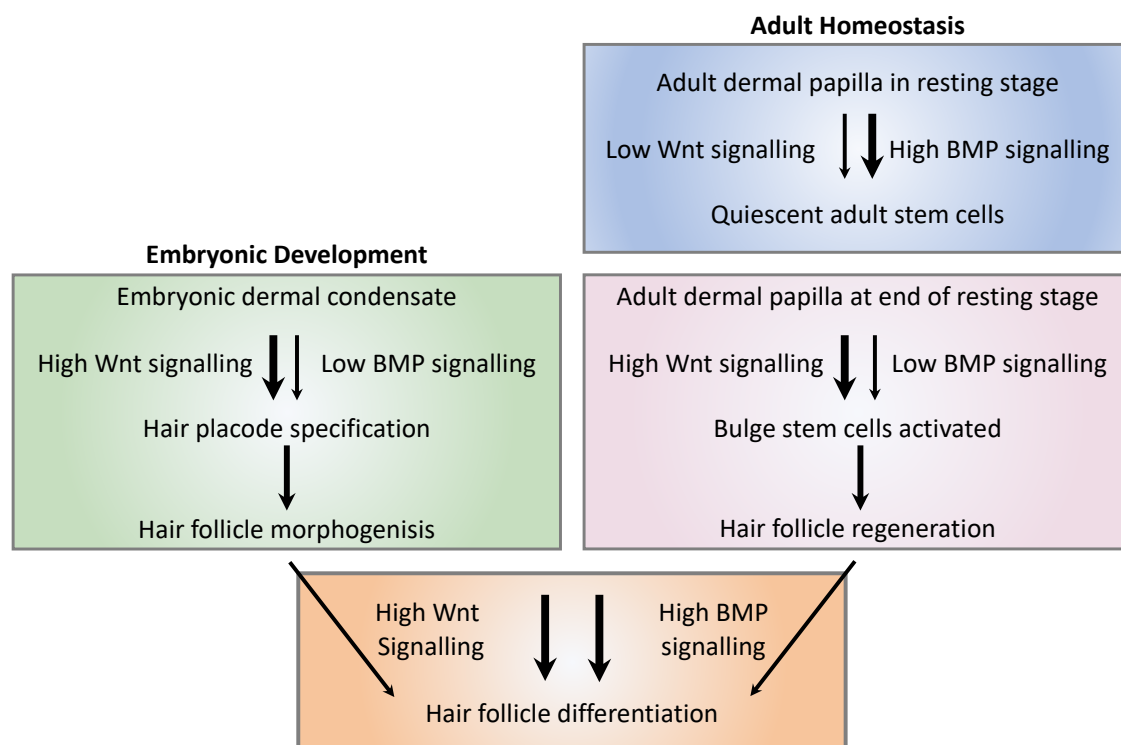


Figure 1.11 Similar mechanisms regulate morphogenesis and homeostasis⁵⁸

Loss and gain-of-function studies in mice highlight the different functions of Wnt/ β -catenin and bone morphogenetic protein (BMP) signalling during morphogenesis and adult skin homeostasis. During hair follicle development, Wnt/ β -catenin signalling is required to specify the hair follicle fate in the undifferentiated basal epidermis. During the adult hair cycle, increased Wnt signalling promotes stem cell (SC) activation to initiate the growth of a new hair during the transition from resting to growing stage. An even more robust response to Wnt signalling is involved later as matrix cells specifically commit to terminal differentiation along the hair shaft lineage. BMP signals are transmitted from the underlying mesenchyme to the epidermis. As dermal condensates form, the prospective dermal papilla expresses the BMP inhibitor noggin. Noggin is required for normal follicle development and stimulates lymphoid enhancer-binding factor 1 (LEF1) expression and Wnt signalling. During the adult hair cycle, active BMP signalling propels bulge SC quiescence during the resting stage. During the transition from resting to growing stage of the hair cycle, the levels of dermal BMPs and BMP inhibitors change, resulting in a net inhibition of BMP signalling and SC activation. As the new follicle matures, activation of BMP receptor signalling is essential for the differentiation of transit-amplifying matrix cells to form the hair shaft and its channel.

During the hair follicle's anagen phase, hair fibre production results from a finely, timely, and spatially tuned choreography of gene expression, which is highly sensitive to stimulatory and inhibitory signals.⁵⁹ A number of signalling pathways,⁷² cytokines,⁷³ hormones,^{74,75} prostaglandins,⁷⁶ and growth factors are known to modulate the duration of anagen.⁷⁷ (For more details, see Table 1.5).

During the telogen phase, the follicle is under the influence of factors that would repress the onset of the neogen phase and factors that would trigger it. Specifically, a strong expression of bone morphogenetic protein (BMP) and fibroblast growth factor (FGF)-18 defines the refractory period during which the neogen onset is prevented. The progressive increase in the production of the BMP antagonist noggin, Wnt/ β -catenin pathway activators, and transforming growth factor (TGF)- β 2 then reach a critical threshold that shifts the telogen follicle to a competency status, receptive to FGF-7, secreted by the nearby dermal papilla, and, ultimately, triggering the onset of the neogen phase.^{35,78} (For more details, see Table 1.5).

It should be noted that animal models are crucial for advancing our knowledge about the molecular pathways involved in human biology and diseases. However, to what extent tissue expression of pathways in healthy individuals is conserved between species remains unclear. In addition, information on pathways in humans is often lacking. Within these limitations, Table 1.5 summarises, without distinction, publicly available literature on the role of molecular pathways in hair growth, cycling and disorders. In this Table 1.5, the need for a complex choreographed interplay of signals to maintain homeostasis can be seen, where any potential disturbances or mistiming of a signal could adversely affect hair follicle productivity, induce catagen, and potentially prevent an involuted follicle from reactivating from its telogen phase or in the worst case, induce scarring.

Table 1.5: Molecular pathways in hair growth, cycling and disorders.

This table identifies relevant adverse molecular pathway signalling in the hair follicle that can lead to impaired follicle productivity, catagen induction, prolonged telogen and various pathological effects such as scarring and other hair disorders.

Function	Pathway	Adverse Signal	Effect on the hair follicle
Stem Cell Activation & Differentiation			<i>An undifferentiated cell can give rise to infinitely more cells of the same type, from which certain other kinds of cells arise by differentiation.</i>
1	Wnt/ β -catenin	↓	Wnt/ β -catenin is a group of signal transduction pathways made of proteins that pass signals into a cell through cell surface receptors. It is a highly studied signalling pathway and is considered a principal, yet not a master regulator during hair follicle morphogenesis and maintenance. It is required to give stem cells the ability to change into various cell types and to allow exhausted stem cells the ability to self-renew. Wnt/ β -catenin signalling declines with age. Wnt/ β -catenin increases cell proliferation and promotes anagen in the dermal papilla. DHT and various inflammatory signals downregulate Wnt/ β -catenin signalling, inducing catagen and prolonging telogen. ^{57,61,64,65,72,79-91}
2	Shh	↓	Sonic hedgehog (Shh) is one of three Hedgehog intercellular signalling proteins in the skin. Shh expression and the ability of skin cells to respond to Shh signalling is spatially and temporally regulated. Shh plays a role in activating the dermal papilla (DP) to induce the anagen (growth phase) in telogen hair follicles. Shh is a morphogen known for regulating hair follicle epithelial growth. It also regulates specific DP signatures to maintain DP maturation and the Shh–Noggin signalling loop to drive hair follicle morphogenesis. ^{69,72,92-94}
3	BMP	↑↓	Bone morphogenetic protein (BMP) is a family of 20 proteins involved in controlling the differentiation of stem cells to form different types of cells as well as various cellular functions. Under normal circumstances, as follicle growth is activated, waves of BMP (especially BMP-4) shut down the stem cells in hair follicles, halting growth. Inhibiting BMP-4 signalling promotes normal hair growth and inhibits hair loss, while androgens downregulate BMP-2 and BMP-4 in dermal papilla cells (DPC). Initiation of a new hair growth phase in postnatal skin requires neutralisation of the inhibitory activity of BMP-4 by the BMP antagonist noggin. Upregulation of BMP receptors and nuclear β -catenin accumulation are also indicative of Wnt/ β -catenin pathway activation. BMP-2 is an androgen-downregulated paracrine factor contributing to DPC inductivity and favours DPC-induced HFSC differentiation to hair lineage cells through crosstalk with the Wnt/ β -catenin pathway. ^{69,95-97}
4	Noggin	↓	Noggin is a development protein that promotes certain growth in tissues. It induces anagen by modulating certain BMPs, including inhibiting BMP-4, which helps in counteracting BMP mediated β -catenin inhibition. The interplay of signalling helps in Shh signal amplification. ^{69,98,99}
5	Notch	↓	Notch signalling determines stem cell fate. The dermal Wnt pathway helps in the upregulation of epithelial Notch expression. ³⁸

6	TERT	↓	Telomerase reverse transcriptase (TERT) is a component of an enzyme called telomerase. TERT functions to add a protective structure that “caps” chromosome ends to prevent damage that occurs either naturally from ageing or from the environment. TERT is expressed in stem cells and serves a critical role in stem cell function and tissue homeostasis. It causes a rapid transition from telogen to anagen, facilitating robust hair growth. TERT promotes this developmental transition by causing the proliferation of quiescent stem cells in the hair follicle bulge region. It controls tissue progenitor cells via transcriptional regulation of a developmental program that converges on the Wnt pathway. ^{100,101}
Angiogenesis			<i>An often-overlooked contributor to normal hair health is circulation. Even a perfectly designed solution for correcting hair loss would be ineffective without adequate blood flow, delivering the necessary nutrients to the hair follicle.</i>
7	VEGF	↓	Vascular endothelial growth factor (VEGF) is a signal and growth factor protein produced by cells that stimulate blood vessel growth. It is part of the system that restores the oxygen supply to tissues when blood circulation is inadequate. VEGF’s normal function is to create new blood vessels. Numerous studies demonstrate that increasing VEGF improves blood flow. In fact, studies in normal hair biology demonstrate that when hair follicles transition from anagen to telogen, new blood vessel creation is stimulated in and around the follicle. ¹⁰²⁻¹⁰⁵
Growth Promotion			<i>Growth promoters and factors are naturally occurring substances within the body capable of stimulating cellular growth, maturation, proliferation and differentiation. Growth factors are proteins, hormones or molecules called cytokines or messengers important for regulating various cellular processes.</i>
8	FGF	↑↓	Fibroblast growth factor (FGF) family members possess the broad ability to encourage cell division and cell survival activities. They are involved in various biological processes, including cell growth and tissue repair. FGFs work in a paracrine manner. Where they are produced by a cell, and the FGF has its effect on a nearby or neighbouring cell. Two FGF family members have been implicated in helping slow the loss of hair and promoting normal healthy hair growth by activation of FGF-7, also known as keratinocyte growth factor (KGF), and inhibit of the formation of FGF-18. ^{96,106,107}
9	HGF	↓	Hepatocyte growth factor (HGF) is a multifunctional protein growth factor which acts to stimulate cell division. HGF is a paracrine hormone found to influence hair length by prolonging anagen, promoting follicular growth and stimulating DNA synthesis in hair follicles. HGF stimulates Wnt/ β -catenin activity in the human hair matrix by upregulating in the dermal papilla. ¹⁰⁸⁻¹¹⁰
10	IGF	↓	Insulin-like growth factors (IGFs) are proteins very similar to insulin used by cells to communicate with their environment. IGFs are important for the regulation of normal physiology. IGFs play an important role in promoting cell proliferation and inhibiting normal or programmed cell death (apoptosis). Research has demonstrated that one form of IGF called IGF-1 affects the proliferation of the hair follicle and the normal hair growth cycle. ¹¹¹⁻¹¹⁵
11	mTORC1	↑↓	The mammalian target of rapamycin (mTOR) is a central regulator of cell proliferation and survival. mTORC1 is a protein complex that senses the need for nutrients, energy and oxygen in cells and controls protein synthesis. mTORC1 is responsible for activating proteins in the body. Hair is primarily composed of the protein keratin. Hair cells must have adequate energy resources, nutrient availability, oxygen abundance, and proper growth factors to develop and mature the keratin. Studies have shown that mTORC1 also limits the negative effects of BMP-4 signalling, allowing hair follicle stem cell activation and has functional

			relevance in anagen entry. mTORC1 cell signalling autonomously regulates the Wnt/ β -catenin pathway to influence stem cell maintenance. However sustained mTORC1 activation can impair Wnt/ β -catenin. ^{116,117}
12	PPAR	↓	Peroxisome proliferator-activated receptors (PPAR) are a group of molecules that are expressed in the skin and play an essential role in regulating cell differentiation, development and metabolism. PPARs play a role in hair follicle growth and melanocyte differentiation. PPAR α plays a role in the formation of the epidermal barrier and in sebocyte differentiation. PPAR β , regulates the late stages of sebaceous cell differentiation and is the most effective isotype in stimulating lipid production in these cells. PPAR γ has been extensively studied due to its role as a “master regulator” of inflammation, and a decrease in PPAR γ is associated with hair loss. The PPAR γ also plays a role in stimulating sebocyte development and lipogenesis. ^{118,119}
Immune Modulation and Inflammation			<i>This microenvironment of the hair follicle will naturally protect the hair from loss by preserving or restoring the ‘immune privilege’. This process actively modulates, directs and adjusts immune responses to the required levels to protect follicles from immune reactions. Autoimmune reactions happen in the hair follicle that can cause loss of existing hair and inhibit normal hair growth. Inflammatory responses are a complex biological response of body tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. Although protective under normal circumstances, uncontrolled inflammation can lead to tissue injury.</i> ^{115,120}
13	COX	↑	Cox is an enzyme that is responsible for the formation of prostaglandins and for promoting inflammation. Uncontrolled inflammation can lead to tissue injury. Numerous studies have shown that inflammation of the scalp exacerbates hair loss. More specifically, COX levels rise when the hair cycle enters catagen and telogen. ¹²¹
14	IFN	↑	Interferons (IFNs) are a group of signalling molecules called cytokines used to communicate between cells. Under normal circumstances, IFNs trigger the protective defences of the immune system to help eradicate foreign invaders such as viruses and bacteria. However, when uncontrolled, they can lead to tissue damage and hair loss. Abnormal levels of IFN- α are found in scalps with various hair loss disorders. Elevation of IFN- γ inhibits hair growth, increases apoptosis, induces catagen faster than TGF- β , and switches off melanogenesis. ¹²²⁻¹²⁴
15	IL-1,6,10,12	↑↓	Interleukins (IL) are a group of over 35 secreted proteins and signalling molecules known to regulate cell growth and differentiation. They are situation-specific and work by stimulating or inhibiting the activity of the immune system. IL-1 is a human hair growth inhibitor and induces hair loss. IL-6 and IL-12 also induce hair loss, and IL-10 promotes hair growth. ^{73,103,125-127}
16	JAK/Stat	↑	Janus kinases (JAKs) and signal transducers and activators of transcription (STATs) are an inflammation cytokine pathway. The JAK/STAT pathway activation promotes quiescence in the hair cycle. Scalp tissue affected by androgenetic alopecia and alopecia areata shows higher STAT3 gene expression levels compared to unaffected areas. Inhibition of the JAK/STAT pathway has shown hair regrowth. ¹²⁷⁻¹³²
17	NF- κ B	↑	The transcription factor NF- κ B regulates multiple aspects of innate and adaptive immune functions and serves as a key mediator of inflammatory responses. It plays a critical role in regulating the survival, activation and differentiation of innate immune cells and inflammatory T cells. Dysregulated NF- κ B activation contributes to the pathogenic processes of various inflammatory diseases and has been implicated in various hair disorders. ^{69,133}

18	Prostaglandins	↑	Prostaglandins are a group of molecules that have hormone-like effects that help with injury and illness by regulating inflammation. Prostaglandin D2 (PGD2) causes baldness in men. PGD2 prevents hair follicles from maturing and therefore growing hair. Prostaglandin E2 (PGE2) increases intracellular cAMP levels and has important immunoregulatory functions. It exerts a suppressive effect on macrophage functions. It also decreases T Helper 1 (TH1) cell proliferation and expression or synthesis of the TH1 cytokines, IL-1, IL-2, IFN- γ , and TNF- α . Therefore, PGE2 suppresses the cellular immune response generated by TH1 cells that is common in hair loss, particularly alopecia areata. ¹³⁴⁻¹³⁶
19	TGF- β	↑↓	TGF- β is a pleiotropic cytokine (cell messenger). It is secreted by fibroblasts and epithelial cells in a tissue-specific manner and functions in a context-dependent fashion. TGF- β member of a cytokine super-family that has approximately 30 members that carry out their actions via specific message-receiving molecules. It is secreted by fibroblasts and epithelial cells in a tissue-specific manner and functions in a context-dependent fashion. These cytokines regulate cell proliferation or destruction in many cell types and are central to the inflammation process. Studies have shown that TGF- β is associated with hair follicle shrinking and eventual hair loss. ¹³⁷⁻¹³⁹
20	TNF- α	↑	TNF- α is a chemical messenger (cytokine) produced by many cells in the body that, under normal circumstances, is required for a healthy immune response. Under abnormal situations (elevated levels), it can cause the immune system to attack healthy hair cells. TNF- α has been shown to modulate hair cycles and plays a central role in apoptosis of hair cells. It has been suggested to be the most significant factor in hair cell death, possibly more significant than DHT. ^{37,125,140}
Hormone Regulation			<i>Many hormones, directly and indirectly, impact the hair follicle, such as thyroid, adrenal, and sex (estrogen and testosterone) hormones. This section focuses on the hormones and cofactors directly influencing androgen receptors and iron metabolism in the hair follicles.</i>
21	DHT/5 α -Reductase	↑	Dihydrotestosterone (DHT) is a more powerful form of the steroid hormone testosterone, and 5 α -Reductase (5 α -R) is a naturally occurring enzyme. DHT production involves converting testosterone into DHT by an enzyme called 5 α -R. In addition, a cofactor known as NADPH (Nicotinamide adenine dinucleotide phosphate) is required by 5 α -R to convert testosterone to DHT effectively. DHT diffuses into hair follicle cells and plays paradoxical role. It induces hair loss over the galea aponeurotica area of the scalp and promotes hair growth in secondary body and facial hair, while it is not needed for androgen-dependent hair growth in pubic and axillae regions. ^{114,141}
22	Hepcidin/FPN	↑↓	The hepatic bactericidal protein (Hepcidin) is the major regulator of systemic iron homeostasis. It is a hepatic hormone that controls dietary iron absorption and plasma iron concentration. When Hepcidin binds to the only known iron export protein, ferroportin (FPN), it inhibits the bioavailability of iron. It plays a critical role in oxygen binding to haemoglobin, mitochondrial function and bioenergetics of hair formation. Systemic iron levels require a tight balanced to prevent iron deficiency which causes anaemia or oxidative stress due to iron accumulation. Regulatory cues such as iron, inflammation, hypoxia or estrogen affect the hepcidin response and impairment of the hepcidin/FPN regulatory system causes iron-induced disorders. The hair follicle is a highly metabolically active mini-organ requiring high availability of both iron and oxygen to remain in anagen. Hepcidin expression is up-regulated by chronic inflammation through the interleukin-6 (IL-6)-Janus kinase 2 (JAK2)-signal transducer and activator of transcription 3 (STAT3) and BMP-mothers against decapentaplegic homolog (SMAD) pathways. A decrease in estrogen also upregulates hepcidin. ^{127,142-146}

1.5 Changes of Gene Expression in Hair Disorders

Several studies have explored the genes associated with various hair disorders. In one study, a total of 1200 differentially expressed genes in AGA and 1360 in SA were identified compared to controls.¹⁴⁷ Of these, 442 genes were unique to AGA, 602 genes were unique to SA, and 758 genes were common to both AGA and SA.¹⁴⁷ Many of the genes required for hair follicle homeostasis were significantly under-expressed in AGA but not in SA when compared to normal scalp tissue. The Androgen Receptor (AR) was upregulated in AGA but not SA. The most significant pathway altered in AGA was Notch signalling, which consisted of 29 genes. A reciprocal negative feedback regulation exists between Notch and AR-dependent pathways. The activation of AR and the concomitant loss of Notch signalling may be contributing factors to hair follicle miniaturisation. Thus, modulating the Notch signalling pathway in AGA may lead to future therapies.¹⁴⁷

In another study in men with AGA, the strongest gene association signal was located intronically in Wnt10A.¹⁴⁸ Wnt10A promotes anagen induction and maintenance of the anagen state.⁶⁸ A reduction in Wnt10A expression leads to the deregulation of the healthy hair cycle, a delay in telogen to anagen transition, and a shortening of the anagen phase, as observed in AGA hair follicles. This suggests that the expression of the Wnt/ β -catenin associated gene plays a major role in hair generation and maintenance in men.¹⁴⁸ However, the same findings could not be confirmed in women with AGA.¹⁴⁹

In a genome wide meta-analysis study in AA, susceptibility gene expression analysis demonstrated expression in relevant immune cells and the hair follicle, and alignment was found with several other autoimmune diseases. The study identified molecular pathways disrupted in AA, including autophagy/apoptosis, TGF- β /Tregs and JAK kinase signalling.¹⁵⁰

Legacy genetic models of hair disorders are not precise, and the post-expression effects of molecular pathophysiological signalling currently appear more reliable.^{151,152}

1.6 A Multi-Molecular Targeting Strategy for Hair Follicle Homeostasis

Having documented hair, the anatomy of the hair follicle, hair and follicle disorders, the biology of the hair follicle and the need for therapies beyond those approved for preventing hair loss, this research now turns its focus towards therapeutic solutions that can regrow hair and restore a person's normal scalp hair phenotype.

Given the limited efficacy and related side effects of the available pharmaceutical-based therapies, there is a steadily growing demand for plant-based medicines and functional cosmetics. Various plants and plant extracts used worldwide for hair care are acclaimed to have hair growth-promoting activity. Further, many of these botanical treatments use formulation strategies consisting of mixtures of herbal extracts designed to simultaneously target more than one of the proposed molecular causes of hair loss.¹⁵³

Therefore, the concept of using a multi-targeting formulation of botanical mixtures could potentially simultaneously regulate a broad spectrum of molecular pathways. Applying this approach for purposes of maintaining or inducing homeostasis in hair follicles could influence the required choreography of cell signalling favourably. The intended effects should result in inducing normal growth, follicle cycling, anagen prolongation, and possibly reversing miniaturised hair. Continuous effective treatment might eventually lead to the safe regeneration of the individual's normal scalp hair phenotype.

1.6.1 Hypothesis

A multi-molecular targeting treatment comprising botanicals, vitamins and minerals regenerates terminal hair in human subjects with androgenetic alopecia.

1.6.2 Aims

To evaluate the effects of multi-molecular targeting botanical extracts, vitamins and mineral (BVM) formulations on the regrowth of hair in adult male and female alopecic scalps over time as measured by:

- Changes in terminal hair density.
- Changes in hair diameter and terminal to vellus hair ratios.
- Sources of changes in terminal hair density.
- Comparisons of terminal hair density changes to other treatments.

2 Materials and Methods

2.1 Materials

A multi-molecular targeting BVM agent, ALRV5XR, was developed to induce restoration of the HF's normal biological homeostasis. In addition, it was designed to target various underlying physiological causes of HL and to promote growth and regrowth of HFs. This multi-molecular targeting design also aimed to induce a counter or normo-regulation of the relevant adverse molecular pathway signals in the hair follicle, as identified Table 1.5, that are thought to impair follicle productivity, inhibit anagen induction or prolongation, and to inhibit and possibly reverse various pathological effects such as scarring and immune or inflammatory effects associated with hair follicle disorders as per Table 2.1. The extracts from botanicals were selected where evidence of effect in hair regrowth or a relevant mechanism of action (MOA) could be determined from the literature. In the particular requirement to replicate, activate and differentiate dermal papilla stem cells and stimulate the Wnt/ β -catenin pathway, various candidate screening and MOA studies were performed, which can be found in more detail in the referenced patent manuscript.¹⁵⁴ The BVM test agent was formulated to be delivered orally or topically as per Table 2.2.

Randomised placebo-controlled clinical trials (RCTs) were designed to observe the intervention outcomes of ALRV5XR in humans. The RCTs received ethics approval and were registered with Clinicaltrials.gov (details are provided in the introductions to Chapters 3 – 5). The results of each RCT were analysed and published in peer-reviewed journals. (See Results in Chapters 3 and 4). In addition, a comparison of outcomes between women and men was published in Chapter 5. Finally, a systematic review (SR) and network meta-analysis (MA) was designed and conducted to compare the outcomes of all treatments in the literature and published as Chapter 6.

2.1.1 Botanical formulation strategy

Table 2.1: Botanicals regulating molecular pathways in hair growth

This table identifies botanical candidates with evidence of regulating relevant molecular pathway signalling in the hair follicle.

Function	Signal/Pathway	Compounds
Stem Cell Activation & Differentiation		
1	Wnt/ β -catenin	Eclipta prostrata ^{154,155} , Polygonum multiflorum ^{156,157} , Scutellaria baicalensis ¹⁵⁸ , Sophora flavescens ¹⁵⁹ , Vitis vinifera ¹⁶⁰
2	Shh	Polygonum multiflorum ¹⁵⁶
3	BMP	Astragalus membranaceus ¹⁶¹ , Pisum sativum ¹⁶²
4	Noggin	Pisum sativum ¹⁶³
5	Notch	Scutellaria baicalensis ¹⁶⁴
6	TERT	Astragalus membranaceus ¹⁶⁵
7	mTORC1	Astragalus membranaceus ¹⁶⁶
Angiogenesis		
8	VEGF	Angelica sinensis ¹⁶⁷ , Astragalus membranaceus ¹⁶⁶ , Caffeine ¹⁶⁸
Growth Promotion		
9	FGF	FGF-7: Eclipta alba ¹⁶⁹ , Polygonum multiflorum ¹⁵⁷ , Pisum sativum ¹⁶² , Sophora flavescens ¹⁷⁰ . FGF-18: Momordica charanita ¹⁷¹
10	HGF	Astragalus membranaceus ¹⁷² , Momordica charanita ¹⁷³
11	IGF	Sophora flavescens ¹⁷⁰
12	PPAR	Rosmarinus officinalis ¹⁷⁴
Immune Modulation and Inflammation		
13	COX	Punica granatum ¹⁷⁵ , Scutellaria baicalensis ¹⁷⁶ , Sophora flavescens ¹⁷⁷
14	IFN	Ganoderma lucidum ¹⁷⁸
15	IL-1,6,10,12	Ganoderma lucidum ¹⁷⁸ , Rosmarinus officinalis ¹⁷⁹ , Scutellaria baicalensis ¹⁸⁰
16	JAK/Stat	Scutellaria baicalensis ¹⁸¹
17	NF- κ B	Astragalus membranaceus ¹⁸² , Ganoderma lucidum ¹⁸³ , Malus domestica ¹⁸⁴ , Rosmarinus officinalis ¹⁸⁵ , Scutellaria baicalensis ¹⁸⁶ , Sophora flavescens ¹⁷⁷
18	Prostaglandins	Rosmarinus officinalis ¹⁸⁷ , Scutellaria baicalensis ¹⁸⁸
19	TGF- β	Astragalus membranaceus ¹⁸⁹ , Eclipta alba ¹⁹⁰ , Vitis vinifera ¹⁹¹
20	TNF- α	Ganoderma lucidum ¹⁷⁸ , Rosmarinus officinalis ¹⁸⁷ , Scutellaria baicalensis ¹⁸⁸
Hormone Regulation		
21	5 α -Reductase	Sophora flavescens ¹⁷⁰ , Caffeine ¹⁹² , Ocimum basilicum ¹⁹³ , Ganoderma lucidum ¹⁹⁴ , Polygonum multiflorum ¹⁹⁴
22	DHT	Rosmarinus officinalis ¹⁹⁵ , Scutellaria baicalensis ¹⁹⁶
23	Hepcidin/ FPN	Angelica sinensis ¹⁹⁷
Treatment Resistance		
24	P-glycoprotein	Astragalus membranaceus ¹⁹⁸ , Rosmarinus officinalis ¹⁹⁸
25	Cytochrome P450	Angelica sinensis ¹⁹⁸ , Astragalus membranaceus ¹⁹⁸ , Scutellaria baicalensis ¹⁹⁸

2.1.2 Vitamin, mineral and cofactor formulation strategy

A range of vitamins, minerals and cofactors have been selected based on various monographs associated with relevant aspects of supporting normal hair physiology.^{199,200} A reference of approved claim monographs from Health Canada's Natural Products Directorate was used. Dosages were determined from recommended daily allowances from Health Canada and the USA National Institutes of Health (NIH).^{199,200}

2.1.3 Formulating ALRV5XR and methods of administration

The candidate botanical components, as per Table 2.1, were evaluated for respective oral and topical use with dosages, ratios and concentrations developed from an extensive literature review that can be found in the reference patent.¹⁵⁴ The supplement formulation was concentrated into an acid-resistant oral capsule dosage form to maximise absorption and bioavailability of the polyphenols from the botanicals through the intestines.²⁰¹ Each capsule was formulated to be taken as one 842 mg capsule BID. The topicals were formulated with dimethyl sulfone as a common agent used to enhance percutaneous delivery of lipophilic compounds into the scalp and hair follicles. Similarly, studies administering natural hydrophilic compounds into humans through hair follicles were found in blood within five minutes after administration, peaking at about one hour, and their presence has been reported to remain in the hair follicle for up to 48 hours.^{202,203} The three topical dosage forms were designed to be administered to the scalp and hair follicles once daily as part of a normal personal care regimen as Shampoo (3–7 ml), Conditioner (3–7 ml) and Serum (1 ml). Practical intervals between administrations were estimated at 12 hours due to estimated half-life ranges of Wnt/ β -catenin stimulation under different conditions from ~1 to <8 hours.²⁰⁴

Table 2.2: BVM investigational agent active ingredients

Table 2.2 summarises the active botanical, vitamin, mineral and cofactor ingredients in each dosage form.

	Active Ingredients
Oral (capsule)	Angelica sinensis, Ascophyllum nodosum, Astragalus membranaceus, Coenzyme Q10, Copper, Eclipta prostrata, Folate, Ganoderma lucidum, Malus domestica, Moringa oleifera, Niacin, Pantothenic acid, Polygonum multiflorum, Punica granatum, Riboflavin, Rosmarinus officinalis, Scutellaria baicalensis, Selenium, Silicon, Sophora flavescens, Vitamin A, Vitamin B6, Vitamin B7, Vitamin B12, Vitamin D, Vitis vinifera, and Zinc
Shampoo	Caffeine, Eclipta prostrata, Lupinus, Momordica charantia, Moringa oleifera, Pyrus malus, Rosmarinus officinalis, Vitis vinifera, Scutellaria baicalensis, Vitamin B7
Conditioner	Caffeine, Eclipta prostrata, Lupinus, Momordica charantia, Moringa oleifera, Pyrus malus, Rosmarinus officinalis, Vitis vinifera, Scutellaria baicalensis, Vitamin B7
Follicle serum	Caffeine, Eclipta prostrata, Lupinus, Momordica charantia, Moringa oleifera, Ocimum basilicum, Pisum sativum, Pyrus malus, Rosmarinus officinalis, Vitis vinifera, Scutellaria baicalensis, Vitamin B7

See patent reference for further details.¹⁵⁴

2.2 Studies Design

2.2.1 Rationale for selecting subjects with androgenetic alopecia

Androgenetic alopecia (AGA), also known as male or female pattern hair loss, is the most common form of hair loss in men and women.⁴⁴ It is a polygenic, heritable, age-dependent process that results in a progressive, non-scarring decline in scalp hair density, and is characterised by gradual loss of terminal hair (TH) and follicular miniaturisation to vellus-like hair fibers in a generally sex-dependent pattern, leading to the eventual irreversible loss of functional potential of hair follicles (HFs).^{34,44,205} AGA results in a reduction in total hair count and a terminal hair to vellus hair (TH/VH) ratio that is clinically identifiable when 15–30% of hair is lost or when the TH/VH ratio approaches 4 to 1.^{15,44,206-208}

The aetiology of AGA is unclear, however observations from ancient times that castrati did not lose their hair was linked to testosterone in 1942.²⁰⁹ The anti-androgen drug, finasteride, a 5 α -reductase inhibitor that restricts the conversion of testosterone to dihydrotestosterone (DHT), was initially approved for the treatment of benign prostate hyperplasia. It was also found to

have a hair regrowth effect, and was subsequently approved for the treatment of male patterned hair loss in the 1990's.¹² This further developed the androgen receptor sensitivity hypothesis as the primary cause of male AGA. Studies suggest that androgen-mediated mechanisms are probably involved in female AGA in some women, but the evidence is weaker than in male AGA. Finasteride is contraindicated for women of childbearing age, and it is thought that non-androgenic pathways may be more dominant to their aetiology. Finasteride has some hair regrowth effect in men, but does not reverse miniaturisation.²¹⁰ Interestingly, minoxidil, which is effective in treating AGA in both men and women, is not known to have anti-androgenic properties. One of the observed effects of AGA is the increased frequency of hair cycling accompanied by a shortened duration of the anagen and the prolonging of telogen often in a state of kenogen, which have all been linked to many of the dysregulated pathways of Table 1.5 that also include 5 α -Reductase/DHT inhibition.

Hair follicle maintenance and regeneration is a complex process governed by the coherent expression of more than 1000 genes and the expression of the Wnt/ β -Catenin associated genes has been shown to play a major role in hair generation and maintenance.^{147-149,211} Although AGA is thought to be familial, 300 genetic risk loci have been identified for male AGA but little is known about the exact molecular mechanisms through which the associated variants exert their effects on male AGA pathophysiology.¹⁴⁹ Susceptibility locus/gene studies for female AGA have been restricted to candidate gene approaches, and could not clearly identify any candidates, suggesting that the aetiology differs substantially between male and female AGA.^{211,212} Interestingly, AGA is thought to have many commonalities with senescence of the hair follicle. A study in males examined gene expression in scalp biopsies by measuring RNA and found 442 genes were unique to AGA, 602 genes were unique to senescent alopecia (SA), and 758 expressed genes were found common to both AGA and senescent alopecia,

nevertheless the dominant expressions suggest male AGA and SA may represent two independent hair disorders and that non-androgen pathways may also contribute to hair loss.^{57,147}

Telogen effluvium (TE) is a common form of hair loss, which generally self-resolves once the underlying trigger is addressed. It is more common in women than men and differential diagnosis between AGA and TE in women can be challenging. The cardinal feature of TE is increased hair shedding, usually as premature exogen. The inability to maintain an anagen cycle is linked to many of the dysregulated pathways of Table 1.5, particularly those required for stem cell activation and differentiation such as Wnt/ β -catenin, Shh, BMP, Noggin, Notch, TERT and mTORC1.

AGA occurs in approximately half the population, and together, AGA and TE account for approximately 95-98% of the hair loss prevalence rate. SA is often diagnosed as AGA.

Having considered the complexity of AGA aetiology, some similarities to that of TE and SA, the large, affected population and the potential for regulatory viability as a dietary supplement or functional cosmetic, it appeared feasible that both men and women with AGA or idiopathic TE should be studied for hair regrowth effects with the multi-targeting BVM agent.

Therefore, the research results in Chapters 3 – 5 are based on recruiting subjects with AGA or TE. All subjects studied in Chapters 3 – 5 were diagnosed with AGA, although some women were diagnosed with both AGA and TE. Chapter 6 compares outcomes from the RCTs in Chapters 3 and 4 to RCTs in the literature of subjects with AGA treated with other

interventions. The primary results of these studies report outcomes of changes in terminal hair as the principal marker of the scalp hair phenotype.

2.2.2 Randomised placebo-controlled clinical trials

Two randomised placebo-controlled clinical trials were designed—one in women and one in men. The hypothesis was that a multi-molecular targeting treatment comprising botanicals, vitamins and minerals regenerates terminal hair in human subjects with androgenetic alopecia. The primary objective of these 24-week trials was to evaluate the safety and efficacy of the BVM, ALRV5XR, in terminal hair (TH) regrowth in women and men with AGA or TE.

Enrollment eligibility included healthy women and men of 18-65 years of age with AGA or TE, Fitzpatrick skin type I-VI, of female hair loss pattern of Ludwig I-II or male hair loss pattern of Hamilton- Norwood I–VII.

Exclusion criteria included all other types of alopecia, or subjects with different hair, scalp or skin disorders, infestations, undernutrition, poor hygiene, abnormalities, surgical correction, injury in or around assessment areas, history of hair transplants or a known history of HIV/AIDS or autoimmune conditions. In addition, subjects or their partners who planned on becoming pregnant during the study or who could not commit to the protocol regimen or follow-up visits or, in the opinion of the investigating dermatologist, were ineligible and excluded.

Each trial was designed with 46 subjects randomised 1:1 into the ALRV5XR treatment or placebo arms. The duration of the trials was 24 weeks with a 12-week mid-point. At baseline, 12 and 24 weeks, independent dermatologists completed a physical evaluation of the subjects,

including the hair and scalp; global photos were taken of the scalp in a stereotactic device; phototrichograms were taken at the trichoglyph after clipping the hair in the target area to < 1mm, dying the hair black for light-skinned subjects and red for dark-skinned subjects, and applying a tattoo for repeat measures; anthropometrics, blood and urine labs were taken; and a quality of life instrument was completed. Subjects were issued diaries for recording daily treatment administration and adverse events. Study administration materials were dispensed and recorded with subject diaries at each visit. Blood, urine and phototrichograms were sent to independent laboratories for analysis.

The Primary outcomes of these two RCTs measured changes in TH density defined as hair with a diameter >40 μm . Vellus or vellus-like hair (VH) was defined as hair with a diameter <40 μm . The commonly used 40 μm parameter in RCTs can observe transitions between terminal and vellus-like hair. This also helps accommodate for inherent marginal procedural errors, the varying oval shapes and orientations of the hair shafts, and the estimated increase of 5 μm in diameter due to the hair dying procedure of the phototrichogram.

2.2.3 Sex differences study

This study analysed the pertinent similarities and differences in outcomes between women and men using the primary and secondary data from the two placebo-controlled randomised clinical trials of the BVM, ALRV5XR vs Placebo. New trichometric data were reported using novel graphic methods that provided insight into the different hair regrowth dynamics observed in women and men.

This study hypothesised that ALRV5XR, an effective treatment in regenerating and maintaining TH in women and men, achieves these effects by both reactivating dormant telogen HFs and thickening of AGA miniaturised (vellus-like) hairs.

2.2.4 Systematic review and Bayesian network meta-analysis

The objective of this study was to examine, in women and men, the relative 24-week efficacy of all commercially available AGA treatments using standard dosage and administration routes. In addition, treatment outcomes were followed over time to determine treatment resistance effects and the continuous efficacy of each treatment in maintaining or improving an existing phenotype.

Eligible studies included RCTs of men or women diagnosed with AGA using either an approved or off-label drug, device, or commercially relevant treatment such as platelet-rich plasma (PRP) and natural products for at least 24 weeks, and which measured density changes of scalp TH, or non-vellus hair of diameter $> 30 \mu\text{m}$ per unit area. Methods had to include performing macrophotography or phototrichogram techniques on hair clipped to approximately 1 mm at the same position on the scalp, typically marked by tattoo, for repeated measures. Only RCTs were eligible to maximise the quality of evidence.

Studies were excluded if results were not separated for women or men, outcomes of interest were not measured or not reported, study size had fewer than 20 subjects or if a treatment arm had less than ten subjects. Studies were also excluded when hair was counted manually or from biopsies or phototrichoscopies of unclipped hair, or when results were measures of total hair count that included vellus hair, percentage changes, or if there was no standard error that was either reported or that could be derived at 24 weeks, or when the design had a treatment

crossover before 24 weeks, or when the intervention was experimental or not commercially available. The most recent article was selected from studies in which interim or partial results were previously published. Studies published in languages other than English or German were excluded.

The primary outcome measured the efficacy of each treatment vs Placebo at 12 and 24 weeks using Bayesian network meta-analysis, measured in TH/cm² with 95% credible intervals (CrI). Secondary outcomes were treatment vs treatment efficacy comparisons, frequentist network meta-analysis with 95% confidence intervals (CI) and weighted averages. The quality of evidence was evaluated using qualitative Cochrane risk of bias assessments and quantitative Confidence in network meta-analysis (CiNeMA) techniques.

3 Results – Paper 1: Safety and efficacy of ALRV5XR in women with androgenetic alopecia or telogen effluvium: A randomised, double-blinded, placebo-controlled clinical trial.

Feldman, Peter R., Klaus M. Fiebig, Charles Piwko, Boris M. Mints, Dennis Brown, Deborah J. Cahan, and Jaime Guevara-Aguirre. “Safety and Efficacy of ALRV5XR in Women with Androgenetic Alopecia or Telogen Effluvium: A Randomised, Double-Blinded, Placebo-Controlled Clinical Trial.” *EclinicalMedicine* 37 (2021/07/01/ 2021): 100978. <https://dx.doi.org/https://doi.org/10.1016/j.eclinm.2021.100978>.²¹³ Supplementary materials are accessible from the link provided in the publication and appended to this chapter.

3.1 Introduction to Paper 1

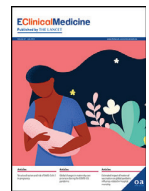
In women, scalp hair loss (alopecia) is a common ageing and senescing condition. It usually presents as androgenetic alopecia (AGA) or telogen effluvium (TE). ALRV5XR is a novel BVM treatment aiming to regenerate a normal hair phenotype by targeting multiple molecular pathways linked to hair growth promotion and hair follicle stem cell activation. The primary objectives of this 24-week trial were to evaluate the safety and efficacy of ALRV5XR in terminal hair (TH) regrowth in women with AGA or TE.

The protocol for this clinical trial was registered on ClinicalTrials.gov, Identifier: NCT04450602. <https://clinicaltrials.gov/ct2/show/NCT04450602>



Contents lists available at ScienceDirect

EClinicalMedicine

journal homepage: <https://www.journals.elsevier.com/eclinicalmedicine>

Research paper

Safety and efficacy of ALRV5XR in women with androgenetic alopecia or telogen effluvium: A randomised, double-blinded, placebo-controlled clinical trial

Peter R. Feldman^{a,*}, Klaus M. Fiebig^{a,*}, Charles Piwko^b, Boris M. Mints^c, Dennis Brown^d, Deborah J. Cahan^a, Jaime Guevara-Aguirre^{e,f,g,h}

^a Arbor Life Labs, Toronto, Ontario, Canada^b CHP Pharma Inc, Thornhill, Ontario, Canada^c BG&B International, Vilnius, Lithuania^d Valent Technologies, Menlo Park, California, USA^e Universidad San Francisco de Quito (USFQ), Quito, Ecuador^f Institute of Endocrinology, Metabolism, and Reproduction (IEMR), Quito, Ecuador^g Maastricht University, Maastricht, The Netherlands^h College of Medicine, University of Florida, Gainesville, Florida, USA

ARTICLE INFO

Article History:

Received 26 February 2021

Revised 20 May 2021

Accepted 3 June 2021

Available online 24 June 2021

Keywords:

Androgenetic alopecia
Telogen effluvium
Female pattern hair loss
Hair restoration
Minoxidil
Finasteride
Botanical
Women's health
Menopause
Senescence
Aging
Wnt/beta-catenin
Multi-targeting
Stem cell
Hair regeneration
Regenerative medicine
COVID-19
PRP

ABSTRACT

Background: Scalp hair loss (alopecia) in women is a common ageing and senescing condition. It usually presents as androgenetic alopecia (AGA) or telogen effluvium (TE) and often has pronounced psychological consequences. ALRV5XR is a novel treatment aiming to regenerate a normal hair phenotype by targeting multiple molecular pathways linked to hair growth promotion and hair follicle stem cell activation. The primary objectives of this 24-week trial were to evaluate the safety and efficacy of ALRV5XR in terminal hair (TH) regrowth in women with AGA or TE.

Methods: This randomised, double-blind, placebo-controlled trial was performed in a USA community clinic. Healthy women 18–65 years of age with AGA or TE of Ludwig classification I–II and Fitzpatrick skin type I–VI were enrolled. They were allocated in a 1:1 ratio into ALRV5XR or placebo treatment groups using a random number table. Masked dermatologist assessments, phototrichograms and blood samples were obtained at baseline, 12 and 24 weeks. Subjects were given a masked treatment regimen of oral capsules, shampoo, conditioner and follicle serum for daily administration. Main outcomes were absolute and per cent changes in TH density and response rates. The trial was registered with clinicaltrials.gov (NCT04450602) and is completed.

Findings: 46 subjects (23 ALRV5XR, 23 placebo) were enrolled between April 3 and October 20, 2018. Five subjects dropped out and two were non-compliant. Thirty-nine subjects completed the trial (18 ALRV5XR, 21 placebo). At 24 weeks, the absolute change in TH density improved by 30.1THs/cm² (95% CI: 15.1–45.1; p=0.0002), and the relative density increased by 19.7% (95% CI: 8.0%–31.4%; p=0.0016). The odds ratio for being a responder (≥0 change) was 2.7. Efficacy increased 133% from week 12 to 24. Efficacy outcomes were similar in AGA and TE subjects. 66.7% of the ALRV5XR group responded by regrowing 40THs/cm² or more hair. No adverse events were reported.

Interpretation: In women with AGA or TE, ALRV5XR treatment significantly increased hair regrowth without adverse events. ALRV5XR displayed a multi-fold improved efficacy and response rate when compared to published trials of standard therapy. Progressive acceleration of TH regrowth suggests regeneration of the structure and function of non-productive telogen follicles and prolonged treatment may restore a normal hair phenotype.

Funding: This clinical trial was funded by Arbor Life Labs, Toronto, Ontario, Canada.

© 2021 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>)

Funding: Arbor Life Labs.

* Corresponding authors

E-mail addresses: petefeldman@gmail.com (P.R. Feldman), klausfiebig@gmail.com (K.M. Fiebig).

<https://doi.org/10.1016/j.eclinm.2021.100978>

2589-5370/© 2021 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>)

1. Introduction

Alopecia or hair loss (HL) of the scalp is a common clinical finding associated with ageing and cellular senescence [1,2]. It is emotionally

Research in context

Evidence before this study

Hair loss (HL) is a sign of normal ageing. It is associated with impaired quality of life in women, metabolic syndrome and increased mortality. Most pharmaceuticals or botanical, vitamin and mineral (BVM) formulations use high-dose compounds targeting few pathways, which lead to limited effectiveness and adverse effects. Similarly, minoxidil, the only FDA approved treatment for HL in women, is effective in only 13–40% of women. A literature review of hair follicle (HF) biology, compounds influencing hair growth and regrowth, and clinical trials, showed that HF regeneration and maintenance is associated with the expression of more than 1,000 genes, and is dependent on HF stem cell (HFSC) activation. It is also influenced by a complex, rhythmic behaviour of multiple pathways that often have opposing influences. Therefore, a single pathological cause of HL is unlikely, and present perspectives suggest that the next generation of HL therapies should aim to address multiple targets by systemic, topical or a combination delivery regimen. While activation of the Wnt/ β -Catenin signalling pathway appears to be a dominant factor for hair regrowth and maintenance, the modulation of certain cytokines, growth factors and hormones are also key components of hair physiology. We researched the mechanisms of many BVMs relevant to HF physiology. After screening candidate BVMs in HFSCs *in vitro* and HFs *in vivo*, we developed ALRV5XR from multiple BVMs for oral or topical administration, with the aim of modulating >20 of the accepted pathways involved in healthy HF physiology.

Added value of this study

ALRV5XR, studied as an oral and topical regimen in women with androgenetic alopecia (AGA) or telogen effluvium (TE), demonstrated a 4-fold improvement in terminal hair regrowth and response rates when compared to published results of trials using standard therapy, with no side effects. ALRV5XR's multi-molecular targeting BVM approach induces homeostasis in the ageing HF and might restore a woman's normal scalp hair phenotype.

Implications of all the available evidence

The multi-targeting compound ALRV5XR might potentially become a new standard of care for women with HL. Future research should focus on evaluation of its long-term safety and efficacy. Moreover, further elucidation of the mechanisms underlying ALRV5XR action might justify its utility in other ageing tissues.

distressing for both sexes, but women are psychologically more affected than men as suggested by parameters of self-esteem, body image and quality of life [3]. The most common forms of female hair loss are androgenetic alopecia (AGA), also called female-pattern hair loss (FPHL), and telogen effluvium (TE) [1]. AGA is thought to have many commonalities with senescence of the hair follicle (HF) and 758 expressed genes were found common to both AGA and senescent alopecia [2,4]. AGA is a heritable, age-dependent process that results in a progressive decline in scalp hair density in a sex-dependent pattern [5]. While onset of hair thinning in women begins between the ages of 12 and 40, it becomes noticeable in about half of the population by the fifth decade [6]. Alopecia has been related to hormonal disturbances, chronic medical conditions, use of certain drugs, topical

scalp treatments, and chemo and radiotherapy [1]. It has also been linked to sudden weight loss, low protein intake, stress and pollution [1]. Miscellaneous hair disorders and alopecia have also been associated with deficiencies in minerals such as copper, iron, selenium, and zinc as well as of vitamins such as niacin and vitamin D [7].

The healthy adult scalp has an area of approximately 700 cm² with a total hair count ranging from 100,000 – 150,000 hairs [1,8]. Ninety per cent of scalp hairs are large, pigmented, and are known as terminal hairs (TH) [9]. They have a shaft diameter of 30 – 150 μ m [9]. The remaining ten per cent are called vellus hairs (VH), and are barely visible, depigmented hairs [9]. Individual hairs are produced by HFs, which are epithelial skin mini-organs that modulate and sustain cyclic hair regrowth over repeated hair cycles [2]. The human scalp has the greatest density of HFs on the body, ranging from 118 to 350 HFs per cm² [9]. Hair density in women is highest from age 20 – 30 years and decreases thereafter at an increasing rate [10].

AGA and TE in women have different clinical features and prognoses [1]. AGA is diagnosed by a high diversity in hair shaft diameter, while TE is diagnosed by an excess of telogen follicles [1,11]. Although most women with AGA have diffuse thinning of the crown, the frontal hairline remains intact, and they rarely become bald [1]. AGA is linked to the metabolic syndrome and is an independent predictor of mortality from diabetes mellitus and heart disease [12]. TE is a medical sign related to underlying conditions rather than a discrete disorder, and can resolve spontaneously in healthy women [1]. Triggering events for TE include acute physical stressors such as surgery, illness, or weight loss, hormonal imbalances, psychological stress, post-partum state, drugs, nutritional deficiencies and scalp inflammation [1]. Interestingly, cases of TE onset have been reported 3 weeks to 3 months after diagnosis of COVID-19 [13]. In some individuals, TE has been associated with senescent thinning, autoimmune disease, or nutritional deficiencies, all conditions which may confuse its proper distinction from subjects affected by AGA [1].

There is increasing evidence that AGA is the consequence of organ-specific premature ageing of HFs [1]. Indeed, age-related hair regrowth reduction and diminished hair shaft diameter, along with abnormalities of the hair cycle, including decreased duration of the growth phase (anagen), increased time between the loss of hair (exogen) in the resting stage (telogen), and the emergence of a replacement hair (neogen) have all been reported [14]. Affected HFs miniaturize, thereby leading to a gradual replacement of THs by VHs, eventually resulting in HF deletion [15,16].

A literature review on HF biology, compounds influencing hair growth and in pathology studies, showed at the molecular level, that hair growth and the physiological HF cycle are influenced by a complex and rhythmic behaviour of multiple intricate signalling pathways, often with opposing influences, including those of various cytokines, hormones, prostaglandins, and growth factors [17]. This implies that it is unlikely to ascertain a single and discrete cause of HL. Consequently, and to improve efficacy, the next generation of HL therapies will likely be a combination therapy consisting of agents that maintain normal growth and promote regrowth, combined with agents that delay catagen entry, while relieving negative pressure on the follicle [18]. Most relevant and as a dominant physiological influence, the canonical Wnt/ β -Catenin signalling in HF stem cells controls hair cycle induction and maintenance [2]. This signalling cascade is less active in aged vs. young telogen HF stem cells; [2] moreover, inactivation of the Wnt/ β -Catenin signalling pathway has been implicated in AGA [17,18].

At present, most available pharmaceutical and non-pharmaceutical hair regrowth treatments target a single or a limited number of pathways by using high-dose active ingredients. They have relatively limited efficacy or are effective only in a minority of women, while associated adverse effects occur at non-negligible incidence rates [18,19], possibly due to the elevated doses needed to attain effective results. Topical minoxidil, at concentrations of 2% and 5%, was approved for treatment

of AGA in women in 1991 and 2014, respectively. It remains as the only FDA approved pharmaceutical to treat women affected with this hair disorder. Currently, there is no FDA approved treatment for TE. The average improvement in hair density (minoxidil vs. placebo) in published trials in women with AGA ranged from 12.4 to 15.1 THs/cm² after 48 weeks of treatment with 2% and 5% minoxidil, respectively [19–21]. Only 13–40% of female subjects with AGA responded to minoxidil treatment [21,22]. While various adverse events were observed, increased facial hair growth was reported by 22% and 46% of participants using 2% and 5% minoxidil, respectively [21]. Minoxidil is a potassium channel opener and has been associated with several mechanisms of actions (MOAs) such as increased HGF, PGE and VEGF expression, EGF inhibition and is also thought to stimulate canonical Wnt/ β -Catenin signalling [18,19,23]. Yet despite these multiple MOAs, TH density changes in AGA subjects treated with minoxidil tend to peak at 12–16 weeks, declining thereafter [24]. Of note, following treatment cessation, there is rapid regression to a density lower than at baseline [24]. This treatment response has been explained as a triggering of the HF in the latent part of telogen into anagen without a prolongation of anagen or reversal of follicular miniaturization [24]. Anti-androgen therapy using finasteride, which inhibits the conversion of testosterone to dihydrotestosterone, while effective in men, has shown no efficacy in randomised placebo-controlled trials (RCTs) in women and is contraindicated in women of childbearing potential [19]. Nevertheless, several uncontrolled studies in women have reported improvements in hair growth [19]. Recombinant human insulin-like growth factor-I (rhIGF-I) treatment has induced the appearance of normal hair and recovery of hairline in subjects with growth hormone insensitivity and concomitant IGF deficiency [25]. Combination therapies of topical minoxidil with pharmaceuticals and supplements have been studied in women, showing no significant difference in TH density changes to those of minoxidil monotherapy [19]. RCTs of Platelet-Rich Plasma (PRP) for the treatment of AGA resulted in significant increases in hair density in men [19]. While for women no significant efficacy has yet been demonstrated for PRP in RCTs, several non-RCTs showed promising results [19]. Combination therapies of PRP supplemented by autologous stem cell transplants, micro-needle wounding and low level laser therapy (LLLT), in men as well as in women, have reported significant improvements in hair regrowth [26]. RCTs with anti-androgen molecules, vitamins, minerals, botanical extracts, collagen and various marine extracts have shown limited efficacy in TH regrowth in women [19]. As it can be appreciated from these experiences, there is an unmet need for safe and effective proven therapies for a wide range and severity of hair loss in women.

ALRV5XR is a novel multi-molecular targeting agent aimed to restore the normal biological homeostasis of the HF. The agent is composed of specific compounds obtained from standardized botanical extracts, vitamins and minerals (BVM), and can be delivered orally as a dietary supplement, or topically. In this RCT, and to maximize HF exposure to the treatment, we administered ALRV5XR, both orally and topically as a regimen of dietary supplementation, shampoo, conditioner, and follicle serum. ALRV5XR was designed to support normal biochemical signalling, thereby contributing to normal HF physiology. ALRV5XR is intended to prolong anagen of HFs and induce neogen in involuted HFs. This combination of actions should aid to regenerate the normal scalp hair phenotype. ALRV5XR was designed to modulate, directly or indirectly, several molecular pathways of the HF, which are associated with hair growth and regrowth promotion as well as hair loss inhibition. Among them, activation of the Wnt/ β -Catenin cascade in HF stem cells and dermal papilla cells appears to be the dominant required effect for new hair formation and maintenance of growth [2,17,18,26,27]. Inhibition of targets linked to reducing hair growth is also relevant, as is the case of BMP-4, DHT and androgen regulator 5α -Reductase [17,18]. In addition, increase of VEGF signalling, aimed to enhance blood flow to HFs, is also linked to promotion of hair growth [17,18]. Similarly the regulation of other growth factors such as FGF-7, FGF-18, HGF, IGF, KGF,

and cytokines such as mTORC1, PKC, PPAR γ , Shh, COX, INF- α , INF- γ , Interleukins 1b, 6 and 12, NF- κ B, Prostaglandin D2, TNF- α , and TGF- β are likely to be influenced directly or indirectly by ALRV5XR [17]. Our hypothesis is that ALRV5XR, a multi-molecular targeting approach designed to modulate multiple pathways, is a highly effective treatment in regenerating and maintaining TH in women.

2. Methods

2.1. Study design

This 24-week randomised, double-blind, placebo-controlled trial was conducted in San Francisco, California, USA in a single centre community clinic. The study protocol was granted ethics approval on March 22, 2018, by IRB Services, registered with the FDA #IRB00000776. The trial was registered with ClinicalTrials.gov (NCT04450602), and is completed. A CONSORT checklist has been completed and we declare adherence.

2.2. Sample size calculation

While designing this study we determined that a sample size of 36 subjects, 18 in each group (before dropout), was required to achieve a power of 80% and a significance level of 5%. This sample size should be able to demonstrate a clinically meaningful difference between the ALRV5XR and placebo groups, as reflected in the increase in total hair count from baseline to Week 24. Based on previous studies of minoxidil vs placebo, we calculated this sample size using a two-sample t test power calculation for a one-sided hypothesis test based on a mean difference of 20 hairs/cm², and a standard deviation of 23 hairs/cm². We anticipated a dropout rate of 20% and therefore adjusted the sample size to 23 per group, for a total sample size of 46.

2.3. Study participants

Eligible subjects were healthy adult females, 18 – 65 years of age with AGA, TE, or with self-reported hair thinning, or hair loss, occurring for more than 3 months prior to screening. Hair loss was confirmed by physical examination performed by a certified dermatologist, who eliminated ineligible types of alopecia, hair or scalp conditions, and assigned a clinical diagnosis of AGA or TE, which was later confirmed by phototrichometry. AGA was determined clinically by observing hair thinning and miniaturisation in the region of the scalp that is consistent with Ludwig classification. AGA diagnosis was confirmed by phototrichogram analysis of diversity of TH diameters and graded with the Anisotrichosis score (the ratio of one standard deviation of TH diameters to the mean TH diameter) >20%. TE was diagnosed by a combination of subject history, reports of self-reported thinning hair, shedding and by evaluating signs of general thinning or diffuse hair loss of the scalp, and directly ruling out underlying treatable causes. Phototrichogram analysis of TH telogen rate >20% was used to confirm the TE diagnosis. Ludwig classification was verified from global photographs. Any differences in diagnosis between the dermatologist and phototrichometry were resolved with the available evidence. For remaining subjects with dubious ascertainment, the diagnosis given by the dermatologist was used. For purposes of this study, only a primary diagnosis of AGA or TE was assigned. 72 women were assessed for eligibility and 46 were enrolled in the trial after AGA or TE diagnoses. All subjects signed IRB approved Informed Consent Forms prior to participation (See Fig. 1).

Exclusion criteria included scarring forms of alopecia, chemical or physical burns and traumatic lesions. Psoriasis, scaling, and follicular dermatitis. Fungal or bacterial infection, lice, and flea infestation of the scalp were also cause for exclusion. In addition, subjects with unusual thinning patches, traction alopecia or trichokryptomania, trichothiodystrophy, pili annulati, monilethrix or clear signs of trichodysmorphia, undernutrition or poor hygiene were excluded. Subjects with

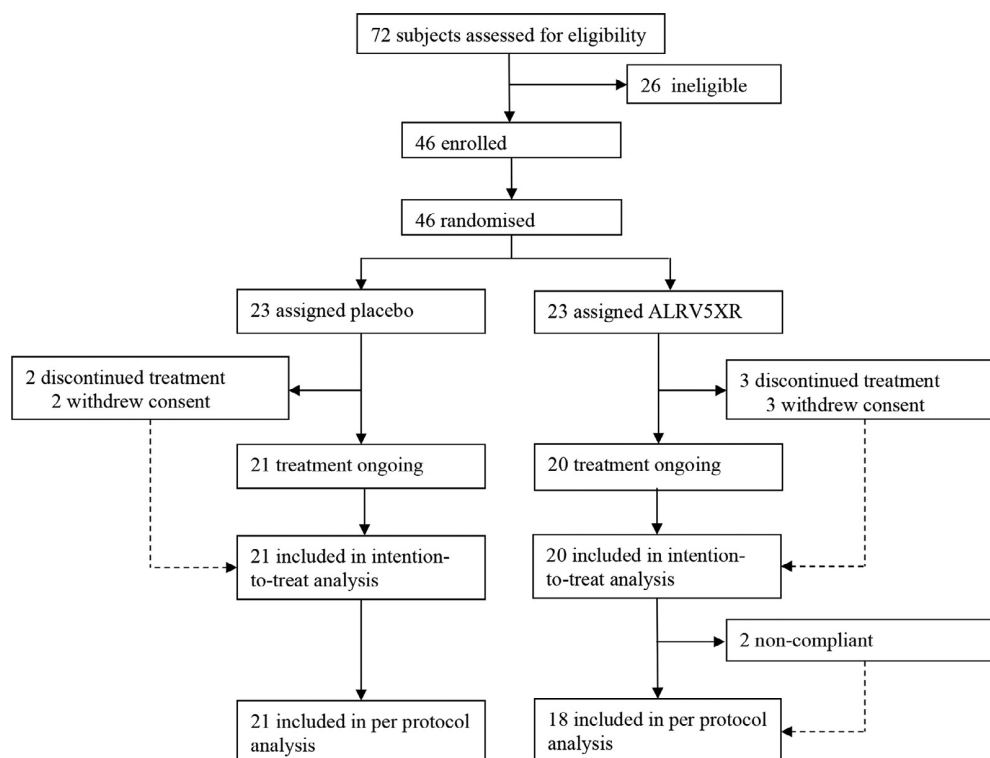


Fig. 1. Profile of Randomised Controlled Trial. 72 subjects were assessed for eligibility which resulted in 21 subjects evaluated in the placebo group and 18 subjects evaluated in the ALRV5XR (treatment) group.

abnormalities such as skin damage in or around assessment areas, and scalp hair loss on treatment surfaces due to disease, injury, or medical therapy as well as those with history of surgical correction of scalp hair loss, hair transplants, and hair weave were also excluded.

Participating women were not allowed to use depilatories, razors, or wax on the scalp if the investigators considered that it might interfere with study assessments. Pregnant or planning to become pregnant women, those who were breastfeeding, or not using recommended contraceptives for the duration of the study, were excluded. Participation of subjects diagnosed with diabetes, endocrine, cardiovascular, renal, or liver disease, hypertension, and those with neurological disease such as Parkinson's disease, stroke, and traumatic brain injury was not allowed. Similarly, women who have recently started (<6 months) hormone replacement therapy were not included.

Women were not allowed to use any medications within 30 days prior to the baseline visit, including natural health products, if these were known to affect hair growth. Subjects with history of malignancy in the past 5 years or undergoing chemo or radiation therapy, and those with a known history of autoimmune disease (e.g., HIV/AIDS, systemic lupus erythematosus, inflammatory bowel disease, alopecia areata, alopecia totalis) were not included. Subjects with uncontrolled thyroid disease or any other disorders that may interfere with the study treatment were also excluded. Evidence of hepatic or renal dysfunction were additional reasons for exclusion.

Eligible subjects with a diagnosis of AGA or TE, Fitzpatrick skin type I-VI, Ludwig hair loss classification I, II or III as clinically established by a certified dermatologist, were enrolled if willing to follow study requirements and procedures, and after signing an IRB approved informed consent form.

2.4. Randomisation and masking

Randomisation and masked allocation into two blinded groups were done as follows: All screened subjects were allocated a

sequential screening number by one dermatologist. A second dermatologist assigned the screening numbers of the eligible subjects sequentially to a biostatistician generated simple randomisation table. This resulted in subjects being randomised in a 1:1 ratio into one of two blinded treatment groups: Group A or B. Both dermatologists were masked from subject identity, characteristics, and diagnosis, to avoid potential bias during the randomisation process.

Study materials and label codes were masked for study subjects, site dermatologists, site personnel, tricho-analyst, and biostatistician. Upon completion of the biostatistician's report, the trial was unblinded and group A became the Placebo group and Group B the ALRV5XR group. An assessment of masking success was evaluated by various factors including baseline characteristics statistics and global photographs, test material dispensing records and inventory, and by observing positive responders in the placebo group.

2.5. Procedures

Similar looking test materials for both groups included oral capsules, shampoo, conditioner, and follicle serum. The ALRV5XR treatment contained botanical extracts, vitamins, and minerals as listed in Table S2 of the supplementary materials. Placebo test preparations contained identical vehicle compounds as those in the active ALRV5XR group products. ALRV5XR and placebo were provided by Arbor Life Labs.

All subjects were required to ingest one oral supplement capsule twice per day, along with daily use of a shampoo, conditioner, and follicle serum for a period of 24 weeks. Subjects were required to maintain a diary of study agent use and report any adverse events.

The duration of the study was approximately 28 weeks, including a screening period designed for eligibility assessment, a baseline visit, and visits at 12 and 24 weeks during the 24-week comparison period.

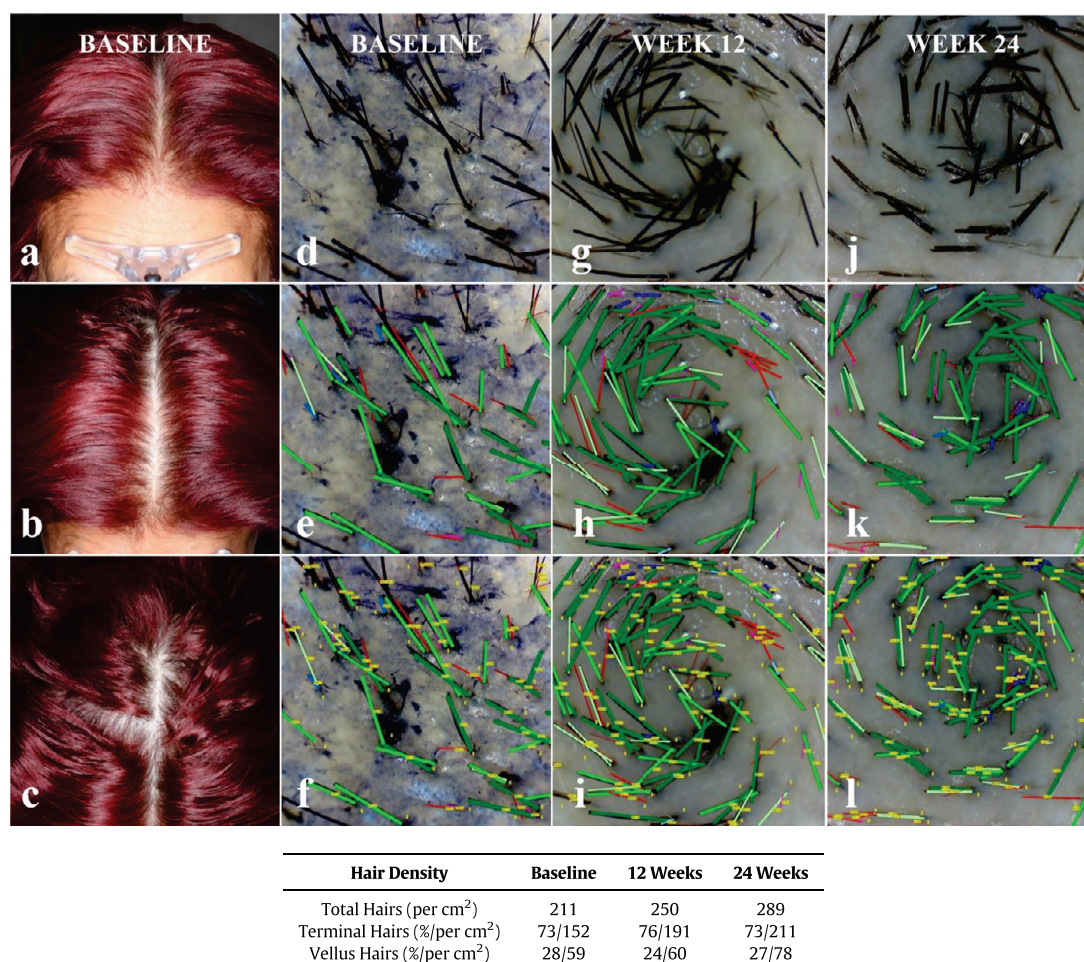


Fig. 2. Sample Global Photographs and Corresponding Phototrichoscopy Computer Image Analysis at baseline, week 12 and week 24. Phototrichoscopy images for Subject: F008 were analysed with the TrichoSciencePro software (version 1.6): a: Baseline (BL) Global Frontal; b: BL Global Top; c: BL Global Vertex; d: BL Phototrichoscopy (PT); e: BL PT Processed (PR); f: BL PT PR & Tagged (TG); g: Week 12 (W12) PT Raw; h: W12 PT PR; i: W12 PT PR & TG; j: Week 24 (W24) PT Raw; k: W24 PT PRD; l: W24 PT PR & TG. m: This analysis resulted in the following sample measurements. Subject: F008; Sex: Female; Age: 54; Ethnicity: Hispanic; Ethnic Hair Type: Latin/Indian/Semitic; Fitzpatrick Skin Type: III; Diagnosis: Androgenetic Alopecia; Ludwig Class-Type: I-3; Phototrichoscopy Area of View: 5.51mm²; Phototrichoscopy Magnification: 35x.

Subjects were evaluated by a certified dermatologist for eligibility, diagnosis of the hair loss condition, and hair loss pattern classification. At weeks 12 and 24, subjects were assessed for changes in health and safety parameters. A 35 mm Nikon digital camera mounted on a Canfield Scientific stereotactic device was used to take 8MP resolution global photographs of the frontal, mid-scalp and vertex of the subject's head, at 0, 45 and 90 degrees (See Fig. 2).

Target area hair counts (TAHC) were performed at baseline, and at week 12 and 24. At baseline, a circular area at the centre of the trichoglyph, located on the vertex region of the scalp was chosen for the TAHC. A permanent ink dot tattoo was placed for precise localization of the target area on subsequent evaluations. Hairs in the target area were clipped to less than 0.5 mm and then dyed. Mineral oil was applied to the target area.

Phototrichoscopic images of the target area were taken at each visit, using a Firefly DE330T Trichoscope with 2MP resolution at 35X magnification, and a 55-10 mm² field of view. A cover glass was fixed to the lens of the trichoscope to orient the hair shafts in the target area, and at 90 degrees to the lens.

Phototrichoscopy images were evaluated by a blinded tricho-analyst using the TrichoSciencePro version 1.6 computer software. Terminal (non-vellus) hair density was determined by counting hairs in the field of view and by measuring their thickness. It was required that hairs be fully visible from the trimmed end to the exiting position of a scalp

pore. THs were identified by a hair diameter greater than or equal to 40 μ m, while VHs were those with a diameter of less than 40 μ m. Digital images were automatically computer analysed to determine hair count and to measure hair shaft diameter. This was repeated and compared using semi-automatic and manual detection, and by diameter measurement performed by a tricho-analyst. TH density was calculated based on the number of THs in the TAHC per cm². (See Fig. 2).

2.6. Outcomes

The purpose of this study was to evaluate the efficacy and safety of ALRV5XR used for hair regrowth in women with AGA, TE, or self-reported thinning hair, over a 24-week period. The primary efficacy objective was measurement of the terminal (non-vellus) hair density change from baseline to week 24, as measured by terminal hair count (THC) and evaluation of TH regrowth per cent change. Secondary objectives included evaluation of the TH density change from baseline to week 12 and assessment of ALRV5XR response rates, as measured in graduated intervals of TH regrowth changes at weeks 12 and 24. Non-responders were individuals whose TH density declined from baseline. Quality of life (QoL) questionnaires were completed by subjects at each visit. Baseline QoL data were recorded using an instrument that was not compatible with the instrument used for weeks 12 and 24. Therefore, only changes in QoL between weeks 12 and 24 were evaluable. Safety

Table 1
Baseline characteristics by treatment group (ITT Population).

	Placebo (N=21)	ALRV5XR (N=20)
Age (years)		
Mean ($\pm$ SD)	51.6 ($\pm$ 10.7)	50.1 ($\pm$ 9.1)
Range	25 - 64	24 - 62
Anthropometrics		
Height (cm)	159.0 ($\pm$ 4.5)	161.1 ($\pm$ 8.2)
Weight (kg)	63.0 ($\pm$ 12.0)	63.9 ($\pm$ 11.9)
BMI (kg/m^2)	24.9 ($\pm$ 4.6)	24.6 ($\pm$ 4.3)
Blood Pressure (mmHg)	118.1/74.7 ($\pm$ 10.8/5.7)	118.8/76.5 ($\pm$ 8.3/7.0)
Trichometry		
Terminal Hair Density (per cm^2)	141.6 ($\pm$ 32.5)	147.0 ($\pm$ 31.3)
Vellus Hair Density (per cm^2)	47.9 ($\pm$ 22.6)	33.1 ($\pm$ 20.2)
Ethnicity		
Caucasian	3 (14%)	1 (5%)
Chinese	12 (57%)	13 (65%)
Filipino	0 (0%)	1 (5%)
Hispanic	4 (19%)	3 (15%)
Japanese	2 (10%)	1 (5%)
Mongolian	0 (0%)	1 (5%)
Fitzpatrick Skin Type		
I	3 (14%)	4 (20%)
II	8 (38%)	8 (40%)
III	7 (33%)	8 (40%)
IV	2 (10%)	0 (0%)
VI	1 (5%)	0 (0%)
Diagnosis		
AGA	11 (52%)	16 (80%)
TE	10 (48%)	4 (20%)
Ludwig Classification-Type		
I-1	3 (14%)	2 (10%)
I-2	4 (19%)	11 (55%)
I-3	9 (43%)	5 (25%)
I-4	1 (5%)	1 (5%)
II-2	0 (0%)	1 (5%)
Missing	4 (19%)	0 (0%)

Abbreviations: $\pm$ or SD=standard deviation.

was reviewed by dermatologist assessment, subject reporting, and laboratory testing of blood and urine samples.

2.7. Statistical analysis

Baseline comparisons of demographic and physical characteristics of treatment and placebo groups were performed using Welch's two sample t-test which allows comparison of group means with differing variances. This method was also used to compare changes within each treatment group from baseline to week 12 and from baseline to week 24, and then confirmed with analysis of variance (ANOVA) using Fisher's F-test. For comparison of categorical variables between treatment groups at baseline, Pearson's chi-squared test (with simulated p-value) was used [28,29].

Table 3
Summary of efficacy: mean of changes in terminal hair.

Study Period	Placebo (N=21)				ALRV5XR (N=18)				Efficacy (95% CI)			Effect Size
	Mean	SD	Min	Max	Mean	SD	Min	Max	Δ -Mean	95% CI	p-value	
Terminal (Hairs/cm^2)												
Week 0-12	3.1	$\pm$ 7.5	-7	22	16.1	$\pm$ 16.6	-9	41	12.9	4.8 - 21.1	0.0028	1.8
Week 12-24	0.7	$\pm$ 9.5	-9	32	17.9	$\pm$ 15.5	-5	38	17.2	9.0 - 25.4	0.0001	10.7
Week 0-24 [†]	3.9	$\pm$ 15.8	-9	54	33.9	$\pm$ 29.8	-6	79	30.1	15.1 - 45.1	0.0002	2.7
Terminal Hairs (%)												
Week 0-12	2.6%	$\pm$ 6.3%	-5.3%	18.3%	10.9%	$\pm$ 12.5%	-6.0%	36.3%	8.3%	2.0 - 14.6%	0.0110	1.8
Week 12-24	0.8%	$\pm$ 8.0%	-8.1%	26.7%	12.1%	$\pm$ 11.2%	-3.2%	33.6%	11.4%	5.1 - 17.6%	0.0007	10.7
Week 0-24 [†]	3.4%	$\pm$ 13.5%	-8.1%	45.0%	23.1%	$\pm$ 22.2%	-4.9%	69.9%	19.7%	8.0 - 31.4%	0.0016	2.7

Abbreviations: [†] = Primary Efficacy Objective; Week 0 = baseline; N=Per Protocol Evaluable Population; SD=standard deviation; Δ -Mean=Difference of Means between ALRV5XR and Placebo; 95% CI = 95% Confidence Interval.**Table 2**
Summary of terminal hair counts per visit (Hairs/ cm^2).

Week	Placebo (N=21)		ALRV5XR (N=18)	
	Mean	SD	Mean	SD
0	141.6	$\pm$ 32.5	145.8	$\pm$ 32.9
12	144.7	$\pm$ 31.5	161.9	$\pm$ 39.2
24	145.4	$\pm$ 32.9	179.8	$\pm$ 48.9

Abbreviations: Week 0 = baseline; N=Per Protocol Evaluable Population; SD or $\pm$ = standard deviation.

Longitudinal hair variations over time were assessed using a linear mixed effects model which examines the change in THs from baseline to week 24; adjusting for all covariates, and incorporating the demographic and physical subject characteristics (age, weight, ethnicity, skin type, diagnosis and Ludwig classification) [30,31]. All statistical analyses were performed using the R software version 4.0.3 (2020-10-10) [32].

2.8. Role of the funding source

The funder of the study had no role in the recruitment of subjects or data collection. The funder had a role in study design, data analysis, data interpretation and writing of the report. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

3. Results

3.1. Study participants

A total of 46 women were enrolled in the study and 23 were randomly assigned to each group between April 3 and October 20, 2018. After enrolment, 2 subjects in the placebo and 3 in the ALRV5XR group withdrew consent during baseline assessments and were therefore excluded from the study. The intent to treat population (ITT) data originated from the 41 remaining subjects and was used for baseline characteristics and safety analyses. There were 21 subjects receiving placebo and 20 subjects receiving ALRV5XR. Two non-compliant subjects in the ALRV5XR group were excluded from the efficacy analyses. In summary, data from 21 subjects receiving placebo and 18 receiving ALRV5XR materials were analysed, as per protocol, for primary and secondary efficacy parameters. (See Fig. 1)

Baseline demographic and physical characteristics were similar between the ALRV5XR and placebo groups. No significant differences between the groups were found in categorical variables, such as age, height, weight, body mass index (BMI), blood pressure, TH

Table 4

Response Rates by Absolute and Relative Change in Terminal Hair Density. For the ALRV5XR treatment group, this table shows a significant shift of subjects' **Response Rates by Change in Terminal Hairs/cm²** from a relatively even distribution of 0–40 TH/cm² at week 12 to higher values of 30 THs/cm² and above at week 24. **Response Rates by % Change in Terminal Hairs** for the ALRV5XR treatment group, shows a significant shift of subjects' hair % changes at week 12 to higher percentage ranges at week 24.

	Placebo			ALRV5XR		
	Week 0-12	Week 12-24	Week 0-24	Week 0-12	Week 12-24	Week 0-24
Positive Responders %	67.7%	42.9%	42.9%	77.8%	88.9%	66.7%
Change in Terminal Hairs/cm²						
<0	33.3%	57.1%	57.1%	22.2%	11.1%	33.3%
0-10	47.6%	33.3%	28.6%	16.7%	22.2%	-
10-20	9.5%	-	4.8%	16.7%	11.1%	-
20-30	9.5%	4.8%	-	16.7%	27.8%	-
30-40	-	4.8%	-	16.7%	27.8%	5.6%
40-50	-	-	4.8%	11.1%	-	22.2%
≥50	-	-	4.8%	-	-	38.9%
Change in Terminal Hairs %						
<0%	33.3%	57.1%	57.1%	22.2%	11.1%	33.3%
0-10%	52.4%	33.3%	28.6%	22.2%	27.8%	-
10-20%	14.3%	-	4.8%	33.3%	33.3%	11.1%
20-30%	-	4.8%	-	16.7%	22.2%	11.1%
30-40%	-	4.8%	4.8%	5.6%	5.6%	27.8%
40-50%	-	-	4.8%	-	-	11.1%
≥50%	-	-	-	-	-	5.6%
Max Regrowth Response						
Terminal Hairs/cm ²	22.0	32.0	54.0	41.0	38.0	79.0
Terminal Hairs %	18.3%	26.7%	45.0%	36.3%	33.6%	69.9%

density, ethnicity, skin type, diagnosis, and Ludwig classification (See Table 1).

3.2. Outcomes

Results are summarized in Tables 2–4. They depict the mean changes in THs/cm² and by per cent, and are stratified per visit, per treatment group, and by response rates.

Fig. 3 depicts a strong and significant correlation of ALRV5XR efficacy with treatment duration. The ALRV5XR group showed a significant change in THC from baseline over time (33.9 THs/cm² in the 24-week study period). After adjusting for categorical variables (age, weight, ethnicity, diagnosis, Ludwig classification and skin type), none of the covariates affected the outcome in a statistically significant manner. Differences in QoL changes from 12 to 24 weeks between the ALRV5XR and placebo groups were not significant.

3.3. Primary outcomes

For the primary outcome at 24 weeks, efficacy of ALRV5XR over placebo induced significant TH regrowth of 30.1 THs/cm² with an Odds Ratio (OR) of 2.7 [95%CI: 15.1 – 45.1; (p=0.0002)] and 19.7% with OR of 2.7 [95%CI: 8.0% – 31.4%; (p=0.0016)]. Also, 12 (66.7%) of 18 subjects in the ALRV5XR group, compared to nine (42.9%) of 21 subjects in the placebo group, responded favourably.

An ANOVA analysis for the overall trial of ALRV5XR vs placebo found $F = 32.3$ ($p < 0.0001$).

An ITT analysis of the primary outcome, at 24 weeks, including the non-compliant subjects, showed an efficacy of ALRV5XR over placebo of 26.3 THs/cm² with an OR of 2.0 [95%CI: 11.2 – 41.4; (p=0.0011)] and 17.1% with an OR of 2.0 [95%CI: 5.5% – 28.7%; (p=0.0049)], with 12 (60.0%) of 20 subjects in the ALRV5XR group compared with 9 (42.9%) of 21 subjects in placebo group responding favourably.

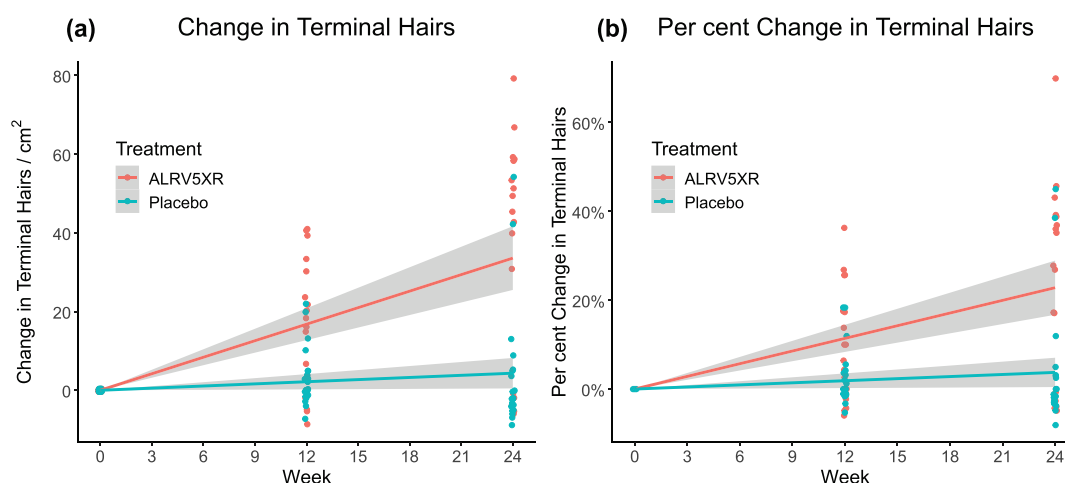


Fig. 3. Changes in Terminal Hair from Baseline Over 24 Weeks. Treatment group ALRV5XR was significantly superior to placebo group for both (a) **Absolute Change in Terminal Hairs** ($P=0.0002$) and (b) **Per Cent Change in Terminal Hairs** ($P=0.0016$). Linear regressions are shown as lines with 95% confidence intervals shown in grey.

Treatment with ALRV5XR was safe, well-tolerated and no adverse effects were observed. Concordantly, no significant changes were observed in physical examinations, blood or urine laboratory parameters (for more details see Tables S4a, S4b and S4c in the supplemental materials).

3.4. Secondary outcomes

At 12 weeks efficacy of ALRV5XR over placebo was 12.9 THs/cm² with an OR of 1.8 [95%CI: 4.8 – 21.1; (p=0.0028)] and 8.3% with an OR of 1.8 [95%CI: 2.0% – 14.6%; (p=0.0110)], with 14 (77.8%) of 18 in the ALRV5XR group responding favourably compared to 14 (66.7%) of 21 subjects in the placebo group.

From 12–24 weeks efficacy was 17.2 THs/cm² with an OR of 10.7 [95%CI: 9.0 – 25.4; (p=0.0001)] and 11.4% with an OR of 10.7 [95%CI: 5.1% – 17.6%; (p=0.0007)], with 16 (88.9%) of 18 responding favourably in the ALRV5XR group compared with nine (42.9%) of 21 subjects in the placebo group.

At 12-weeks, 11.1% of the ALRV5XR group had increased their TH density by more than 40 THs/cm², increasing to 61.1% at 24-weeks with all responders increasing TH by more than 30 THs/cm². Comparable results were achieved when measuring per cent change in TH density: 5.6% of the ALRV5XR group increased their TH density by 30% after week 12 while 44.5% had attained these results after 24-weeks. Moreover, at 12-weeks, the greatest increases in THC in the ALRV5XR group was 41.0 THs/cm² and 36.3%. At 24-weeks, the greatest increases seen were 79.0 THs/cm² and 69.9% respectively. Specifics can be found in Table 4, with additional responder information in Supplementary Table S5 and Figure S1.

Efficacy of total hair counts including all TH and VH at 24 weeks of ALRV5XR vs. placebo was 33.9 hairs/cm² with OR 4.2 [95%CI: 18.1 – 49.8; (p=0.0001)]. Total hair count change within the ALRV5XR group at 24 weeks was 37.9 hairs/cm² (p<0.0001). There was no statistical significance in changes to VH or TH/VH ratios between or within any of the groups at 24 weeks (see Table S3 in the supplemental materials).

3.5. AGA and TE cohort outcomes

Efficacy for the AGA cohort was similar to the overall trial at 24 weeks, with efficacy of ALRV5XR vs. placebo of 31.4 more THs/cm² with an OR of 3.2 [95%CI: 10.3 – 52.6; (p=0.0053)]. Efficacy for the TE cohort was also similar to the overall trial and that of the AGA cohort at 24 weeks with an efficacy of ALRV5XR vs. placebo of 29.0 THs/cm² with an OR of 3.0 [95%CI: 3.3 – 54.7; (p=0.0301)]. An ANOVA analysis showed that differences between the AGA and TE cohorts for efficacy and rate of change of efficacy were not significant.

4. Discussion

During this 24-week clinical trial, ALRV5XR was well-tolerated by all female subjects, and displayed an identical safety profile to that of placebo. Compliance with the treatment regimen of one oral supplement capsule twice per day, along with daily use of a shampoo, conditioner, and follicle serum was greater than 95% with only two non-compliant subjects. ALRV5XR's efficacy demonstrated superiority vs. placebo in the treatment of AGA or TE. Efficacy in the AGA and TE cohorts was similar to that observed in the overall trial. The effect size of the efficacy found in all groups, and presented as odds ratios, was largely in favour of the ALRV5XR treatment.

When compared to placebo, the use of ALRV5XR in subjects affected with AGA or TE significantly increased THC and per cent change of TH from baseline to week 12. The larger effects observed at week 24 suggested cumulative effects of the ALRV5XR treatment over time. These positive effects were observed regardless of age, weight, ethnicity, diagnosis, skin type or hair loss classification.

Baseline trichometric differences between study groups were not statistically or clinically relevant and did not affect the study outcome, especially considering that the balance of the differences had no influence in results in either group.

The response profile of the placebo group remained generally low at weeks 12 and 24. In sharp contrast, the ALRV5XR group showed a rather uniform spread of responses at week 12 and a bimodal effect at week 24 (66.7% responders vs. 33.3% non-responders). This bimodal response was not dependent on absolute THC or per cent THC change. In the responders, a noticeable acceleration of hair regrowth from week 12 to week 24 was documented.

When comparing efficacy of ALRV5XR vs. placebo over time, there was a significant improvement from 12.9 THs/cm² in the first 12-week period, with an additional 17.2 THs/cm² in the second 12-week period culminating in the overall 30.1 THs/cm² efficacy at week 24. This impressive 133% increase in efficacy in the second 12-week period over the first 12-week period indicates that the accelerated regrowth induced by the ALRV5XR treatment in responders may continue well beyond week 24. This finding suggests that longer and sustained exposure to ALRV5XR may continue to induce anagen in involuted (telogen) HFs and might induce almost complete regeneration of the normal scalp hair phenotype.

Considering that the average human scalp has approximately 700 cm² of hair coverage, the efficacy observed at week 24 (30.1 THs/cm²), indicates a regrowth potential of an additional 21,000 THs for the individual using ALRV5XR. Hypothetically, if the regrowth rate of the ALRV5XR group at week 24 is maintained, 91.6% of responders may gain more than 28,000 new THs, and 58.3% of responders more than 35,000 new THs.

There were two outliers in the placebo group with unexpected high positive responses at 24 weeks where a subject with AGA increased their THC by 42 THs/cm² (39%) and a TE subject increased theirs by 54 THs/cm² (45%). These subjects were included in the final analysis. There are few trials studying regrowth in healthy TE subjects and no treatments are approved for TE. Given the clinical view that TE in a healthy subject typically self resolves, there was a risk that the TE placebo group might diminish or confound the efficacy of the overall trial. The randomisation table assigned approximately equal proportions of AGA subjects to the ALRV5XR and placebo groups, however the TE cohort had by chance, a disproportionate assignment to the placebo group, which may have further confounded the trial outcome. The similar efficacy in each of the AGA and TE cohorts, and its similarity to that of the overall trial, suggest that TE is a treatable condition and ALRV5XR is an effective option for women with TE.

In published clinical trials and meta-analyses, the current standard therapy (minoxidil) after 48 weeks of treatment, resulted in average gains of hair density of 12.4 (2% minoxidil) to 15.1 (5% minoxidil) THs/cm² [19–21]. The efficacy of ALRV5XR reported in this study compares favourably to these results. At 12 weeks, with an efficacy of 12.9 THs/cm², ALRV5XR had similar efficacy than that of 2% minoxidil at 48 weeks. At 24 weeks, with an efficacy of 30.1 THs/cm², ALRV5XR reached gains that almost double those induced by 5% minoxidil treatment at 48 weeks. Furthermore, the 66.7% responder rate seen with ALRV5XR is far superior to the 13–40% reported for minoxidil [21,22]. In addition, ALRV5XR has a clearly improved safety profile when compared to that of minoxidil, which was associated to a miscellaneous range of adverse events in the reported studies [19,21].

Interestingly, when compared to published RCTs of standard approved therapy, the overall efficacy of ALRV5XR indicates that 2% minoxidil requires 4 times longer treatment to achieve the same efficacy of ALRV5XR. Similarly, ALRV5XR doubles the efficacy of 5% minoxidil in half the time, and the per cent of responders to ALRV5XR approximately quadruples the number of responders in minoxidil trials.

In the pursuit of improving the efficacy of hair regrowth in women, various studies have investigated combination therapies including one or more topical and/or oral agents with and without topical minoxidil, reporting unremarkable improvements [19]. ALRV5XR is consistent with these combination approaches, yet with its multi-molecular targeting, designed to modulate >20 of the accepted pathways involved in healthy HF biology, it has achieved a synergistic effect as documented by its superior outcome.

When evaluating the totality of the evidence in the literature and the results of this clinical trial, our hypothesis of ALRV5XR, proved to be a highly effective treatment in regenerating and maintaining TH in women. The significant clinical effect achieved in this trial, without side effects, and the significant and accelerating increases in TH density from week 12 to 24, can be explained clinically as prolonging anagen while also inducing anagen in involuted or non-productive telogen hair follicles. These clinical effects have been explained in the literature as HF regenerative and homeostatic physiological effects of activating HF stem cells via the Wnt/ β -catenin signalling pathway, and by regulation and/or modulation of multiple cytokines, growth factors and hormone mediators, and likely relieving pressure on the HF [2,16–18,25–27]. These mechanisms of action are similar to those observed in PRP scalp treatments which have been linked to multiple similar pathways [26]. Future research should focus on elucidating the main mechanisms of action to refine ALRV5XR delivery and applications in other ageing tissues. Other interesting aspects of research should aim to explore the potentiating and longevity effects of ALRV5XR as a combination treatment with LLLT, PRP, or transplantation of stem cells and follicle units. More importantly, additional and longer-term studies of ALRV5XR as topical, oral or combination treatments, are required to evaluate various regimen options to maintain adherence for optimal efficacy and the long-term potential in regeneration of a woman's normal scalp hair phenotype of the third decade [10].

ALRV5XR appears to provide a viable option for the unmet need of an effective, safe, and well-tolerated treatment for women of all ages, ethnicities and skin types with premature ageing or senescent hair loss associated with AGA and TE. The ALRV5XR multi-molecular targeting approach for hair regrowth significantly increased TH density and appeared to regenerate the normal structure and function of involuted and non-productive hair follicles and prolong anagen. Besides having an improved safety profile over the current FDA approved standard of care, ALRV5XR achieved a multi-fold improvement in efficacy and response rates. While longer studies are needed to demonstrate the complete potential of ALRV5XR, progressive acceleration of TH regrowth over 24 weeks suggests that ALRV5XR may be a highly effective long-term treatment for hair loss in women, while reasonably expecting that a prolonged ALRV5XR treatment might restore a normal hair phenotype.

Funding

This clinical trial was funded by Arbor Life Labs, Toronto, Ontario, Canada.

Contributors

PRF and DB contributed to the study concept and design. PRF obtained funding. BMM contributed to recruitment of subjects, investigation, supervision, data acquisition and data curation. PRF, KMF, CP, BMM and JGA contributed to data analysis and interpretation. PRF, KMF and CP contributed to the validation, statistical analysis and visualization. CP and KMF drafted the manuscript. PRF, KMF, CP, DB, DJC and JGA critically revised the manuscript for important intellectual content.

Data sharing

Data collected for the study, including individual participant data and a data dictionary, will be made available. Data available includes de-identified participant data, study protocol and informed consent form. These data will be available beginning 6 months and ending 3 years after publication. Data will be shared with investigator support to researchers who provide a methodologically sound proposal with a signed data access agreement. Contact corresponding author.

Declaration of Competing Interest

PRF is the director of Arbor Life Labs (ALL), PRF, DJC and DB are coinventors of patents filed for ALRV5XR and have a share in ALL. PRF, KMF, CP, BMM, DJC and JG, received honoraria from ALL and proposed submission for publication. PRF, DB and DJC have a share in ALL. All authors had full access to the data in the study.

Acknowledgment

The authors express their profound appreciation and acknowledgement to Dr Luciano Eugenio Marra, PhD for his contribution to developing ALRV5XR – we dedicate this publication to Dr Marra, who died shortly after the commencement of this trial; Dr Kurt Stenn MD for reviewing the data and his guidance on reporting and interpreting results; Dr Elizabeth Renouf, PhD for Biostatistical analysis; and Deborah Cahan, without whom ALRV5XR would not have been developed due to her persistent and complex alopecia which dramatically changed her life. This trial was funded by Arbor Life Labs.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:[10.1016/j.eclinm.2021.100978](https://doi.org/10.1016/j.eclinm.2021.100978).

References

- [1] McMichael AJ, Hordinsky MK. Hair and scalp disorders: medical, surgical, and cosmetic treatments, second edition. Boca Raton: CRC Press; 2018.
- [2] Matsumura H, Mohri Y, Binh NT, et al. Hair follicle aging is driven by transepidermal elimination of stem cells via COL17A1 proteolysis. *Science* 2016;351(6273):aad4395.
- [3] Cash TF, Price VH, Savin RC. Psychological effects on androgenetic alopecia on women: comparisons with balding and female control subjects. *J Am Acad Dermatol* 1993;29(4):568–75.
- [4] Karnik P, Shah S, Dvorkin-Wininger Y, Oshtry S, Mirmirani P. Microarray analysis of androgenetic and senescent alopecia: comparison of gene expression shows two distinct profiles. *J Dermatol Sci* 2013;72(2):183–6.
- [5] Norwood OT. Incidence of female androgenetic alopecia (female pattern alopecia). *Dermatol Surg* 2001;27(1):53–4.
- [6] Price VH. Treatment of hair loss. *N Engl J Med* 1999;341(13):964–73.
- [7] Guo EL, Katta R. Diet and hair loss: effects of nutrient deficiency and supplement use. *Dermatol Pract Concept* 2017;7:1–10.
- [8] Olsen EA, Salt II CD. A new take on the Severity of Alopecia tool (SALT) for determining percentage scalp hair loss. *J Am Acad Dermatol* 2016;75(6):1268–70.
- [9] Whiting DA. The structure of the human hair follicle: light microscopy of vertical and horizontal sections of scalp biopsies. NY: Canfield; Fairfield; 2004.
- [10] Robbins C, Mirmirani P, Messenger AG, et al. What women want - quantifying the perception of hair amount: an analysis of hair diameter and density changes with age in caucasian women. *Br J Dermatol* 2012;167(2):324–32.
- [11] Trüeb RM. Nutrition for healthy hair: guide to understanding and proper practice. Switzerland: Springer Nature; 2020.
- [12] Su LH, Chen LS, Lin SC, Chen HH. Association of androgenetic alopecia with mortality from diabetes mellitus and heart disease. *JAMA Dermatol* 2013;149(5):601–6.
- [13] Olds H, Liu J, Luk K, Lim HW, Ozog D, Rambhatla PV. Telogen effluvium associated with COVID-19 infection. *Dermatol Ther* 2021:e14761.
- [14] Courtois M, Loussouarn G, Hourseau C, Grollier JF. Ageing and hair cycles. *Br J Dermatol* 1995;132(1):86–93.
- [15] Messenger AG, Sinclair R. Follicular miniaturization in female pattern hair loss: clinicopathological correlations. *Br J Dermatol* 2006;155(5):926–30.
- [16] Trüeb RM. Molecular mechanisms of androgenetic alopecia. *Exp Gerontol* 2002;37(8–9):981–90.

- [17] Bernard BA. Advances in understanding hair growth. *F1000Res*. 2016;5.
- [18] Vasserot AP, Geyfman M, Poloso NJ. Androgenetic alopecia: combing the hair follicle signaling pathways for new therapeutic targets and more effective treatment options. *Expert Opin Ther Targets* 2019;23(9):755–71.
- [19] Kanti V, Messenger A, Dobos G, et al. Evidence-based (S3) guideline for the treatment of androgenetic alopecia in women and in men – short version. *J Eur Acad Dermatol Venereol* 2018;32(1):11–22.
- [20] Adil A, Godwin M. The effectiveness of treatments for androgenetic alopecia: a systematic review and meta-analysis. *J Am Acad Dermatol* 2017;77(1):136–41 e135.
- [21] Lucky AW, Piacquadio DJ, Ditre CM, et al. A randomized, placebo-controlled trial of 5% and 2% topical minoxidil solutions in the treatment of female pattern hair loss. *J Am Acad Dermatol* 2004;50(4):541–53.
- [22] Roberts J, Desai N, McCoy J, Goren A. Sulfotransferase activity in plucked hair follicles predicts response to topical minoxidil in the treatment of female androgenetic alopecia. *Dermatol Ther* 2014;27(4):252–4.
- [23] Kwack MH, Kang BM, Kim MK, Kim JC, Sung YK. Minoxidil activates beta-catenin pathway in human dermal papilla cells: a possible explanation for its anagen prolongation effect. *J Dermatol Sci* 2011;62(3):154–9.
- [24] Messenger AG, Rundegren J. Minoxidil: mechanisms of action on hair growth. *Br J Dermatol* 2004;150(2):186–94.
- [25] Guevara-Aguirre J, Torres C, Pena G, et al. IGF-I deficiency and enhanced insulin sensitivity due to a mutated growth hormone receptor gene in humans. *Mol Cell Endocrinol* 2021;519:111044.
- [26] Gentile P, Garcovich S. Systematic review of platelet-rich plasma use in androgenetic alopecia compared with Minoxidil(R), Finasteride(R), and Adult stem cell-based therapy. *Int J Mol Sci* 2020;21(8):2702.
- [27] Herman A, Herman AP. Mechanism of action of herbs and their active constituents used in hair loss treatment. *Fitoterapia* 2016;114:18–25.
- [28] Dupont WD. Statistical modeling for biomedical researchers: a simple introduction to the analysis of complex data. New York: Cambridge Press; 2002.
- [29] Venables WN, Ripley BD. Modern applied statistics with S. 4th edition New York: Springer-Verlag; 2002.
- [30] Fitzmaurice GM, Laird N, Ware JH. Applied longitudinal analysis. 2nd ed. New Jersey: Wiley; 2011.
- [31] Lang W. Mixed effects models for complex data. New York: CRC Press; 2010.
- [32] R Core Team. A language and environment for statistical computing. Vienna, Austria: R foundation for statistical computing; 2012. Available at: <http://cran.r-project.org>.

Supplementary Tables and Figures

Table S1. Abbreviations

Text Abbreviations	
AGA	Androgenetic Alopecia
BVM	Botanical, Vitamin and Mineral
FDA	Food and Drug Administration
FPHL	Female Pattern Hair Loss
HF	Hair Follicle
HFSC	Hair Follicle Stem Cell
HL	Hair Loss
LLLT	Low Level Laser Therapy
MOA	Mechanism of Action
PRP	Platelet-Rich Plasma
QoL	Quality of Life
RCT	Randomised Controlled Clinical Trial
SC	Stem Cell
TAHC	Target Area Hair Count
TE	Telogen Effluvium
TH	Terminal Hair
THC	Terminal Hair Count
VH	Vellus Hair
Units of Measure	
CI	Confidence Interval
cm	Centimetre
µm	Micrometre
mm	Millimetre
OR	Odds Ratio
%	Per cent or percent
p	Statistical Probability – T-Test
F	Fisher Statistic - ANOVA Test
Growth Factors	
EGF	Epidermal Growth Factor
FGF-7	Fibroblast Growth Factor – 7
FGF-18	Fibroblast Growth Factor – 18
HGF	Hepatocyte Growth Factor
IGF	Insulin Growth Factor
KGF	Keratinocyte Growth Factor
VEGF	Vascular Endothelial Growth Factor
Dermal Papilla cell and Stem Cell stimulation	
BMP	Bone Morphogenic Proteins
mTORC1	Mammalian Target of Rapamycin – 1
PKC	Protein Kinase C
PPAR γ	Peroxisome Proliferator-Activated Receptor Gamma
Shh	Sonic Hedgehog
Wnt	Wingless-related integration site
Immunomodulation	
COX	Cyclooxygenase
IFN- α	Interferon-alpha
IFN- γ	Interferon-gamma
IL-1b, IL-6 and IL-12	Interleukins -1b, 6, -12
NF- κ B	Nuclear Factor Kappa Beta
PGE2	Prostaglandin E2
PGD2	Prostaglandin D2
TNF- α	Tumour Necrosis Factor alpha
TGF- β	Transformation Growth Factor beta
Androgens & Mediators	
5 α -R	5 α -Reductase
DHT	Dihydrotestosterone

Table S2. ALRV5XR Material Active Ingredients

Active Ingredients*	
Oral (capsule)	Angelica sinensis, Ascophyllum nodosum, Astragalus membranaceus, Biotin, Coenzyme Q10, Copper, Eclipta prostrata, Folate, Ganoderma lucidum, Malus domestica, Moringa oleifera, Niacin, Pantothenic acid, Polygonum multiflorum, Punica granatum, Riboflavin, Rosmarinus officinalis, Scutellaria baicalensis, Selenium, Silicon, Sophora flavescens, Vitamin A, Vitamin B6, Vitamin B7(Biotin), Vitamin B12, Vitamin D, Vitis vinifera, and Zinc
Shampoo	Caffeine, Eclipta prostrata, Lupinus, Momordica charantia, Moringa oleifera, Pyrus malus, Rosmarinus officinalis, Vitis vinifera, Scutellaria baicalensis, Vitamin B7(Biotin)
Conditioner	Caffeine, Eclipta prostrata, Lupinus, Momordica charantia, Moringa oleifera, Pyrus malus, Rosmarinus officinalis, Vitis vinifera, Scutellaria baicalensis, Vitamin B7(Biotin)
Follicle serum	Caffeine, Eclipta prostrata, Lupinus, Momordica charantia, Moringa oleifera, Ocimum basilicum, Pisum sativum, Pyrus malus, Rosmarinus officinalis, Vitis vinifera, Scutellaria baicalensis, Vitamin B7(Biotin)

*Patents pending

Table S3. Summary of Efficacy: Mean of Changes in Vellus, Total Hair and Terminal/Vellus Ratio. Efficacy of ALRV5XR vs placebo in women with AGA or TE showed a significant increase in total hair density at 24 weeks (33·9 hairs/cm², p = 0·0001 and 18·6%, p = 0·0004), while vellus hair changes and Terminal/Vellus Ratio changes were not significant at 24 weeks.

	Placebo (N=21)				ALRV5XR (N=18)				Efficacy			Effect Size
Study Period	Mean	SD	Min	Max	Mean	SD	Min	Max	Δ-Mean	(95% CI)	p-value	Odds Ratio
Vellus (Hairs/cm ²)												
Week 0-12	-1·0	±4·3	-11	7	3·1	±7·1	-11	20	4·1	0·1 – 8·1	0·0434	6·5
Week 0-24	0·2	±6·9	-14	17	3·9	±9·5	-18	20	3·7	-1·6 – 9·0	0·1675	2·4
Vellus Hairs (%)												
Week 0-12	-3·3%	±8·8%	-14·5%	17·6%	9·1%	±30·5%	-33·3%	83·3%	12·5%	-1·6 – 26·6%	0·0338	6·5
Week 0-24	0·1%	±12·3%	-18·4%	31·5%	11·8%	±35·0%	-54·5%	93·3%	11·7%	-4·8 – 28·2%	0·0953	2·4
Total (Hairs/cm ²)												
Week 0-12	2·0	±7·4	-8	24	19·1	±16·7	-5	53	17·1	8·9 – 25·3	0·0001	4·9
Week 0-24	4·0	±17·4	-6	58	37·9	±30·6	-4	78	33·9	18·9 – 49·8	0·0001	4·2
Total Hairs (%)												
Week 0-12	1·2%	±4·5%	-4·9%	14·7%	10·7%	±10·7%	-4·8%	36·1%	9·5%	4·3 – 14·7%	0·0006	4·9
Week 0-24	2·6%	±10·9%	-3·7%	35·6%	21·2%	±18·8%	-3·3%	49·7%	18·6%	8·8 – 28·4%	0·0004	4·2
Terminal/Vellus Ratio												
Week 0-12	0·2	±0·5	-0·9	1·1	0·1	±1·9	-3·1	4·0	-0·1	-1·0 – 0·7	0·8012	0·4
Week 0-24	0·1	±0·6	-0·8	1·7	0·9	±3·1	-2·9	10·7	0·7	-0·6 – 2·1	0·2836	0·8
Terminal/Vellus Ratio (%)												
Week 0-12	7·1%	±12·4%	-16·4%	30·9%	2·4%	±35·6%	-1·9%	6·7%	-4·7%	-21·4 – 12·1%	0·9517	0·4
Week 0-24	4·6%	±17·6%	-20·2%	49·4%	15·7%	±61·1%	-31·7%	97·6%	11·2%	-17·1 – 39·4%	0·2487	0·8

Abbreviations: † = Primary Efficacy Objective; Week 0 = baseline; N=Per Protocol Evaluable Population; SD=standard deviation; Δ-Mean=Difference of Means between ALRV5XR and Placebo; 95% CI = 95% Confidence Interval.

Table S4a: Blood Laboratory Results. There were no significant differences between the groups at trial completion. Any significant differences between the groups at baseline or 12 weeks (highlighted in grey) normalised by the end of the trial. Any results with significant differences were within normal ranges.

			Placebo									ALRV5XR									Placebo vs. ALRV5XR			
			BL			12 Weeks			24 Weeks			BL			12 Weeks			24 Weeks			T-Tests P-Value			
Analyte	UOM	Reference Range	N	Mean	Stddev	N	Mean	Stddev	N	Mean	Stddev	N	Mean	Stddev	N	Mean	Stddev	N	Mean	Stddev	BL	BL - 12 Weeks	BL-24 Weeks	12 - 24 Weeks
Blood:																								
Albumin	g/dL	3.5-5.5	16	4.350	0.218	18	4.300	0.323	16	4.363	0.269	15	4.413	0.263	14	4.407	0.194	13	4.446	0.247	0.485	0.463	0.615	0.870
Alkaline Phosphatase	IU/L	39-117	18	69.444	19.143	17	69.471	21.377	18	77.667	21.050	16	64.938	18.988	16	69.875	22.282	17	69.824	18.293	0.509	0.546	0.916	0.594
ALT (SGPT)	IU/L	0-32	17	21.118	8.498	17	23.118	11.256	17	23.588	13.012	16	18.000	6.413	17	20.235	7.207	17	22.471	7.317	0.260	0.776	0.584	0.719
AST (SGOT)	IU/L	0-40	15	22.333	6.600	17	24.588	7.822	15	24.000	6.976	12	19.250	5.988	15	18.867	4.455	15	21.467	3.879	0.238	0.847	0.420	0.284
Baso (Absolute)	x10E3/uL	0.0-0.2	19	0.011	0.031	18	0.011	0.031	18	0.011	0.031	16	0.006	0.024	17	0.006	0.024	17	0.012	0.032	0.664	1.000	0.692	0.639
Basos	%	Not Estab.	10	1.100	0.300	8	1.000	0.000	6	1.000	0.000	8	1.000	0.000	6	1.000	0.000	7	1.000	0.000	0.387	0.855	0.447	0.430
Bilirubin, Total	mg/dL	0.0-1.2	14	0.379	0.137	11	0.382	0.147	15	0.353	0.175	12	0.508	0.198	14	0.421	0.174	13	0.492	0.249	0.071	0.567	0.863	0.761
BUN	mg/dL	6-27	17	13.706	3.426	15	13.867	4.064	15	15.133	3.201	13	13.154	2.627	16	13.250	2.817	15	13.600	2.471	0.645	0.200	0.466	0.581
Calcium	mg/dL	8.7-10.3	18	9.311	0.349	18	9.472	0.300	16	9.519	0.464	15	9.320	0.382	16	9.388	0.260	16	9.375	0.334	0.947	0.773	0.434	0.586
Carbon Dioxide, Total	mmol/L	18-29	15	26.400	3.363	16	26.750	3.631	16	26.250	4.802	14	26.857	2.559	15	25.333	1.619	13	24.923	1.542	0.696	0.799	0.472	0.576
Chloride	mmol/L	96-106	18	99.556	4.512	17	100.529	3.882	18	100.556	4.969	16	100.375	2.759	17	101.471	1.460	17	102.000	2.169	0.546	0.484	0.698	0.720
Cholesterol, Total	mg/dL	100-199	19	208.368	33.186	18	200.389	29.947	18	202.222	32.673	16	190.750	38.512	17	191.353	33.505	17	187.824	38.099	0.167	0.326	0.434	0.858
C-Reactive Protein, Cardiac	mg/L	0.00-3.00	19	5.693	12.934	18	3.373	4.529	18	3.208	6.157	16	1.328	1.158	17	1.612	1.469	17	1.542	1.404	0.200	0.402	0.382	0.937
Creatinine	mg/dL	0.57-1.00	18	0.767	0.157	18	0.763	0.160	18	0.733	0.130	15	0.697	0.100	17	0.715	0.107	17	0.701	0.108	0.158	0.377	0.331	0.898
eGFR If African Am	UNABL1	>59	17	104.647	24.743	17	107.176	22.688	16	109.313	21.400	13	113.231	15.313	17	112.176	15.931	17	112.529	15.263	0.297	0.163	0.176	0.781
eGFR If NonAfrican Am	mL/min/1.73	>59	16	90.313	21.779	16	94.125	19.065	18	93.500	17.964	13	98.077	13.211	17	97.412	13.899	17	97.706	13.265	0.286	0.205	0.512	0.542
Eos	%	Not Estab.	15	2.400	1.665	15	2.400	1.306	17	2.353	2.056	16	3.125	1.932	16	2.688	2.493	16	2.500	1.732	0.289	0.481	0.375	0.616
Eos (Absolute)	x10E3/uL	0.0-0.4	16	0.131	0.058	14	0.136	0.048	15	0.167	0.125	13	0.223	0.125	14	0.171	0.116	13	0.162	0.074	0.018	0.550	0.175	0.280
Glucose	mg/dL	65-99	18	87.222	7.976	18	91.444	13.684	18	88.444	11.663	15	92.733	10.389	16	99.063	19.008	16	91.000	8.881	0.105	0.681	0.872	0.770
HDL Cholesterol	mg/dL	>39	19	60.421	16.869	16	62.063	20.089	15	60.267	20.407	15	61.333	18.539	17	60.588	18.630	16	57.563	14.009	0.885	0.139	0.355	0.858
Hematocrit	%	34.0-46.6	19	39.726	3.168	18	38.794	2.664	18	39.833	2.455	16	40.225	1.865	17	39.671	2.705	17	40.082	2.999	0.594	0.399	0.527	0.925
Hemoglobin	g/dL	11.1-15.9	19	13.111	1.097	18	12.689	0.902	18	13.028	0.820	15	13.387	0.653	17	13.065	0.951	17	13.124	1.158	0.409	0.241	0.364	0.894
INR		0.8-1.2	9	1.033	0.047	7	1.000	0.000	8	1.025	0.043	9	1.122	0.175	9	1.011	0.057	7	1.029	0.070	0.184	0.812	0.583	0.354
Insulin	uIU/mL	2.6-24.9	15	10.313	5.189	17	22.912	23.338	17	14.418	12.165	15	15.167	17.440	16	25.338	27.616	17	12.200	10.213	0.327	0.940	0.329	0.609
LDL Cholesterol Calc	mg/dL	0-99	17	116.647	28.371	16	107.938	30.431	15	114.333	33.198	13	117.231	30.519	16	108.875	29.854	13	105.615	39.599	0.959	0.242	0.857	0.279
Lymphs	%	Not Estab.	19	33.158	7.407	18	30.000	7.295	18	33.167	7.089	16	32.063	9.331	17	30.000	5.369	17	31.059	9.283	0.709	0.428	0.675	0.736
Lymphs (Absolute)	x10E3/uL	0.7-3.1	19	1.989	0.614	18	1.833	0.513	18	2.061	0.678	15	1.847	0.610	15	1.987	0.550	17	1.771	0.410	0.517	0.301	0.600	0.602
Magnesium	mg/dL	1.6-2.3	17	2.159	0.220	17	2.041	0.200	17	2.082	0.150	14	2.150	0.098	13	2.169	0.082	17	2.112	0.132	0.894	0.967	0.285	0.227
MCH	pg	26.6-33.0	19	29.737	1.467	17	29.388	1.646	18	29.556	1.739	16	29.750	1.780	17	29.788	1.769	17	29.635	2.052	0.982	0.168	0.494	0.689
MCHC	g/dL	31.5-35.7	19	32.995	0.561	17	32.747	0.804	17	32.700	0.915	16	33.144	0.523	17	32.941	0.918	16	32.700	0.882	0.439	0.280	0.584	0.701
MCV	fL	79-97	19	90.105	4.179	18	90.222	4.697	18	90.278	4.828	15	90.133	4.965	17	90.529	4.367	17	90.588	4.551	0.986	0.239	0.315	0.999
Monocytes	%	Not Estab.	18	7.944	2.272	17	7.412	1.611	18	7.500	2.088	16	6.375	1.317	16	6.875	1.728	16	6.250	1.561	0.025	0.333	0.823	0.379
Monocytes(Absolute)	x10E3/uL	0.1-0.9	18	0.483	0.150	18	0.456	0.154	17	0.465	0.161	16	0.375	0.083	17	0.429	0.152	16	0.381	0.124	0.018	0.204	0.572	0.512
Neutrophils	%	Not Estab.	18	57.167	7.566	18	60.167	8.408	18	56.722	8.130	16	57.875	10.582	17	60.235	6.916	17	59.941	10.015	0.828	0.716	0.504	0.739
Neutrophils (Absolute)	x10E3/uL	1.4-7.0	18	3.689	1.268	18	3.850	1.304	18	3.572	1.158	15	3.367	1.200	17	3.953	1.270	17	3.806	1.733	0.476	0.233	0.202	0.881
Phosphorus	mg/dL	2.5-4.5	16	3.644	0.387	14	3.686	0.464	16	3.719	0.476	15	3.567	0.580	16	3.769	0.526	14	3.664	0.403	0.675	0.349	0.832	0.215
Platelets	x10E3/uL	150-379	19	282.211	48.804	18	292.056	46.201	18	299.111	72.228	16	289.250	58.499	17	303.882	62.641	17	298.059	52.800	0.709	0.393	0.624	0.765
Potassium	mmol/L	3.5-5.2	18	4.389	0.448	16	4.244	0.480	15	4.333	0.532	11	4.745	0.421	12	4.292	0.247	14	4.386	0.311	0.050	0.601	0.220	0.360
Prothrombin Time	sec	9.1-12.0	19	10.200	0.334	17	10.141	0.303	18	10.183	0.344	15	10.380	0.339	17	10.206	0.352	17	10.253	0.373	0.142	0.179	0.338	0.737
RBC	x10E6/uL	3.77-5.28	19	4.415	0.381	18	4.314	0.346	18	4.417	0.273	16	4.497	0.348	17	4.389	0.240	17	4.428	0.236	0.528	0.444	0.555	0.930
RDW	%	12.3-15.4	18	13.650	0.544	18	13.494	0.611	17	13.835	0.812	16	13.531	0.514	16	13.388	0.889	17	13.512	0.940	0.532	0.998	0.535	0.540
Sodium	mmol/L	134-144	18	140.500	2.267	18	140.389	1.704	17	141.235	2.289	16	141.063	1.784	16	140.563	1.657	17	141.059	1.830	0.445	0.987	0.556	0.432
Triglycerides	mg/dL	0-149	18	146.000	84.339	17	175.176	136.138	17	165.235	120.628	15	95.333	43.518	16	126.750	77.475	16	129.063	124.222	0.050	0.715	0.452	0.855
TSH	uIU/mL	0.450-4.500	19	1.973	0.926	15	1.784	1.031	18	1.841	0.870	15	1.874	1.356	17	2.232	1.504	17	2.071	1.020	0.808	0.039	0.181	0.223
Uric Acid	mg/dL	2.5-7.1	15	4.827	0.642	14	5.000	1.448	15	4.880	1.093	11	4.645	1.188	15	4.567	1.115	14	4.571	1.127	0.636</			

Table S4b: Urine Laboratory Results. There were no significant differences between the groups.

			Placebo									ALRV5XR									Placebo vs. ALRV5XR			
			BL			12 Weeks			24 Weeks			BL			12 Weeks			24 Weeks			T-Tests P-Value			
Analyte	UOM	Reference Range	N	Mean	Stddev	N	Mean	Stddev	N	Mean	Stddev	N	Mean	Stddev	N	Mean	Stddev	N	Mean	Stddev	BL	BL - 12 Weeks	BL-24 Weeks	12 - 24 Weeks
Urine:																								
Alb/Creat Ratio		0.0-30.0	19	21.705	46.354	18	18.767	23.959	18	25.839	39.088	16	7.494	5.648	17	7.965	6.864	17	16.006	26.505	0.245	0.497	0.419	0.850
Albumin, Urine		Not Estab.	19	24.758	73.108	18	21.439	37.381	18	28.661	49.981	16	6.644	6.921	16	6.200	6.405	17	15.294	25.214	0.345	0.712	0.585	0.774
Creatinine, Urine	mg/dL	Not Estab.	19	89.911	55.601	18	98.906	58.051	18	94.778	60.639	16	102.256	71.312	17	80.982	43.746	17	113.771	79.054	0.580	0.500	0.589	0.266
pH		5.0-7.5	16	6.781	1.060	16	6.500	1.262	17	6.706	1.138	11	6.591	0.701	14	6.286	0.977	14	6.107	0.660	0.620	0.324	0.737	0.623
Specific Gravity		1.005-1.030	19	1.016	0.006	18	1.017	0.006	18	1.019	0.008	16	1.018	0.007	17	1.016	0.006	17	1.018	0.007	0.524	0.440	0.498	0.994
Urobilinogen,Semi-Qn	mg/dL	0.2-1.0	14	0.200	0.000	12	0.200	0.000	8	0.200	0.000	11	0.273	0.230	11	0.200	0.000	7	0.200	0.000	0.268	0.758	0.749	1.000

Table S4c: Physical Exam Results. There were no significant differences between the groups.

			Placebo									ALRV5XR									Placebo vs. ALRV5XR			
			BL			12 Weeks			24 Weeks			BL			12 Weeks			24 Weeks			T-Tests P-Value			
Analyte	UOM	Reference Range	N	Mean	Stddev	N	Mean	Stddev	N	Mean	Stddev	N	Mean	Stddev	N	Mean	Stddev	N	Mean	Stddev	BL	BL - 12 Weeks	BL-24 Weeks	12 - 24 Weeks
Physical Exam:																								
Height	cm		21	159.005	4.518	21	159.005	4.518	21	159.005	4.518	20	161.048	8.233	20	161.076	8.211	20	160.677	8.126	0.336	0.103	0.328	0.326
Weight	kg		21	62.998	12.041	21	61.681	11.937	21	60.002	11.308	20	63.884	11.930	20	63.829	10.755	20	63.273	11.193	0.816	0.485	0.896	0.675
BMI	kg/m^2		21	24.936	4.635	21	24.412	4.578	21	23.801	4.621	20	24.643	4.335	20	24.630	3.968	20	24.520	4.063	0.837	0.446	0.930	0.685
Temperature	deg F		21	97.805	0.308	21	97.900	0.748	21	97.890	0.493	20	97.781	0.634	20	97.367	0.937	20	97.660	0.784	0.881	0.073	0.304	0.358
Respiratory Rate	bpm		21	15.857	2.253	21	15.333	1.984	21	15.333	1.084	20	15.524	2.038	20	14.333	2.233	20	15.950	1.883	0.626	0.477	0.870	0.366
BP-L SYS	mmHg		20	118.000	11.054	20	114.900	15.106	20	115.200	15.683	20	120.250	8.431	20	119.350	10.021	19	122.368	11.789	0.485	0.529	0.855	0.635
BP-R SYS	mmHg		20	118.100	10.972	20	119.700	14.273	19	114.789	11.610	20	117.350	9.794	20	121.600	12.659	19	120.579	9.837	0.825	0.444	0.487	0.664
BP-L DIA	mmHg		20	74.800	5.741	20	76.850	9.210	20	77.700	7.267	20	76.350	8.039	20	76.200	9.574	19	77.211	6.902	0.498	0.439	0.168	0.400
BP-R DIA	mmHg		20	74.550	6.712	20	77.050	8.176	19	76.895	9.519	20	76.550	6.644	20	77.500	7.839	19	78.684	7.219	0.362	0.508	0.961	0.837
Mean BP SYS	mmHg		20	118.050	10.778	20	117.300	14.214	20	115.175	12.851	20	118.800	8.288	20	120.475	10.157	19	121.474	9.957	0.811	0.408	0.934	0.640
Mean BP DIA	mmHg		20	74.675	5.726	20	76.950	8.240	20	77.325	7.308	20	76.450	7.002	20	76.850	7.438	19	77.947	6.306	0.398	0.382	0.222	0.427
Heart rate	bpm		21	71.857	7.415	20	75.750	9.433	21	73.619	7.877	21	67.048	8.995	20	70.550	16.246	19	75.579	10.434	0.072	0.981	0.938	0.965

Table S5: ALRV5XR Responder Distribution by Change in Terminal Hair. For the ALRV5XR treatment group, this table shows a significant shift of changes in subjects' terminal hair count per cm² from a relatively even distribution of 0-40 TH/cm² at week 12. The changes from week 12 – 24 show an accelerating regrowth rate that cumulates in a week 24 result of the lowest responders regrowing more than 30 THs/cm² while 91·7% of responders regrow more than 40 THs/cm². This table also shows the ALRV5XR treatment group's significant shift of subjects' terminal hair % changes at week 12, accelerating regrowth from weeks 12 – 24 to overall higher percentage ranges at week 24.

	Week 0 - 12	Week 12 - 24	Week 0 - 24
Change in Terminal Hairs/cm²			
0-10	21·4%	25·0%	-
10-20	21·4%	12·5%	-
20-30	21·4%	31·3%	-
30-40	21·4%	31·3%	8·3%
40-50	14·3%	-	33·3%
≥50	-	-	58·3%
Per cent Change in Terminal Hairs			
0-10%	28·6%	31·3%	-
10-20%	42·9%	37·5%	16·7%
20-30%	21·4%	25·0%	16·7%
30-40%	7·1%	6·3%	41·7%
40-50%	-	-	16·7%
≥50%	-	-	8·3%

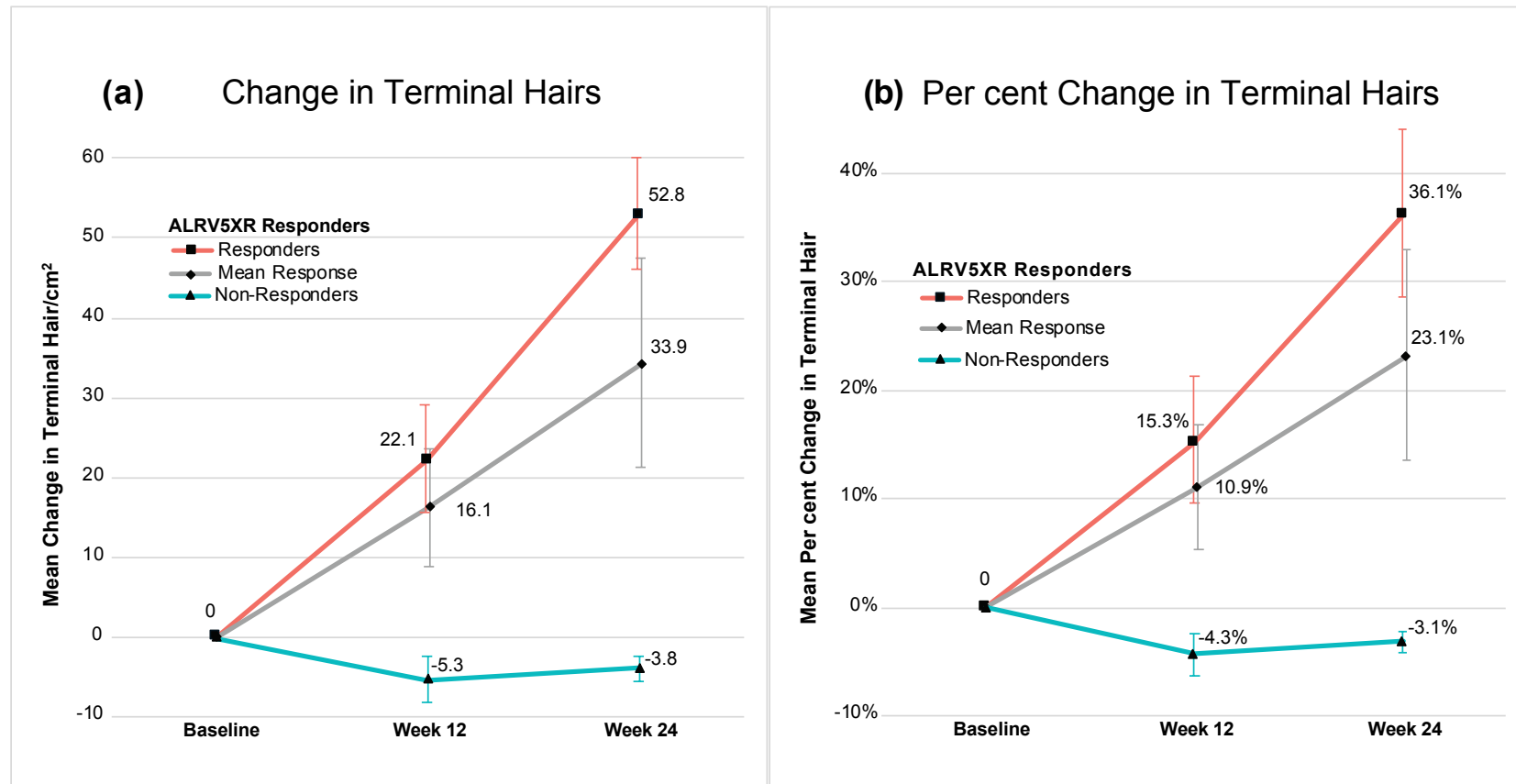


Figure S1. ALRV5XR treatment group changes in Terminal Hair. (a) Changes per cm². The mean regrowth response rate within the ALRV5XR group showed a significant increase from baseline ($P_{12\text{weeks}} = 0.0004$ and $P_{24\text{weeks}} < 0.0001$). Non-responders in the first 12 weeks began to regrow hair in the 12 - 24 week period. **(b) Changes by per cent.** The mean regrowth response rate within the ALRV5XR group showed a significant increase from baseline. Non-responders in the first 12 weeks began to regrow hair in the 12 - 24 week period. Responders showed a significant acceleration in regrowth rate in the 12 - 24 week period over the 0 - 12 week period ($P_{24\text{weeks}} = 0.0001$). 95% Confidence Intervals for each response group are shown.

3.2 Summary of Key Findings of Paper 1

ALRV5XR appears to provide a viable option for the unmet need for an effective, safe, and well-tolerated treatment for women with AGA and TE. All subjects were diagnosed with AGA, with a group of subjects having both AGA and TE. The ALRV5XR multi-molecular targeting approach for hair regrowth significantly increased terminal hair density. At 24 weeks, absolute change in terminal hair density improved by 30.1 THs/cm² (95% CI: 15.1 – 45.1; $p = 0.0002$), and the relative density increased by 19.7% (95% CI: 8.0% – 31.4%; $p = 0.0016$). 66.7% of the treatment group responded (change in TH ≥ 0), and the odds ratio for being a responder was 2.7. At 12 weeks change in terminal hair density improved by 12.9 THs/cm² (95% CI: 4.8 – 21.1; $p = 0.0028$), and the relative density increased by 8.3% (95% CI: 2.0% – 14.6%; $p = 0.0110$). Efficacy of TH density increased by 133% from week 12 to 24. Efficacy results were similar for each subgroup of primary AGA and primary TE. ALRV5XR appeared to regenerate the normal structure and function of involuted and non-productive hair follicles and prolong anagen. While longer studies are needed to demonstrate the full potential of ALRV5XR, progressive acceleration of TH regrowth over 24 weeks suggests that ALRV5XR may be a highly effective long-term treatment for hair loss in women while reasonably expecting that a prolonged ALRV5XR treatment might restore a normal hair phenotype.

4 Results – Paper 2: Safety and efficacy of ALRV5XR in men with androgenetic alopecia: A randomised, double-blinded, placebo-controlled clinical trial.

Feldman, P. R., K. M. Fiebig, C. Piwko, B. M. Mints, D. Brown, D. J. Cahan, and J. Guevara-Aguirre. “Safety and Efficacy of ALRV5XR in Men with Androgenetic Alopecia: A Randomised, Double-Blinded, Placebo-Controlled Clinical Trial.” *EclinicalMedicine* 40 (Oct 2021): 101124. <https://dx.doi.org/10.1016/j.eclinm.2021.101124>.²¹⁴ Supplementary materials are accessible from the link provided in the publication and appended to this chapter.

4.1 Introduction to Paper 2

Androgenetic alopecia (AGA) is the most common hair loss disorder seen in men. It can have an early onset but is also associated with ageing and senescence. ALRV5XR, a new BVM hair loss treatment herein evaluated, was designed to target multiple molecular pathways involved in hair growth and hair follicle stem cell biology. The main objectives of this 24-week study were the assessment of safety and efficacy profiles of ALRV5XR in terminal hair (TH) regrowth in men with AGA or TE.

The protocol for this clinical trial was registered on ClinicalTrials.gov, Identifier: NCT04450589. <https://clinicaltrials.gov/ct2/show/NCT04450589>



Contents lists available at ScienceDirect

EClinicalMedicine

journal homepage: <https://www.journals.elsevier.com/eclinicalmedicine>

Research Paper

Safety and efficacy of ALRV5XR in men with androgenetic alopecia: A randomised, double-blinded, placebo-controlled clinical trial

Peter R Feldman^{a,*}, Klaus M Fiebig^{a,*}, Charles Piwko^b, Boris M Mints^c, Dennis Brown^d, Deborah J Cahan^a, Jaime Guevara-Aguirre^{e,f,g,h}

^a Arbor Life Labs, Toronto, Ontario, Canada^b CHP Pharma Inc, Thornhill, Ontario, Canada^c B&B International, Vilnius, Lithuania^d Valent Technologies, Menlo Park, California, USA^e Universidad San Francisco de Quito (USFQ), Quito, Ecuador^f Institute of Endocrinology, Metabolism, and Reproduction (IEMR), Quito, Ecuador^g Maastricht University, Maastricht, the Netherlands^h College of Medicine, University of Florida, Gainesville, Florida, USA

ARTICLE INFO

Article History:

Received 3 June 2021

Revised 13 August 2021

Accepted 18 August 2021

Available online 11 September 2021

Keywords:

ALRV5XR

Alopecia

Androgenetic alopecia

Male pattern hair loss

Hair restoration

Minoxidil

Finasteride

Botanical

Vitamin

Supplement

Senescence

Ageing

Wnt/beta-catenin

Stem cell

Hair regeneration

Regenerative medicine

Clinical trial

PRP

Terminal hair

Vellus like hair

Terminal vellus ratio

ABSTRACT

Background: Androgenetic alopecia (AGA) is the most common hair loss disorder seen in men. It can have an early onset but has also been associated with ageing and senescence. It often induces pronounced psychological impact. ALRV5XR, a new hair loss treatment herein evaluated, was designed to target multiple molecular pathways involved in hair growth and hair follicle stem cell biology. The main objectives of the study were the assessment of safety and efficacy profiles of ALRV5XR in men.

Methods: This 24-week, parallel randomised, placebo-controlled, double-blinded clinical trial was performed in a USA community clinic. Healthy men (age 22–65) with AGA and belonging to the Hamilton-Norwood (HN) classification I–VII and Fitzpatrick skin type (FST) I–VI, were randomly allocated in a 1:1 ratio into ALRV5XR or placebo treatment groups. Dermatologist assessment, phototrichograms, and blood samples were obtained in a blinded fashion at baseline, 12 and 24 weeks. Subjects were given a masked treatment consisting of oral capsules, shampoo, conditioner, and follicle serum, which was intended for daily use. Efficacy was assessed via absolute and per cent changes in terminal hair (TH) density, and response rates. The trial was registered with clinicaltrials.gov (NCT04450589) and is completed.

Findings: Forty-six subjects were enrolled in the study, 23 allocated to the ALRV5XR treatment and 23 to the placebo group. Enrolment occurred from April 11 to October 23, 2018. Thirty-six subjects completed the trial (17 ALRV5XR, 19 placebo) and 11 subjects in each group were evaluable for TH outcomes. At 24 weeks, the absolute change in TH density improved by 21.0 THs/cm² (95% CI: 9.2–32.8; $p = 0.0014$), and the relative density increased by 16.4% (95% CI: 7.4–25.5%; $p = 0.0012$). The odds ratio for being a responder (≥ 0 change) was 87.4. TH density increased linearly and was not affected by HN, FST, ethnicity, age, or body mass index. All subjects in the ALRV5XR group responded to treatment while 81.8% of the placebo group decreased TH density. ALRV5XR induced statistically significant changes in both decrease in vellus hair (VH) density as well as in concomitant increase of the TH/VH ratio when compared to placebo. ALRV5XR was well tolerated, and no adverse events were observed.

Interpretation: ALRV5XR treatment resulted in clinically significant TH regrowth in men with AGA. Furthermore, it appeared to reverse the characteristic hair miniaturisation seen in this condition. When compared to results of published trials of standard therapy, ALRV5XR showed a multi-fold increase both in efficacy and in response rates. In addition, the continuance of TH regrowth from 12 to 24 weeks suggests that the normal structure and function of non-productive telogen follicles is restored and that a normal hair phenotype may be attained by extended ALRV5XR treatment.

Funding: Arbor Life Labs.

© 2021 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>)

* Corresponding author.

E-mail addresses: petefeldman@gmail.com (P.R. Feldman), klausfiebig@gmail.com (K.M. Fiebig).

<https://doi.org/10.1016/j.eclinm.2021.101124>

2589-5370/© 2021 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>)

Research in context

Evidence before this study

Hair follicle (HF) maintenance and regeneration is a complex process governed by the coherent expression of more than 1000 genes and is dependant on HF stem cell (HFSC) activation and multiple signalling pathways. The Wnt/ β -Catenin pathway in HFSCs is an essential component of hair regrowth and maintenance along with modulation of various cytokines, growth factors and hormones. ALRV5XR was developed after studying botanicals, vitamins and minerals that activate Wnt/ β -Catenin in HFSCs *in vitro* and HFs *in vivo*, and was formulated for oral or topical administration with the aim of influencing more than 20 of the accepted pathways involved in normal HF physiology.

Added value of this study

In men with androgenetic alopecia (AGA), ALRV5XR was found to be clinically effective in preventing HL, regrowing terminal hair (TH) and possibly modifying key pathological features of AGA and senescent alopecia via reversal of hair miniaturisation, all with no side effects. It also displayed a multi-fold increase in response rates and regrowing TH in a shorter time when compared to results of published trials using standard therapies. ALRV5XR's, induction of continuous TH regrowth suggests that its long-term use could restore the normal scalp hair phenotype in men.

Implications of all the available evidence

ALRV5XR may become the first-line standard of care hair regeneration treatment for men with HL. Evaluation of its long-term safety and efficacy profile should be addressed in future research. Similarly, elucidation of the main mechanisms underlying ALRV5XR action might lead to identification of distinct features as well as targets of importance for the regeneration of other tissues.

1. Introduction

Alopecia or hair loss (HL) of the scalp and most notably, androgenetic alopecia (AGA) and senescent alopecia (SA) are common clinical signs associated with ageing and cellular senescence [1,2]. Throughout history and to the present, luxuriant hair has been a symbol of health, youthfulness, and virility [3]. The effects of HL on emotional distress and quality of life can have psychological impact, as suggested by studies on self-esteem and body image [3]. HL in men generally presents as AGA, or telogen effluvium (TE) and in men over 60 it also presents as SA [1]. AGA, also called male-pattern hair loss (MPHL), has various commonalities with SA and with the senescence of the hair follicle (HF) [1,2]. While the expression of over 750 genes were found to be common in both AGA and SA, the expression of the Wnt/ β -Catenin associated genes has been shown to play a major role in hair generation and maintenance [4,5]. AGA is a heritable, benign, and age-dependent process that displays an appearance-altering trait characterised by a progressive decline in scalp hair density in a sex-dependent pattern [1,3]. While onset of hair thinning begins between the ages of 12 and 40, AGA is present in more than half of men by the age of 50 and in 75% of men over 80 [6,7]. AGA is linked to the metabolic syndrome and its comorbidities, and is an independent predictor of mortality from diabetes mellitus, heart disease and COVID-19 [8,9]. TE is a medical sign related to underlying conditions rather than to a discrete disorder and can resolve spontaneously in

otherwise healthy men [1]. Triggering events for TE include acute physical stressors such as surgery, illness, or weight loss, hormonal imbalances, psychological stress, drugs, nutritional deficiencies and scalp inflammation [1]. Miscellaneous and less-common hair disorders and alopeciae have also been associated with deficiencies of minerals such as zinc, selenium, iron and copper as well as of vitamins such as B₃, D, and occasionally biotin [10]. Several drugs often interact with absorption or activity of various nutrients relevant to hair health as in the cases of the commonly used metformin which interferes with vitamin B₁₂, statins with selenium and coenzyme Q10, and ACE inhibitors, diuretics and antacids that interfere with zinc [10].

HFs are epithelial skin mini-organs that produce individual hairs by modulating and sustaining hair regrowth over repeated hair cycles [2]. The greatest HF density on the human body is on the scalp, and the normal healthy adult scalp phenotype has a total hair count ranging from 100,000–150,000 hairs. Corresponding density ranges from 118 to 350 HFs per cm² [1,11]. A normal scalp averages seven terminal hairs (TH) per each vellus hair (VH) [1]. THs are large, pigmented, and have a shaft diameter of 30–150 μ m, while diameter of VHs are less than 30 μ m, and are short, depigmented and barely visible [1,11].

There is accumulating evidence that AGA is the result of organ-specific premature ageing of HFs [1]. Age-related reduction in hair regrowth and miniaturised hair shaft diameter along with hair cycle abnormalities, including increased time in the resting stage (telogen), and more specifically from the loss of hair (exogen) through the empty follicle phase (kenogen), until the emergence of a replacement hair (neogen) have been noted [12,13]. Similarly, decreased duration of the growth phase (anagen) has been reported [12]. In general, AGA leads to HF miniaturisation resulting in the gradual replacement of THs by vellus-like hairs and the eventual deletion of the HF [1,12]. Furthermore, HF miniaturisation appears indistinguishable between SA and MPHL [1]. In any case, both clinical entities display similar reductions in total hair count and TH/VH ratio [10].

At the molecular level, hair growth and the physiological cycle of the HF are determined by the complex and rhythmic interaction of multiple pathways [13]. Interestingly, these elements often receive modulating and even apparently opposing influences such as those of cytokines, hormones, prostaglandins and growth factors [13]. Positive and negative forces acting simultaneously on HFs, simply reflect the various aspects of a complex and dynamic phenomenon occurring in a specific physiological period [13], thereby suggesting multiple and miscellaneous pathological causes of HL. In consequence, to improve efficacy, the next generation of HL therapies should include a combination of agents that promote growth and regrowth, along with agents that delay catagen entry while relieving stress on the follicle [14]. Contextually, the canonical Wnt/ β -Catenin signalling in HF stem cells is seen as the central component controlling hair cycle induction and maintenance [2]. This cascade is less active in aged vs. young telogen HF stem cells [2]. Furthermore, reduced activity of the Wnt/ β -Catenin pathway has been aetiologically linked to hair senescence and AGA [2].

Most available pharmaceutical and non-pharmaceutical hair regrowth treatments target a single, or a limited number of pathways, using high-dose active ingredients. They are effective in only a subset of individuals and, possibly due to the elevated doses needed to attain effective results, are associated with miscellaneous adverse effects [14,15]. Topical minoxidil, at concentrations of 2% and 5%, and oral finasteride (1 mg) were approved for treatment of AGA in men in 1986, 1993 and 1992, respectively [15]. These agents remain as the only FDA approved pharmaceuticals to treat men affected with AGA. Currently, there is no FDA approved treatment for TE or SA. According to a recent meta-analysis, the average improvement due to minoxidil or finasteride vs. placebo in men's hair density was 11.5, 14.9 and 14.8 THs/cm² after 48 weeks of treatment with 2%, 5% minoxidil or

finasteride (1 mg), respectively [7,15,16]. Although 34%, 58% and 52% of male patients with AGA responded to minoxidil 2%, 5% and finasteride (1 mg) treatment, respectively, various adverse events were observed, including sexual dysfunction, suicidality, depression, and anxiety while using finasteride [15,17–19].

Minoxidil is a potassium channel opener that has been associated with miscellaneous mechanisms of action (MOAs) such as increased HGF, PGE and VEGF expression as well as EGF inhibition. Minoxidil is also thought to stimulate the Wnt/ β -Catenin pathway [14,15,20]. Finasteride is a type 2 and 3 5α -reductase inhibitor which decreases the conversion of testosterone to dihydrotestosterone (DHT) in younger men by about 65% [15]. It induces similar actions in the prostate gland and scalp [15]. Nevertheless, in men older than 60 years, finasteride is not an effective treatment for MPHL [6]. Despite multiple MOAs of minoxidil, TH density changes in AGA patients tend to peak at 12–16 weeks and decline thereafter [21]. Furthermore, upon treatment discontinuation, there is a rapid regression to a density lower than that seen at baseline [21]. This response to treatment has been explained as a triggering of follicles in the latent part of telogen into anagen without prolongation of anagen or reversal of follicular miniaturisation [21]. There are several studies evaluating terminal hair regrowth with finasteride (1 mg). Most of them were done in men between the ages of 18 and 41 [7,16]. Total hair density peaked at 12 months and was maintained in the second year [6]. After treatment cessation, regression to a density lower than that at baseline was observed, although at a slower rate than observed in minoxidil studies [6]. Fifty-two percent of treated men with early stages of HL had a total hair regrowth response, and the odds ratio (OR) of finasteride vs. placebo was 1.67 [7,19]. Interestingly, insulin-like growth factor-I (rhIGF-1) treatment induced the appearance of normal hair and recovery of hairline in IGF-1 deficient populations, thereby highlighting the wide range of molecules that act on the physiology of hair [22]. Combination therapies of topical minoxidil with finasteride, other pharmaceuticals and or supplements have been studied in randomised placebo-controlled trials (RCTs) in men. However, no statistically significant differences with minoxidil or finasteride monotherapies were found [15]. RCTs using Platelet-Rich Plasma (PRP) for the treatment of AGA showed a significant increase in hair density in men [15]. Combination therapies of PRP supplemented by autologous stem cell transplants, micro-needle wounding and low-level laser therapy (LLLT) have also reported significant improvements in hair regrowth [23]. RCTs with vitamins, minerals, botanical extracts, collagen and various marine extracts have shown limited efficacy of TH regrowth in men [15]. Unfortunately a large fraction of men with AGA do not respond to standard treatments, clearly highlighting an unmet need for safe and effective HL therapies.

Aiming to restore normal HF homeostasis, ALRV5XR was designed to target various of the multiple underlying physiological causes of HL. The agent is a formulation of standardised botanical extracts, vitamins, and minerals that can be delivered both systemically, as a supplement, or topically, as a shampoo, conditioner, or follicle serum. ALRV5XR is intended to support normal biochemical signalling, thereby contributing to normal HF physiology. ALRV5XR aims to induce neogen in involuted HFs and prolong anagen of HFs. This combination of actions should aid to regenerate the normal scalp hair phenotype. ALRV5XR was also designed to modulate, directly or indirectly, several molecular pathways of the HF associated with the promotion of hair growth and regrowth, as well as those involved in HL inhibition. Amongst them, activation of the Wnt/ β -Catenin cascade in HF stem cells and dermal papilla cells appears to be a key driver of new hair formation and growth maintenance [2,4,5,13,14,24]. While activation of the Wnt/ β -Catenin signalling pathway with botanical extracts and compounds has been demonstrated [25], inhibition of targets linked to HL is also important as in the case of BMP-4, DHT, or the enzymatic androgen regulator 5α -reductase. In addition, increasing VEGF signalling to enhance blood

flow to HFs, which is linked to the promotion of hair growth is relevant for attainment of a healthy hair physiology [13,14]. Similarly the regulation of additional growth factors such as FGF-7, FGF-18, HGF, IGF, KGF, and cytokines such as mTORC1, PKC, PPAR γ , Shh, COX, INF- α , INF- γ , Interleukins 1b, 6 and 12, NF- κ β , Prostaglandin D2, TGF- β , and TNF- α [13,26] are likely to be influenced at least indirectly by several of the ingredients of ALRV5XR. We demonstrated that ALRV5XR vs. placebo has a clinically significant TH regrowth effect in women with AGA or TE over 24 weeks, where absolute TH density improved by 30.1 THs/cm² ($p = 0.0002$), and relative TH density increased by 19.7% ($p = 0.0016$) [27]. It acts by regenerating the structure and function of non-productive telogen follicles and by prolonging the anagen phase of TH [27]. Our hypothesis is that the wide molecular targeting approach of ALRV5XR, aimed to modulate miscellaneous pathways, results in a safe and efficacious treatment that achieves regeneration and maintains TH in men.

2. Methods

2.1. Study design

This 24-week parallel, randomised, double-blind, placebo-controlled trial was conducted in San Francisco, California, USA in a single centre community clinic. The study protocol was granted ethics approval on March 22, 2018, by IRB Services, identified with the FDA #IRB00000776. The trial was also registered with ClinicalTrials.gov as [NCT04450589](#) and is completed. A CONSORT checklist has been completed and we declare adherence.

2.2. Sample size calculation

The study design was based on the expected efficacy reported in previous minoxidil studies, and required a sample size of 36 subjects, 18 in each group (before dropouts), to achieve a power of 80% and a significance level of 5%. This sample size was predicted to demonstrate clinically meaningful differences between ALRV5XR and placebo groups, as assessed by total hair counts from baseline to week 24. For sample size calculation, the primary objective of absolute difference in hairs/cm² took primacy over the second primary objective of relative difference in per cent. Sample size was calculated using a two-sample t -test power calculation for a one-sided hypothesis test based on a mean absolute target difference of 20 total hairs/cm² (± 23). We anticipated a dropout rate of 20% thereby adjusted the sample size to 23 per group, for a total sample size of 46. Using the same power and significance levels, we further estimated a sample size of evaluable subjects based on previous studies of mean absolute TH differences of 14.7 TH/cm² (± 3.1) for 5% minoxidil and 17.5 TH/cm² (± 8.7) finasteride (1 mg) vs placebo [7]. Hence, a sample size of 10 evaluable subjects in each group was suitable to determine primary efficacy outcomes.

2.3. Study participants

Eligible subjects were healthy 18–65-year-old males with self-reported hair thinning, AGA, TE, or hair loss occurring for more than 3 months prior to screening. Hair loss was confirmed by physical examination done by a certified dermatologist who excluded ineligible types of alopecia, hair, or scalp disease, and assigned a clinical diagnosis of AGA or TE, later confirmed by phototrichometry. AGA was determined clinically by observing hair thinning and miniaturisation in the region of the scalp that was consistent with the Hamilton-Norwood (HN) classification I–VII. AGA diagnosis was confirmed by phototrichogram analysis of diversity of TH diameters and then graded with the anisotrichosis score (the ratio of one standard deviation of TH diameters to the mean TH diameter) of more than 20% and a TH/VH ratio of $< 4:1$. TE was diagnosed by subject history including

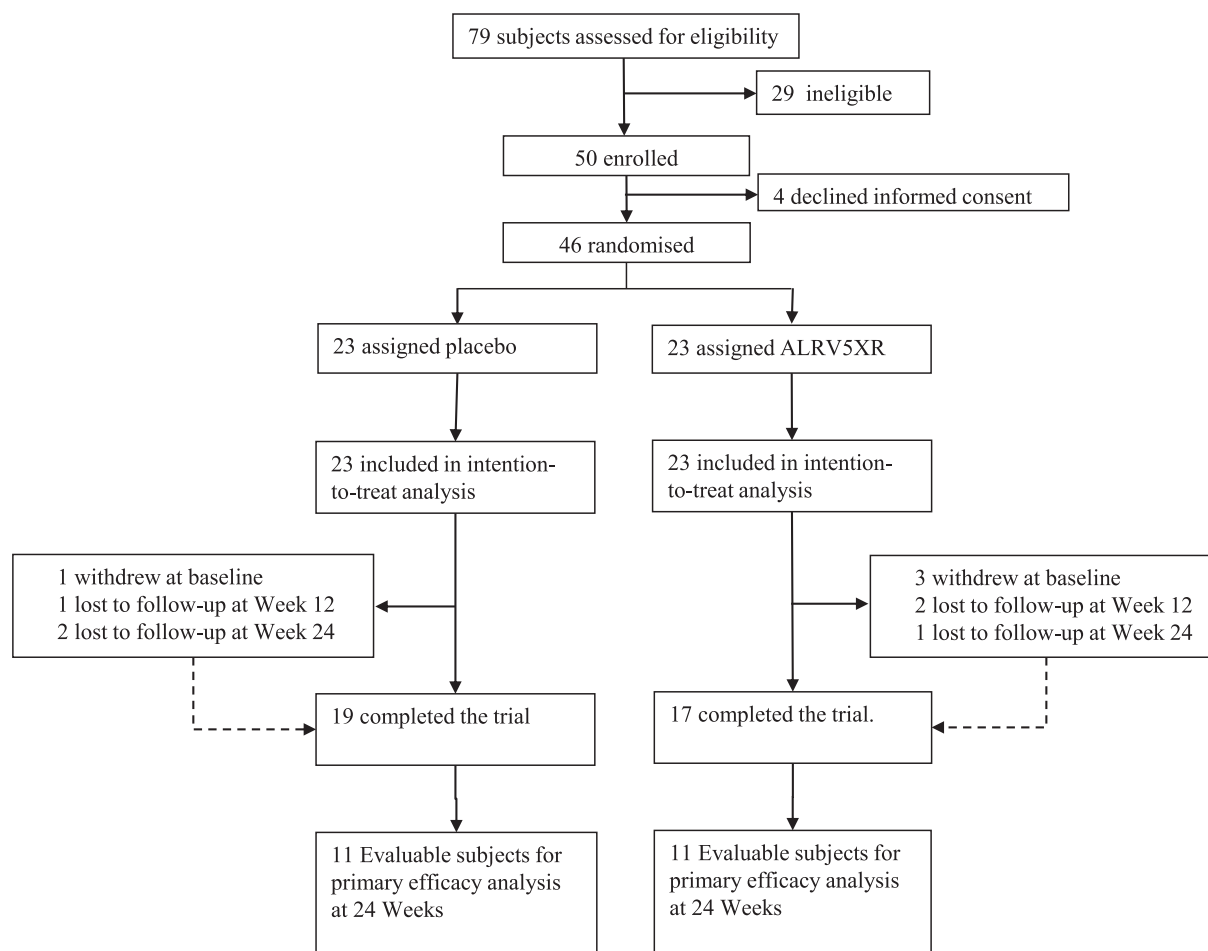


Fig. 1. Profile of Randomised Controlled Trial. 79 subjects were assessed for eligibility which resulted in 19 subjects in the placebo group and 17 subjects in the ALRV5XR (treatment) group completing the trial and were included in the safety analysis. Evaluable subjects for primary efficacy analysis at 24 weeks resulted in 11 from the placebo group and 11 from the ALRV5XR group.

reports of self-reported thinning hair and shedding, by evaluating signs of general thinning or diffuse hair loss of the scalp, and by directly ruling out underlying treatable causes. Phototrichogram analysis of TH telogen rate greater than 20% was used to confirm the TE diagnosis. HN classification was assigned by the clinical dermatologist and verified from global photographs. For this trial, a primary diagnosis of AGA or TE was a prerequisite before subjects were randomised to a given study arm. As seen in Fig. 1, seventy-nine subjects were assessed; 50 deemed as eligible for enrolment in the trial with a primary diagnosis of AGA, and 46 subjects were further enrolled and randomised.

Exclusion criteria included scarring types of alopecia, chemical or physical burns, and traumatic lesions. Reasons for exclusion also included psoriasis, scaling, and follicular dermatitis, fungal or bacterial infection, lice, and flea infestation of the scalp. Additional reasons for exclusion included subjects with unusual thinning patches, traction alopecia or trichokryptomania, trichothiodystrophy, pili annulati, monilethrix or clear signs of trichodysmorphia, undernutrition or poor hygiene. Subjects with abnormalities in or around assessment areas, and scalp hair loss on treatment surfaces due to disease, injury, skin damage or medical therapy as well as those with history of hair transplants, surgical correction of scalp hair loss, and hair weave were also excluded. Men with a known history of HIV/AIDS, or autoimmune diseases such as systemic lupus erythematosus, inflammatory bowel disease, alopecia areata, and alopecia totalis, were not included.

Participating men were not allowed to use razors, depilatories, or wax on the scalp if, in the opinion of the investigators, it might interfere with study assessments. Subjects diagnosed with neurological diseases such as Parkinson's disease, stroke, and traumatic brain injury and those with diabetes, endocrine, cardiovascular disease, or hypertension were excluded. Evidence of hepatic or renal dysfunction were additional reasons for exclusion. Subjects with history of malignancy in the past 5 years or undergoing chemo or radiation therapy were also not included. Men who were on hormone replacement therapy within the past six months were also excluded. Subjects with uncontrolled thyroid disease or any other disorders that may interfere with the treatment were additional reasons for exclusion. Participants were not allowed to use any medications within 30 days prior to the baseline visit, including natural health products, if these were known to affect hair growth.

Forty-six eligible subjects with a diagnosis of AGA (FST I–VI, HN hair loss classification I–VII), as clinically established by a certified dermatologist, were enrolled if willing to follow study requirements and procedures, and after signing an IRB approved informed consent form.

2.4. Randomisation and masking

Randomisation and masked allocation into two blinded groups were conducted as follows: All screened subjects were allocated a sequential screening number by a dermatologist. A second

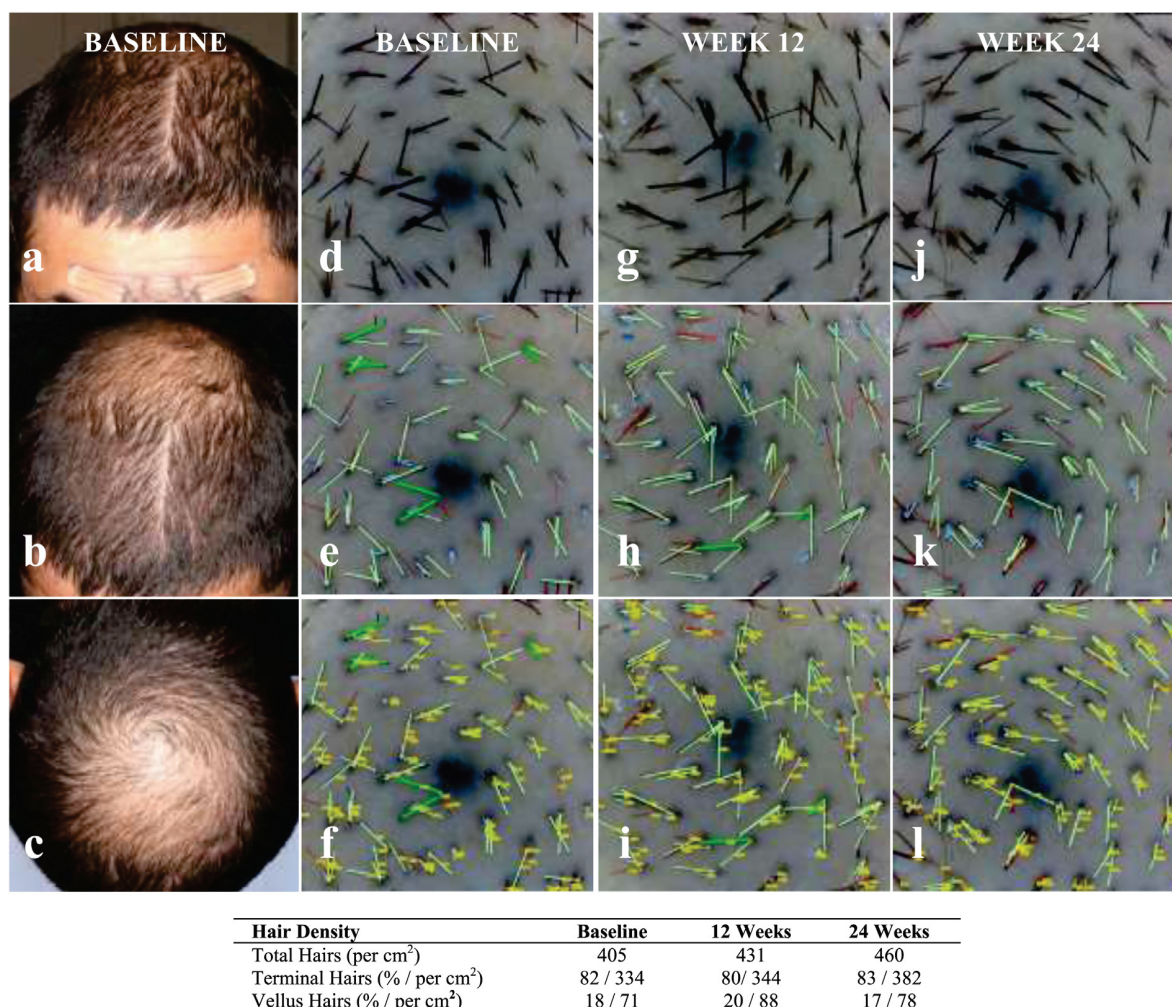


Fig. 2. Sample Global Photographs and Corresponding Phototrichoscopy Computer Image Analysis at baseline, week 12 and week 24. Phototrichoscopy images for Subject: M013 were analysed with the TrichoSciencePro software (version 1.6): a: Baseline (BL) Global Frontal; b: BL Global Top; c: BL Global Vertex; d: BL Phototrichoscopy (PT); e: BL PT Processed (PR); f: BL PT PR & Tagged (TG); g: Week 12 (W12) PT Raw; h: W12 PT PR; i: W12 PT PR & TG; j: Week 24 (W24) PT Raw; k: W24 PT PRD; l: W24 PT PR & TG. This analysis resulted in the following sample measurements: Subject: M013; Sex: Male; Age: 34; Ethnicity: Hispanic; Ethnic Hair Type: Latin/Indian/Semitic; Fitzpatrick Skin Type: IV; Diagnosis: Androgenetic Alopecia; Hamilton-Norwood Class: IIIV; Trichoscopy Area of View: 42.19 mm²; Trichoscopy Magnification: 40x.

dermatologist assigned the screening numbers of the eligible subjects sequentially to a biostatistician generated simple randomisation table. This resulted in subjects being randomised in a 1:1 ratio into one of two parallel blinded treatment groups: Group A or B. Both dermatologists were masked from subject identity, characteristics, and diagnosis, to avoid potential bias during the randomisation process.

Study materials and label codes were masked for study subjects, site dermatologists, site personnel, tricho-analyst, and biostatistician. Upon completion of the biostatistician's report, the trial was unblinded and group A became the Placebo group and Group B the ALRV5XR group. An assessment of randomisation and masking success was evaluated by various factors including baseline characteristics, global photographs, test material dispensing records and inventory, and by observing positive responders in the placebo group.

2.5. Procedures

Similar looking test materials for both groups included oral capsules, shampoo, conditioner, and follicle serum. The ALRV5XR treatment contained botanical extracts, vitamins, and minerals (BVM) as listed in Table S2 of the supplementary materials. Each of the BVMs

was dosed well within their safe limits or recommended daily allowance. Placebo test preparations contained identical vehicle compounds as those in the ALRV5XR group materials. Arbor life labs provided the ALRV5XR and placebo study materials.

All subjects were required to ingest one supplement capsule (842 mg) twice per day, along with daily use of a shampoo (3–7 ml), conditioner (3–7 ml), and follicle serum (1 ml) for a period of 24 weeks. Subjects were required to maintain a diary of the study materials used and to report adverse events. Study duration encompassed 28 weeks, including a screening period designed for eligibility assessment, a baseline visit, and visits at 12 and 24 weeks during the 24 week comparison period.

Subjects were evaluated by a certified dermatologist for eligibility, hair loss diagnosis, and hair loss pattern classification. At weeks 12 and 24, subjects were assessed for changes in safety parameters. A 35 mm Nikon digital camera mounted on a Canfield Scientific stereotactic device was used to take 8MP resolution global photographs of the frontal, mid-scalp, and vertex of the subject's head, at 0, 45 and 90° (See Fig. 2).

Target area hair counts (TAHC) were performed at baseline, and at week 12 and 24. At baseline, a circular area at the centre of the trichoglyph located on the vertex region of the scalp was chosen for the

TAHC. A permanent ink dot tattoo was placed for precise localisation of the target area on subsequent evaluations. Hairs in the target area were clipped to less than 0.5 mm and then dyed. Mineral oil was applied to the target area to enhance the image contrast between hair and scalp.

Phototrichoscopic images of the target area were taken at each visit, using a Firefly DE330T Trichoscope with 2MP resolution at 40x magnification, and a 42.19 mm² field of view. A cover glass was fixed to the lens of the trichoscope to protect the lens and to orientate hair shafts in the target area at 90° to the lens. Evaluable phototrichoscopies for outcomes analysis required that all the procedures be executed, and that the field of view was in focus and the target area was uncontaminated of undyed hair, fluorescence, excess mineral oil, hair clippings or confounding artefacts.

Phototrichoscopy images were evaluated by a blinded tricho-analyst using the TrichoSciencePro version 1.6 computer software. Terminal (non-vellus) hair density was determined by counting hairs in the field of view and by measuring their thickness. It was required that hairs be fully visible from the trimmed end to the exiting position of a scalp pore. THs were identified by a hair diameter greater than or equal to 40 µm, while VHs were those with a diameter of less than 40 µm. Automatic computer analysis of digital images was used to determine hair count and to measure hair shaft diameter. This was repeated and compared using semi-automatic and manual detection, and by diameter measurement performed by a tricho-analyst. TH density was calculated from the number of THs in the TAHC per cm². (See Fig. 2).

2.6. Outcomes measures

The purpose of this study was to evaluate the efficacy and safety of ALRV5XR used for hair regrowth in men with AGA and TE. The primary efficacy objective was the quantification of terminal (non-vellus) hair density change from baseline to week 24, as measured by TH count (THC), and evaluation of TH regrowth by per cent change. Secondary objectives included evaluation of the TH density change from baseline to week 12 and ALRV5XR response rates, as measured in graduated intervals of TH regrowth changes at weeks 12 and 24. Individuals with no TH loss were classified as responders and were grouped by graduated levels of changes in TH density at weeks 12 and 24. A quality of life (QoL) questionnaire was completed by subjects at each visit.

2.7. Safety outcomes

Safety of ALRV5XR was evaluated based on dermatologist assessments and subject reporting. Safety analysis also included physical examinations and blood and urine laboratory tests performed on subjects completing each visit.

2.8. Statistical analysis

Comparisons of treatment and placebo groups were performed using Welch's two-sample *t*-test which allows comparison of group means with differing variances. This method was also used to compare changes within each treatment group from baseline to week 12 and from baseline to week 24, and then confirmed with analysis of variance (ANOVA) using Fisher's *F*-test. For comparison of categorical variables between treatment groups at baseline, Pearson's chi-squared test (with simulated *p*-value) was used [28,29]. Statistical significance was determined as *p* values to be less than or equal to 0.05.

Longitudinal hair variations over time were assessed using a linear mixed-effects model which examines the change in THs from baseline to week 24. All covariates were adjusted, and demographic and physical subject characteristics such as age, weight, ethnicity,

skin type, diagnosis and Hamilton-Norwood classification were considered [30–32]. See Appendix S1 in the Supplementary materials for further details. All statistical analyses were performed using the R software version 4.0.5 (2021–3–31) [33].

2.9. Role of the funding source

The funder of the study had no role in the recruitment of subjects or data collection. The funder had a role in the study design, data analysis and interpretation, as well as writing the report. All authors had full access to all the data in the study and had final responsibility for the decision to submit the report for publication.

3. Results

3.1. Study participants

A total of 46 men with an age range of 22 to 65 years and a primary diagnosis of AGA were enrolled in the study. Twenty-three men were randomly assigned to each group between April 11 and October 23, 2018, and were included in the intent-to-treat population (ITT). After baseline assessment, one subject in the placebo and three in the ALRV5XR group withdrew. The placebo group had one subject who missed the week 12 visit and two subjects the week 24 visit, and were therefore excluded. Two subjects in the ALRV5XR group were lost to follow-up at the week 12 visit and one subject at the week 24 visit, and were also excluded. In summary, 19 subjects receiving placebo and 17 receiving ALRV5XR completed the trial. All subjects completing each visit were compliant with study procedures and with proper use of the materials provided. Missing data or non-evaluable trichoscopic images required to determine a difference from baseline were excluded from the outcomes analysis. Phototrichoscopies from 11 subjects in each group were evaluable at 24 weeks to determine primary efficacy outcomes (see Fig. 1). Baseline demographic and physical characteristics of the ALRV5XR and placebo groups including sex, age, height, weight, body mass index (BMI), blood pressure, TH density, ethnicity, FST, diagnosis, or HN classification can be seen in Table 1.

3.2. Outcomes

Results of hair density analysis were determined per protocol from evaluable phototrichoscopies and are summarised in Tables 2 and 3. They depict the mean changes in THs/cm², and by per cent changes, and are stratified by visit, treatment group, and response rates. The main results are shown in Figs. 3–5. Fig. 3 depicts a strong and significant correlation between treatment duration and ALRV5XR efficacy, measured as increases in TH density. Also, during the trial, there was a statistically significant decrease in VH density when comparing ALRV5XR to placebo (Fig. 4). It was accompanied by a statistically significant increase in the TH to VH ratio (see Fig. 5). After adjusting for categorical variables (age, weight, ethnicity, diagnosis, HN classification and FST), none of the covariates affected the outcome in a statistically significant manner. Differences in QoL changes at 24 weeks between the ALRV5XR and placebo groups were not statistically significant. There is no multiplicity for secondary outcomes.

3.3. Primary outcomes

For the primary outcomes at 24 weeks, ALRV5XR induced a statistically significant TH regrowth of 21.0 THs/cm² more than placebo with an Odds Ratio (OR) of 87.4 [95% CI: 9.2–32.8; (*p* = 0.0014)], and 16.4% more than placebo with an OR of 87.4 [95% CI: 7.4%–25.5%; (*p* = 0.0012)]. Notably, 11 (100%) of evaluable subjects responded favourably in the ALRV5XR group in comparison with two (18.2%) of

Table 1
Baseline Characteristics by Treatment Group (ITT Population).

	Placebo (N = 23)	ALRV5XR (N = 23)
Sex		
Male	23 (100%)	23 (100%)
Female	0 (0%)	0 (0%)
Age (years)		
Mean (± SD)	45.7 (± 12.9)	48.3 (± 7.9)
Range	22–65	32–63
Anthropometrics		
Height (cm) [†]	172.7 (170.3–185.5)	175.3 (170.5–177.8)
Weight (kg) [†]	80.2 (69.3–90.8)	81.4 (73.7–93.6)
BMI (kg/m ²) [†]	25.1 (22.6–31.2)	27.1 (23.7–31.8)
BP (mmHg)	128.3/84.1 (± 13.5/9.9)	128.1/84.4 (± 21.5/17.9)
Trichometry		
Terminal hair density (per cm ²)	141.6 (± 66.2)	139.1 (± 68.6)
Vellus hair density (per cm ²)	139.2 (± 62.2)	123.7 (± 66.2)
Ethnicity		
African American	4 (17.4%)	4 (17.4%)
Caucasian	10 (43.5%)	7 (30.4%)
Chinese	5 (21.7%)	6 (26.1%)
Hispanic	1 (4.3%)	3 (13%)
Indian	1 (4.3%)	0 (0%)
Filipino	0 (0%)	1 (4.3%)
Korean	1 (4.3%)	0 (0%)
Mongolian	0 (0%)	1 (4.3%)
Thai	0 (0%)	1 (4.3%)
Vietnamese	1 (4.3%)	0 (0%)
Fitzpatrick Skin Type		
I	3 (13%)	1 (4.3%)
II	3 (13%)	2 (8.7%)
III	10 (43.5%)	15 (65.2%)
IV	2 (8.7%)	1 (4.3%)
V	2 (8.7%)	1 (4.3%)
VI	2 (8.7%)	3 (13%)
Unknown	1 (4.3%)	0 (0%)
Diagnosis		
AGA	23 (100%)	23 (100%)
TE	0 (0%)	0 (0%)
Hamilton–Norwood Classification		
I	4 (17.4%)	0 (0%)
II	2 (8.7%)	1 (4.3%)
IIA	2 (8.7%)	1 (4.3%)
III	1 (4.3%)	0 (0%)
IIIA	0 (0%)	1 (4.3%)
IIIV	5 (21.7%)	6 (26.1%)
IV	1 (4.3%)	4 (17.4%)
V	0 (0%)	1 (4.3%)
VA	3 (13%)	2 (8.7%)
VI	1 (4.3%)	0 (0%)
VII	2 (8.7%)	3 (13%)
Missing	2 (8.7%)	4 (17.4%)

Abbreviations: ± or SD=standard deviation; † Height, Weight and BMI are reported as Median (IQR).

11 evaluable subjects in the placebo group (see Table 2 and Fig. 3). An ANOVA analysis of the primary efficacy for terminal hair changes for the overall trial of ALRV5XR vs. placebo found a statistically significant Fisher's *F*-test statistic of $F = 13.48$ with $p < 0.0001$. Treatment with ALRV5XR was safe, well-tolerated and no adverse effects were observed. Concordantly, no statistically significant changes were observed in physical examinations or urine laboratory parameters. Any statistically significant changes in blood laboratory parameters were not clinically relevant (see Tables S3a, S3b and S3c in the supplemental materials).

3.4. Secondary outcomes

At 12 weeks the efficacy of ALRV5XR over placebo was 10.9 THs/cm² with an OR of 10.0 [95% CI: 3.4–18.4; ($p = 0.0065$)], and 9.3%

with an OR of 10.0 [95% CI: 2.7%–15.9%; ($p = 0.0084$)]. Ten (90.9%) of 11 evaluable subjects in the ALRV5XR group responded favourably in comparison to five (50.0%) of ten evaluable subjects in the placebo group (see Table 2 and Fig. 3).

3.5. Vellus hair density outcomes

At 24 weeks the average VH density of the ALRV5XR group when compared to placebo changed by -10.5 VHs/cm² with OR 0.1 [95% CI: -1.9 – -19.1 ; ($p = 0.0196$)], and -12.0 with OR 0.1 [95% CI: -2.5 – -21.5 ; ($p = 0.0158$)]. Only three (30.0%) of ten evaluable subjects in the ALRV5XR group had increasing VH density compared to eight (80.0%) of ten evaluable subjects in the placebo group (see Table 2 and Fig. 4).

3.6. Terminal to vellus hair ratio outcomes

At 24 weeks, the average TH/VH ratio in the ALRV5XR group increased significantly by 0.5 compared to placebo, with an OR of 81.0 [95% CI: 0.2–0.9; ($p = 0.0097$)], and 33.0% with an OR of 81.0 [95% CI: 12.5%–53.4%; ($p = 0.0033$)] (see Table 2 and Fig. 5).

3.7. Total hair density outcomes

As described above, when compared to placebo, ALRV5XR induced statistically significant increases in TH density, reductions in VH density, and a higher TH/VH ratio. In addition, there was a non-statistically significant increase of 13.4 hairs/cm² or 5.0% in total hair density.

3.8. Responders

At 24 weeks, a post-hoc analysis showed 100% of the ALRV5XR group vs 18.2% of the placebo responded favourably in TH outcomes. At 12 weeks, 36.4% of the ALRV5XR group increased TH density by more than 10 THs/cm², further increasing to 54.5% at 24 weeks with no subjects losing THs. In contrast, the placebo group reported no increases in TH density at 12 weeks above the 10 THs/cm² level and at 24 weeks, the maximum gain reported, was within the 0–5 THs/cm² segment, and 81.8% of the placebo subjects lost THs. When comparing results measured as the per cent change in TH density, at 12 weeks 18.2% of the ALRV5XR group increased their TH density by more than 10%, further increasing to 45.5% of the group attaining these results at 24 weeks. The placebo group had no increased TH density beyond the levels of 10% at 12 weeks and 5% at 24 weeks. Moreover, at 12 weeks, the greatest increases in TH density in the ALRV5XR group was 25.0 THs/cm² and 22.9% vs. 9.0 THs/cm² and 7% in the placebo group. At 24 weeks, the greatest increases seen in the ALRV5XR group were 51.0 THs/cm² and 46.8% vs 3.0 THs/cm² and 1.6% in the placebo group. (More details are presented in Table 3, Supplementary Fig. S1 and Supplementary Table S4).

4. Discussion

During this 24 week clinical trial, ALRV5XR was well-tolerated by all subjects and displayed a comparable safety profile to that of placebo. Overall, ALRV5XR demonstrated superiority vs. placebo in the treatment of AGA. The net efficacy effect, as reflected by odds ratio determination, was favourable to the ALRV5XR treatment and increased by a factor of 9 from 12 to 24 weeks. Outcomes were not affected by covariates such as age, weight, ethnicity, FST, diagnosis or HN classification.

Baseline trichoscopic and characteristic differences between study groups were not statistically or clinically relevant and did not affect study outcome, especially considering that the balance of the differences had no influence on the results of either group. When

Table 2

Summary of Efficacy: Mean Changes in Hair. This table shows statistically significant efficacy in primary outcomes of the ALRV5XR group measured by increases in absolute and per cent Terminal Hair at week 24. Secondary outcomes all show statistically significant changes in favour of the ALRV5XR group at 24 weeks and in absolute and per cent Terminal Hair at week 12. The effect size shows highly favourable odds ratios in favour of ALRV5XR at 12 weeks, with multi-fold increases from 12 to 24 weeks.

	Placebo					ALRV5XR					Efficacy			Effect size
	N	Mean	SD	Min	Max	N	Mean	SD	Min	Max	Δ-Mean	(95% CI)	p-value	
Terminal hairs/cm ²														
Week 0–12	10	−2.9	± 8.0	−17	9	11	8.0	± 8.4	−3	25	10.9	3.4 – 18.4	0.0065	10.0
Week 0–24 (Primary)	11	−5.8	± 5.5	−12	3	11	15.2	± 18.0	0	51	21.0	9.2 – 32.8	0.0014	87.4
Terminal Hairs (%)														
Week 0–12	10	−2.5%	± 6.0%	−12.1%	7.0%	11	6.7%	± 8.2%	−3.4%	22.9%	9.3%	2.7 – 15.9%	0.0084	10.0
Week 0–24 (Primary)	11	−4.5%	± 4.1%	−12.3%	1.6%	11	11.9%	± 13.8%	0	46.8%	16.4%	7.4 – 25.5%	0.0012	87.4
Vellus Hairs/cm ²														
Week 0–12	8	−0.3	± 7.8	−12	−8	10	−2.8	± 9.6	−19	17	−2.6	−11.4 – 6.3	0.5511	0.2
Week 0–24	10	4.8	± 6.9	−8	15	10	−5.7	± 11.0	−24	12	−10.5	−19.1 – 1.9	0.0196	0.1
Vellus Hairs (%)														
Week 0–12	8	−0.3%	± 7.2%	−10.1%	11.1%	10	−1.7%	± 10.1%	−11.1%	23.9%	−2.1%	−11.2 – 7.0%	0.6314	0.2
Week 0–24	10	−4.9%	± 7.5%	−6.7%	20.5%	10	−7.1%	± 12.2%	−23.6%	9.9%	−12.0%	−21.5 – 2.5%	0.0158	0.1
Terminal to Vellus Ratio														
Week 0–12	8	−0.0	± 0.2	0.3	0.2	10	0.1	± 0.4	−0.8	0.65	0.1	−0.2 – 0.4	0.3955	6.7
Week 0–24	10	0.2	± 0.2	0.5	0.1	10	0.4	± 0.6	−0.0	1.8	0.5	0.1 – 0.9	0.0097	81.0
Terminal to Vellus Ratio (%)														
Week 0–12	8	−2.5%	± 13.3%	−19.2%	19.0%	10	10.3%	± 15.0%	−16.9%	32.6%	12.8%	−1.6 – 27.2%	0.0769	6.7
Week 0–24	10	−8.6%	± 9.0%	−23.6%	8.9%	10	24.4%	± 29.4%	−1.3%	92.2%	33.0%	12.5 – 53.4%	0.0033	81.0

Abbreviations: Primary = Primary Outcomes; Week = Baseline; N = Per Protocol evaluable population paired with baseline; SD = standard deviation; Mean = Mean of Differences between Week 12 or 24 and Baseline; Δ-Mean = Difference of Means between ALRV5XR and Placebo.

compared to placebo, the use of ALRV5XR in subjects affected with AGA increased TH density and TH per cent change from baseline to week 12 with statistical significance. The larger effect observed at week 24, with no plateauing or flattening, suggest actions of the ALRV5XR treatment that should be considered as cumulative (See Fig. 3).

The entire ALRV5XR group responded favourably at 24 weeks and 90.9% of the group increased their TH density at 12 weeks. At 12 weeks of treatment, there was a high responder subgroup within the ALRV5XR group. This circumstance generated a bimodal effect that could be appreciated within the responders. This bimodality was not

dependant on absolute TH or % TH density changes. By week 24, the TH density response profile of the ALRV5XR treated group displayed a trimodal distribution of hair regrowth response, with 45.5% of the group behaving as low responders (0–5 THs/cm²), 36.4% as moderate responders (10–20 THs/cm²), and 18.2% as high responders (> 25 THs/cm²). Nevertheless, when evaluated on a percentage basis at 24 weeks, all ALRV5XR subjects were more uniformly distributed. This response pattern suggests that approximately 45% of the ALRV5XR subjects will at least maintain their baseline TH while mildly improving their TH density. Of note, approximately 55% of men will have important favourable responses, regardless of individual characteristics. In clear contrast, 50% and 81.8% of the placebo group subjects decreased their TH density at weeks 12 and 24, respectively. (See Table 3, Supplementary Table S4 and Supplementary Fig. S1).

In addition to statistically significant improvements in TH density, ALRV5XR's effect on vellus hair at 24 weeks was remarkable. Indeed, a statistically significant decrease in VH density and a simultaneous statistically significant increase in the ratio of TH to VH occurred. This effect suggests that ALRV5XR treatment is not only inducing anagen in non-productive telogen hair follicles, but also that it is possibly reversing the miniaturisation of hair, a feature that is a characteristic hallmark of AGA. This transformation of VH to TH occurred from baseline to 24 weeks. (See Figs. 4 and 5). The reversal of the miniaturisation of hair herein reported, while not seen with minoxidil use [21], was observed during the second year of treatment with finasteride in men under 40 years of age [6]. Of note, since AGA and SA have TH miniaturisation as a common feature, and also share parallel derangements in gene expression [1,4], future research on ALRV5XR-induced miniaturisation reversal in SA may show similar results.

The uniform and statistically significant increase in TH in the ALRV5XR group at weeks 12 and 24 was associated with a nine-fold increase, from 10.0 to 87.4 in the OR, from 12 to 24 weeks. This suggests that prolonged treatment with ALRV5XR will probably result in significant TH regrowth well beyond 24 weeks. This finding implies that sustained exposure to ALRV5XR for longer periods may continuously induce anagen in involuted (or telogen) HF and that it might result in substantial regeneration leading to a healthy and normal scalp hair phenotype.

Results from published clinical trials and meta-analyses of current standard therapy showed that 48 weeks of treatment resulted in average gains of hair density of 11.5 THs/cm² for 2% minoxidil, 14.9 THs/cm² for 5% minoxidil, and 14.8 THs/cm² for 1 mg finasteride

Table 3

Response Rates: Change in Terminal Hairs from Baseline. This table shows the post hoc stratified response by treatment group with a substantial increase in response in hair growth in the ALRV5XR group over time, while the placebo group's hair loss continues to progress. The ALRV5XR group separates into a trimodal responder pattern of low, moderate, and high responders when measured by absolute changes in Terminal Hair density however, these response rates are more uniform when stratified by per cent changes in Terminal Hair density. 54.5% of the ALRV5XR group increased their TH density by 10 or more THs/cm² by week 24 and 18.2% of the group increased density by more than 25 THs/cm².

	Placebo		ALRV5XR	
	Week 12	Week 24	Week 12	Week 24
Positive responders	50.0%	18.2%	91.9%	100%
Terminal hairs/cm ²				
<0	50.0%	81.8%	9.1%	—
0–5	40.0%	18.2%	27.3%	45.5%
5–10	10.0%	—	27.3%	—
10–15	—	—	18.2%	27.3%
15–20	—	—	9.1%	9.1%
20–25	—	—	—	—
>25	—	—	9.1%	18.2%
Max regrowth	9	3	25	51
Min regrowth	−17	−12	−3	0
Terminal hairs (%)				
<0%	50.0%	81.8%	9.1%	0.0%
0–5%	40.0%	18.2%	45.5%	45.5%
5–10%	10.0%	—	27.3%	9.1%
10–15%	—	—	—	18.2%
15–20%	—	—	—	9.1%
20–25%	—	—	18.2%	9.1%
> 25%	—	—	—	9.1%
Max regrowth	7.0%	1.6%	22.9%	46.8%
Min regrowth	−12.1%	−12.3%	3.4%	0%

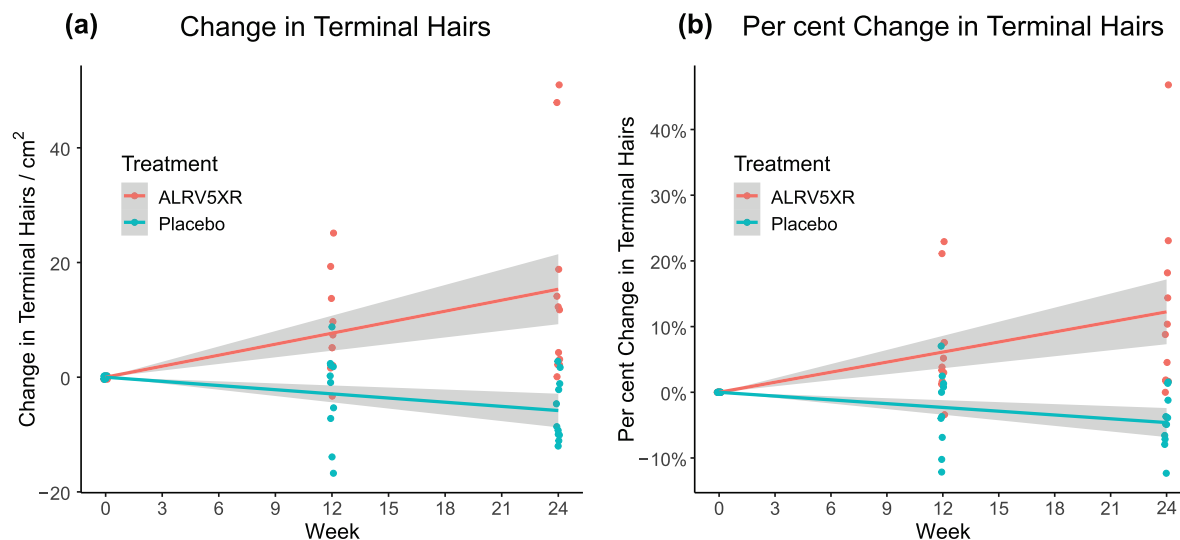


Fig. 3. Changes in Terminal Hair Density from Baseline Over 24 Weeks. Treatment group ALRV5XR was statistically significantly superior to the placebo group for both (a) Absolute Change in Terminal Hairs: 21.0 THs/cm² with an Odds Ratio (OR) of 87.4 [95% CI: 9.2–32.8; ($p = 0.0014$)] and (b) Per Cent Change in Terminal Hairs: 16.4% with an OR of 87.4 [95% CI: 7.4% – 25.5%; ($p = 0.0012$)]. Linear regressions are shown as lines with 95% confidence intervals shown in grey.

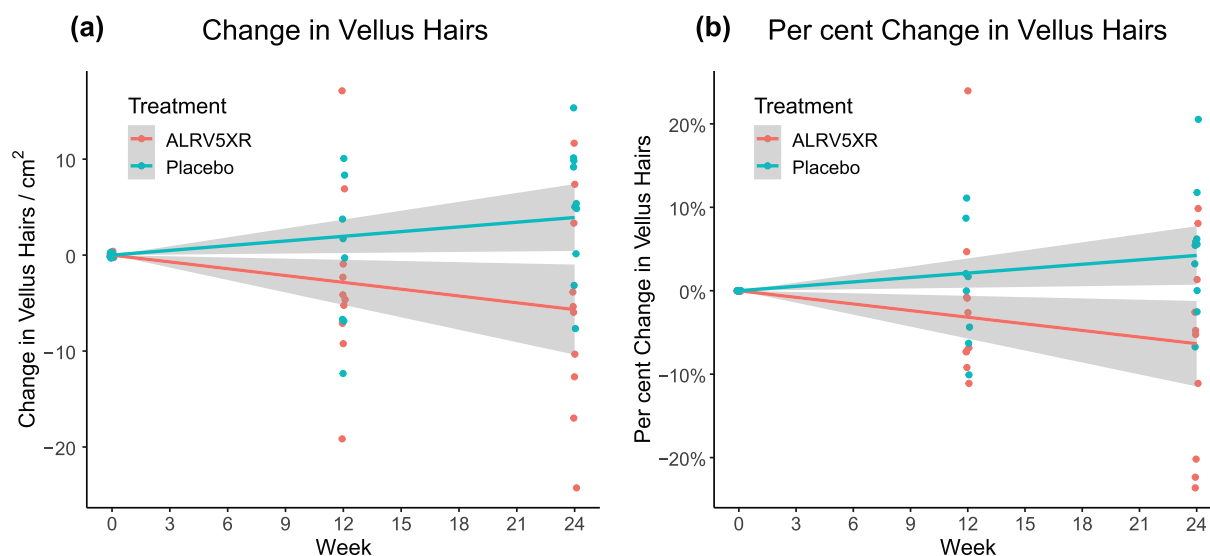


Fig. 4. Changes in Vellus Hair Density from Baseline Over 24 Weeks. Treatment group ALRV5XR decreased statistically significantly to placebo group for both (a) Absolute Change in Vellus Hairs: -10.5 VHs/cm² with OR 0.1 [95% CI: -1.9–19.1; ($p = 0.0196$)] and (b) Per Cent Change in Terminal Hairs: -12.0% with OR 0.1 [95% CI: -2.5%–21.5%; ($p = 0.0158$)]. Linear regressions are shown as lines with 95% confidence intervals shown in grey.

[7,15,16]. The efficacy of ALRV5XR reported in this study displays clear superiority over those results. Indeed, after 12 weeks, ALRV5XR's efficacy of 10.9 THs/cm² reaches 73 to 94% of the 48 week efficacy of standard therapy. At 24 weeks and with an efficacy of 21.0 THs/cm², ALRV5XR reached gains that are 141 to 182% of those corresponding to the efficacy established for standard therapies at 48 weeks. Longer-term studies are needed to further confirm the apparent linear progression of the linear effect of ALRV5XR treatment. The current results suggest that TH regrowth to more than 40 THs/cm² over 48 weeks may be possible. This would be a 2.8 to 3.6 fold efficacy improvement of ALRV5XR over standard therapies. Furthermore, the 100% response rate seen with ALRV5XR is clearly superior to the approximately 34%, 58% and 52% reported for 2%, 5% minoxidil and finasteride (1 mg), respectively [18,19]. Furthermore, ALRV5XR treatment has a clearly improved safety profile when compared to standard therapies, which have been aetiologically associated with a range of miscellaneous adverse events [15].

Attempts to improve hair regrowth in men have included research in various combination therapies using various topical or oral agents. Results showed minimal improvements if any when compared to individual treatments [15], and positive effects are attributed to the addition of either minoxidil or finasteride to such agents. ALRV5XR, while in accord with the combination approach, focuses on multi-molecular targeting designed to directly, or indirectly, modulate various of the known pathways involved in healthy HF biology. Results of its use suggest a synergistic effect that leads to a superior outcome.

An expert opinion on the effects of PRP and autologous stem cell therapy noted that hair regeneration and wound healing share many common molecular pathways, especially the stimulation of Wnt/ β -catenin signalling [26], that are similarly influenced by ALRV5XR. A meta-analysis of clinical trials of PRP in men and women found a mean efficacy of change in total hair count of 23.5 (95%CI: 9.9–37.1) hairs/cm², however within 9–12 months of

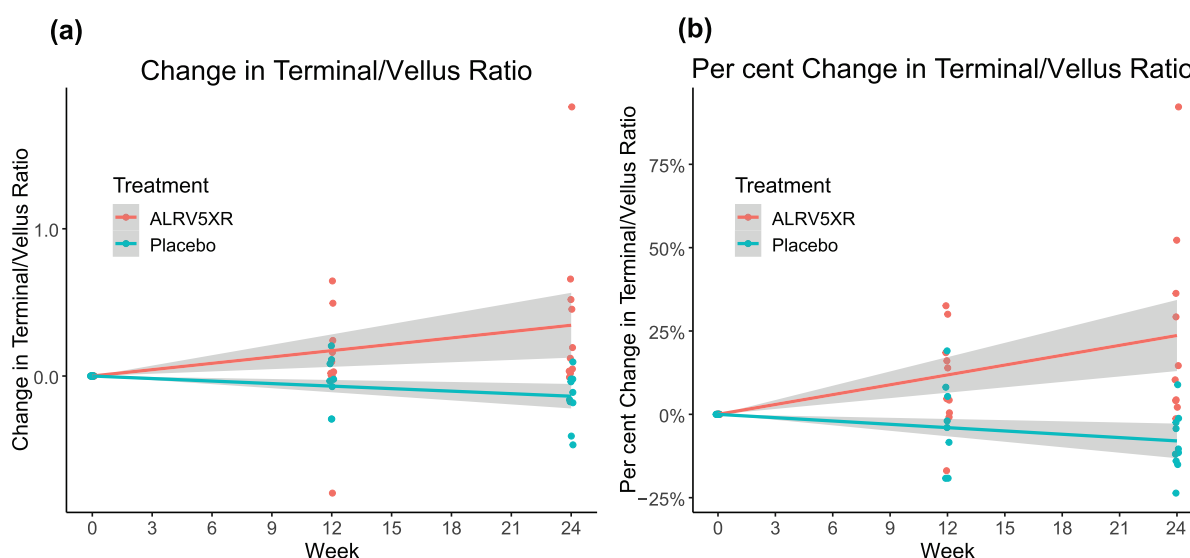


Fig. 5. Changes in Terminal to Vellus Hair Ratio from Baseline Over 24 Weeks. Treatment group ALRV5XR increased statistically significantly to the placebo group for both (a) Absolute Change in Terminal to Vellus Hair Ratio: 0.5 compared to placebo with OR of 81.0 [95% CI: 0.2–0.9; ($p = 0.0097$)] and (b) Per Cent Change in Terminal to Vellus Hair Ratio: 33.0% with an OR of 81.0 [95% CI: 12.5%–53.4%; ($p = 0.0033$)]. Linear regressions are shown as lines with 95% confidence intervals shown in grey.

completing PRP or stem cell therapy, the gains in hair density decline by approximately 50% [16,26]. When considering the initial rapid hair regrowth effect followed by a decline in hair density after PRP and stem cell therapy [23,26], studying the use of ALRV5XR as an adjuvant for pre and post PRP or stem cell therapy, may offer a potentiating effect to enhance or sustain the hair density improvement. Furthermore, this commonality of growth factors and cytokines suggests that ALRV5XR should be investigated for its wound healing and skin maintenance effects.

Due to ALRV5XR's inherent widespread actions, and as hypothesised, it was proven to be a highly effective treatment in regenerating and maintaining TH in men. The statistically significant clinical effect without side effects and the consistent increase in TH density from week 12–24, is likely due to induction of anagen in involuted hair follicles and prolongation of anagen while reversing miniaturisation of hair follicles. These effects can be regarded as regenerative and homeostatic physiological effects on the HF, and have been linked to the activation of hair follicle stem cells via the Wnt/ β -catenin pathway as well as to modulation of multiple cytokines, growth factors and hormone mediators, possibly relieving stress on the HF [2,13,14,22,24]. Besides exploring the events occurring in hair physiology, future research should also focus on defining the main mechanisms of action of ALRV5XR for the potential development of applications in other tissues.

ALRV5XR promises to fill an unmet need for an effective, safe, and well-tolerated treatment for men impacted by HL and within all HN degrees and classifications, regardless of age and ethnicities as well as in all FSTs with premature ageing or senescent hair loss associated with AGA. The multi-molecular targeting approach of ALRV5XR for hair regrowth increased TH density with clinical significance and likely induced normal structure and function of hair follicles via regeneration of non-productive telogen hair follicles, by prolonging anagen and by reversing hair follicle miniaturisation. Besides exhibiting an improved safety profile over the FDA approved standard of care drugs, minoxidil and finasteride, ALRV5XR achieved improvement in efficacy and a 100% response rate in half the time (24 weeks). While longer, larger and multi-centred studies are needed to demonstrate the complete potential and generalisable safety of ALRV5XR in men, the progressive increase of TH regrowth over 24 weeks suggests that ALRV5XR will be an effective long-term treatment for hair

loss in men, and that its prolonged use may restore a normal hair phenotype.

Contributors

PRF and DB contributed to the study concept and design. PRF obtained funding. BMM contributed to recruitment of subjects, investigation, supervision, data acquisition and data curation. PRF, KMF and BMM accessed and were responsible for the raw data associated with the study. PRF, KMF, CP, BMM and JG contributed to data analysis and interpretation. PRF, KMF and CP contributed to the validation, statistical analysis, and visualisation. CP and KMF drafted the manuscript. PRF, KMF, CP, BMM, DB, DJC and JG critically revised the manuscript for important intellectual content.

Declaration of Competing Interest

PRF is the director of Arbor Life Labs (ALL). PRF, DJC and DB are coinventors of patent pending PCT/US2020/067585 titled Botanicals as Wnt/ β -Catenin Activators, Molecular Pathway Regulators, Tissue Regenerators and Health Biomarker Regulators, filed for ALRV5XR and have a share in ALL. PRF, KMF, CP, BMM, DJC and JG, received honoraria from ALL and proposed submission for publication. DJC is an employee of ALL. All authors had full access to the data in the study.

Data sharing

Data collected for the study, including individual participant data and a data dictionary, will be made available. Data available includes de-identified participant data, study protocol and informed consent form. These data will be available beginning 6 months and ending 3 years after publication. Data will be shared with investigator support to researchers who provide a methodologically sound proposal with a signed data access agreement. Contact corresponding author.

Funding

This clinical trial was funded by Arbor Life Labs, Toronto, Ontario, Canada.

Acknowledgments

The authors express their profound appreciation and acknowledgement to Dr Luciano Eugenio Marra, PhD for his contribution to developing ALRV5XR—we dedicate this publication to Dr Marra, who died shortly after the commencement of this trial; Dr Kurt Stenn MD for reviewing the data and his guidance on reporting and interpreting results; Dr Elizabeth Renouf, Ph.D for Biostatistical analysis and Deborah Cahan, without whom ALRV5XR would not have been developed due to her persistent and complex alopecia which dramatically changed her life. This trial was funded by Arbor Life Labs.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:[10.1016/j.eclinm.2021.101124](https://doi.org/10.1016/j.eclinm.2021.101124).

References

- [1] McMichael AJ, Hordinsky MK. Hair and scalp disorders: medical, surgical, and cosmetic treatments. 2nd ed Boca Raton: CRC Press; 2018.
- [2] Matsumura H, Mohri Y, Binh NT, et al. Hair follicle aging is driven by transepidermal elimination of stem cells via COL17A1 proteolysis. *Science* 2016;351(6273):aad4395.
- [3] Cash TF. The psychosocial consequences of androgenetic alopecia: a review of the research literature. *Br J Dermatol* 1999;141(3):398–405.
- [4] Karnik P, Shah S, Dvorkin-Wininger Y, Oshtory S, Mirmirani P. Microarray analysis of androgenetic and senescent alopecia: comparison of gene expression shows two distinct profiles. *J Dermatol Sci* 2013;72(2):183–6.
- [5] Heilmann S, Kiefer AK, Fricker N, et al. Androgenetic alopecia: identification of four genetic risk loci and evidence for the contribution of WNT signalling to its etiology. *J Invest Dermatol* 2013;133(6):1489–96.
- [6] Price VH. Treatment of hair loss. *N Engl J Med* 1999;341(13):964–73.
- [7] Adil A, Godwin M. The effectiveness of treatments for androgenetic alopecia: a systematic review and meta-analysis. *J Am Acad Dermatol* 2017;77(1):136–41 e135.
- [8] Su LH, Chen LS, Lin SC, Chen HH. Association of androgenetic alopecia with mortality from diabetes mellitus and heart disease. *JAMA Dermatol* 2013;149(5):601–6.
- [9] Wambier CG, Vano-Galvan S, McCoy J, Pai S, Dhurat R, Goren A. Androgenetic alopecia in COVID-19: compared to age-matched epidemiologic studies and hospital outcomes with or without the Gabrin sign. *J Am Acad Dermatol* 2020;83(6):e453–4.
- [10] Trüeb RM. Nutrition for healthy hair: guide to understanding and proper practice. Switzerland: Springer Nature; 2020.
- [11] Whiting DA. The structure of the human hair follicle: light microscopy of vertical and horizontal sections of scalp biopsies. NY Canfield: Fairfield; 2004.
- [12] Courtois M, Loussouarn G, Hourseau C, Grollier JF. Ageing and hair cycles. *Br J Dermatol* 1995;132(1):86–93.
- [13] Bernard BA. Advances in understanding hair growth. *F1000Res* 2016;5-147/v1. doi: [10.12688/f1000research.7520.1](https://doi.org/10.12688/f1000research.7520.1).
- [14] Vasserot AP, Geyfman M, Poloso NJ. Androgenetic alopecia: combing the hair follicle signalling pathways for new therapeutic targets and more effective treatment options. *Expert Opin Ther Targets* 2019;23(9):755–71.
- [15] Kanti V, Messenger A, Dobos G, et al. Evidence-based (S3) guideline for the treatment of androgenetic alopecia in women and in men - short version. *J Eur Acad Dermatol Venereol* 2018;32(1):11–22.
- [16] Gupta AK, Bamimore MA, Foley KA. Efficacy of non-surgical treatments for androgenetic alopecia in men and women: a systematic review with network meta-analyses, and an assessment of evidence quality. *J Dermatolog Treat* 2020;1–11.
- [17] Nguyen DD, Marchese M, Cone EB, et al. Investigation of suicidality and psychological adverse events in patients treated with finasteride. *JAMA Dermatol* 2021;157(1):35–42.
- [18] Olsen EA, Dunlap FE, Funicella T, et al. A randomized clinical trial of 5% topical minoxidil versus 2% topical minoxidil and placebo in the treatment of androgenetic alopecia in men. *J Am Acad Dermatol* 2002;47(3):377–85.
- [19] Leyden J, Dunlap F, Miller B, et al. Finasteride in the treatment of men with frontal male pattern hair loss. *J Am Acad Dermatol* 1999;40(6):930–7 Pt 1.
- [20] Kwack MH, Kang BM, Kim MK, Kim JC, Sung YK. Minoxidil activates beta-catenin pathway in human dermal papilla cells: a possible explanation for its anagen prolongation effect. *J Dermatol Sci* 2011;62(3):154–9.
- [21] Messenger AG, Rundegren J. Minoxidil: mechanisms of action on hair growth. *Br J Dermatol* 2004;150(2):186–94.
- [22] Guevara-Aguirre J, Torres C, Pena G, et al. IGF-I deficiency and enhanced insulin sensitivity due to a mutated growth hormone receptor gene in humans. *Mol Cell Endocrinol* 2021;519:111044.
- [23] Gentile P, Garcovich S. Systematic review of platelet-rich plasma use in androgenetic alopecia compared with minoxidil(R), finasteride(R), and adult stem cell-based therapy. *Int J Mol Sci* 2020;21(8):2702.
- [24] Herman A, Herman AP. Mechanism of action of herbs and their active constituents used in hair loss treatment. *Fitoterapia* 2016;114:18–25.
- [25] Choi BY. Targeting Wnt/beta-catenin pathway for developing therapies for hair loss. *Int J Mol Sci* 2020;21(14).
- [26] Gentile P, Alves R, Cole JP, et al. AIRMESS – academy of international regenerative medicine & surgery societies: recommendations in the use of platelet-rich plasma (PRP), autologous stem cell-based therapy (ASC-BT) in androgenetic alopecia and wound healing. *Expert Opin Biol Ther* 2021:1–7.
- [27] Feldman P, Fiebig K, Piwko C, et al. Safety and efficacy of ALRV5XR in women with androgenetic alopecia or telogen effluvium: a randomized, double-blinded, placebo-controlled clinical trial. *EClinicalMedicine* 2021;37:100978.
- [28] Dupont WD. Statistical modeling for biomedical researchers: a simple introduction to the analysis of complex data. New York: Cambridge Press; 2002.
- [29] Venables WN, Ripley BD. Modern applied statistics with s-plus. 4th ed Verlag, New York: Springer; 2002.
- [30] Fitzmaurice GM, Laird N, Ware JH. Applied longitudinal analysis. 2nd ed New Jersey: Wiley; 2011.
- [31] Lang W. Mixed effects models for complex data. New York: CRC Press; 2010.
- [32] Hackl S, Koch A, Lasch F. Empirical evaluation of the implementation of the EMA guideline on missing data in confirmatory clinical trials: specification of mixed models for longitudinal data in study protocols. *Pharm Stat* 2019;18(6):636–44.
- [33] R Core Team. A language and environment for statistical computing. Vienna, Austria: R foundation for statistical computing. 2012; available at: <http://cran.r-project.org>.

SUPPLEMENTARY MATERIALS

EClinicalMedicine 40 (2021) 101124

<https://doi.org/10.1016/j.eclinm.2021.101124>

Safety and Efficacy of ALRV5XR in Men with Androgenetic Alopecia: A Randomised, Double-Blinded, Placebo-Controlled Clinical Trial

Authors: Peter R Feldman^{a*}, Klaus M Fiebig^{a*}, Charles Piwko^b, Boris M Mints^c, Dennis Brown^d, Deborah J Cahan^a
and Jaime Guevara-Aguirre^{e,f,g,h}

Author Affiliations:

^a Arbor Life Labs, Toronto, Ontario, Canada

^b CHP Pharma Inc, Thornhill, Ontario, Canada

^c B&B International, Vilnius, Lithuania

^d Valent Technologies, Menlo Park, California, USA

^e Universidad San Francisco de Quito (USFQ), Quito, Ecuador

^f Institute of Endocrinology, Metabolism, and Reproduction (IEMR), Quito, Ecuador

^g Maastricht University, Maastricht, The Netherlands

^h College of Medicine, University of Florida, Gainesville, Florida, USA

* Corresponding authors.

E-mail address: petefeldman@gmail.com (P. Feldman), klausfiebig@gmail.com (K. Fiebig)

Supplementary Tables

Table S1: Abbreviations

Table S2: ALRV5XR Material Active Ingredients

Table S3: Laboratory and Physical Exam Results.

Table S3a: Blood Laboratory Results.

Table S3b: Urine Laboratory Results.

Table S3c: Physical Exam Results.

Table S4: Adverse Events.

Table S5: ALRV5XR Responder Distribution.

Supplementary Figures

Figure S1: ALRV5XR treatment group changes in Terminal Hair.

(a) Changes per cm².

(b) Changes by per cent.

Supplementary Content

Appendix S1: Supplementary Statistical Methods

Supplementary Tables and Figures

Table S1. Abbreviations

Text Abbreviations	
AGA	Androgenetic Alopecia
BVM	Botanical, Vitamin and Mineral
FDA	Food and Drug Administration
FPHL	Female Pattern Hair Loss
HF	Hair Follicle
HFSC	Hair Follicle Stem Cell
HL	Hair Loss
MOA	Mechanism of Action
MPHL	Male Pattern Hair Loss
QoL	Quality of Life
SC	Stem Cell
TAHC	Target Area Hair Count
TE	Telogen Effluvium
TH	Terminal Hair
THC	Terminal Hair Count
VH	Vellus Hair
Units of Measure	
CI	Confidence Interval
cm	Centimetre
µm	Micrometre
mm	Millimetre
OR	Odds Ratio
%	Per cent
p	Statistical Probability – T-Test
F	Fisher Statistic - ANOVA Test
Growth Factors	
EGF	Epidermal Growth Factor
FGF-7	Fibroblast Growth Factor – 7
FGF-18	Fibroblast Growth Factor – 18
HGF	Hepatocyte Growth Factor
IGF	Insulin Growth Factor
KGF	Keratinocyte Growth Factor
VEGF	Vascular Endothelial Growth Factor
Dermal Papilla cell and Stem Cell stimulation	
BMP	Bone Morphogenic Proteins
mTORC1	Mammalian Target of Rapamycin – 1
PKC	Protein Kinase C
PPARγ	Peroxisome Proliferator-Activated Receptor Gamma
Shh	Sonic Hedgehog
Wnt	Wingless-related integration site
Immunomodulation	
COX	Cyclooxygenase
IFN-α	Interferon-alpha
IFN-γ	Interferon-gamma
IL-1b, IL-6 and IL-12	Interleukins -1b, 6, -12
NF-κβ	Nuclear Factor Kappa Beta
PGE2	Prostaglandin E2
PGD2	Prostaglandin D2
TNF-α,	Tumour Necrosis Factor alpha
TGF-β	Transformation Growth Factor beta
Androgens & Mediators	
5α-R	5α-Reductase
DHT	Dihydrotestosterone

Table S2. ALRV5XR Material Active Ingredients

	Active Ingredients*
Oral (capsule)	Angelica sinensis, Ascophyllum nodosum, Astragalus membranaceus, Biotin, Coenzyme Q10, Copper, Eclipta prostrata, Folate, Ganoderma lucidum, Malus domestica, Moringa oleifera, Niacin, Pantothenic acid, Polygonum multiflorum, Punica granatum, Riboflavin, Rosmarinus officinalis, Scutellaria baicalensis, Selenium, Silicon, Sophora flavescens, Vitamin A, Vitamin B6, Vitamin B7(Biotin), Vitamin B12, Vitamin D, Vitis vinifera, and Zinc
Shampoo	Caffeine, Eclipta prostrata, Lupinus, Momordica charantia, Moringa oleifera, Pyrus malus, Rosmarinus officinalis, Vitis vinifera, Scutellaria baicalensis, Vitamin B7(Biotin)
Conditioner	Caffeine, Eclipta prostrata, Lupinus, Momordica charantia, Moringa oleifera, Pyrus malus, Rosmarinus officinalis, Vitis vinifera, Scutellaria baicalensis, Vitamin B7(Biotin)
Follicle serum	Caffeine, Eclipta prostrata, Lupinus, Momordica charantia, Moringa oleifera, Ocimum basilicum, Pisum sativum, Pyrus malus, Rosmarinus officinalis, Vitis vinifera, Scutellaria baicalensis, Vitamin B7(Biotin)

*Patents pending

Table S3: Laboratory and Physical Exam Results. At trial completion, there were statistically significant differences between the groups (highlighted in grey) for serum Albumin, HDL Cholesterol and Platelets. These differences were within the normal range, except for Albumin, which was slightly above the normal range for both groups, however, the ALRV5XR group was trending towards the normal range. These differences were determined not clinically relevant.

Table S3a: Blood Laboratory Results.

			Placebo									ALRV5XR									Placebo vs ALRV5XR			
			Baseline			12 Weeks			24 Weeks			Baseline			12 Weeks			24 Weeks			T-Tests P-Value (Placebo vs. ALRV5XR)			
Analyte	UOM	Reference Range	N	Mean	Stddev	N	Mean	Stddev	N	Mean	Stddev	N	Mean	Stddev	N	Mean	Stddev	N	Mean	Stddev	BL	BL - 12 Weeks	BL - 24 Weeks	12 - 24 Weeks
Blood:																								
Albumin	g/dL	3.4-4.2	18	4.47	0.213	16	4.54	0.247	17	4.55	0.245	21	4.42	0.291	16	4.40	0.262	16	4.33	0.316	0.535	0.186	0.015	0.045
Alkaline Phosphatase	IU/L	46-119	18	69.44	13.929	16	68.31	13.190	17	68.24	12.996	21	71.05	19.800	16	69.81	19.793	16	74.38	17.663	0.781	0.384	0.771	0.176
ALT (SGPT)	IU/L	0-29	18	25.06	18.969	16	25.31	13.967	17	23.24	12.120	21	36.81	40.014	16	34.69	31.492	16	31.75	18.663	0.274	0.406	0.796	0.737
AST (SGOT)	IU/L	0-120	18	24.89	9.533	16	24.56	8.888	17	22.94	7.787	21	24.57	6.884	16	29.69	19.924	16	23.75	7.980	0.907	0.291	0.945	0.516
Baso (Absolute)	x10E3/uL	0.0-0.6	18	0.02	0.037	16	0.03	0.043	17	0.02	0.038	21	0.01	0.035	16	0.02	0.039	16	0.03	0.046	0.842	0.564	0.441	0.186
Basos	%	Not Estab.	18	0.67	0.577	16	0.50	0.500	17	0.47	0.499	21	0.57	0.495	16	0.69	0.583	16	0.63	0.484	0.592	0.103	0.173	0.410
Bilirubin, Total	mg/dL	0.0-1.2	18	0.54	0.254	16	0.50	0.250	17	0.59	0.253	21	0.57	0.334	16	0.49	0.287	16	0.49	0.245	0.786	0.584	0.146	0.262
BUN	mg/dL	6-24	18	14.83	2.774	16	15.63	2.997	17	15.71	3.706	21	15.33	4.663	16	16.50	2.449	16	15.00	3.354	0.700	0.952	0.502	0.174
Calcium	mg/dL	8.6-10.4	18	9.40	0.269	16	9.45	0.341	17	9.33	0.349	21	9.48	0.384	16	9.48	0.283	16	9.39	0.325	0.496	0.294	0.654	0.723
Carbon Dioxide, Total	mmol/L	16-29	18	24.89	1.882	16	24.56	2.474	17	24.06	2.100	21	25.62	1.588	16	24.69	2.256	16	22.88	1.166	0.209	0.969	0.109	0.432
Chloride	mmol/L	96-106	18	100.39	1.976	16	101.19	2.242	17	101.35	1.780	21	100.81	2.788	16	102.19	1.590	16	102.44	1.456	0.605	0.084	0.104	0.756
Cholesterol, Total	mg/dL	100-169	18	174.78	38.888	16	188.69	28.504	17	174.47	30.817	21	203.19	34.436	16	207.38	40.009	16	185.56	36.975	0.024	0.151	0.274	0.936
C-Reactive Protein, Cardiac	mg/L	0.00-3.00	17	1.78	3.038	16	1.59	2.468	17	1.70	2.044	21	1.99	2.031	16	2.46	3.632	16	2.24	2.666	0.807	0.769	0.408	0.649
Creatinine	mg/dL	0.44-1.19	18	0.93	0.114	16	0.99	0.107	17	0.96	0.148	21	0.94	0.170	16	0.98	0.167	16	0.91	0.143	0.791	0.688	0.888	0.902
eGFR IF Africn Am	mL/min/1.73	>59	18	111.83	13.777	16	104.75	12.467	16	109.63	17.084	21	108.48	17.565	16	105.75	19.337	16	111.25	17.181	0.527	0.480	0.778	0.805
eGFR IF Nofricn Am	mL/min/1.73	>59	18	96.78	11.914	16	90.50	10.671	16	94.69	14.696	21	94.00	15.191	16	91.44	16.677	16	96.38	14.887	0.545	0.480	0.730	0.832
Eos	%	Not Estab.	18	2.72	1.520	16	2.63	2.088	17	2.12	1.409	21	2.90	1.269	16	3.25	1.199	16	2.88	1.364	0.693	0.646	0.217	0.602
Eos (Absolute)	x10E3/uL	0.0-0.6	18	0.16	0.117	16	0.19	0.175	17	0.12	0.094	21	0.19	0.106	16	0.23	0.083	16	0.21	0.103	0.347	0.600	0.380	0.284
Glucose	mg/dL	65-99	18	94.50	8.946	16	93.56	28.346	17	94.29	16.619	21	93.95	8.952	16	96.06	16.690	16	103.00	14.405	0.854	0.346	0.120	0.642
HDL Cholesterol	mg/dL	>39	18	49.00	7.930	16	48.69	9.231	17	49.29	6.875	21	51.00	8.690	16	48.75	12.070	16	45.25	6.505	0.472	0.517	0.044	0.166
Hematocrit	%	31.9-57.2	18	43.34	2.959	16	43.70	3.671	17	42.41	3.435	21	44.22	2.867	16	44.35	2.295	16	43.99	2.369	0.362	0.420	0.426	0.301
Hemoglobin	g/dL	10.7-20.5	18	14.51	1.068	16	14.54	1.243	17	14.21	1.301	21	14.75	1.213	16	14.71	1.036	16	14.59	1.067	0.536	0.638	0.378	0.355
Immature Grans (Abs)	x10E3/uL	Not Estab.	18	0.00	0.000	15	0.00	0.000	17	0.00	0.000	21	0.00	0.021	16	0.01	0.024	16	0.01	0.024	0.361	1.000	1.000	1.000
Immature Granulocytes	%	Not Estab.	18	0.06	0.229	15	0.00	0.000	17	0.06	0.235	21	0.19	0.499	16	0.19	0.390	16	0.13	0.331	0.311	0.948	0.677	0.170
INR	N	0.8-1.2	18	1.03	0.047	16	1.03	0.043	17	1.05	0.061	21	1.02	0.039	15	1.01	0.034	16	1.04	0.048	0.321	0.326	0.477	0.106
Insulin	uIU/mL	2.6-24.9	19	10.07	6.510	16	21.41	24.915	16	16.90	16.183	19	12.78	9.982	16	30.81	31.045	16	32.73	35.052	0.342	0.648	0.336	0.624
LDL Cholesterol Calc	mg/dL	0-109	18	104.11	35.559	16	109.25	28.904	16	98.06	24.608	21	126.62	31.347	15	123.73	36.547	15	101.20	22.956	0.048	0.295	0.452	0.849
Lymphs	%	Not Estab.	18	33.28	6.296	16	30.81	7.764	17	30.12	7.925	21	34.00	7.764	16	33.75	8.151	16	32.75	10.103	0.760	0.175	0.260	0.761
Lymphs (Absolute)	x10E3/uL	0.9-5.0	18	1.82	0.484	16	2.02	0.526	17	1.71	0.484	21	2.20	0.627	16	2.23	0.618	16	2.15	0.654	0.047	0.123	0.572	0.303
Magnesium	mg/dL	1.6-2.4	18	2.14	0.111	16	2.08	0.151	17	2.07	0.102	21	2.17	0.145	16	2.13	0.148	16	2.10	0.132	0.455	0.852	0.879	0.421
MCH	pg	26.1-38.7	18	29.28	2.523	16	29.32	2.731	17	29.26	2.605	21	29.81	2.332	16	29.73	2.380	16	29.63	2.512	0.506	0.725	0.149	0.168
MCHC	g/dL	31.9-36.8	18	33.48	1.052	16	33.28	1.063	17	33.48	0.741	21	33.32	0.883	16	33.16	1.055	16	33.15	1.074	0.619	0.687	0.795	0.706
MCV	fL	79-110	18	87.33	6.791	16	87.94	6.995	17	87.35	6.721	21	89.43	5.786	16	89.63	6.071	16	89.31	6.400	0.317	0.950	0.292	0.123
Monocytes	%	Not Estab.	18	7.67	2.082	16	7.81	1.424	17	7.76	2.263	21	8.00	1.799	16	7.44	1.731	16	7.50	1.458	0.604	0.142	0.391	0.690
Monocytes(Absolute)	x10E3/uL	0.2-1.3	18	0.41	0.110	16	0.51	0.122	17	0.43	0.118	21	0.54	0.191	16	0.49	0.130	16	0.50	0.137	0.021	0.001	0.062	0.061
Neutrophils	%	Not Estab.	18	55.61	7.447	16	57.81	7.410	17	59.47	9.300	21	54.33	8.374	16	54.69	9.432	16	56.13	10.816	0.629	0.329	0.285	0.903
Neutrophils (Absolute)	x10E3/uL	1.2-6.1	18	3.12	0.991	16	3.88	1.101	17	3.45	1.026	21	3.62	1.124	16	3.67	0.887	16	3.77	1.012	0.161	0.011	0.063	0.151
Phosphorus	mg/dL	2.5-7.1	18	3.27	0.421	16	3.50	0.582	17	3.16	0.511	21	3.20	0.453	16	3.29	0.597	16	2.94	0.579	0.644	0.424	0.311	0.808
Platelets	x10E3/uL	140-396	18	270.44	59.770	16	272.13	56.253	17	247.88	43.710	21	267.52	43.177	16	251.25	40.172	16	277.75	44.463	0.864	0.139	0.102	0.013
Potassium	mmol/L	3.7-5.2	18	4.51	0.205	16	4.47	0.293	17	4.50	0.243	21	4.42	0.374	16	4.38	0.248	16	4.34	0.333	0.370	0.938	0.621	0.577
Prothrombin Time	sec	10.2-15.4	18	10.48	0.289	16	10.44	0.341	17	10.60	0.485	21	10.27	0.360	15	10.19	0.360	16	10.38	0.419	0.059	0.512	0.521	0.176
RBC	x10E6/uL	3.68-5.77	18	4.98	0.413	16	4.99	0.432	17	4.87	0.374	21	4.97	0.447	16	4.96	0.253	16	4.95	0.406	0.937	0.407	0.807	0.569
RDW	%	12.1-16.9	18	13.89	1.142	16	14.24	1.163	17	13.98	0.859	21	13.90	0.961	16	13.75	0.839	16	13.77	0.820	0.975	0.002	0.418	0.283
Sodium	mmol/L	134-144	18	140.72	1.557	16	140.81	2.038	17	140.76	2.237	21	141.52	2.038	16	140.50	1.768	16	141.06	0.827	0.193	0.315	0.859	0.529
Triglycerides	mg/dL	0-74	18	108.50	57.696	16	153.38	65.116	17	148.94	97.428	21	128.00	60.768	16	180.69	97.521	16	188.63	122.040	0.326	0.907	0.958	0.852
TSH	uIU/mL	0.700-15.200	18	1.50	0.864	16	1.57	1.023	17	1.30	0.689	21	1.85	0.842	16	1.71	0.768	16	1.50	0.772	0.225	0.633	0.356	0.697
Uric Acid	mg/dL	3.3-8.4	18	6.31	1.610	16	6.13	1.419	17	5.85	1.128	21	6.22	1.353	16	6.05	0.973	16	5.94	1.061	0.851	0.680	0.742	0.978

Table S3b: Urine Laboratory Results.

			Placebo									ALRV5XR									Placebo vs ALRV5XR			
			Baseline			12 Weeks			24 Weeks			Baseline			12 Weeks			24 Weeks			T-Tests P-Value (Placebo vs. ALRV5XR)			
Analyte	UOM	Reference Range	N	Mean	Stddev	N	Mean	Stddev	N	Mean	Stddev	N	Mean	Stddev	N	Mean	Stddev	N	Mean	Stddev	BL	BL - 12 Weeks	BL - 24 Weeks	12 - 24 Weeks
Urine:																								
Alb/Creat Ratio	mg/g creat	0.0-30.0	18	4.74	4.535	16	4.36	3.242	17	3.86	2.480	21	12.90	29.818	16	5.96	7.800	16	14.06	24.084	0.270	0.495	0.722	0.191
Albumin, Urine	ug/mL	Not Estab.	18	7.97	8.961	16	5.76	4.177	17	5.58	4.439	21	18.10	44.045	16	8.40	11.427	16	24.47	42.448	0.356	0.732	0.779	0.198
Creatinine, Urine	mg/dL	Not Estab.	18	174.93	82.364	16	151.26	75.552	17	158.31	80.166	21	138.19	67.787	16	141.09	72.827	16	157.72	62.722	0.145	0.610	0.326	0.954
pH		5.0-7.5	18	5.86	0.847	16	5.69	0.527	17	6.26	0.987	21	5.98	0.932	16	5.84	0.896	16	6.19	0.827	0.698	0.949	0.314	0.646
Specific Gravity		1.005-1.030	18	1.02	0.007	16	1.02	0.007	17	1.02	0.008	21	1.02	0.007	16	1.02	0.007	16	1.02	0.006	0.236	0.970	0.417	0.956
Urobilinogen, Semi-Qn	mg/dL	0.2-1.0	18	0.29	0.251	16	0.25	0.194	17	0.34	0.305	21	0.24	0.170	16	0.25	0.194	16	0.30	0.265	0.471	0.964	0.521	0.680

Table S3c: Physical Exam Results.

			Placebo									ALRV5XR									Placebo vs ALRV5XR			
			Baseline			12 Weeks			24 Weeks			Baseline			12 Weeks			24 Weeks			T-Tests P-Value (Placebo vs. ALRV5XR)			
Analyte	UOM	Reference Range	N	Mean	Stddev	N	Mean	Stddev	N	Mean	Stddev	N	Mean	Stddev	N	Mean	Stddev	N	Mean	Stddev	BL	BL - 12 Weeks	BL - 24 Weeks	12 - 24 Weeks
Physical Exam:																								
Height (cm)	cm		22	175.94	12.783	22	175.94	12.783	22	175.95	12.733	22	173.70	9.522	22	173.59	10.298	22	173.93	9.183	0.523	0.927	0.628	0.784
Weight (kg)	kg		22	84.47	23.029	21	84.17	22.782	19	85.38	22.886	22	84.77	17.277	20	87.13	15.915	18	88.68	13.889	0.962	0.711	0.858	0.903
BMI	kg/m^2		22	27.25	6.109	21	27.35	5.939	19	27.44	5.515	22	28.26	6.153	20	29.05	5.459	18	29.52	5.534	0.596	0.766	0.791	0.863
Temperature	deg F		22	96.94	1.600	21	97.72	0.751	19	97.79	0.634	22	97.17	0.679	20	97.81	0.795	18	97.76	0.569	0.552	0.744	0.602	0.575
RespiratoryRate	bpm		22	15.32	1.519	21	15.43	1.620	19	16.37	2.454	22	16.05	2.602	20	15.75	2.256	17	16.06	1.392	0.275	0.092	0.364	0.743
BP-L DIA	mmHg		22	85.91	11.623	20	82.30	11.424	18	81.17	10.194	22	84.95	18.428	20	88.55	15.781	18	85.56	19.511	0.842	0.350	0.477	0.388
BP-L SYS	mmHg		22	128.41	12.405	21	127.00	15.850	19	125.11	15.960	22	128.82	21.670	20	133.60	17.656	18	131.94	20.101	0.941	0.579	0.753	0.650
BP-R DIA	mmHg		20	82.20	10.328	21	83.81	9.205	19	79.68	10.563	22	83.77	18.218	20	86.85	12.167	18	83.56	17.765	0.742	0.994	0.817	0.826
BP-R SYS	mmHg		20	129.00	15.821	21	124.48	14.381	19	127.37	15.401	22	127.32	21.007	20	128.70	18.725	18	129.28	18.535	0.778	0.800	0.945	0.497
Mean BP DIA	mmHg		22	84.11	9.628	21	83.07	9.963	19	81.42	9.967	22	84.36	17.561	20	87.70	12.743	18	84.56	15.404	0.955	0.640	0.765	0.453
Mean BP SYS	mmHg		22	128.34	13.229	21	125.98	13.616	19	126.26	15.076	22	128.07	21.015	20	131.15	15.750	18	130.61	13.141	0.960	0.653	0.859	0.502
Heart rate	bpm		22	77.27	11.258	21	78.29	12.279	19	82.58	12.504	22	77.05	14.537	20	75.10	10.094	17	77.76	9.478	0.955	0.335	0.106	0.496

Table S4: Adverse Events. No adverse events (AE's) or serious adverse events (SAE's) were reported in either the ALRV5XR or Placebo groups. A list of AE's typically reported in clinical trials of AGA are identified.

Possible Adverse Events
Acne
Burning/Stinging
Decreased Libido
Desquamation/Dandruff
Elevated Blood Pressure
Erythema
Feeling of Skin Tension
Folliculitis
Gastrointestinal Complaints/Stomach Upset
Headache
Joint Stiffness
Malaise and Fatigue
Mood Disorders
Neurology-related Complaints
Papules
Peripheral Edema/Fluid Retention
Pruritus
Sexual Dysfunction
Skin Disorders

Table S5: ALRV5XR Responder Distribution. For the ALRV5XR treatment group, the **Change in Terminal Hairs/cm²** shows a significant shift of subjects' hair count changes with a relatively even distribution of 0-20 TH/cm² at week 12. Non-responders from week 12 began to respond by week 24 increasing the proportion of low responders. The overall changes at week 24 show an increased terminal regrowth rate separating into a trimodal effect of low responders increasing density between 0 and 5 THs/cm² (45·5%), moderate responders increasing density 10-20 THs/cm² (36·4%), and high responders regrowing more than 25 THs/cm² (18·2%). The **Per Cent Change in Terminal Hairs** also shows a significant shift of subjects' hair % changes at week 12, increasing regrowth at week 24 to more uniform distribution for moderate and high responders and increasing the proportion of low responders from the population of non-responders at 12 weeks.

	Week 0-12	Week 0-24
Change in Terminal Hairs/cm²		
0-5	30·0%	45·5%
5-10	30·0%	-
10-15	20·0%	27·3%
15-20	10·0%	9·1%
20-25	-	-
≥25	10·0%	18·2%
Per Cent Change in Terminal Hairs		
0-5%	50·0%	45·5%
5-10%	30·0%	9·1%
10-15%	-	18·2%
15-20%	-	9·1%
20-25%	20·0%	9·1%
≥25%	-	9·1%

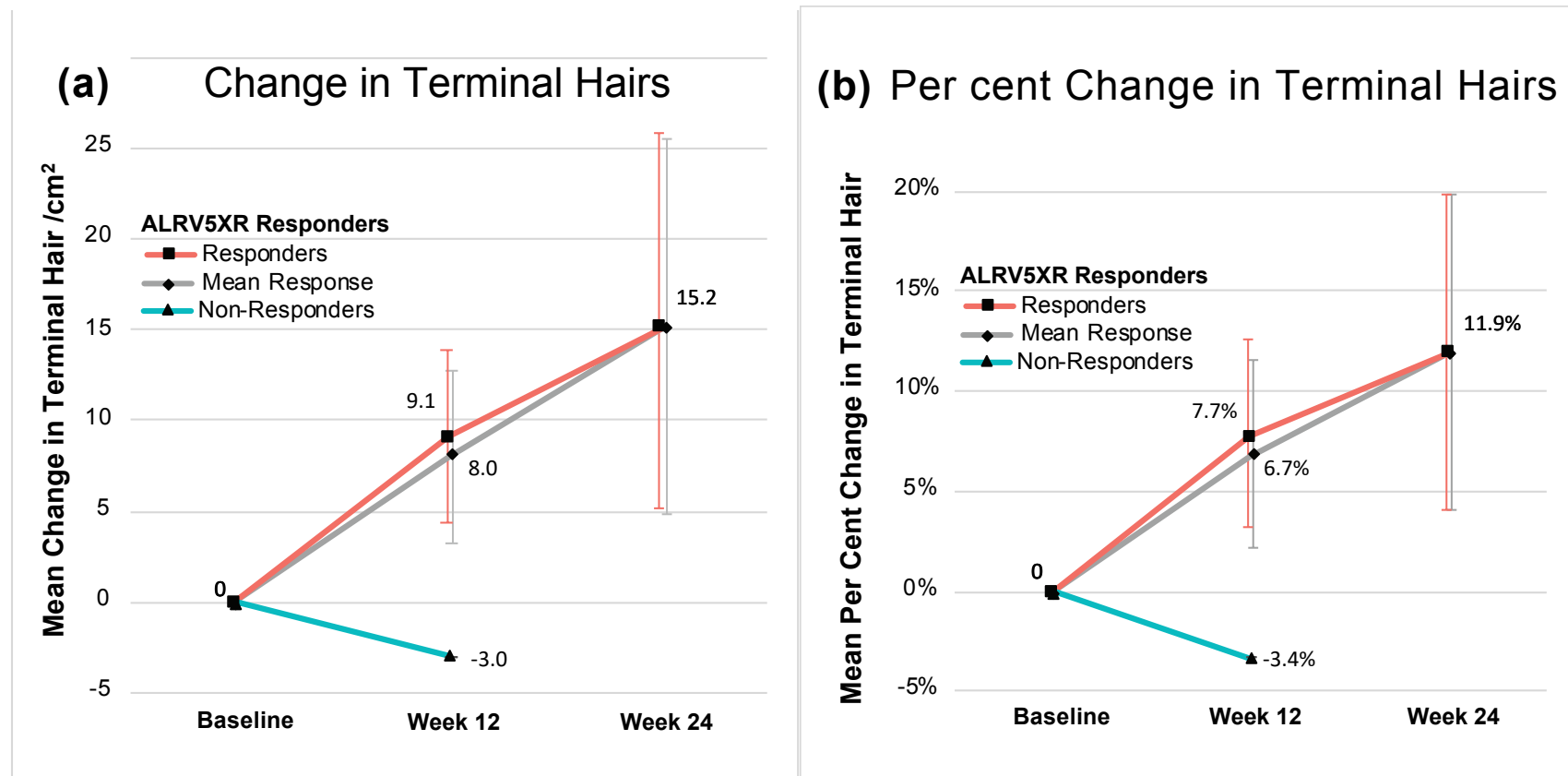


Figure S1. ALRV5XR treatment group changes in Terminal Hair. (a) Changes per cm². (b) Changes by per cent. The mean regrowth response rate within the ALRV5XR group showed a statistically significant increase from baseline ($P_{12\text{weeks}} = 0.0099$ and $P_{24\text{weeks}} < 0.0190$). Non-responders in the first 12 weeks began to regrow hair in the 12 - 24 week period and by week 24, all initial non-responders had become responders. 95% Confidence Intervals for each response group are shown.

Appendix S1: Supplementary Statistical Methods

Mean changes from baseline were analysed using a restricted maximum likelihood based repeated measures approach in combination with the Newton Raphson Algorithm. Analyses include the fixed, categorical effects of treatment, visit, and treatment-by-visit interaction, as well as the continuous, fixed covariates of baseline score and baseline score-by-visit interaction. A common unstructured covariance structure was used to model the within-patient errors. When this analysis failed to converge, structures were tested in a subsequent order until model-convergence was achieved. The Kenward-Roger approximation was used to estimate denominator degrees of freedom. Significance tests were based on least-squares means using a two-sided $\alpha = 0.05$ (two-sided 95% confidence intervals). The primary treatment comparisons, were the contrast between treatments at the endpoint visit. (Hackl S, et al, 2019)

4.2 Summary of Key Findings of Paper 2

ALRV5XR promises to fill an unmet need for an effective, safe, and well-tolerated treatment for men impacted by hair loss associated with AGA. All subjects evaluated were diagnosed with AGA. After 24 weeks, the multi-molecular targeting approach of ALRV5XR for hair regrowth increased terminal hair (TH) density with clinical significance. Efficacy of absolute change in TH was 21.0 THs/cm² (95% CI: 9.2 – 32.8; $p = 0.0014$), and relative efficacy in density was 16.4% (95% CI: 7.4% – 25.5%; $p = 0.0012$). 100% of the treatment group responded (change in TH ≥ 0), and the odds ratio for being a responder was 87.4. Efficacy of the absolute TH/VH ratio was 0.5 (95% CI: 0.2–0.9; $p = 0.0097$). Efficacy of TH density increased linearly from week 12 to 24. Efficacy of relative changes in the TH/VH ratio was 33.0% (95% CI: 12.5%–53.4%; $p = 0.0033$) with an odds ratio of 81.0, as a change in TH/VH ratio ≥ 0 . These regenerative effects induced function of non-productive telogen hair follicles, prolonged anagen and possibly reversed hair follicle miniaturisation. ALRV5XR showed a 100% response rate over the 24-week trial. While more extensive and multi-centred studies are needed to demonstrate the full potential and generalisable safety of ALRV5XR in men, the progressive increase of TH regrowth over 24 weeks suggests that ALRV5XR could be an effective long-term treatment for hair loss in men and that its prolonged use may restore a normal hair phenotype.

5 Results – Paper 3: Sex differences in clinical trials of ALRV5XR treatment of androgenetic alopecia and telogen effluvium.

Feldman, Peter R., Oriel J. Feldman, Jaime Guevara-Aguirre, and Klaus M. Fiebig. “Sex Differences in Clinical Trials of ALRV5XR Treatment of Androgenetic Alopecia and Telogen Effluvium.” Brief Research Report, *Frontiers in Medicine* 9 (2022-August-04 2022). <https://dx.doi.org/10.3389/fmed.2022.918058>.²¹⁵ Supplementary materials are accessible from the link provided in the publication and appended to this chapter.

5.1 Introduction to Paper 3

ALRV5XR treatment of androgenetic alopecia (AGA) and telogen effluvium (TE) has early evidence of regenerating a normal scalp hair phenotype in both sexes. Previous reports found treatment with ALRV5XR showed clinically significant terminal hair (TH) regrowth with no adverse signals or events in two double-blinded randomised placebo-controlled trials (RCTs). This paper analyses the pertinent similarities and differences in the outcomes of the trials between women and men. In addition, new secondary trichometric data provides insight into the different hair regrowth dynamics observed in women and men. This regrowth dynamic determines if new hair was derived from reactivating dormant telogen HFs or the thickening of AGA miniaturised (vellus-like) hairs.

The protocols for this clinical trial were registered on ClinicalTrials.gov, Identifiers: NCT04450602 and NCT04450589. <https://clinicaltrials.gov/ct2/show/NCT04450602> and <https://clinicaltrials.gov/ct2/show/NCT04450589>



OPEN ACCESS

EDITED BY

Manuel Valdebran Canales,
Medical University of South Carolina,
United States

REVIEWED BY

Joanna Czuwara,
Medical University of Warsaw, Poland
Sahar Ghannam,
Alexandria University, Egypt

*CORRESPONDENCE

Peter R. Feldman
petefeldman@gmail.com

SPECIALTY SECTION

This article was submitted to
Dermatology,
a section of the journal
Frontiers in Medicine

RECEIVED 12 April 2022

ACCEPTED 13 July 2022

PUBLISHED 04 August 2022

CITATION

Feldman PR, Feldman OJ,
Guevara-Aguirre J and Fiebig KM
(2022) Sex differences in clinical trials
of ALRV5XR treatment of androgenetic
alopecia and telogen effluvium.
Front. Med. 9:918058.
doi: 10.3389/fmed.2022.918058

COPYRIGHT

© 2022 Feldman, Feldman,
Guevara-Aguirre and Fiebig. This is an
open-access article distributed under
the terms of the [Creative Commons
Attribution License \(CC BY\)](#). The use,
distribution or reproduction in other
forums is permitted, provided the
original author(s) and the copyright
owner(s) are credited and that the
original publication in this journal is
cited, in accordance with accepted
academic practice. No use, distribution
or reproduction is permitted which
does not comply with these terms.

Sex differences in clinical trials of ALRV5XR treatment of androgenetic alopecia and telogen effluvium

Peter R. Feldman^{1*}, Oriel J. Feldman^{1,2},
Jaime Guevara-Aguirre^{3,4,5,6} and Klaus M. Fiebig¹

¹Arbor Life Labs, Toronto, ON, Canada, ²Faculty of Science, Wilfrid Laurier University, Waterloo, ON, Canada, ³College of Medicine, Universidad San Francisco de Quito, Quito, Ecuador, ⁴Faculty of Health, Medicine and Life Sciences, Maastricht University, Maastricht, Netherlands, ⁵Institute of Endocrinology, Metabolism, and Reproduction, Quito, Ecuador, ⁶College of Medicine, University of Florida, Gainesville, FL, United States

Introduction: ALRV5XR treatment of androgenetic alopecia (AGA) and telogen effluvium (TE) has early evidence of regenerating a normal scalp hair phenotype in both sexes.

Design: We performed two 24-week double-blinded placebo-controlled comparison trials, one in each sex, on the ALRV5XR treatment effect on hair regeneration, in AGA and TE, in 92 AGA subjects (24 also had TE). Forty-six women (age 24–64 years) and 46 men (age 22–63 years) were randomized 1:1 to either ALRV5XR or placebo regimens (one b.i.d. oral capsule and daily administration of shampoo, conditioner, and follicle serum).

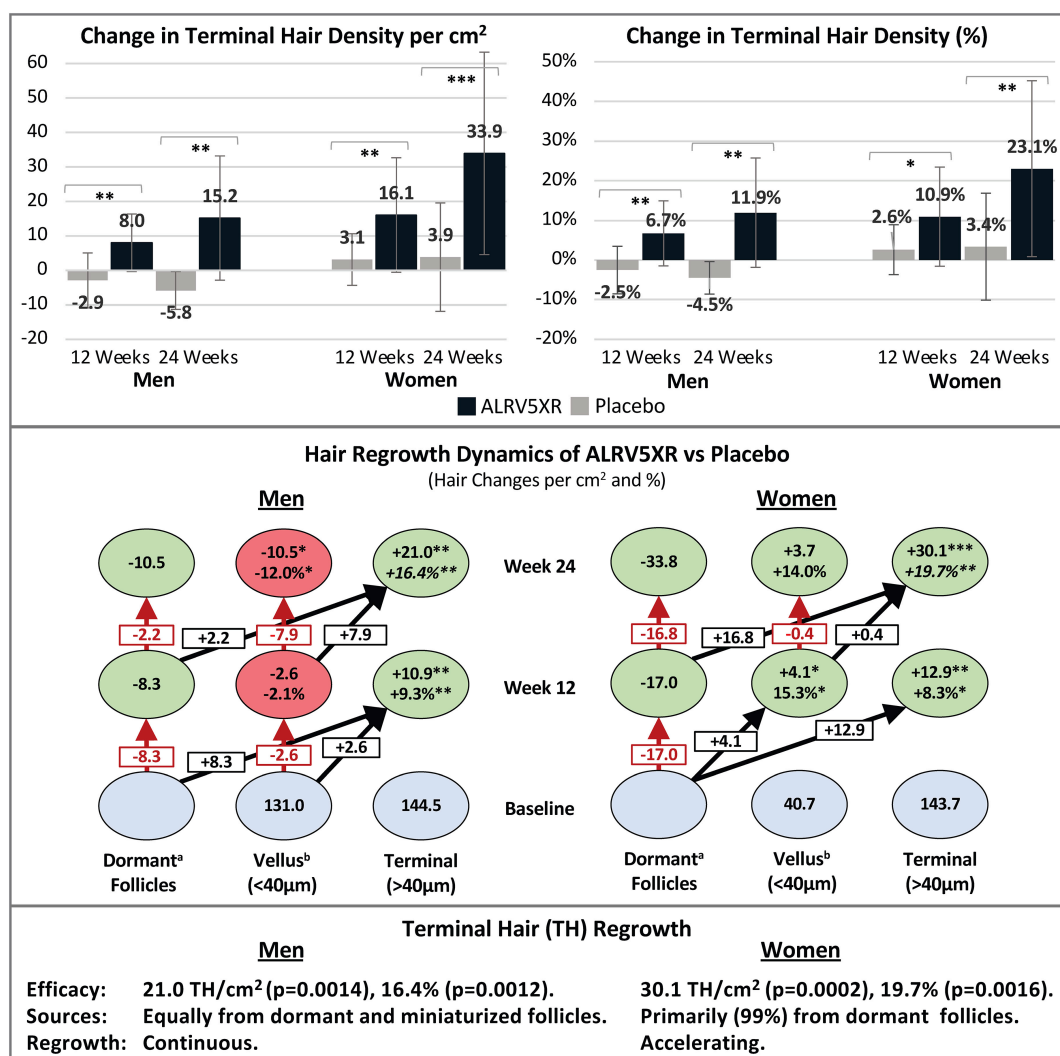
Evaluation: *Primary outcomes:* Absolute and relative changes in terminal hair (TH) density. *Secondary outcomes:* Response rate, changes in vellus hair (VH) density, TH/VH ratio, hair diameter, growth, and shedding rate.

Results: Forty-one women (20 ALRV5XR, 21 placebo) and 36 men (17 ALRV5XR, 19 placebo) completed the trials. TH outcome was evaluable for 18 and 21 women and 11 and 11 men (ALRV5XR, placebo, respectively). Efficacy in women: 30.1 THs/cm² ($p = 0.0002$) and 19.7% ($p = 0.0016$). Efficacy in men: 21.0 THs/cm² ($p = 0.0014$) and 16.4% ($p = 0.0012$). 66.7% of women and 100% of men responded to ALRV5XR. TH/VH ratio for men increased 33.0% ($p = 0.0033$). Growth rate in women increased by 30.7 $\mu\text{m}/24\text{ h}$ ($p < 0.0001$) and 10.0% ($p < 0.0001$). There were no adverse events reported.

Conclusion and relevance: ALRV5XR induced significant regrowth of TH. Accelerating regrowth by reactivation of dormant telogen follicles were the dominant effects in women. Thickening of miniaturized hair and regrowth of dormant telogen follicles contributed equally to the increased TH seen in men (see [Graphical Abstract](#)).

KEYWORDS

ALRV5XR, androgenetic alopecia, pattern hair loss, hair restoration, telogen effluvium, regenerative medicine, miniaturization reversal, hair regrowth dynamics



GRAPHICAL ABSTRACT

Sex differences in hair regrowth with ALRV5XR. Blue ovals are absolute Terminal or Vellus hair counts at baseline. Green ovals represent increases of hair density, and red ovals are reductions. (a) Changes in dormant follicles account for less empty follicles due to new hair regrowth. (b) Vellus Hairs include True Vellus, Vellus-Like and New Hairs. Arrows in black show new hairs from dormant follicles regrowing into Vellus or Terminal hairs, and Vellus hairs thickening into Terminal hairs. Arrows in red are reductions in Vellus hairs or dormant follicles. Statistical significance between ALRV5XR and placebo: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Introduction

ALRV5XR is a novel natural treatment of standardized botanicals, vitamins, and minerals designed to regenerate a normal hair phenotype by targeting more than 20 molecular pathways in the hair follicle (HF) linked to HF stem cell activation, promotion of healthy hair growth, and normal HF physiology. It was designed to modulate multiple cytokines, growth factors, and hormone mediators while relieving stress on the HF and, more specifically, to upregulate growth promotion pathways of the HF while inhibiting targets linked to hair loss. Among these actions, the Wnt/ β -Catenin cascade in HF stem cells and dermal papilla cells is regarded as the

dominant effector for new hair formation and maintenance of growth. Similarly, ALRV5XR includes botanicals known to modulate growth factors such as FGF-7, FGF-18, HGF, IGF, KGF, and VEGF, which are well-characterized in the induction and maintenance of anagen. Additionally, DHT and androgen regulator 5 α -Reductase, as well as cytokines such as mTORC1, PKC, PPAR γ , Shh, BMP-4, COX, INF- α , INF- γ , Interleukins 1b, 6, and 12, NF- κ B, Prostaglandin D2, TNF- α , and TGF- β , are all targets which have been reported to have been influenced directly or indirectly by one or more ingredients of ALRV5XR. ALRV5XR was developed for use as oral supplementation and topical applications in shampoo, conditioner, and serum (see [Supplementary Material](#)).

We previously reported clinically significant terminal hair (TH) regrowth in treatment with ALRV5XR with no adverse signals or events in two double-blinded randomized placebo-controlled trials (RCTs) in women and men with androgenetic alopecia (AGA) and telogen effluvium (TE) (1, 2). We report herein the pertinent similarities and differences in outcomes of the trials between women and men. We also report new secondary trichometric data that provides insight into the different hair regrowth dynamics observed in women and men. In these RCTs, TH was reported as hair with a diameter $> 40 \mu\text{m}$, and vellus or vellus-like hair (VH) with a diameter $< 40 \mu\text{m}$ (1, 2).

We hypothesize that ALRV5XR, a highly effective treatment in regenerating and maintaining TH in women and men, achieves these effects by both reactivating dormant telogen HF and thickening of AGA miniaturized (vellus-like) hairs.

Materials and methods

Two RCTs were conducted in women and men with AGA over 24 weeks. One hundred fifty-one volunteer subjects between the ages of 18 and 65 were screened for eligibility resulting in 92 healthy subjects being enrolled. All subjects were of Fitzpatrick skin type I–VI and had AGA. A primary or secondary diagnosis of AGA or TE was assigned. Forty-six women having Ludwig classification I–II–2 and 46 men with Hamilton–Norwood classification I–VII were randomized 1:1 to ALRV5XR or placebo treatment groups. Scalp and physical evaluation, vertex phototrichograms, blood and urine samples were taken at baseline, 12 and 24 weeks. Subjects were given a masked treatment of ALRV5XR or placebo as one oral capsule (842 mg) taken twice per day and daily administration of shampoo (3–7 ml), conditioner (3–7 ml), and follicle serum (1 ml) over the 24-week study duration (see [Supplementary Material](#)). Primary outcomes were absolute and relative changes in TH densities. Secondary outcomes were response rates, changes in VH density, TH/VH ratios, hair diameter, and growth and shedding rates. The trials were conducted in a community clinic in San Francisco, United States, between April 3 and October 23 of 2018. Primary findings were published with CONSORT adherence in July (women) and October (men) 2021. Trial registration with [ClinicalTrials.gov](#) was NCT04450589 and NCT04450602.

Results at 24 weeks

Participants and baseline trichometry

Ninety-two healthy subjects were enrolled in the two RCTs, with 46 women and 46 men randomly assigned (1:1) into ALRV5XR or placebo groups with mean($\pm$ SD) age

50.1($\pm$ 9.1) and 51.6($\pm$ 10.7) years for women, and 48.3($\pm$ 7.9) and 45.7($\pm$ 12.9) for men, respectively. Forty-one subjects completed the women's trial (20 ALRV5XR, 21 placebo), and 36 subjects completed the men's trial (17 ALRV5XR, 19 placebo), all evaluable for safety. All subjects were diagnosed with AGA, and 23 women (8 ALRV5XR, 15 placebo) also had TE. Two women were non-compliant, resulting in 18 ALRV5XR and 21 placebo evaluable for TH outcomes, and 11 male subjects in each group were evaluable for TH outcomes. At baseline, both sexes had a similar TH density of approximately 140 THs/cm², while men had 3–4 times more VH than women. TH/VH ratios were 3–4.5 for women and 1–1.1 for men. Total hair density for women at baseline was approximately 90 Hairs/cm² less than the men (185 vs. 275 Hairs/cm²). Mean diameters of all hairs and THs for women were 61 and 69 μm , and for men, 43 and 54 μm , respectively. Hair growth rate for women was 307 $\mu\text{m}/24 \text{ h}$, and for men, 232 $\mu\text{m}/24 \text{ h}$ (see [Supplementary Table S1](#)).

Primary results and safety

ALRV5XR increased TH density in both women and men with statistical significance compared to placebo. Women increased their average TH density by 30.1 THs/cm² ($p = 0.0002$) and 19.7% ($p = 0.0016$) with odds ratio (OR) 2.7. Men increased their average TH density by 21.0 THs/cm² ($p = 0.0014$) and 16.4% ($p = 0.0012$) with OR 87.4 ([Table 1](#) and [Figure 1](#)). Having a similar TH density at baseline, the absolute increase in men's TH was 2/3 of the women, and the percentage TH increase for men was 4/5 of women. Efficacy was clinically significant in subgroups of women with a primary diagnosis of AGA or TE, and each was similar to the efficacy of the overall cohort of women. The combined overall TH regrowth efficacy of the two RCTs was 26.3 THs/cm² ($p < 0.0001$) and 18.2% ($p < 0.0001$). In both sexes, these results were not affected by age, ethnicity, hair loss pattern, Fitzpatrick skin type, or body mass index. No clinically relevant adverse signals or adverse events were reported (see [Table 1](#) and [Figure 1](#)).

Secondary results

Response rates

Sixty seven percent of women responded to ALRV5XR compared to 100% of men. In general, the women responders regrew approximately double the amount of TH than the men. In the ALRV5XR women's group, all responders increased TH density by 30 THs/cm² or more, and 44.5% increased their TH density by at least 30%. In the ALRV5XR men's group, 54.5% of responders increased TH by 10 THs/cm² or more, and 18.2% increased TH by more than 25 THs/cm². 45.5% of men increased their TH density by at least 10%, and 18.2% increased TH density by more than 20% (see [Supplementary Table S2](#)).

TABLE 1 Summary of results after 24 weeks.

Summary of efficacy results after 24 weeks of treatment between ALRV5XR and placebo

Efficacy	Men				Women			
	Δ -Mean	95% CI	P-value	OR	Δ -Mean	95% CI	P-value	OR
Hair density changes								
Terminal (per cm ²) ^a	21.0	9.2–32.8	0.0014	87.4	30.1	15.1–45.1	0.0002	2.7
Terminal (%) ^a	16.4	7.4–25.5	0.0012	87.4	19.7	8.0–31.4	0.0016	2.7
Terminal response rate (%)	100.0				67.0			
Vellus (per cm ²)	–10.5	–19.1–1.9	0.0196	0.1	3.7	–1.6–9.0	>0.05	2.4
Vellus (%)	–12.0	–21.5–2.5	0.0158	0.1	11.7	–4.9–28.2	>0.05	2.4
Total (per cm ²)	13.4	–0.9–27.7	>0.05	4.0	33.9	18.1–49.8	0.0001	4.2
Total (%)	5.0	–0.7–10.7	>0.05	4.0	18.6	8.8–28.4	0.0004	4.2
Terminal/vellus ratio changes								
Absolute	0.5	0.1–0.9	0.0097	81.0	0.7	–0.6–2.1	>0.05	0.8
Relative (%)	33.0	12.5–53.4	0.0033	81.0	16.3	–11.9–44.6	>0.05	0.8
Hair diameter changes								
Terminal (μ m)	0.2	–2.9–3.3	>0.05	1.8	–0.7	–3.6–2.2	>0.05	1.8
Total (μ m)	2.0	–0.6–4.7	>0.05	4.1	–1.2	–4.4–2.1	>0.05	1.2
Growth rate changes								
Absolute (μ m per 24 h)	10.4	–0.3–17.6	0.0552	0.8	30.7	19.1–42.3	<0.0001	12.8
Relative (%)	4.8	–0.2–9.8	>0.05	0.8	10.0	6.1–14.0	<0.0001	12.8
Shed rate changes								
Absolute (Scalp per day) ^b	–127.2	–260.1–5.6	0.0594	0.2	22.9	–7.0–52.8	>0.05	0.3
Relative (%)	–21.5	–38.5–4.5	0.0158	0.2	21.8	–6.5–50.2	>0.05	0.3
Safety								
	N				N			
Adverse signals	0				0			
Adverse events	0				0			

^aPrimary objectives results.^bAverage scalp area (700 cm²). Δ -Mean, difference of means between ALRV5XR and placebo; OR, odds ratio; Vellus hair includes true vellus, vellus-like and new hair (all <40 μ m in diameter); N, number of adverse signals or events.

Regrowth rate

The TH regrowth rate in women accelerated during the second 12-week treatment period and was 133% of the first 12-week period. The men's regrowth rate remained constant throughout the 24 weeks (see [Table 1](#)).

Terminal to vellus hair ratio changes

Vellus hair density increased without significance in women, while in men, VH density declined significantly by –10.5 VHs/cm² ($p = 0.0196$) and –12.0% ($p = 0.0158$) with OR 0.1. Consequently, men's TH/VH ratio increased by 33.0% ($p = 0.0033$) with OR 81.0. In women, the TH/VH ratio increased by 11.2% without significance (see [Table 1](#) and [Figure 1](#)).

Total hair density regrowth

Women reported a significant increase in total hair density of 33.9 Hairs/cm² ($p = 0.0001$) with OR 4.2 when comparing ALRV5XR to placebo, while men reported a non-significant increase in total hair density (see [Table 1](#)).

Growth and shedding rates

The growth rate for women treated with ALRV5XR increased by 30.7 μ m/24 h ($p = 0.0001$) or 10.0% ($p = 0.0001$) when compared to placebo, and the men's increase was borderline significant. Hair shedding rates in men decreased by 21.5% ($p = 0.0158$) and in women were trending down relative to the increased density but were not significant (see [Table 1](#)).

Hair regrowth dynamics results

In women, 29.8 TH/cm² or 98.7% of the 30.1 TH/cm² increase in hair density emerged from dormant hair follicles, and 0.4 TH/cm² or 1.3% came from thickening of VH. In men, the density increase of 21.0 TH/cm² was derived equally from dormant hair follicles 10.5 TH/cm² (50%) and 10.5 TH/cm² (50%) were derived from thickening of VH (see [Figure 2](#)).

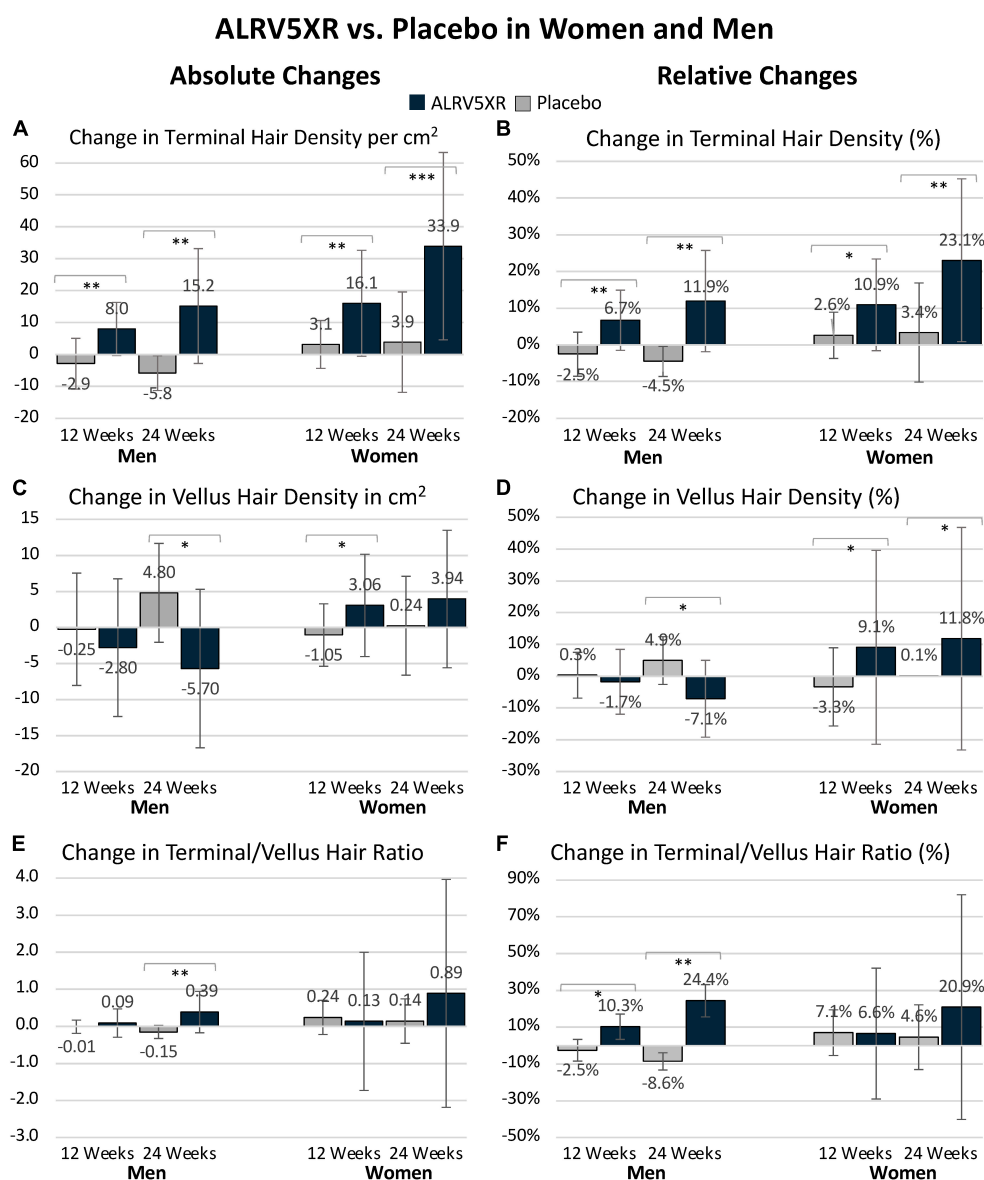


FIGURE 1

ALRV5XR vs. Placebo in women and men with AGA and TE over 24 weeks. (A) Absolute Changes (AC) in Terminal Hairs (TH) per cm²; (B) Relative Changes (RC) in TH in percent (%); (C) AC in Vellus Hair (VH); (D) RC in VH%; (E) AC in TV/VH ratio; (F) RC in TH/VH ratio in %. Statistical significance of ALRV5XR vs. Placebo: **P* < 0.05, ***P* < 0.01, and ****P* < 0.001. Error bars are 95% confidence intervals.

Discussion

Previously, for ALRV5XR, we reported remarkable and clinically significant increases in TH regrowth when compared to other treatments (1, 2). In these studies, both sexes had common TH but highly differential VH densities at baseline (see [Supplementary Table S1](#)). The observed data indicates that both the reactivation of dormant TH follicles and thickening of AGA miniaturized (vellus-like) hairs are sources of the new TH; however, there are significant differences between the sexes (see [Table 1](#) and [Figures 1, 2](#)).

In women, the observed significant increase in TH and total hair densities and non-significant increase in VH and TH/VH ratio suggest that the women's TH regrowth was primarily from activation of dormant TH follicles as seen by the double-digit shift in hair density toward TH at 12 and 24 weeks in [Figure 2B](#). Similar substantial shifts (>15 Hairs/cm²) toward TH are seen in the regrowth dynamics within the ALRV5XR group ([Figure 2D](#)), whereas changes in the placebo group were much smaller (<2.5 Hairs/cm²) as seen in [Figure 2F](#). Furthermore, the non-significant changes in diameters of total hair and TH suggest there was also some thickening of AGA miniaturized

Hair Regrowth Dynamics

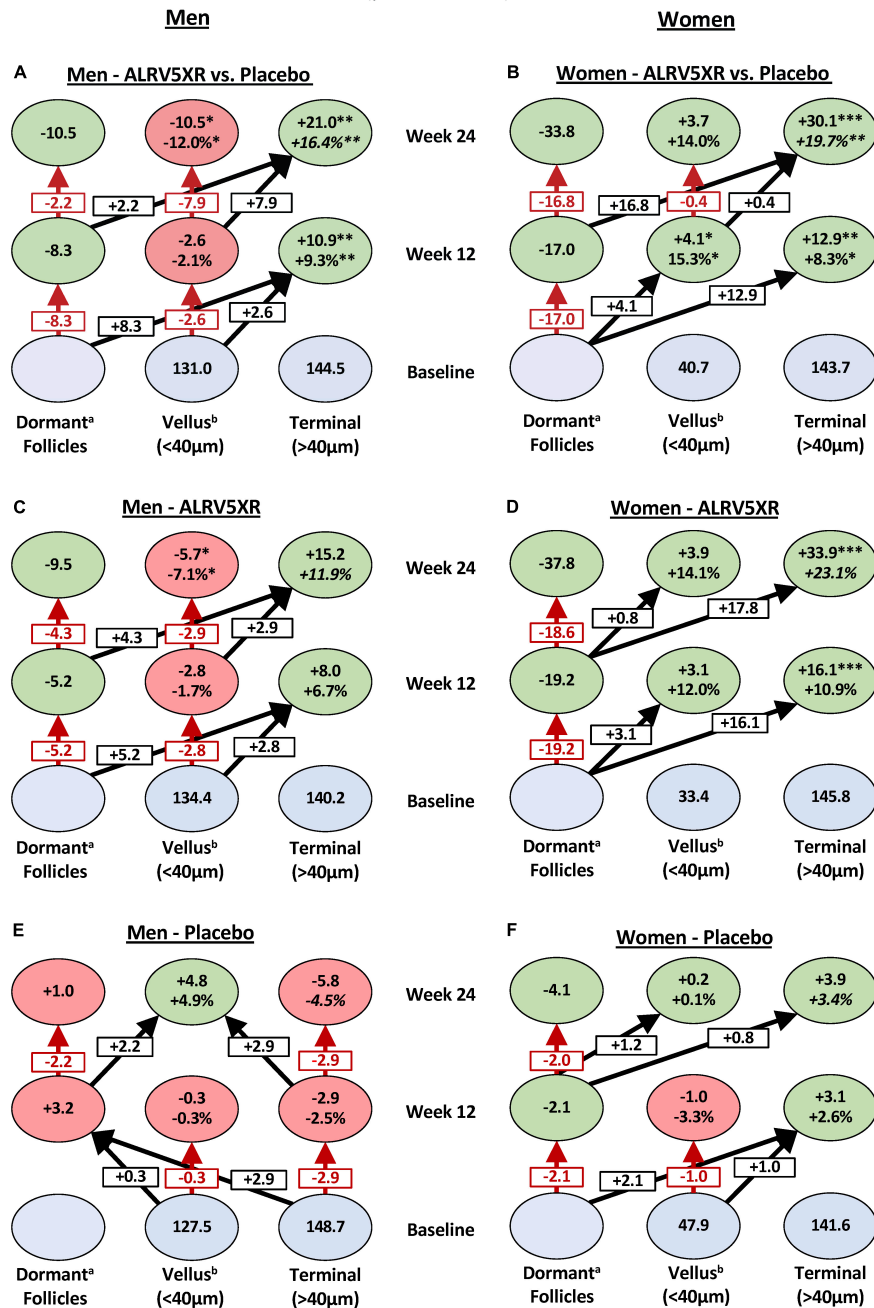
(per cm² and %)

FIGURE 2

Hair regrowth dynamics of ALRV5XR vs. placebo in women and men with AGA and TE over 24 weeks. ALRV5XR vs. Placebo (A,B), ALRV5XR (C,D), placebo (E,F). The dynamics show for women, new hair growth is primarily from dormant hair follicles, and in men, terminal hair is mostly regenerated via miniaturization reversal of vellus-like hair. Baseline values in blue ovals are absolute counts of vellus and terminal hair. Values at 12 and 24 weeks are absolute and relative changes in hair counts from baseline. Decreases in dormant follicles represent the total of new hairs grown. Vellus hair includes true vellus, vellus-like, and new hair. Dormant follicle changes are the sum of vellus and terminal hair changes, and any small differences in these results compared to total hair density changes are due to rounding or methods of analyzing evaluable subjects for total hair density. Dormant follicle counts at baseline are not known. Values at 12 and 24 weeks are absolute and relative changes in hair counts from baseline. For vellus and terminal hairs, green ovals represent increases in hair, and red ovals represent decreases. Decreases in dormant follicles are colored green. a: Changes in dormant follicles account for less empty follicles due to new hair regrowth. b: Vellus Hairs include True Vellus, Vellus-Like, and New Hairs. Arrows in black show new hairs from dormant follicles regrowing into vellus or terminal hairs and vellus hairs thickening into terminal hairs. Arrows in red are reductions in vellus hairs or dormant follicles. Statistical significance between ALRV5XR and placebo: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

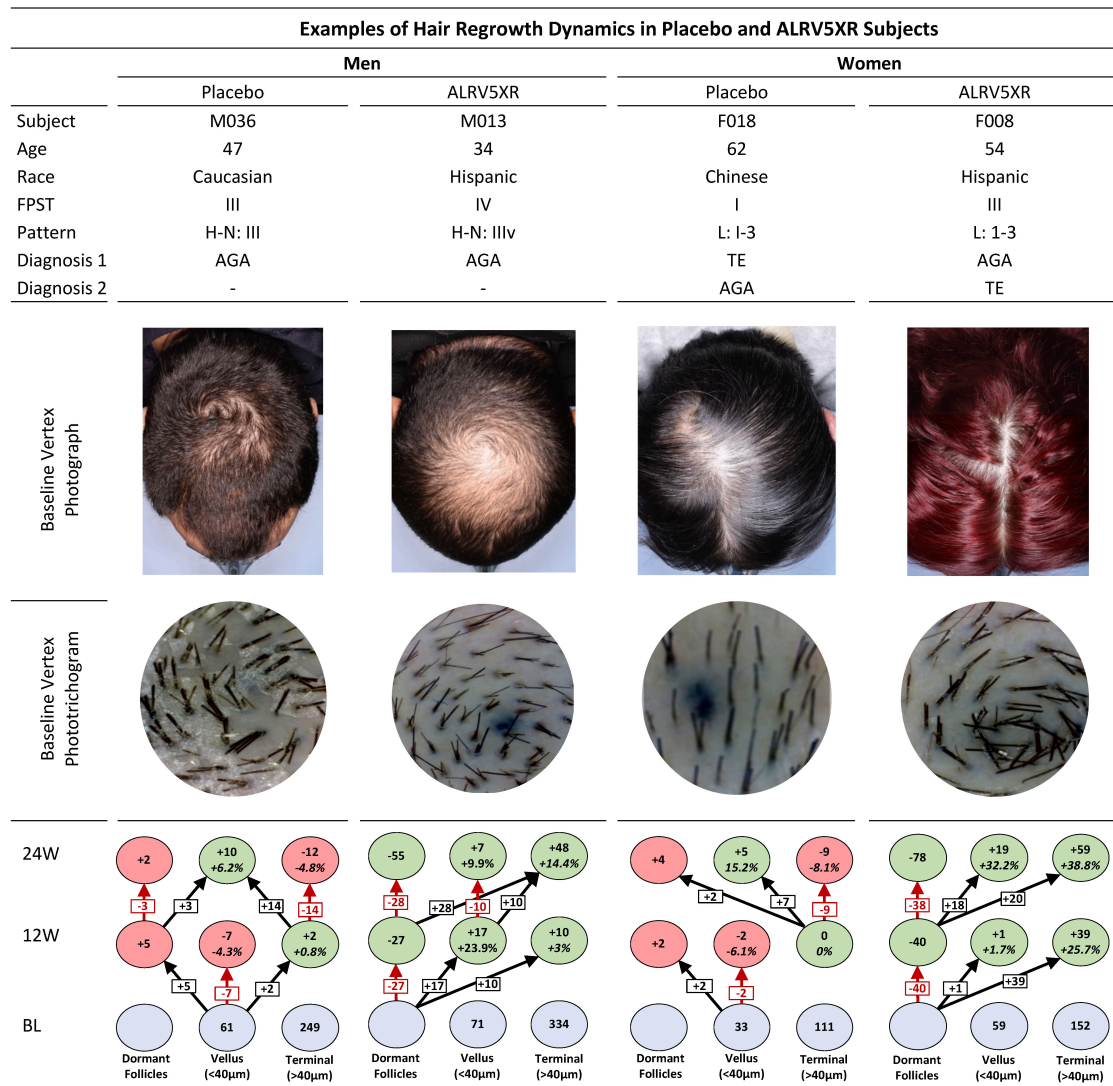


FIGURE 3

Examples of hair regrowth dynamics of ALRV5XR vs. Placebo in subjects over 24 weeks. This figure shows the individual hair regrowth dynamics of four subjects, one male and one female in placebo and ALRV5XR treatment arms. The clinical context of each subject is presented with baseline global vertex photographs and phototrichogram images. The corresponding hair regrowth dynamics chart after 12 and 24 weeks shows the placebo subjects with a progressive reduction in terminal hair density, miniaturizing of terminal hair to vellus hair, and the increasing of empty hair follicles. The ALRV5XR subjects show a completely opposite dynamic of progressive terminal hair regrowth, miniaturization reversal, and decreasing empty follicles. FPST, fitzpatrick skin type; Pattern, H-N: Hamilton Norwood; L, Ludwig; Diagnosis 1 and 2, primary and secondary diagnosis; BL, baseline, 12W and 24 are 12 and 24 weeks, respectively. Blue ovals are absolute terminal or vellus hair counts at BL. Dormant follicle counts at baseline are not known. Values at 12 and 24 weeks are absolute and relative changes in hair counts from baseline. For vellus and terminal hairs, green ovals represent increases in hair, and red ovals represent decreases. Decreases in dormant follicles are colored green. Changes in dormant follicles account for less empty follicles due to new hair regrowth. Vellus hairs include true vellus, vellus-like, and new hairs. Arrows in black show new hairs from dormant follicles regrowing into vellus or terminal hairs, vellus hairs thickening into terminal hairs, terminal hair miniaturizing into vellus. Arrows in red are reductions in vellus hairs, terminal hairs, or increases in dormant follicles.

TH follicles that contributed to the diameter distribution with a thinner caliber of TH (Table 1). Therefore, in women, the dominant effect of TH regrowth was from reactivating dormant TH follicles (Figure 2).

In contrast, ALRV5XR in men saw a significant reduction in VH density along with a significant increase in TH density as seen by a net shift to TH of $> +10$ Hairs/cm² originating

primarily from dormant HF during the first 12 weeks and mainly from VH during the second 12 weeks as seen in Figure 2A. This same effect is seen in the ALRV5XR group, where arrows mostly point to the right in Figure 2C, but net hair shifts are <10 Hairs/cm². In the placebo group, regrowth dynamics are very different, as seen in Figure 2E, with net hair loss of 3.2 Hairs/cm² during the first 12 weeks and a shift

toward miniaturization of TH to VH (+2.9 Hairs/cm²) and regrowth of VH (+2.2 Hairs/cm²) during the second 12-week period. Consequently, the TH/VH ratio increased significantly. In contrast, an increase in total hair density was not significant, indicating that the men's TH regrowth was derived in equal parts from the reversal and thickening of AGA miniaturized TH follicles and regeneration of new THs from dormant HFs. Furthermore, there were no significant changes in average total and TH diameter, supporting the fact that half of the new TH came from empty TH follicles (Table 1). Therefore, in men, TH regrowth was equally from thickening of AGA miniaturized TH and regeneration of new TH from dormant HFs (Figure 2).

For purposes of clinical context, four subjects, one from each trial arm were analyzed with global photographs, phototrichogram images, and individual hair regrowth dynamics charts over 24 weeks of treatment (see Figure 3).

Similar to ALRV5XR in women, the current first-line treatments for AGA (oral finasteride in men and topical minoxidil in women and men) result in TH regrowth mostly from dormant telogen TH follicles (3–6). Nevertheless, these first-line treatments are unable to reverse the miniaturization of vellus-like hairs and attain normal THs as ALRV5XR does in men (3–5). This unique property suggests a novel mechanism of action for ALRV5XR. Interestingly, during induction of hair and skin regeneration, platelet-rich plasma (PRP), autologous stem cell therapy (ACST), and ALRV5XR act as activators of similar growth factor and cytokine subcellular routes, including the Wnt/ β -catenin signaling pathway (1, 2, 7). Moreover, in a related but different matter, it can be observed that senescent alopecia (SA) and AGA display similar miniaturization and histologic features (8). Consequently, the mechanism by which ALRV5XR attains reversal of those changes might illustrate distinct and specific features belonging to physiological regeneration of senescing tissues, thereby allowing the identification of potential sites for pharmacologic intervention.

The above observations suggest that despite clinically significant TH regrowth in both sexes, ALRV5XR is likely to reactivate dormant telogen follicles faster than it can reverse miniaturization of hair. Consequently, women have the potential to restore a more normal scalp hair phenotype quicker than men.

Limitations

The inability to differentiate new hairs amongst true vellus and vellus-like hair diameters <40 μ m by phototrichogram may have contributed to a more conservative TH density and an inflated VH density. Also, more extensive trials are needed to provide more statistically robust data on hair regrowth dynamics.

Findings

ALRV5XR significantly increased TH density in both sexes without adverse effects. Regeneration of structure and function of both dormant telogen and impaired anagen follicles were documented. In women, an accelerated growth rate was observed over two 12-week treatment periods, and reactivation of dormant telogen follicles appeared to be the dominant influence leading to increased TH density. In men, while reactivation of dormant telogen follicles similarly contributed to increased TH density, a novel equally contributing ALRV5XR influence was observed. Indeed, a newfound effect, the thickening of the AGA miniaturized hair, contributed toward normalization of the physiologic hair phenotype. Prolonged treatment may restore a more normal scalp hair phenotype in women in a shorter time than in men.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by IRB-Institutional Review Board Services. The patients/participants provided their written informed consent to participate in this study.

Author contributions

PRF and KMF contributed to the study concept and design. PRF obtained funding and drafted the manuscript. JG-A, OJF, and KMF critically revised the manuscript for important intellectual content. All authors accessed and were responsible for the data associated with the study, contributed to data validation, data analysis, statistical analysis, interpretation, and visualization, and approved the submitted version.

Funding

This study received funding from Arbor Life Labs of Toronto, Canada. The funder was involved in the study design, interpretation of data, the writing of this article, or the decision

to submit it for publication. The funder was not involved in the data collection.

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Acknowledgments

The authors express their profound appreciation and acknowledgment to Dr. Luciano Eugenio Marra, Ph.D., for his contribution to developing ALRV5XR, who died shortly after the commencement of these trials and Deborah Cahan, without whom ALRV5XR would not have been developed due to her persistent and complex alopecia which dramatically changed her life.

Conflict of interest

PRF was employed by Arbor Life Labs (ALL). OJF and KMF received honoraria from ALL.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmed.2022.918058/full#supplementary-material>

References

1. Feldman PR, Fiebig KM, Piwko C, Mints BM, Brown D, Cahan DJ, et al. Safety and efficacy of ALRV5XR in women with androgenetic alopecia or telogen effluvium: a randomised, double-blinded, placebo-controlled clinical trial. *EClinicalMedicine*. (2021) 37:100978.
2. Feldman PR, Fiebig KM, Piwko C, Mints BM, Brown D, Cahan DJ, et al. Safety and efficacy of ALRV5XR in men with androgenetic alopecia: a randomised, double-blinded, placebo-controlled clinical trial. *EClinicalMedicine*. (2021) 40:101124. doi: 10.1016/j.eclinm.2021.101124
3. Rushton DH, Westgate GE, Van Neste DJ. Following historical "tracks" of hair follicle miniaturisation in patterned hair loss: Are elastin bodies the forgotten aetiology? *Exp Dermatol*. (2022) 31:102–9. doi: 10.1111/exd.14393
4. Van Neste D. Exhaustive analysis of scalp hair regression: subjective and objective perception from initial hair loss to severe miniaturisation and drug-induced regrowth. *Plast Aesthet Res*. (2021) 8:16. doi: 10.20517/2347-9264.2020.220
5. Van Neste D. Placebo-controlled dose-effect studies with topical minoxidil 2% or 5% in male-patterned hair loss treated with oral finasteride employing an analytical and exhaustive study protocol. *Skin Res Technol*. (2020) 26:542–57. doi: 10.1111/srt.12827
6. Tamashunas NL, Bergfeld WF. Male and female pattern hair loss: treatable and worth treating. *Cleve Clin J Med*. (2021) 88:173–82. doi: 10.3949/ccjm.88a.20014
7. Gentile P, Alves R, Cole JP, Andjelkov K, Van Helmelyck T, Fernandez J, et al. AIRMESS – academy of international regenerative medicine & surgery societies: recommendations in the use of platelet-rich plasma (PRP), autologous stem cell-based therapy (ASC-BT) in androgenetic alopecia and wound healing. *Expert Opin Biol Ther*. (2021) 21:1443–9.
8. Mirmirani P. Age-related hair changes in men: mechanisms and management of alopecia and graying. *Maturitas*. (2015) 80:58–62. doi: 10.1016/j.maturitas.2014.10.008

SUPPLEMENTARY MATERIALS

Article Title

Sex differences in clinical trials of ALRV5XR treatment of androgenetic alopecia and telogen effluvium

Peter R Feldman¹, Oriel J Feldman^{1,2}, Jaime Guevara-Aguirre^{3,4,5,6}, Klaus M Fiebig¹

¹Arbor Life Labs, Toronto, Ontario, Canada

²Faculty of Science, Wilfrid Laurier University, Waterloo, Ontario, Canada

³College of Medicine, Universidad San Francisco de Quito (USFQ), Quito, Ecuador

⁴Faculty of Health, Medicine and Life Sciences, Maastricht University, Maastricht, The Netherlands

⁵Institute of Endocrinology, Metabolism, and Reproduction (IEMYR), Quito, Ecuador

⁶College of Medicine, University of Florida, Gainesville, Florida, USA

*** Correspondence:**

Corresponding Author: Peter R Feldman

petefeldman@gmail.com

Accepted: 13 July 2022

Contents:

Table S1. Baseline Characteristics

Table S2. Response Rates as Change in Terminal Hairs from Baseline.

Table S3. ALRV5XR Material Active Ingredients

Table S1. Baseline Characteristics

	Men		Women	
	Placebo N=23(11) ^a	ALRV5XR N=23(11) ^a	Placebo N=21(21) ^a	ALRV5XR N=20(18) ^a
Sex				
Male	23 (100%)	23 (100%)	0 (0%)	0 (0%)
Female	0 (0%)	0 (0%)	21 (100%)	20 (100%)
Age (years)				
Mean (±SD)	45.7 (±12.9)	48.3 (±7.9)	51.6 (±10.7)	50.1 (±9.1)
Median (IQR)	48.0 (34.0 – 54.3)	47.0 (45.5 – 52.5)	48.0 (33.0 – 52.0)	47.0 (45.5 – 57.8)
Range	22 – 65	32 – 63	25 – 64	24 – 62
Anthropometrics				
Height (cm) ^b	172.7 (170.3 – 185.5)	175.3 (170.5 – 177.8)	158.8 (157.5 – 160.0)	160.3 (154.8 – 165.0)
Weight (kg) ^b	80.2 (69.3 – 90.8)	81.4 (73.7 – 93.6)	63.6 (58.1 – 72.3)	60.1 (55.6 – 73.9)
BMI (kg/m ²) ^b	25.1 (22.6 – 31.2)	27.1 (23.7 – 31.8)	25.3 (21.6 – 31.3)	24.4 (21.7 – 27.2)
BP (mmHg)	128.3/84.1 (±13.5/9.9)	128.1/84.4 (±21.5/17.9)	118.1/74.7 (±10.8/5.7)	118.8/76.5 (±8.3/7.0)
Trichometry				
Terminal Hair Density (per cm ²)	148.7 (±69.5)	140.2 (±71.4)	141.6 (±32.5)	145.8 (±32.9)
Vellus Hair Density (per cm ²)	127.5 (±57.9)	134.3 (±70.4)	47.9 (±22.6)	33.4 (±21.1)
Terminal/Vellus Hair Ratio	1.1	1.5	3.5	5.6
Diameter of All Hair (µm)	44.7 (±8.3)	40.9 (±7.0)	56.2 (±7.9)	66.3 (±7.6)
Diameter of Terminal Hair (µm)	55.1 (±7.0)	53.3 (±6.9)	65.3 (±7.3)	73.6 (±6.7)
Growth Rate (µm/24 hours)	225.2 (±45.1)	237.8 (±49.7)	312.0 (±32.1)	302.3 (±33.6)
Shed Rate (Hairs/24 hours) ^c	411.0 (±183.0)	513.7 (±286.2)	131.2 (±44.0)	125.7 (±50.0)
Diagnosis				
AGA Primary	23 (100%)	23 (100%)	11 (52%)	16 (80%)
AGA Secondary	0 (0%)	0 (0%)	10 (48%)	4 (20%)
TE Primary	0 (0%)	0 (0%)	10 (48%)	4 (20%)
TE Secondary	0 (0%)	1 (4.3%)	5 (24%)	4 (20%)
HN / Ludwig Classification				
I / I-1	4 (17%)	0 (0%)	3 (14%)	2 (10%)
II / I-2	2 (9%)	1 (4%)	4 (19%)	11 (55%)
IIA / I-3	2 (9%)	1 (4%)	9 (43%)	5 (25%)
III / I-4	1 (4%)	0 (0%)	1 (5%)	1 (5%)
IIIA / II-2	0 (0%)	1 (4%)	0 (0%)	1 (5%)
IIIV	5 (22%)	6 (26%)		
IV	1 (4%)	4 (17%)		
V	0 (0%)	1 (4%)		
VA	3 (13%)	2 (9%)		
VI	1 (4%)	0 (0%)		
VII	2 (9%)	3 (13%)		
Missing	2 (9%)	4 (17%)	4 (19%)	0 (0%)
Fitzpatrick Skin Type				
I	3 (13%)	1 (4%)	3 (14%)	4 (20%)
II	3 (13%)	2 (9%)	8 (38%)	8 (40%)
III	10 (44%)	15 (65%)	7 (33%)	8 (40%)
IV	2 (9%)	1 (4%)	2 (10%)	0 (0%)
V	2 (9%)	1 (4%)	0 (0%)	0 (0%)
VI	2 (9%)	3 (13%)	1 (5%)	0 (0%)
Unknown	1 (4%)	0 (0%)	0 (0%)	0 (0%)
Ethnicity				
African American	4 (17%)	4 (17%)	0 (0%)	0 (0%)
Caucasian	10 (44%)	7 (30%)	3 (14%)	1 (5%)
Chinese	5 (24%)	6 (26%)	12 (57%)	13 (65%)
Filipino	0 (0%)	1 (4%)	0 (0%)	1 (5%)
Hispanic	1 (4%)	3 (13%)	4 (19%)	3 (15%)
Indian	1 (4%)	0 (0%)	0 (0%)	0 (0%)
Japanese	0 (0%)	0 (0%)	2 (10%)	1 (5%)
Korean	1 (4%)	0 (0%)	0 (0%)	0 (0%)
Mongolian	0 (0%)	1 (4%)	0 (0%)	1 (5%)
Thai	0 (0%)	1 (4%)	0 (0%)	0 (0%)
Vietnamese	1 (4%)	0 (0%)	0 (0%)	0 (0%)

Legend: a: Evaluable for Hair Efficacy (Per Protocol); b: Median (IQR); ± or SD=standard deviation; c: Scalp Shed Rate based on 700cm² shedding assumed uniformly from phototrichogram area on vertex; Trichometry measures are per protocol; AGA: Androgenetic Alopecia; TE: Telogen Effluvium HN: Hamilton-Norward classification of hair loss pattern for men. Ludwig classification of hair loss pattern for women. Vellus hair includes true vellus hair, vellus-like and new hair.

Table S2. Response Rates as Change in Terminal Hairs from Baseline. This table shows the stratified response by treatment group with a substantial increase in response in hair growth in the ALRV5XR groups over time. **In Women**, the ALRV5XR group's change in terminal hair (TH) /cm² shows a bimodal responder pattern of non-responders and very high responders with a significant shift of responding subjects' hair count changes from a relatively even distribution in women of 0-40 TH/cm² at week 12 to higher values of 30 THs/cm² and above at week 24. Response Rates by % Change in THs for the ALRV5XR treatment group show a significant shift of subjects' hair % changes at week 12 to higher percentage ranges at week 24. **In Men**, the ALRV5XR group separates into a trimodal responder pattern of low, moderate, and high responders when measured by absolute changes in TH density; however, percent changes in TH density are more uniform. 54.5% of the ALRV5XR group increased their TH density by 10 or more THs/cm² by week 24 and 18.2% of the group increased their TH density by more than 25 THs/cm². Overall, men have a perfect response rate, while women grow significantly more hair than men.

	Men				Women				
	Placebo		ALRV5XR		Placebo		ALRV5XR		
	Week 12	Week 24	Week 12	Week 24	Week 12	Week 24	Week 12	Week 24	
Positive Responders	50.0%	18.2%	91.9%	100%	67.7%	42.9%	77.8%	66.7%	
Terminal Hairs/cm ²									
<0	50.0%	81.8%	9.1%	-	<0	33.3%	57.1%	22.2%	33.3%
0-5	40.0%	18.2%	27.3%	45.5%	0-10	47.6%	28.6%	16.7%	-
5-10	10.0%	-	27.3%	-	10-20	9.5%	4.8%	16.7%	-
10-15	-	-	18.2%	27.3%	20-30	9.5%	-	16.7%	-
15-20	-	-	9.1%	9.1%	30-40	-	-	16.7%	5.6%
20-25	-	-	-	-	40-50	-	4.8%	11.1%	22.2%
≥25	-	-	9.1%	18.2%	≥50	-	4.8%	-	38.9%
Min Regrowth	-17	-12	-3	0	-7	-9	-9	-6	
Max Regrowth	9	3	25	51	22	54	41	79	
Terminal Hairs %									
<0%	50.0%	81.8%	9.1%	0.0%	<0%	33.3%	57.1%	22.2%	33.3%
0-5%	40.0%	18.2%	45.5%	45.5%	0-10%	47.6%	28.6%	22.2%	-
5-10%	10.0%	-	27.3%	9.1%	10-20%	9.5%	4.8%	33.3%	11.1%
10-15%	-	-	-	18.2%	20-30%	9.5%	-	16.7%	11.1%
15-20%	-	-	-	9.1%	30-40%	-	4.8%	5.6%	27.8%
20-25%	-	-	18.2%	9.1%	40-50%	-	4.8%	-	11.1%
≥25%	-	-	-	9.1%	≥50%	-	-	-	5.6%
Min Regrowth	-12.1%	-12.3%	3.4%	0%	-5.3%	-8.1%	-6.0%	-4.9%	
Max Regrowth	7.0%	1.6%	22.9%	46.8%	18.3%	45.0%	36.3%	69.9%	

Table S3. ALRV5XR Material Active Ingredients

	Active Ingredients*
Oral (capsule)	Angelica sinensis, Ascophyllum nodosum, Astragalus membranaceus, Coenzyme Q10, Copper, Eclipta prostrata, Folate, Ganoderma lucidum, Malus domestica, Moringa oleifera, Niacin, Pantothenic acid, Polygonum multiflorum, Punica granatum, Riboflavin, Rosmarinus officinalis, Scutellaria baicalensis, Selenium, Silicon, Sophora flavescens, Vitamin A, Vitamin B6, Vitamin B7(Biotin), Vitamin B12, Vitamin D, Vitis vinifera, and Zinc
Shampoo	Caffeine, Eclipta prostrata, Lupinus, Momordica charantia, Moringa oleifera, Pyrus malus, Rosmarinus officinalis, Vitis vinifera, Scutellaria baicalensis, Vitamin B7(Biotin)
Conditioner	Caffeine, Eclipta prostrata, Lupinus, Momordica charantia, Moringa oleifera, Pyrus malus, Rosmarinus officinalis, Vitis vinifera, Scutellaria baicalensis, Vitamin B7(Biotin)
Follicle serum	Caffeine, Eclipta prostrata, Lupinus, Momordica charantia, Moringa oleifera, Ocimum basilicum, Pisum sativum, Pyrus malus, Rosmarinus officinalis, Vitis vinifera, Scutellaria baicalensis, Vitamin B7(Biotin)

*Patents pending

5.2 Summary of Key Findings of Paper 3

ALRV5XR significantly increased TH density in both sexes without adverse effects. The combined overall TH regrowth efficacy of the two RCTs in both women and men was 26.3 THs/cm² ($p < 0.0001$) and 18.2% ($p < 0.0001$). In general, the women responders regrew approximately double the amount of TH than the men. In the ALRV5XR women's group, all responders increased TH density by 30 THs/cm² or more, and 44.5% increased their TH density by at least 30%. In the ALRV5XR men's group, 54.5% of responders increased TH by 10 THs/cm² or more, and 18.2% increased TH by more than 25 THs/cm². 45.5% of men increased their TH density by at least 10%, and 18.2% increased TH density by more than 20%. Hair fibre growth rate efficacy in women increased by 30.7 $\mu\text{m}/24\text{ hr}$ ($p = 0.0001$) or 10.0% ($p = 0.0001$), and the men's increase was borderline significant. Hair shedding rates in men decreased by 21.5% ($p = 0.0158$). Regeneration of structure and function of dormant telogen and impaired anagen follicles were documented. In women, an accelerating TH regrowth rate was observed over two 12-week treatment periods. Reactivation of dormant telogen follicles appeared to be the dominant influence leading to increased TH density by contributing 99% of the new TH. In men, while reactivation of dormant telogen follicles similarly contributed 50% of increased TH density, a novel equally contributing ALRV5XR influence was observed. A newfound effect, the thickening of the AGA miniaturised vellus-like hair, contributed 50% toward the normalisation of the physiologic scalp hair phenotype. Prolonged treatment with ALRV5XR may restore a more normal scalp hair phenotype in women in a shorter time than in men.

6 Results – Paper 4: Hair regrowth treatment efficacy and resistance in androgenetic alopecia: A systematic review and continuous Bayesian network meta-analysis.

Feldman, Peter R., Pietro Gentile, Charles Piwko, Hendrik M. Motswaledi, Samantha Gorun, Jacob Pesachov, Michael Markel, Maxwell I. Silver, Megan Brenkel, Oriel J. Feldman, Corey L. Kamen, Elizabeth Uleryk, Jaime Guevara-Aguirre, and Klaus M. Fiebig. “Hair Regrowth Treatment Efficacy and Resistance in Androgenetic Alopecia: A Systematic Review and Continuous Bayesian Network Meta-Analysis.” Systematic Review, *Frontiers in Medicine* 9 (2023-January-23 2023). <https://dx.doi.org/10.3389/fmed.2022.998623>.²¹⁶ Supplementary materials are accessible from the link provided in the publication and appended to this chapter.

6.1 Introduction to Paper 4

Androgenetic alopecia (AGA) affects almost half the population, and several treatments intending to prevent hair loss and regenerate a normal scalp hair phenotype are used. This study is the first published systematic review and network meta-analysis study, in women or men, to compare the efficacy and resistance of all commercially available non-surgical AGA treatments, including pharmaceuticals, medical devices and natural treatments that use standardised terminal hair outcomes at 12 and 24 weeks or evaluates continuous efficacy profiles over the study period. Furthermore, this is also the first study to compare clinical trials that measure efficacy using standardised methods for the treatment of AGA and to compare Bayesian to frequentist outcomes. The relevance of this study in this thesis is to show a rigorous comparison of the results reported from the two randomised placebo-controlled clinical trials

in Chapters 3 and 4 and the sex differences in outcomes from Chapter 5 to the body of AGA literature using systematic review and quantitative network meta-analysis techniques.

The protocol for this systematic review was registered on Prospero: CRD42021268040.

www.crd.york.ac.uk/PROSPERO/display_record.asp?ID=CRD42021268040



OPEN ACCESS

EDITED BY

Manuel Valdebran Canales,
Medical University of South Carolina,
United States

REVIEWED BY

Animesh A. Sinha,
University at Buffalo, United States
Alireza Firooz,
Tehran University of Medical Sciences,
Iran

*CORRESPONDENCE

Peter R. Feldman
✉ petefeldman@gmail.com

SPECIALTY SECTION

This article was submitted to
Dermatology,
a section of the journal
Frontiers in Medicine

RECEIVED 20 July 2022

ACCEPTED 07 December 2022

PUBLISHED 23 January 2023

CITATION

Feldman PR, Gentile P, Piwko C,
Motswaledi HM, Gorun S, Pesachov J,
Markel M, Silver MI, Brenkel M,
Feldman OJ, Kamen CL, Uleryk E,
Guevara-Aguirre J and Fiebig KM
(2023) Hair regrowth treatment
efficacy and resistance in
androgenetic alopecia: A systematic
review and continuous Bayesian
network meta-analysis.
Front. Med. 9:998623.
doi: 10.3389/fmed.2022.998623

COPYRIGHT

© 2023 Feldman, Gentile, Piwko,
Motswaledi, Gorun, Pesachov, Markel,
Silver, Brenkel, Feldman, Kamen,
Uleryk, Guevara-Aguirre and Fiebig.
This is an open-access article
distributed under the terms of the
[Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/)
(CC BY). The use, distribution or
reproduction in other forums is
permitted, provided the original
author(s) and the copyright owner(s)
are credited and that the original
publication in this journal is cited, in
accordance with accepted academic
practice. No use, distribution or
reproduction is permitted which does
not comply with these terms.

Hair regrowth treatment efficacy and resistance in androgenetic alopecia: A systematic review and continuous Bayesian network meta-analysis

Peter R. Feldman^{1,2*}, Pietro Gentile³, Charles Piwko⁴,
Hendrik M. Motswaledi⁵, Samantha Gorun^{6,7},
Jacob Pesachov⁸, Michael Markel⁸, Maxwell I. Silver^{9,10},
Megan Brenkel¹¹, Oriel J. Feldman^{1,12}, Corey L. Kamen⁸,
Elizabeth Uleryk¹³, Jaime Guevara-Aguirre^{14,15,16,17} and
Klaus M. Fiebig¹

¹Arbor Life Labs, Toronto, ON, Canada, ²Norwich Medical School, University of East Anglia, Norwich, United Kingdom, ³Surgical Science Department, University of Rome Tor Vergata, Rome, Italy, ⁴CHP Pharma Inc., Thornhill, ON, Canada, ⁵Department of Dermatology, Sefako Makgatho Health Sciences University, Pretoria, South Africa, ⁶Faculty of Epidemiology and Biostatistics, Western University, London, ON, Canada, ⁷School of Mathematics and Statistics, University of Glasgow, Glasgow, United Kingdom, ⁸Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel, ⁹Faculty of Dentistry, University of Toronto, Toronto, ON, Canada, ¹⁰Faculty of Medicine and Dentistry, University of Alberta, Edmonton, AB, Canada, ¹¹Faculty of Medicine, University of Ottawa, Ottawa, ON, Canada, ¹²Faculty of Science, Wilfrid Laurier University, Waterloo, ON, Canada, ¹³Uleryk Consulting, Mississauga, ON, Canada, ¹⁴College of Medicine, Universidad San Francisco De Quito (USFQ), Quito, Ecuador, ¹⁵Faculty of Health, Medicine and Life Sciences, Maastricht University, Maastricht, Netherlands, ¹⁶Institute of Endocrinology, Metabolism, and Reproduction (IEMR), Quito, Ecuador, ¹⁷College of Medicine, University of Florida, Gainesville, FL, United States

Background: Androgenetic alopecia (AGA) affects almost half the population, and several treatments intending to regenerate a normal scalp hair phenotype are used. This is the first study comparing treatment efficacy response and resistance using standardized continuous outcomes.

Objective: To systematically compare the relative efficacy of treatments used for terminal hair (TH) regrowth in women and men with AGA.

Methods: A systematic literature review was conducted (from inception to August 11, 2021) to identify randomized, Placebo-controlled trials with ≥ 20 patients and reporting changes in TH density after 24 weeks. Efficacy was analyzed by sex at 12 and 24 weeks using Bayesian network meta-analysis (B-NMA) and compared to frequentist and continuous outcomes profiles.

Results: The search identified 2,314 unique articles. Ninety-eight were included for full-text review, and 17 articles met the inclusion criteria for data extraction and analyses. Eligible treatments included ALRV5XR, Dutasteride 0.5 mg/day, Finasteride 1 mg/day, low-level laser comb treatment (LLLT),

Minoxidil 2% and 5%, Nutrafol, and Viviscal. At 24 weeks, the B-NMA regrowth efficacy in TH/cm² and significance (**) in women were ALRV5XR: 30.09**, LLLT: 16.62**, Minoxidil 2%: 12.13**, Minoxidil 5%: 10.82**, and Nutrafol: 7.32**, and in men; ALRV5XR: 21.03**, LLLT: 18.75**, Dutasteride: 18.37**, Viviscal: 13.23, Minoxidil 5%: 13.13**, Finasteride: 12.38, and Minoxidil 2%: 10.54. Two distinct TH regrowth response profiles were found; Continuous: ALRV5XR regrowth rates were linear in men and accelerated in women; Resistant: after 12 weeks, LLLT, Nutrafol, and Viviscal regrowth rates attenuated while Dutasteride and Finasteride plateaued; Minoxidil 2% and 5% lost some regrowth. There were no statistical differences for the same treatment between women and men. B-NMA provided more accurate, statistically relevant, and conservative results than the frequentist-NMA.

Conclusion: Some TH regrowth can be expected from most AGA treatments with less variability in women than men. Responses to drug treatments were rapid, showing strong early efficacy followed by the greatest resistance effects from flatlining to loss of regrowth after 12–16 weeks. Finasteride, Minoxidil 2% and Viviscal in men were not statistically different from Placebo. LLLT appeared more efficacious than pharmaceuticals. The natural product formulation ALRV5XR showed better efficacy in all tested parameters without signs of treatment resistance (see Graphical abstract).

Systematic review registration: www.crd.york.ac.uk/PROSPERO/display_record.asp?ID=CRD42021268040, identifier CRD42021268040.

KEYWORDS

hair loss, ALRV5XR, Dutasteride, Finasteride, LLLT, Minoxidil, Viviscal, Nutrafol

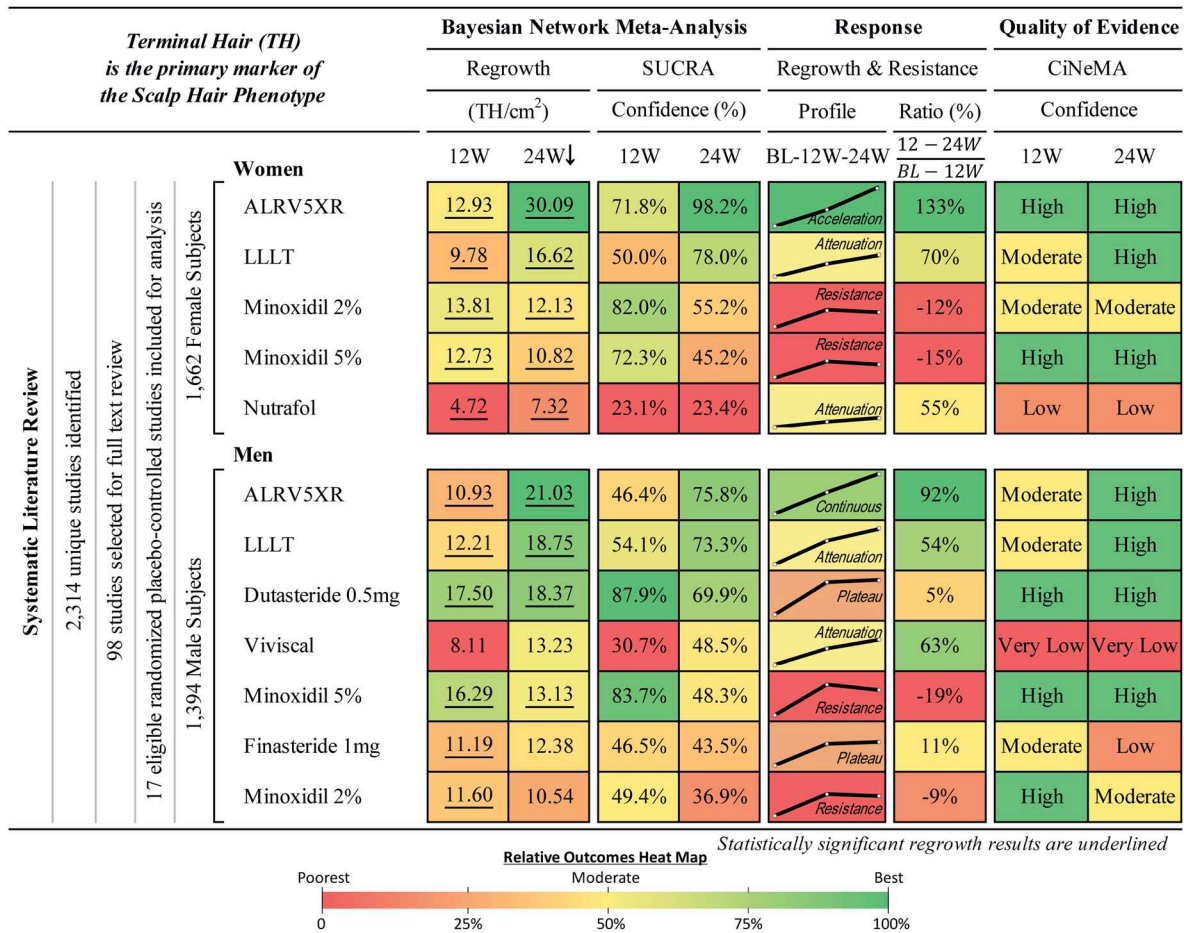
1. Introduction

Androgenetic alopecia (AGA), also known as male or female pattern hair loss, is the most common form of hair loss in men and women (1). AGA is a polygenic, heritable, age-dependent process that results in a progressive, non-scarring decline in scalp hair density, and is characterized by gradual loss of terminal hair (TH) and follicular miniaturization to vellus-like hair fibers in a generally sex-dependent pattern, leading to the eventual irreversible loss of functional potential of hair follicles (HFs) (1–3). THs are large pigmented hairs with a shaft diameter of 30–150 μ m, while vellus hairs (VHs) have less than 30 μ m diameter, are short (< 30 mm), unpigmented, and barely visible (4–6). A normal scalp averages seven THs per each VH (4). AGA results in a reduction in total hair count and a TH/VH ratio that is clinically identifiable when 15–30% of hair is lost or when the TH/VH ratio approaches 4 to 1 (1, 5, 7–9). TH contributes to more than 85% of total hair density, and constitutes almost all visible scalp hair. In consequence, its count is the single most important marker of the normal scalp hair phenotype.

Identifying and counting TH can be challenging. Manual hair counting methods used prior to 1990 were inaccurate (10).

While phototrichoscopies of unclipped and undyed hair are useful for patient diagnosis and follow-up in the clinic, they are not precise and can lead to miscount. The 4 mm punch biopsy is highly effective at measuring and counting hair; however, few clinical trials have used this methodology. Of relevance, results from biopsies can anticipate, by weeks, visible scalp hair regrowth. Notably, in the last 30 years, improvements in imaging technology have increased the accuracy of hair diameter measurements and TH counting reliability. These newer methods are accurate, repeatable, and cost-effective. Among them, the contrast-enhanced phototrichogram (CEPT) technique, performed on hair clipped to < 1 mm and dyed, generates highly precise results. Consequently, CEPT linked to specialized analysis software is the currently accepted gold standard for measuring hair regrowth in clinical trials (5, 8, 9).

At the present, first-line treatments for AGA include the type I 5 α -reductase androgen inhibitor, oral Finasteride 1 mg/day for men, and the potassium channel opener, topical Minoxidil 2% and 5%, for both sexes (1, 5, 8, 9, 11). TH regrowth induced by these agents was found to be derived from activation of dormant telogen terminal follicles (5, 8, 9). In any case, it has been noted that AGA-miniaturized vellus-like hairs, are not



GRAPHICAL ABSTRACT

Hair regrowth treatment efficacy and resistance in androgenetic alopecia. Systematic literature review and Bayesian network meta-analysis of terminal hair regrowth efficacy and resistance in women and men with androgenetic alopecia using different treatments vs. Placebo at 12 and 24 weeks. Women and men are listed in descending order of efficacy at 24 weeks of network meta-analysis (NMA) after 250,000 Markov Chain Monte Carlo simulations. Regrowth measures efficacy as mean difference (MD) in TH/cm² between each treatment and Placebo using direct and indirect comparisons. 12 and 24W refer to 12 and 24 week treatment duration. SUCRA: surface under the cumulative ranking area measures relative confidence. Response Profile is a line graph connecting the treatment changes from baseline (BL) to MD at 12 and 24W. Y-axes (MD) are scaled 0–31 for women and 0–22 for men. Response Ratio (%) is change in regrowth of 12–24W compared to BL-12W. Quality of evidence is CiNeMA results using Cochrane risk of bias, GRADE scores, and study data. Heat maps are shared between each sex's numerical result block and manually assigned to categorical results.

definitively influenced by these drugs; consequently, they cannot be converted back to THs (5, 8, 9). In contrast, ALRV5XR, a novel botanical natural product treatment for AGA, showed significant TH regrowth and appeared to reverse miniaturized hairs (12, 13). Individuals affected by AGA seeking therapy would benefit from both a safe option aimed to prevent further hair loss and, potentially, a treatment that might regenerate their normal scalp hair phenotype.

There have been several double-blinded randomized Placebo-controlled trials (RCTs) that lasted at least 24 weeks. This timeframe appears appropriate since it allows for reasonable measurement of continuous efficacy parameters. In addition, this period permits midpoint evaluations at 12 weeks to detect earlier changes. Therefore, a network meta-analysis

(NMA) performed at 12 and 24 week time points, and an analysis of continuous treatment outcomes could better allow for a proper comparison of the regrowth profiles seen in different trials. They will also permit a more precise evaluation of discrete responses as well as of resistance dynamics.

Hair regrowth outcomes for AGA treatments have been compared in 15 meta-analyses (14–28). These evaluations included studies that performed total hair counting and other non-comparable hair counting methodologies. Notably, they compared a single outcome endpoint for treatments of different duration, including non-RCTs. In summary, among these 15 meta-analyses, which included studies with inaccurate hair counting methods and non-RCTs, only one, performed in men, compared the TH outcome of three drugs.

To our knowledge, no published NMA study, in women or men, has compared the efficacy or resistance of all commercially available non-surgical AGA treatments using standardized outcomes such as determination, at precise times, of TH characteristics, or with assessment of hair density by unit area and evaluation of continuous efficacy profiles over the study period. Furthermore, there are no reports of NMAs comparing pharmaceuticals, medical devices and natural treatments, or Bayesian to frequentist outcomes, or studies that measure efficacy using standardized methods for the treatment of AGA.

Under the above cited premises, the objective of this study was to examine, in women and men, the relative 24 week efficacy of all commercially available AGA treatments using standard dosage and administration routes. Efficacy was derived from determining the outcome of the most appropriate parameter: TH regrowth per unit area over an identical treatment duration period. In addition, treatment outcome was followed over time aiming to determine treatment resistance effects and the continuous efficacy of each treatment in maintaining or improving an existing phenotype.

2. Methods

2.1. Study eligibility

A professional librarian performed a database search on MEDLINE, Medline-in-Process, Medline ePub Ahead of Print, EMBASE databases (OvidSP) and Cochrane, initially on July 8, 2020, without date restrictions. The search was updated on August 11, 2021. Both subject headings and text word terms were used to search for RCTs on alopecia and current standard of care therapies (e.g., Minoxidil or Finasteride or Dutasteride or biotin or low-level laser light therapy or supplements or transplant, etc.). Manual and gray literature searches were also conducted, and studies included in prior meta-analyses were evaluated for study eligibility (see Search Strategy in [Supplementary Appendix 2](#)).

Eligible studies included RCTs of men or women diagnosed with AGA using either an approved or off-label drug, device, or commercially relevant treatment such as platelet-rich plasma (PRP) and natural products for a duration of at least 24 weeks, and which measured density changes of scalp TH, or non-vellus hair of diameter $> 30 \mu\text{m}$ per unit area. Methods had to include performance of macrophotography or phototrichogram techniques on hair clipped to approximately 1 mm, at the same position on the scalp, and typically marked by tattoo, for repeated measures. Only RCTs were eligible to maximize the quality of evidence (29).

Studies were excluded if results were not separated for women or men, outcomes of interest were not measured or not reported, study size had fewer than 20 subjects or if a treatment arm had less than 10 subjects. Also excluded were those studies

containing diagnosis of scarring alopecia, alopecia areata, and non-specific diagnoses that could not be interpreted explicitly as AGA—such as self-reported hair thinning, thinning hair, or patchy hair loss. Studies were also excluded when hair was counted manually or from biopsies or phototrichoscopies of unclipped hair, or when results were measures of total hair count that included vellus hair, percentage changes, or if there was no standard error that was either reported or that could be derived at 24 weeks, or when the design had a treatment crossover before 24 weeks, or when the intervention was experimental or not commercially available. In studies in which interim or partial results were previously published, the most recent article was selected. Studies published in languages other than English or German were excluded.

2.2. Study screening and data extraction

Two research teams independently screened titles, reviewed abstracts and full-text articles, extracted data, and assessed the risk of bias of included studies. The adjudicating reviewer panel resolved discrepancies, and external experts were consulted when necessary. Missing data were obtained by contacting authors and sponsors, and from additional data reported on clinicaltrials.gov. Extracted information included: Author, date, treatment, dose, country of study, sex, race, Fitzpatrick skin type, hair loss pattern and severity, scalp site studied, age, number of subjects randomized into each treatment group, number of subjects evaluated for outcomes at, or subsequent to 12 and 24 weeks, TH count at baseline (BL), change in TH density from BL at, or near 12 weeks, 24 weeks and study mid or endpoints, variances of changes from baseline as standard deviations (SD) and *p*-values. Imputed data and missing SD for changes from baseline were estimated using Cochrane's methodology (30). All eligible studies were included in the NMA and the continuous outcomes analysis (COA). The number of subjects and TH changes were extracted for continuous efficacy evaluation at each available study timepoint. All TH counts and densities were standardized to a unit area of 1 cm^2 at 12 and 24 weeks by interpolation between timepoints. Studies and cohorts were separated by sex into groups of women or men only for analysis.

2.3. Efficacy measures and synthesis

Each study cohort was assessed for efficacy at 12 and 24 weeks with 95% confidence intervals (CI) and *p*-values as the mean difference (MD) between treatment vs. Placebo measured in TH/cm^2 . Placebo, sham, or vehicle comparators for each study cohort are referred to as Placebo in the data synthesis and results. The primary outcome measure of the Bayesian NMA was MD of treatment vs. Placebo, measured in TH/cm^2 with 95% credible intervals (CrI).

2.4. Statistical analysis methods

2.4.1. Network meta-analyses

NMA outcomes were reported in forest plots, league tables, composite heat-mapped outcomes picto-tables, and netgraphs.

For each efficacy outcome, an NMA was conducted using Bayesian random-effects models. Studies with more than one cohort of the same treatment, with the same effective dose that was compared to the same Placebo cohort, were combined using Cochrane methods (30). Bayesian NMAs were seeded with uniform priors and simulated 250,000 times in a Markov Chain Monte Carlo (MCMC) model (30–32). For each outcome, the network of treatments was depicted through a netgraph as an integrated diagram with nodes representing each treatment and edges where treatments were compared to Placebo or another treatment in a head-to-head trial (30, 33).

League tables for women and men at 12 and 24 weeks were produced to present the relative efficacy for every possible direct and indirect pairwise combination. The point estimate for league tables was the MD, with 95% CrIs, CIs and significance (32).

Surface Under the Cumulative Rank Area (SUCRA) scores for each treatment to determine the relative confidence were estimated and ranked with the highest value corresponding to the most effective treatment (34).

2.4.2. Quality of evidence within studies and across networks

Each eligible study was evaluated for risk of bias using the Cochrane Collaboration risk of bias assessment tool using qualitative judgment for five domains: randomization, deviations from intended intervention, missing outcomes data, measurement of the outcome, and selective reporting (30, 33). A separate overall risk of bias assessment was conducted (30). Each domain was judged as low, some concern, moderate, or high risk of bias (30).

For each NMA outcome, the quality of evidence across a network was assessed using the Confidence in Network Meta-Analysis (CiNeMA) framework based on the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) approach (29, 33). Under the CiNeMA framework, qualitative and quantitative judgments were made across six domains: Within-study Cochrane risk of bias, reporting bias, indirectness, imprecision, heterogeneity, and incoherence (33, 35). In the CiNeMA framework, a confidence rating is an overall qualitative assessment that summarizes judgment that assesses the quality of evidence; Confidence ratings are high, moderate, low, or very low (33).

2.4.3. Continuous outcomes analysis (COA)

Weekly data points were imputed for each study cohort via linear extrapolation between reported timepoints.

Weekly outcomes for each treatment were determined using a weighted MD. Results were plotted on a standard outcome in TH/cm² vs. time graph for each treatment's COA. Error analysis was not performed for the COA due to sparse availability of change error data on the interim data points.

2.4.4. Frequentist analysis

Frequentist NMA and MA analyses were performed for comparison purposes through a random effects model. Frequentist results were provided as MD with 95% CI, *p*-values, and z-scores (30). A statistical significance of *p* < 0.05 was used.

2.4.5. Composite outcomes evaluation

A composite range of outcomes in women and men were evaluated at 12 and 24 weeks. Continuous outcomes were determined to at least 24 weeks. Domains of outcomes were evaluated as an estimate of treatment efficacy, SUCRA score, regrowth rate, regrowth profile, and quality of evidence.

2.4.6. Statistical analysis software

R studio software version 4.1.2 (2021-11-01) was used for all analyses using a significance level of $\alpha = 0.05$ (36). R package “meta” was used for meta-analysis, “netmeta” and “BUGSnet” were used for NMA, “ggplot2” was modified for Kilim plots, Bayesian forest plots, netgraphs, and CiNeMA plots (32, 33, 35–38). Microsoft Excel was used for continuous outcomes imputation and analysis.

2.5. Safety

Safety data as reported in the included studies were summarized.

2.6. Study registration

Study registration on PROSPERO: CRD42021268040. Ethics approval was not required since there were no direct human treatments or observations that used medical records. The authors declare adherence to PRISMA reporting guidelines for NMAs.

3. Results

3.1. Eligible studies

The search identified 3,157 records. After removing 843 duplicates, 2,314 unique records were screened, and 98 studies were eligible for full-text review. Seven studies were not retrievable, 2 had repeated data in other studies or with

Eligible Studies, Characteristics and Results - Women																						
Eligible Studies		Characteristics			Treatment ↓	Baseline				12 Week Changes from Baseline				24 Week Changes from Baseline				MD Chart				
#	Author, Year	Country Race	Skin Type HL Pattern	Scalp Site Age		N	TH per cm ²	SD	RSD	N	TH per cm ²	SD	Corr	MD (95% CI) P-value	N	TH per cm ²	SD	Corr	MD (95% CI) P-value	12W	24W	
1	Feldman et al, 2021 ¹²	US <i>A, W, H</i>	I-VI <i>L:I-I-II2</i>	Vertex <i>24 - 64</i>	ALRV5XR vs. Placebo	20 21	147.0 141.6	31.3 32.5	21.3% 23.0%	18 21	16.1 3.1	16.6 7.5	0.91 0.97	12.92 (4.60 - 21.24) <i>p = 0.0023</i>	18 21	33.9 3.9	29.4 15.7	0.81 0.88	30.08 (14.93 - 45.23) <i>p < 0.0001</i>			
2	Jimenez et al, 2014 ⁴⁷	US <i>W, B, A, P, O</i>	II-IV <i>L:I-4 - II-2</i>	Vertex <i>29 - 60</i>	LLLT (9-beam) vs. Placebo	43 22	162.6 155.7	46.2 43.5	28.4% 27.9%	43 22	11.1 1.0	7.2 11.1	<i>N/A</i> <i>N/A</i>	10.13 (5.01 - 15.24) <i>p = 0.0001</i>	42 21	19.1 2.5	10.7 16.2	<i>N/A</i> <i>N/A</i>	16.62 (8.99 - 24.25) <i>p < 0.0001</i>			
3		US <i>W, B, A, P, O</i>	I-IV <i>L:I-4 - II-2</i>	Vertex <i>26 - 61</i>	LLLT (12-beam) vs. Placebo	39 18	142.2 168.4	40.5 41.1	28.5% 24.4%	39 18	8.9 -0.6	8.5 5.9	<i>N/A</i> <i>N/A</i>	9.53 (5.73 - 13.32) <i>p < 0.0001</i>	39 18	18.9 2.2	11.5 9.0	<i>N/A</i> <i>N/A</i>	16.62 (11.12 - 22.12) <i>p < 0.0001</i>			
4	Olsen et al, 1991 ³⁹	US <i>N/A</i>	<i>N/A</i> <i>L:I-II</i>	Fronto-Parietal <i>19 - 45</i>	Minoxidil 2% vs. Placebo	14 14	160.1 154.2	34.6 36.0	21.6% 23.3%	<i>N/A</i> <i>N/A</i>	<i>N/A</i> <i>N/A</i>	<i>N/A</i> <i>N/A</i>	<i>N/A</i> <i>N/A</i>	<i>N/A</i>	14 14	37.6 15.5	29.8 21.3	<i>N/A</i> <i>N/A</i>	22.13 (2.95 - 41.30) <i>p = 0.0237</i>			
5	Whiting et al, 1992 ⁴⁰	US <i>W, O</i>	<i>N/A</i> <i>L:I-II</i>	Vertex <i>22 - 44</i>	Minoxidil 2% vs. Placebo	17 16	169.0 161.0	41.0 40.0	24.3% 24.8%	16 16	20.5 8.5	23.0 15.0	0.85 0.93	12.00 (-1.45 - 25.45) <i>p = 0.0805</i>	14 14	21.0 17.0	31.0 12.0	0.71 0.96	4.00 (-13.41 - 21.41) <i>p = 0.6525</i>			
6	Jacobs et al, 1993 ⁴¹	FR, UK, BE, DK, NL, DE, CH <i>W, O</i>	<i>N/A</i> <i>L:I-II</i>	Vertex <i>18 - 45</i>	Minoxidil 2% vs. Placebo	154 139	136.7 139.8	39.7 44.8	29.0% 32.0%	155 139	35.5 19.8	32.7 25.1	0.72 0.87	15.70 (9.08 - 22.32) <i>p < 0.0001</i>	147 135	26.1 14.0	24.2 27.6	0.82 0.82	12.10 (6.01 - 18.19) <i>p < 0.0001</i>			
7	De Villez et al, 1994 ⁴²	US <i>W, O</i>	<i>N/A</i> <i>L:I-II</i>	Vertex <i>17 - 46</i>	Minoxidil 2% vs. Placebo	126 124	140.4 138.6	43.7 37.1	31.1% 26.8%	121 121	25.5 9.2	25.6 22.0	0.85 0.85	16.30 (10.28 - 22.32) <i>p < 0.0001</i>	125 125	22.2 9.9	22.4 19.6	0.89 0.87	12.30 (7.09 - 17.51) <i>p < 0.0001</i>			
8	Lucky et al, 2004 ⁴³	US <i>W, O</i>	<i>N/A</i> <i>L:I-III</i>	Vertex <i>18 - 49</i>	Minoxidil 2% vs. Placebo	154	150.4	46.1	30.7%	108	27.1	20.4	<i>N/A</i>	11.55 (5.03 - 18.07) <i>p = 0.0005</i>	108	31.3	21.0	<i>N/A</i>	13.70 (7.30 - 20.10) <i>p < 0.0001</i>			
9		US <i>W, O</i>	<i>N/A</i> <i>L:I-III</i>	Vertex <i>18 - 49</i>	Minoxidil 5% vs. Placebo	153 74	141.3 138.4	47.0 44.9	33.3% 32.4%	101 51	28.9 15.6	20.7 19.2	<i>N/A</i> <i>N/A</i>	13.30 (6.67 - 19.93) <i>p < 0.0001</i>	101 51	31.6 17.6	22.8 18.3	<i>N/A</i> <i>N/A</i>	13.95 (7.23 - 20.66) <i>p < 0.0001</i>			
10	Bergfeld et al, 2016 ⁴⁴	US <i>W, B, A, O</i>	<i>N/A</i> <i>S:3-6</i>	Midline <i>22 - 87</i>	Minoxidil 5% vs. Placebo	203 201	158.6 152.7	61.6 59.7	38.8% 39.1%	172 168	16.4 5.3	21.5 15.3	0.94 0.97	11.10 (7.14 - 15.06) <i>p < 0.0001</i>	159 165	13.4 4.3	22.3 16.2	0.95 0.97	9.10 (4.84 - 13.36) <i>p < 0.0001</i>			
11	Ablon et al, 2018 ⁴⁵	US <i>W, H, A</i>	I-IV <i>N/A</i>	Ant. Lat. Triangle <i>21 - 65</i>	Nutrafol vs. Placebo	26 14	141.7 136.5	11.5 12.7	8.1% 9.3%	26 14	9.1 0.9	8.6 5.4	0.86 [†] 0.92 [†]	8.12 (3.79 - 12.45) <i>p = 0.0002</i>	26 14	14.0 4.3	9.2 6.0	0.84 [†] 0.90 [†]	9.74 (4.99 - 14.49) <i>p < 0.0001</i>			
12	Ablon et al, 2021 ⁴⁶	US <i>W, H, A, P, O</i>	I-IV <i>N/A</i>	Ant. Lat. Triangle <i>40 - 65</i>	Nutrafol vs. Placebo	40 30	68.1 69.2	6.5 6.7	9.5% 9.7%	33 27	2.7 -0.3	3.9 2.7	0.86 [†] 0.92 [†]	2.99 (1.32 - 4.65) <i>p = 0.0004</i>	33 27	6.5 0.2	5.0 4.0	0.84 [†] 0.90 [†]	6.23 (3.97 - 8.50) <i>p < 0.0001</i>			

FIGURE 1

Eligible studies, characteristics, and results — Women. ↓, primary sort by treatment (secondary by year of study); Country: US, United States of America; FR, France; UK, United Kingdom; BE, Belgium; DK, Denmark; NL, Netherlands; CH, Switzerland. Race: W, White; H, Hawaiian; A, Asian; B, African; P, Pacific; O, other; Skin Type, Fitzpatrick I-VI; HL, hair loss pattern (Ludwig or Savin scale); Scalp Site is observed position in study; Age, study-cohort age range; N, sample population in each arm; TH, terminal hair (mean count per cm²); SD, standard deviation; RSD, relative SD (SD/TH); Corr, correlation factor; †, imputed corr; MD, mean difference between treatment and Placebo; 95% CI = 95% confidence interval; 12W and 24W, 12 and 24 weeks of treatment; bar chart colors for N, TH, SD, RSD, and MD chart are for illustrative purposes; *N/A* = not available, NS = not statistically significant; *, **, and *** are frequentist statistically significant *P*-values < 0.05, < 0.01, and < 0.001, respectively.

Eligible Studies, Characteristics and Results - Men																						
Eligible Studies		Characteristics			Treatment ↓	Baseline				12 Week Changes from Baseline				24 Week Changes from Baseline				MD Chart				
#	Author, Year	Country Race	Skin Type HL Pattern	Scalp Site Age		N	TH per cm ²	SD	RSD	N	TH per cm ²	SD	Corr	MD (95% CI) P-value	N	TH per cm ²	SD	Corr	MD (95% CI) P-value	12W	24W	
13	Feldman et al, 2021 ¹³	US	I-VI	Vertex	ALRV5XR	23	139.1	68.6	49.3%	11	8.0	8.4	1.00	10.95 (4.14 - 17.76) <i>p</i> = 0.0016	11	15.2	18.0	0.99	21.00 (9.87 - 32.13) <i>p</i> = 0.0002			
		<i>B, W, A, H</i>	<i>HN:I-VII</i>	<i>22 – 65</i>	vs. Placebo	23	141.6	66.2	46.8%	11	-3.0	7.9	0.99		11	-5.8	5.5	1.00				
14	Gubelin Harcha et al, 2014 ⁴⁸		N/A	Vertex	Dutasteride 0.5mg	184	149.0	44.1	29.6%	149	17.1	20.1	N/A	17.5 (12.95 - 22.05) <i>p</i> < 0.0001	152	18.1	21.5	N/A	18.40 (13.58 - 23.22) <i>p</i> < 0.0001			
		CL, PE, TW, US, SG	<i>HN:III-V</i>	<i>20 - 50</i>	vs. Placebo	Shared Placebo with Finasteride (#15)																
15		<i>A, H, W</i>	N/A	Vertex	Finasteride 1mg	179	148.0	37.3	25.2%	134	10.8	20.1	N/A	11.20 (6.53 - 15.87) <i>p</i> < 0.0001	142	12.1	21.4	N/A	12.40 (7.49 - 17.31) <i>p</i> < 0.0001			
			<i>HN:III-V</i>	<i>21 - 50</i>	vs. Placebo	181	148.0	45.1	30.5%	152	-0.4	20.1	N/A		152	-0.3	21.5	N/A				
16	Jimenez et al, 2014 ⁴⁷	US	I-IV	Vertex	LLLT (7-beam)	24	211.5	54.0	25.5%	24	13.3	9.4	N/A	11.18 (6.55 - 15.80) <i>p</i> < 0.0001	24	18.3	13.3	N/A	16.42 (9.54, 23.30) <i>p</i> < 0.0001			
		<i>W, O</i>	<i>HN:III-V</i>	<i>25 - 59</i>	vs. Placebo	14	216.6	34.8	16.1%	14	2.1	5.2	N/A		14	1.8	8.3	N/A				
17		US	I-IV	Vertex	LLLT (9-beam)	21	163.3	69.4	42.5%	21	15.3	10.9	N/A	12.00 (6.65 - 17.35) <i>p</i> < 0.0001	21	20.8	14.0	N/A	12.40 (4.54 - 20.26) <i>p</i> = 0.0020			
		<i>W, A, P</i>	<i>HN:II-V</i>	<i>26 - 61</i>	vs. Placebo	Shared Placebo with (12-beam) (#18)																
18		US	I-IV	Vertex	LLLT (12-beam)	22	151.5	42.4	28.0%	22	17.6	13.3	N/A	14.30 (8.15 - 20.45) <i>p</i> < 0.0001	19	25.3	17.1	N/A	16.90 (7.68 - 26.12) <i>p</i> = 0.0003			
		<i>W, A, P, O</i>	<i>HN:II-V</i>	<i>26 - 61</i>	vs. Placebo	22	171.4	62.3	36.3%	22	3.3	6.3	N/A		21	8.4	11.9	N/A				
19	Leavitt et al, 2009 ⁵⁹	US	I-IV	Vertex	LLLT (9-beam)	71	122.9	51.4	41.8%	N/A	N/A	N/A	N/A	N/A	71	16.0	11.9	N/A	24.18 (19.59 - 28.78) <i>p</i> < 0.0001			
		<i>W, H, O</i>	<i>HN:IIa-V</i>	<i>30 - 60</i>	vs. Placebo	39	120.7	48.6	40.3%	N/A	N/A	N/A	N/A		39	-8.2	11.7	N/A				
20	Olsen et al, 2002 ⁵⁰	US	N/A	Vertex	Minoxidil 2%	142	143.6	45.0	31.3%	141	27.4	18.5	N/A	12.50 (7.24 - 17.76) <i>p</i> < 0.0001	141	26.2	19.6	N/A	14.65 (9.06 - 20.24) <i>p</i> < 0.0001			
		<i>W, H, B</i>	<i>HN:III-VII</i>	<i>20 - 49</i>	vs. Placebo	Shared Placebo with Minoxidil 5% (#21)																
21		US	N/A	Vertex	Minoxidil 5%	139	151.1	45.9	30.4%	139	32.7	18.5	N/A	17.80 (12.52 - 23.08) <i>p</i> < 0.0001	139	32.4	19.6	N/A	20.85 (15.25 - 26.45) <i>p</i> < 0.0001			
		<i>W, H, B, A, O</i>	<i>HN:IIa-VII</i>	<i>21 - 49</i>	vs. Placebo	71	152.4	42.3	27.8%	71	14.9	18.5	N/A		71	11.5	19.6	N/A				
22	Pumthong et al, 2012 ⁵¹	TH	N/A	N/A	Minoxidil 5%	22	173.3	44.4	25.6%	22	23.8	31.1	0.72 [‡]	9.85 (-6.00 - 25.70) <i>p</i> = 0.2231	22	29.4	36.5	0.68 [‡]	7.27 (-10.90 - 25.44) <i>p</i> = 0.4327			
		<i>A</i>	<i>HN:II-VII</i>	<i>21 - 57</i>	vs. Placebo	22	178.6	37.0	20.7%	22	13.9	21.7	0.81 [‡]		22	22.1	23.6	0.78 [‡]				
23	Hillmann et al, 2015 ⁵²	DE	N/A	Fronto-Temporal	Minoxidil 5%	33	208.3	48.5	23.3%	32	20.8	18.6	0.93 [‡]	17.4 (9.99 - 24.81) <i>p</i> < 0.0001	31	7.8	24.1	0.88	9.30 (-0.57 - 19.17) <i>p</i> = 0.0647			
		<i>N/A</i>	<i>HN:III-VI</i>	<i>23 - 66</i>	vs. Placebo	35	217.2	48.2	22.2%	34	3.4	10.9	0.97 [‡]		34	-1.5	15.0	0.95				
24		DE	N/A	Vertex	Minoxidil 5%	34	208.8	35.7	17.1%	32	19.2	18.2	0.87 [‡]	14.78 (7.99 - 22.55) <i>p</i> = 0.0002	32	8.7	24.0	0.78	8.30 (-3.31 - 19.91) <i>p</i> = 0.1610			
		<i>N/A</i>	<i>HN:III-VI</i>	<i>23 - 66</i>	vs. Placebo	33	215.6	48.6	22.5%	33	4.4	13.3	0.96 [‡]		31	0.4	23.0	0.89				
25	Ablon, 2016 ⁵³	US	N/A	Midline	Viviscal	30	122.0	47.0	38.5%	30	5.3	17.0	0.93 [‡]	8.11 (1.19 - 15.03) <i>p</i> = 0.0216	30	8.0	21.8	0.89 [‡]	13.24 (3.77 - 22.71) <i>p</i> = 0.0062			
		<i>N/A</i>	<i>HN:III-V</i>	<i>20 - 65</i>	vs. Placebo	30	104.7	42.8	40.9%	30	-2.8	9.2	0.98 [‡]		30	-5.2	15.1	0.95 [‡]				

FIGURE 2

Eligible studies, characteristics, and results — Men. ↓, primary sort by treatment (secondary by year of study); Country: US, United States of America; CL, Chile; PE, Peru; TW, Taiwan; SG, Singapore; DE, Germany; Race: W, White; H, Hawaiian; A, Asian; B, African; P, Pacific; O, other; Skin Type, Fitzpatrick I-VI; HL, hair loss pattern (Hamilton-Norwood scale); Scalp Site is observed position in study; Age, study-cohort age range; N, sample population in each arm; TH, terminal hair (mean count per cm²); SD, standard deviation; RSD, relative SD (SD/TH); Corr, correlation factor; ‡, derived from 6-month endpoint Corr; †, derived from average Corr; MD, mean difference between treatment and Placebo; 95% CI = 95% confidence interval; 12W and 24W, 12 and 24 weeks of treatment; bar chart colors for N, TH, SD, ratio, and MD chart are for illustrative purposes; N/A, not available; NS, not statistically significant; *, **, and *** are frequentist statistically significant P -values < 0.05 , < 0.01 , and < 0.001 , respectively. Details from studies 14 and 15 (48) were clarified from [ClinicalTrials.gov: NCT01231607](https://clinicaltrials.gov/ct2/show/study/NCT01231607).

different authors, and 72 did not meet the eligibility criteria, leaving a total of 17 eligible studies (12, 13, 39–53), 10 with eligible cohorts in women (12, 39–47, 53) and eight with eligible cohorts in men (13, 47–53). Data were extracted and included in the analysis from 25 treatment cohorts (12 for women, 13 for men) using eight treatments (5 for women, 7 for men), involving a total of 3,056, 2,575, and 2,691 subjects at BL, 12 and 24 weeks, of whom 1,662, 1,429 and 1,431 were women and 1,394, 1,146 and 1,260 were men, respectively. At 12 weeks, women had 832 treatment and 597 Placebo subjects, and men had 736 and 410, respectively. At 24 weeks, women had 812 treatment and 619 Placebo subjects, and men had 835 and 425, respectively (see [Figures 1–3](#) and [Supplementary Appendix 2](#) for more details of studies and treatments searched, screened, and excluded with reasons).

3.2. Treatments in eligible studies

The treatments included in this study were: A) The oral drugs Dutasteride 0.5 mg/day and Finasteride 1 mg/day in men; (B) The topical drugs Minoxidil 2% twice per day and Minoxidil 5% once per day; (C) The LLLT comb devices of the same effective dose; and, (D) The natural treatments ALRV5XR in both sexes, Nutrafol in women, and Viviscal in men (see [Figures 1, 2](#)).

3.3. Extracted data

Data extracted from the eligible studies included study and subject characteristics, subject demographics, sample sizes, and TH/cm² at BL, with changes in TH/cm² and SD at 12 and 24 weeks for each treatment cohort and Placebo for NMA purposes (see [Figures 1, 2](#)). Additional timepoint data of density changes were extracted for up to 48 weeks for continuous analysis (see [Supplementary Appendix 1](#)).

3.4. Imputed data

Missing SDs at 12 weeks in one study were imputed using the Cochrane correlation factors determined from BL and 24 week data within the study (51) (see [Figures 1, 2](#)). Three studies that did not have sufficient data to determine SDs from *p*-values or internal correlation factors, were estimated by taking the average of the correlation factors from the available data in the eligible studies (45, 46, 53). These average correlation factors at 12 and 24 weeks for women were (treatments: 0.855 and 0.837; Placebo 0.918 and 0.901) and for men were (treatments: 0.932 and 0.887; Placebo 0.977 and 0.947), respectively, and were used

to estimate the SDs of change from BL in three studies (45, 46, 53) (see [Figure 1, 2](#)).

3.5. Study characteristics

Studies were conducted internationally. Eleven of the 12 studies in women and eight of the 13 studies in men were exclusively in the USA. A broad range of races were reported in the studies; however, most subjects were Caucasians. Fitzpatrick skin types were not available for most of the studies. All scalp study sites were at the vertex of the scalp, except for four study cohorts in women that were located at the frontoparietal, anterior lateral triangle, and midline. In men, two study cohorts were at the frontotemporal and midline. BL TH densities in women were 68.1–169.0 TH/cm². BL SDs in women ranged from 6.5–61.6 TH/cm² and the BL relative SDs (RSDs) of SD/TH ranged from 8.1 to 39.1%. BL TH densities in men were 104.7–217.2 TH/cm². BL SDs in men ranged from 34.8 to 69.4 TH/cm² and RSDs ranged from 16.1 to 49.3% (see [Figures 1, 2](#)). Dutasteride 0.5 mg, Finasteride 1 mg, Minoxidil 2% and Viviscal were in subjects < 51 years. All other treatments also included subjects > 60 years. Races, ranges of Fitzpatrick skin types, age ranges, and hair loss patterns of participants in these studies are described in [Figures 1, 2](#).

All studies were found to be statistically different from Placebo in women except for one study in Minoxidil 2% at 12 and 24 weeks (40). In men, one cohort at 12 weeks and three cohorts at 24 weeks of Minoxidil 5%, were not statistically significantly different from Placebo (51, 52) (see [Figures 1, 2](#)).

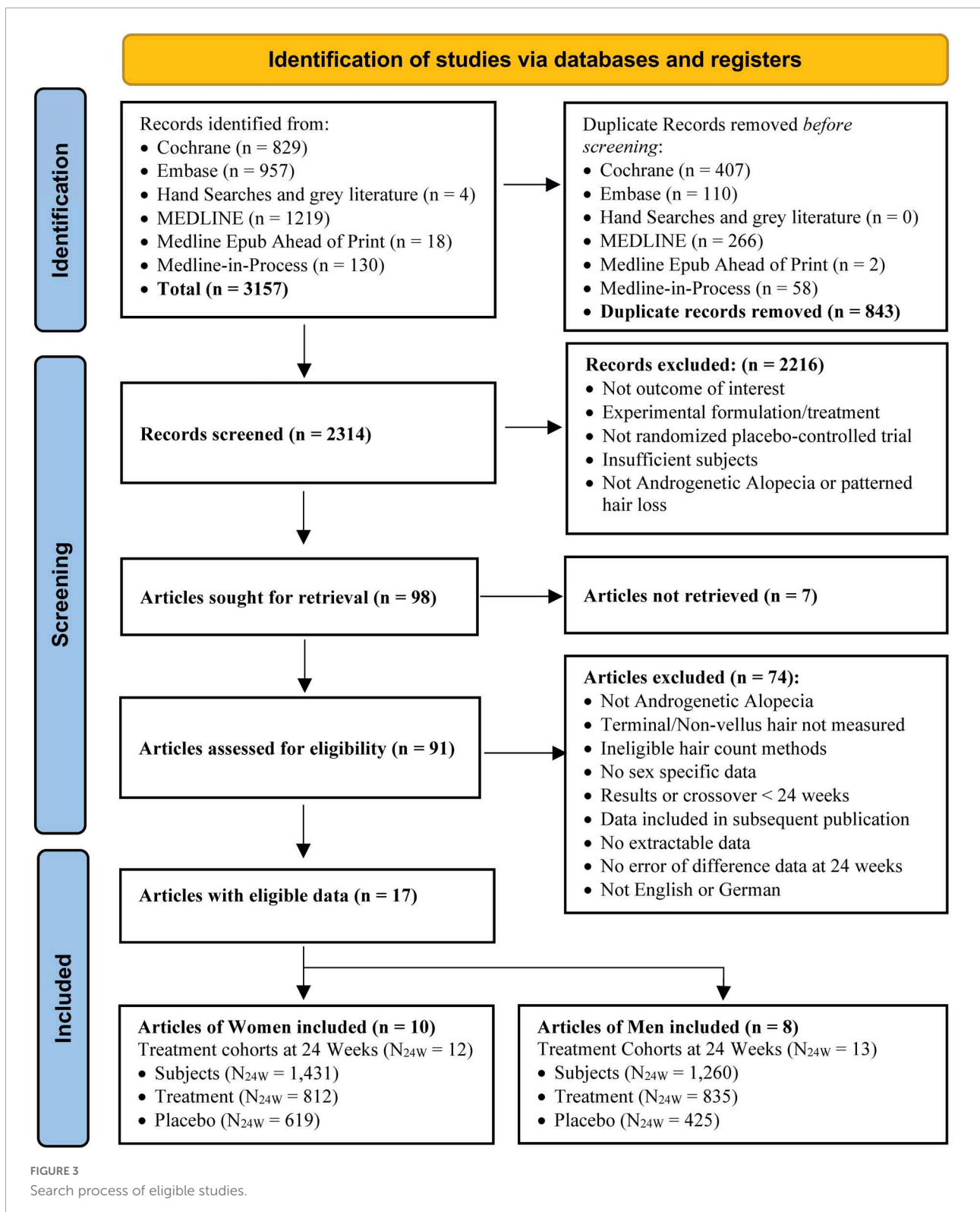
3.6. Bayesian network meta-analyses of treatments vs. Placebo

3.6.1. Women

In women at 24 weeks, the Bayesian NMA, using direct and indirect treatment comparisons, found MD in TH regrowth for each treatment group vs. Placebo in TH/cm² (Bayesian significance = **) ranked as follows: ALRV5XR: 30.09**, LLLT: 16.62**, Minoxidil 2%: 12.13**, Minoxidil 5%: 10.82**, and Nutrafol: 7.32**. At 12 weeks, the ranked women's results were: Minoxidil 2%: 13.81**, ALRV5XR: 12.93**, Minoxidil 5%: 12.73**, LLLT: 9.78**, and Nutrafol 4.72**. MDs in the frequentist NMAs were similar to the Bayesian and at 24 weeks, all frequentist NMA results were significant (see [Figures 4–6](#) and [Supplementary Appendix 1](#)).

3.6.2. Men

In men at 24 weeks, the Bayesian NMA, using direct and indirect treatment comparisons, found the following



ranked MD of each treatment group vs. Placebo in TH/cm² (Bayesian significance = **); ALRV5XR: 21.03**, LLLT: 18.75**, Dutasteride 0.5 mg: 18.37**, Viviscal: 13.23, Minoxidil 5%:

13.13**, Finasteride 1 mg: 12.38, and Minoxidil 2%: 10.54. At 12 weeks, the men's results ranked; Dutasteride 0.5 mg: 17.50**, Minoxidil 5%: 16.29**, LLLT: 12.21**, Minoxidil 2%:

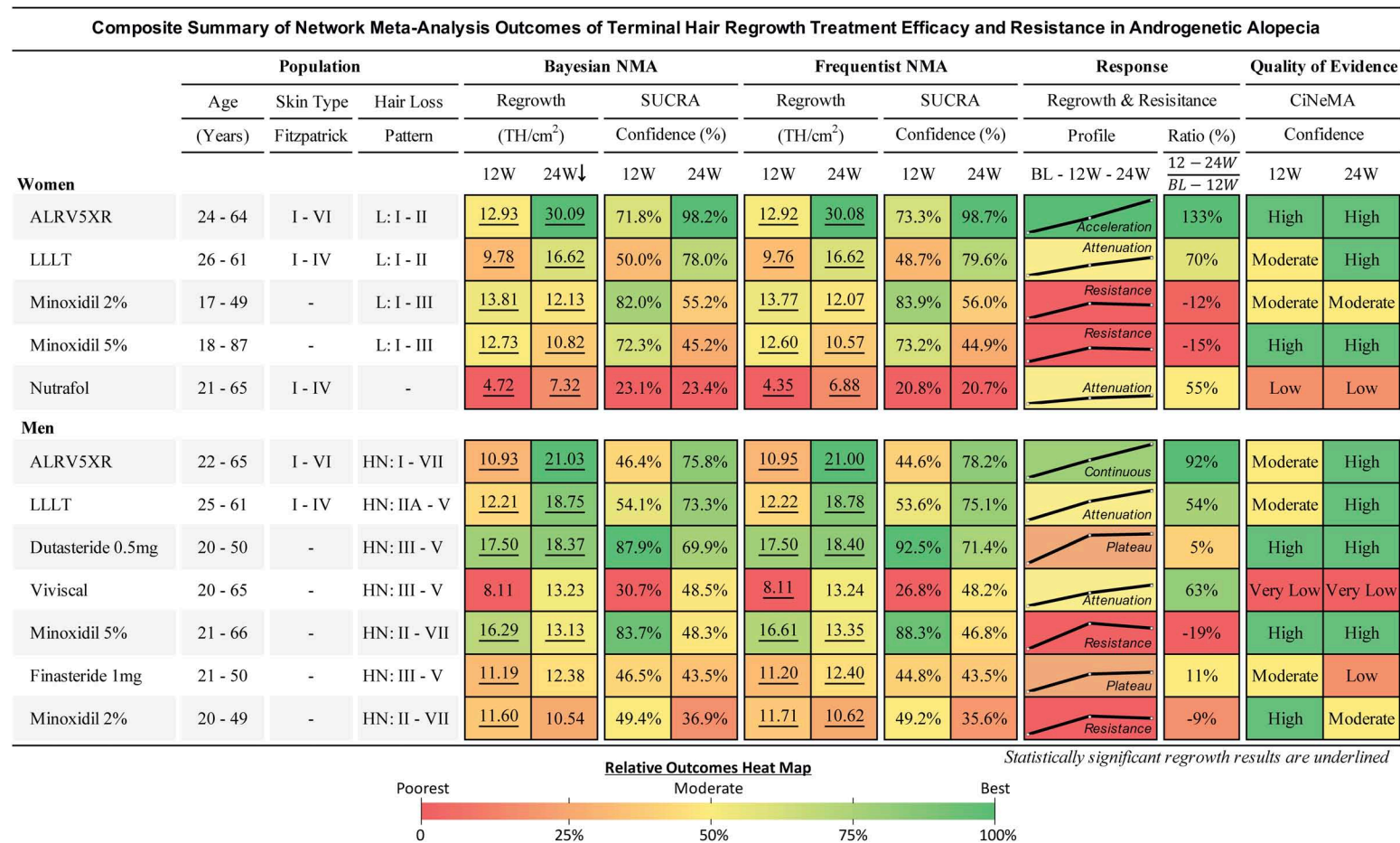
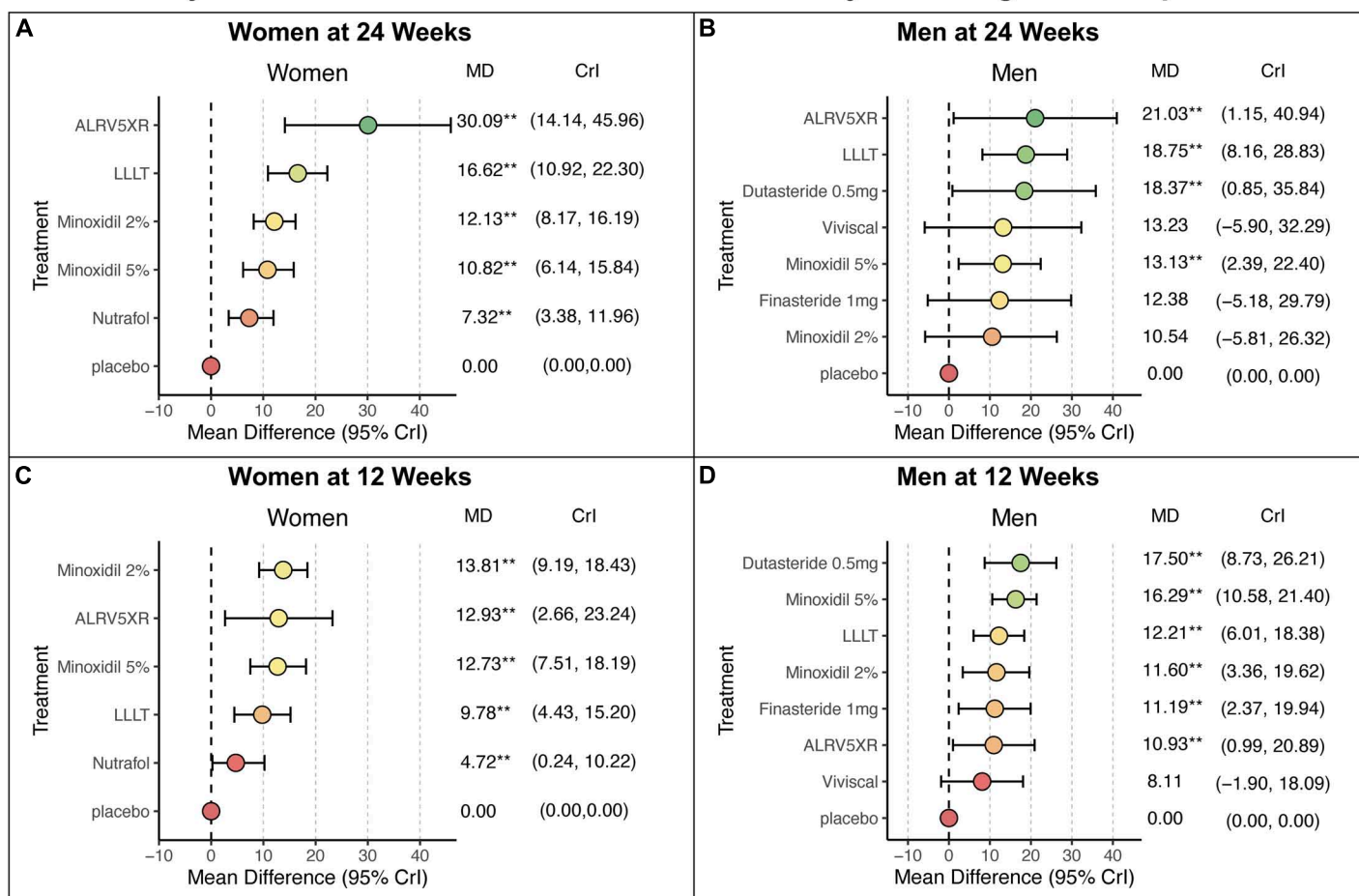


FIGURE 4

Composite summary of terminal hair regrowth and resistance from network meta-analysis outcomes for treatments in androgenetic alopecia. This outcomes table provides an overview of the studied populations, Bayesian and frequentist network meta-analyses (NMAs), response profiles showing regrowth and resistance, and quality of evidence for each treatment. Women and men are ranked in descending order of efficacy at 24 weeks of the Bayesian NMA after 250,000 Markov Chain Monte Carlo simulations. Regrowth measures efficacy as mean difference (MD) in TH/cm² between each treatment and Placebo using direct and indirect comparisons at 12 and 24 weeks. Hair loss pattern: L = Ludwig (women), HN = Hamilton-Norwood (men). BL, 12W, and 24W refer to baseline, 12 weeks, and 24 weeks duration of treatment. SUCRA: surface under the cumulative ranking area measures relative confidence of outcomes of the compared treatments. Response Profile is a line graph connecting the treatment changes from baseline (BL) to MD at 12 and 24W. Y-axes (MD) are scaled 0–31 for women and 0–22 for men. Response Ratio (%) is change in regrowth of 12–24W compared to BL–12W. Quality of evidence is CiNeMA results using Cochrane risk of bias, GRADE scores, and study data. Heat maps are shared between each sex's numerical result block and manually assigned to categorical results.

Bayesian NMA Forest Plots of Treatment Efficacy in Androgenetic Alopecia



Statistically significant mean difference (MD) results are marked **

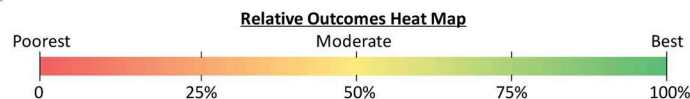


FIGURE 5

Bayesian NMA forest plots. Results of Bayesian network meta-analyses (NMA) forest plots show mean differences (MD) between treatment vs. Placebo for changes in terminal hair per cm² from baseline. **(A,C)** Results for women. **(B,D)** Results for men. Results are ranked in descending order of MD with 95% credible intervals (CrI). Bayesian statistical significance is marked, **. The colors of treatment MD's correspond with the heat map colors of Bayesian NMA results in Figure 4.

11.60**, Finasteride 1 mg: 11.19**, ALRV5XR: 10.93**, and Viviscal: 8.11. Bayesian statistical significance at 12 weeks was lost at 24 weeks for Finasteride 1 mg and Minoxidil 2%, and Viviscal was not significant at either 12 or 24 weeks. MDs in the frequentist NMAs were similar to the Bayesian and at 12 weeks all the treatments were significant, however, at 24 weeks, Viviscal was not significant and Minoxidil 2% was borderline significant ($p = 0.0491$) (see [Figures 4–6](#) and [Supplementary Appendix 1](#)).

3.7. Regrowth from 12 to 24 weeks vs. BL to 12 weeks

Comparing regrowth from 12–24 weeks vs. BL–12 weeks, ALRV5XR showed an accelerating regrowth effect in women (133%) and an effectively continuous regrowth effect in men (92%). Resistance effects were observed in all other treatments after 12 weeks. There was an attenuated regrowth effect from 12–24 weeks vs. BL–12 weeks for LLLT (women: 70%, men: 54%), Nutrafol (women: 55%), and Viviscal (men: 63%). A flatlining effect was observed after 12 weeks for Dutasteride 0.5 mg (men: 5%) and Finasteride 1 mg (men: 11%). After peaking at 12 weeks, some of the regrowth was lost by 24 weeks for Minoxidil 2% (women: -12%, men: -9%) and Minoxidil 5% (women: -15%, men: -19%) (see [Figures 4–6](#) and [Supplementary Appendix 1](#)).

3.8. NMA relative effects of treatment vs. treatment

In women, the Bayesian NMA relative effect results found at 24 weeks that ALRV5XR had a significantly greater MD in TH/cm² (Bayesian significance = **) than Nutrafol by 22.77**, Minoxidil 5% by 19.27** and Minoxidil 2% by 17.97**, and LLLT was greater than Nutrafol by 9.29**. At 12 weeks, Minoxidil 2% and 5% were significantly greater than Nutrafol: 9.09** and 8.00** TH/cm².

In men, there were no significant differences in NMA relative effects between treatments at 12 or 24 weeks. (For more details, see [Figure 6](#) and [Supplementary Appendix 1](#)).

3.9. Surface under the cumulative rank area (SUCRA) scores

SUCRA scores can be found in [Figure 4](#) and cumulative ranking curves can be seen in [Supplementary Appendix 1](#).

3.10. Continuous outcomes analysis

Continuous outcomes can be found in [Figures 7, 8](#) and Section 4 of [Supplementary Appendix 1](#).

3.11. Risk of bias and quality of evidence

The Cochrane risk of bias instrument assessed four studies (41, 45, 46, 53) with a high risk of bias, nine studies (13, 40, 42, 43, 47–49, 51, 52) with some concerns, and four studies (12, 39, 44, 50) with no overall concerns.

The summarized quality of evidence can be found in [Figure 4](#), and the supporting Cochrane risk of bias assessments, CiNeMA results and conflict of interest data can be found in Section 3 of [Supplementary Appendix 1](#).

3.12. Safety

A summary of treatment adverse events as reported in the respective trials can be found in Section 6 of [Supplementary Appendix 1](#).

3.13. Frequentist results

Changes in efficacy from 12 to 24 weeks were similar between Bayesian and frequentist NMAs. Results from frequentist NMAs with SUCRA scores after 12 and 24 weeks of treatment can be found in [Figure 4](#). Additional frequentist results can be found in [Supplementary Appendix 1](#).

4. Discussion

This study is distinctive since it used comparable data from RCTs to determine TH density change as the primary marker of scalp hair phenotype. It used multiple standard analytical methods at key treatment time points, 12 and 24 weeks, and evaluated women and men separately. This approach enabled the detection of discrete insights and treatment nuances. In addition, it used rigorous methods to screen studies to ensure accurate eligibility. Opinions from independent experts, some of whom have led pivotal RCTs in AGA treatments since the late 1980's, helped guide interpretation of eligible studies, methods, and data. Similar confirmatory results were obtained using a comprehensive range of accepted analytical methods, providing a valuable decision-making tool for clinical practitioners and, eventually, for patient self-evaluation of treatment options.

4.1. Treatment resistance effects

For the treatments that showed resistance, the first 12 weeks of treatment was the phase of the highest rates of TH regrowth. Thereafter, resistance affected each treatment at different rates with the drugs showing the most pronounced

League Tables of Bayesian Relative Effects between Treatments in Androgenetic Alopecia

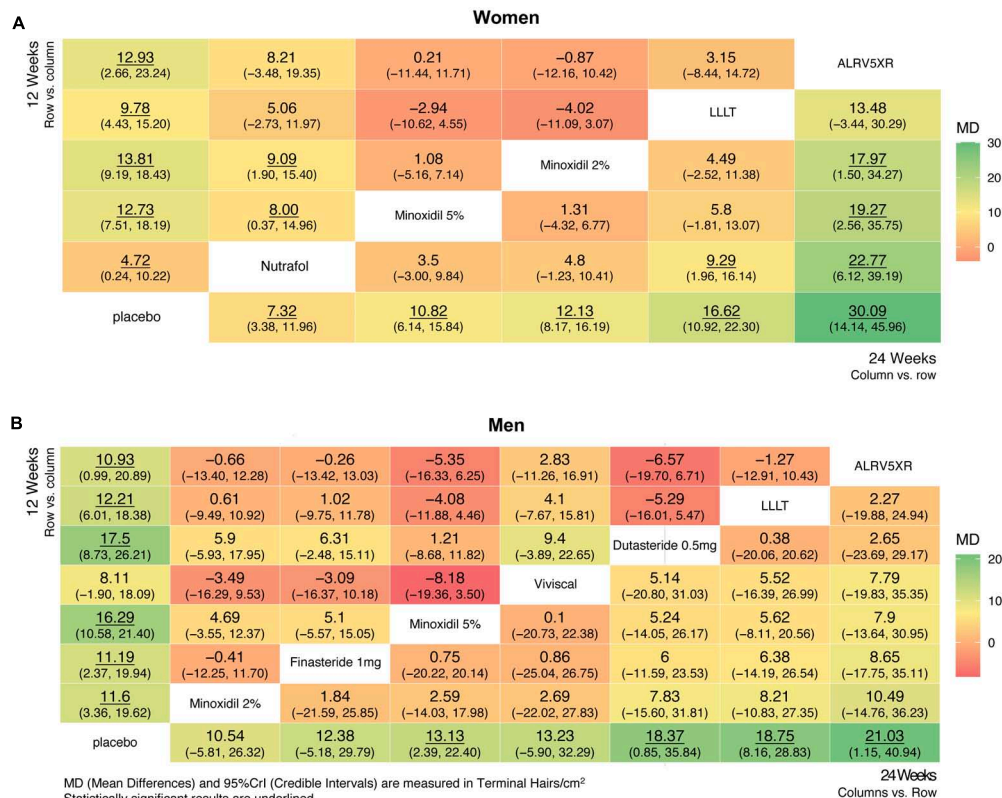


FIGURE 6

League tables showing relative effects between treatments after Bayesian network meta-analysis. These results show mean differences (MD) in regrowth of terminal hairs per cm² (95% Credible Interval) between each treatment at 12 and 24 weeks in women (A) and men (B), with significant differences underlined. League tables are arranged with 24 week results in the lower right diagonal and 12 week results in the top-left diagonal. Twenty four week results are treatment column vs. row, and are ranked in ascending order vs. Placebo from the bottom left row to bottom right. Twelve week results are treatment row vs. column. The largest positive differences are in dark green, and the largest negative differences are in dark red, and intermediate differences are shaded yellow accordingly.

resistance. Men and women showed similar timing of resistance for the same treatments, particularly LLLT and Minoxidil. Nutrafol in women and Viviscal in men showed very similar resistance profiles. LLLT seems to have a reduced resistance effect and its mild attenuation maintained positive regrowth rate. The continuous regrowth effects observed with ALRV5XR appear to bypass or inhibit resistance factors (see [Figures 4–8](#) and [Supplementary Appendix 1](#)). Possible combinations of ALRV5XR with other treatments may therefore be synergistic.

4.2. Bayesian vs. frequentist network meta-analysis

The Bayesian and frequentist NMAs were in agreement with MDs. In contrast, all the Bayesian 95% CrI's were wider than the frequentist 95% CI's by an average of 26.0% in women and 30.8% in men. A sensitivity analysis of the

24-week Bayesian NMA in men for the changes in significance from 12 to 24 weeks simulated 50,000, 150,000, 300,000, and 500,000 MCMC iterations and found no meaningful change to the 250,000 MCMC simulation results (see [Supplementary Tables 1-1, 1-3](#) in [Supplementary Appendix 1](#)). Due to the sparseness of the study data, we believe that a Bayesian NMA provides more accurate, statistically relevant, and conservative results than the frequentist NMA.

4.3. Women vs. men

The MD regrowth profiles were similar between sexes for the same treatment type. The analysis found that the widths of CrI's were narrower in women than in men by 55.9% (13.90 vs. 31.54 TH/cm²). Women had slightly lower efficacy than men for LLLT (11.4%) and Minoxidil 5% (17.6%), while Minoxidil 2% was slightly better in women than men (15.1%), however, ALRV5XR showed a much larger efficacy in women than men

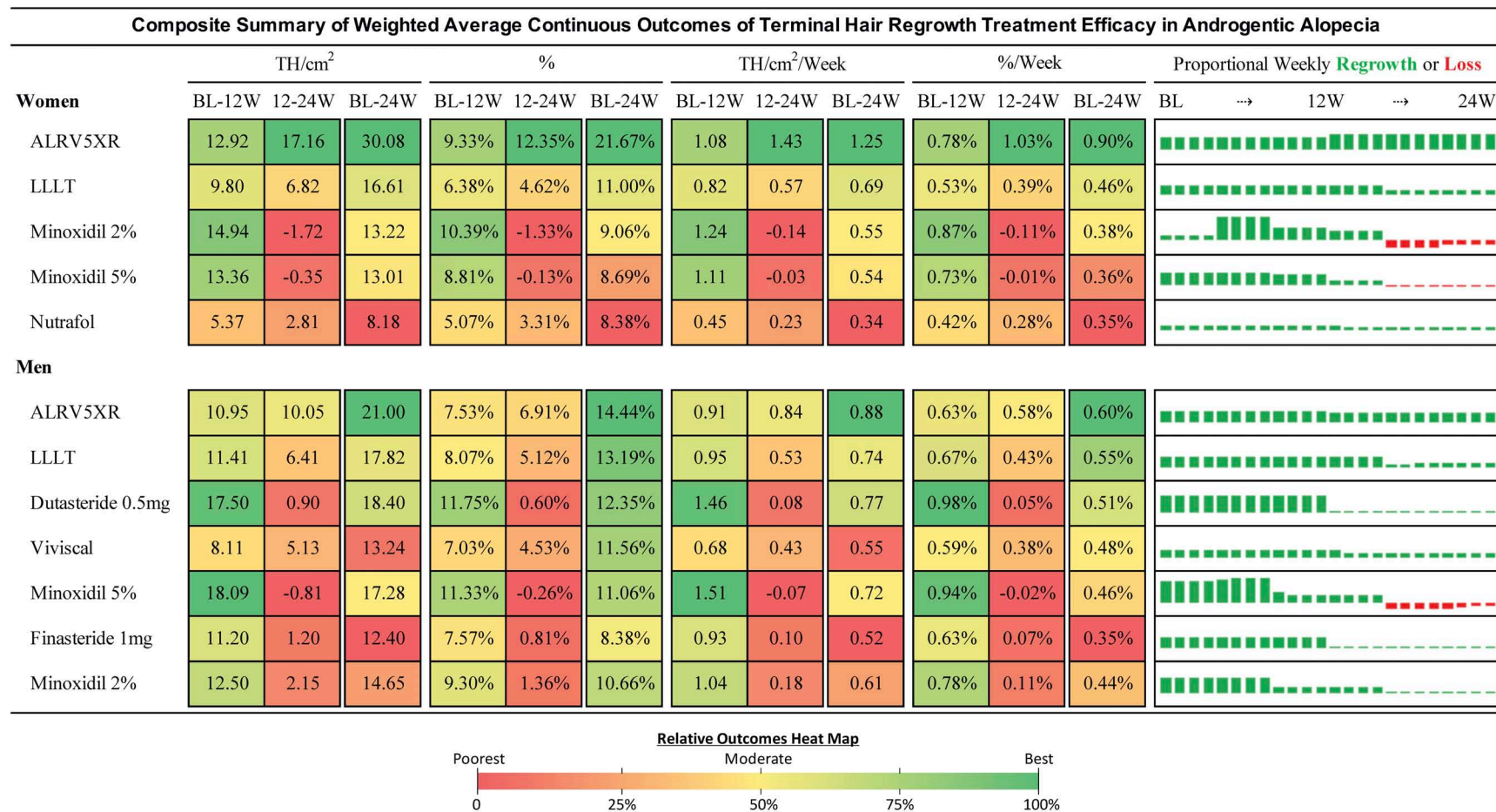


FIGURE 7

Composite summary of terminal hair regrowth from weighted average continuous outcomes analysis. This composite outcomes table provides a comprehensive overview of the treatments from weekly imputed data points for all eligible studies. Efficacy of each treatment is the difference between treatment and Placebo weighted by the N of each respective cohort. Efficacy percent is the change divided by the baseline of each cohort and weighted by the N of each cohort population. The bar charts show weekly regrowth or loss for each of the 24 weeks in TH/cm². Treatments are sorted by the 24 week efficacy determined in the Bayesian NMA. Results may be different from the NMA due to analytical methods. Continuous outcomes have no measures for statistical significance. Heat map colors are green (highest result), yellow (moderate), and red (lowest). Heat maps are shared between BL-12W and 12-24W. The BL-24W column has its own heat map for the overall result. Heat maps are separate for women and men.

Weighted Average Continuous Efficacy and Weekly Growth Rate Plots for Treatments in Androgenetic Alopecia

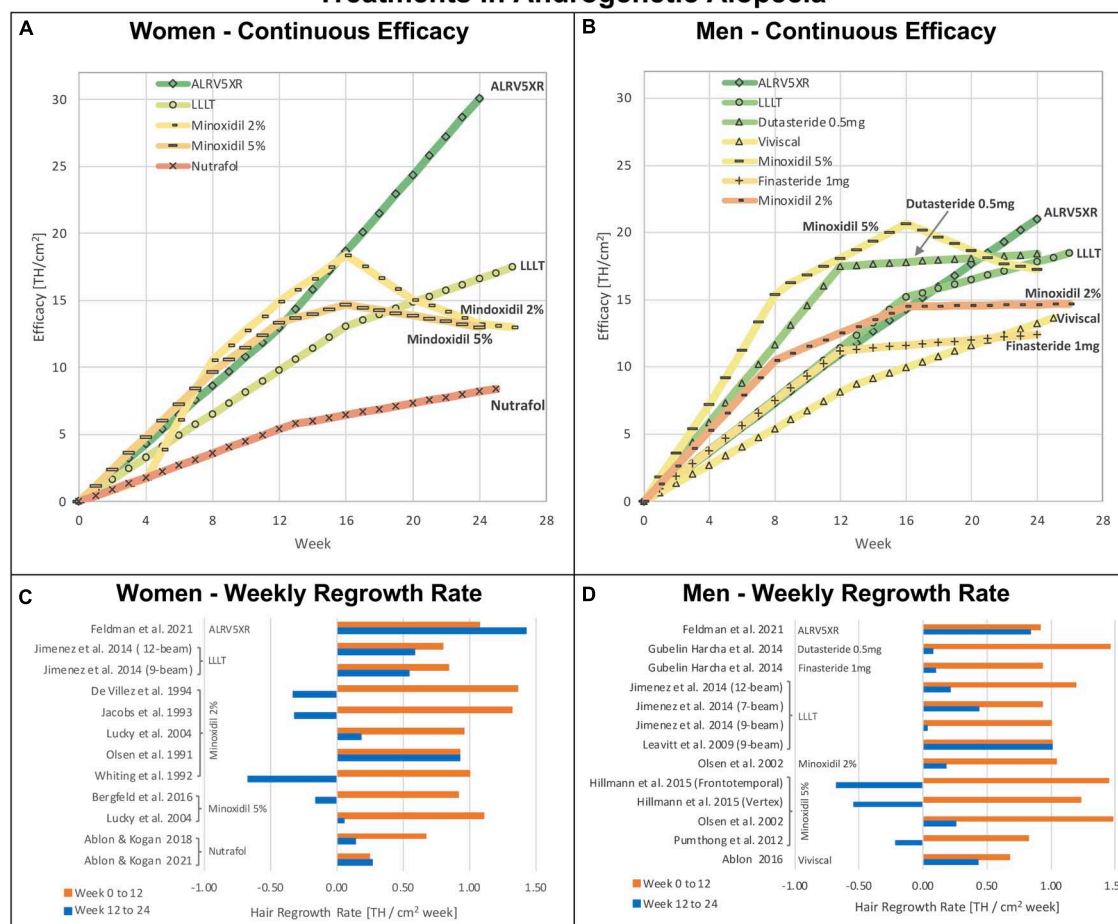


FIGURE 8

Weighted average continuous efficacy and weekly regrowth rate plots: Weighted average continuous efficacy plots are in panels (A) for women and (B) for men. Average weekly regrowth rates from 0 to 12 weeks and 12–24 weeks are in panels (C) for women and (D) for men. The continuous efficacy line graphs show the cumulative changes in efficacy for each treatment by sex. They identify two distinct regrowth response profiles common to each treatment in both sexes: Continuous: (ALRV5XR); Resistant: Dutasteride 0.5 mg, Finasteride 1 mg, LLLT, Minoxidil 2% and 5%, Nutrafol, and Viviscal. The colors of treatments in continuous efficacy response lines correspond with the 24 week heat map colors in the Bayesian network meta-analysis in Figure 4.

by 43.6% (30.11 vs. 20.97 TH/cm²). Analysis of the differences in the same treatment between women and men found no statistical significance (see [Supplementary Tables 1-2, 1-3](#) in [Supplementary Appendix 1](#)).

4.4. Data anomalies, outliers and interpretation

Most studies reported mean BL TH densities consistent with the evaluated scalp sites for the eligible hair loss patterns, except for one study in women that was > 3 SDs less than the mean in both the treatment and Placebo groups (46). Two studies in women had a BL RSD < 10% compared to 21.3–39.1% in the other women's studies (45, 46). Another study in men reported

and referenced three different target area sizes in its methods for the hair density measurements (53). A target area of 1 cm² was assumed since it resulted in normal BL TH density relative to the other studies. All data was extracted as reported according to Cochrane methods without interpretations.

4.5. Confidence, risk of bias and conflicts of interest

The inclusion of only RCTs in this NMA assisted in increasing the overall confidence of the GRADE and CiNeMA assessments. At 24 weeks, the CiNeMA confidence evaluations for each treatment vs. Placebo, found no concerns for women, and in men there were no major concerns,

therefore no investigations into causes of heterogeneity or small study bias were deemed necessary. Studies most impacted by the risk of bias assessment were found wanting in reporting randomization procedures and selected reporting of results. The two largest effectors of the CiNeMA analysis results were the Cochrane risk of bias, which downgraded confidence in almost all studies, while the high magnitude effect upgraded confidence in ALRV5XR, LLLT and Dutasteride treatments.

All studies, except for one (51), declared a conflict of interest relating to study funding by the owner of the investigational treatment. Authors in 11 studies (12, 13, 41, 42, 44–46, 48–50, 52) were affiliated with or employed by the study sponsor. Five studies (40–42, 48, 50) had trichometric data generated by the sponsor or investigators employed by the sponsor (see [Supplementary Table 1-5](#) in [Supplementary Appendix 1](#)).

After considering the results of the risk of bias and CiNeMA, no study conflict was deemed to have a sufficiently differential impact on the outcome of this NMA, that would require its exclusion. To further improve the confidence in this study the AMSTAR 2 (54) and ROBIS (55) appraisal tools have been used as a guide. Therefore, the confidence reported in [Figure 4](#) can be considered a reasonable assessment.

4.6. Ineligible treatments of interest

Stem cells, PRP, oral Minoxidil, topical Finasteride, additional off-label drugs other than dutasteride, combination treatments, other laser, and electrostatic devices, natural treatments and cosmetics with hair loss improvement claims were of interest for this study; however, no eligible studies were found. Recently, the sub lingual use of Minoxidil in AGA with doses ranging from 0.45 to 4.05 mg has been reported, but it did not fit the inclusion criteria for this study (56). Also, a recent review reported PRP preparation and administration protocols have a broad variability, and there is no current consensus on which protocol provides the best results (57). Although no single vitamin, mineral, botanical, collagen, or protein eligible studies were found, many of these ingredients of interest are part of the natural treatments included in this study.

4.7. Future treatment directions addressing the biology of AGA

A need exists for improved AGA hair regrowth treatments using innovative hair follicle (HF) regenerative strategies that aim to prolong anagen, induce neogen in involuted HFs, and reverse the miniaturization of terminal HFs (58). Amongst the main identified biological targets that induce HF regeneration are the Wnt/ β -catenin cascade and

growth factors such fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), transforming growth factor β (TGF- β), insulin-like growth factor (IGF), endothelial cell growth factor (ECGF), and epidermal growth factor (EGF) (57, 58).

At present, multi-targeting treatments with HF regenerative effects have clinical evidence with ALRV5XR, show promise for PRP, and early work in wound induced hair neogenesis (WIHN) indicates potential as a novel treatment option (57–60). A combination of ALRV5XR and PRP may lead to an improved hair regrowth efficacy and a prolonged continuous regenerative response.

4.8. Hard evidence of treatments for AGA

The very small proportion of eligible studies based on the inclusion criteria (17 of the 2,314 unique records or 0.73%) measuring TH indicates there is very limited hard published evidence of TH regrowth. Furthermore, the eight eligible treatments found for this study represent a very small number of the commercially available treatment options claiming the prevention of hair loss or promotion of hair regrowth, suggesting treatments without eligible studies might have no hard published evidence.

4.9. First line treatment

When considering the current first-line therapies for AGA, topical Minoxidil 2% and 5%, and oral Finasteride 1 mg, these Bayesian results appear to challenge the usefulness of these treatments. Even though additional studies are required, LLLT comb devices and the natural treatment ALRV5XR appear as scientifically suitable candidates for inclusion within the AGA first-line of treatment options, alone or in combination.

5. Limitations

A small number of eligible studies reporting changes in TH density were found. Hindrances posed by inconsistencies in TH definition, incomparable hair-counting methods, and lack of error reporting of changes from baseline (particularly for midpoints within longer-term studies) were the most common omissions found in the studies that could not be included. Also, the study design intended to include CONSORT compliant RCTs; nevertheless, this factor was partially represented in the risk of bias and quality of evidence analysis. The lack of treatment population response data and odds ratios in the literature limited key parameters for analysis. Although this meta-analysis used trials published in English or German, it is possible that studies published in other languages may have a

more comprehensive range of treatments, populations, races, skin, and hair types.

6. Conclusion

For most commercially available products claiming to prevent hair loss or to regrow hair, there is only a dearth of scientific proof available in the literature of TH regrowth. This meta-analysis examines the available evidence with scientifically validated methods in the few RCTs with published TH regrowth data. Despite the long-term treatment resistance and statistically significant improvement in TH reported for most treatments, the primary clinical outcome of virtually all of them was the maintenance of existing hair, or the regrowth of a small amount of hair that may decrease AGA pattern severity, with LLLT comb being more efficacious than first-line pharmaceuticals. The exception was ALRV5XR which demonstrated better efficacy without treatment resistance effects, and, in consequence, showed strong characteristics to be among the first-line AGA treatment options for men and women, alone or in combination.

Eligible treatment trade names

Tradenames of eligible treatments in this study are ALRV5XR (Replenology), Dutasteride 0.5 mg (Avodart, generic dutasteride), Finasteride 1 mg (Proscar, Propecia, generic finasteride), LLLT comb (HairMax), Minoxidil (Rogaine, Regaine, generic minoxidil). Nutrafol and Viviscal are trade names.

Plain language summary

Terminal hair (TH) on the scalp is thick, long hair giving a unique visual identity to each person. TH density is the most important marker of the scalp hair phenotype, yet it is one of the least reported hair loss treatment outcomes. Androgenetic alopecia, or patterned balding, affects about half the population. It is characterized by TH diminishing into short, fine vellus-like hair, which eventually stops growing and can lead to baldness. Scalp hair phenotype restoration can only be achieved by increasing TH density. This study compares the continuous efficacy of treatments claiming to promote hair regrowth. A systematic literature search found 2,314 unique published hair regrowth studies. Results could be used from only 17 studies with five different therapies in women and seven in men to statistically compare TH regrowth over 24 weeks. These treatments included drugs, laser devices, and natural products. A network meta-analysis using Bayesian

statistics simulated the real-world effectiveness of each treatment. This study found that most of the included treatments will maintain existing TH density, and some might reduce the hair-loss pattern. However, responses to most treatments had a resistance effect after 12 weeks, where drugs plateaued or lost part of the regrowth, and some were not different from Placebo. Pharmaceutical treatments showed limited regrowth efficacy, and laser comb devices appeared slightly better than pharmaceuticals. The natural treatment ALRV5XR displayed the best results without resistance and might have the potential to restore TH in women and men.

Author contributions

PRF and CP contributed to the study concept and design. PRF obtained funding and drafted the manuscript. EU developed the search strategy, performed the article search, and prepared the article database. SG, JP, MM, MIS, MB, OJE, and CLK screened the studies and extracted the data. PRF, CP, JG-A, PG, HMM, and KMF adjudicated and reviewed the data. PRF, SG, and KMF performed statistical analysis and visualization. PG, CP, HMM, JG-A, and KMF critically revised the manuscript for important intellectual content. All authors reviewed, edited, and approved the manuscript for submission.

Funding

This study was supported by funding from the Government of Canada (grants 2020/017260985, 2021/017813049, and 2022/018358713) and Arbor Life Labs of Toronto, Canada.

Acknowledgments

We express our profound appreciation and acknowledgment for their insights into the data and assistance for study selection to Dr. Dominique Van Neste (DVN) of Skinterface, Belgium; Dr. Hugh Rushton (HR) of University of Portsmouth, UK; Dr. Wilma Bergfeld of Cleveland Clinic, USA; Dr. Mirmirani Paradi of University of California San Francisco and Kaiser Permanente, USA. We also thank DVN and HR for critically reviewing the manuscript.

Conflict of interest

Arbor Life Labs (ALL) is the owner of ALRV5XR. PRF was employed by ALL. SG, JP, MM, MIS, MB, OJE, CLK, JG-A, EU, and KMF received honoraria from ALL. CP was employed by CHP Pharma Inc. EU was employed by Uleryk Consulting.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the

reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmed.2022.998623/full#supplementary-material>

References

1. Tamashunas N, Bergfeld W. Male and female pattern hair loss: treatable and worth treating. *Cleve Clin J Med*. (2021) 88:173–82. doi: 10.3949/ccjm.88a.20014
2. Norwood O. Incidence of female androgenetic alopecia (female pattern alopecia). *Dermatol Surg*. (2001) 27:53–4. doi: 10.1097/00042728-200101000-00015
3. Courtois M, Loussouarn G, Hourseau C, Grollier J. Ageing and hair cycles. *Br J Dermatol*. (1995) 132:86–93. doi: 10.1111/j.1365-2133.1995.tb08630.x
4. McMichael A, Hordinsky M. *Hair and scalp disorders: medical, surgical, and cosmetic treatments*. 2nd ed. Boca Raton, FL: CRC Press (2018). doi: 10.1201/9781351169844
5. Rushton D, Westgate G, Van Neste D. Following historical "tracks" of hair follicle miniaturisation in patterned hair loss: are elastin bodies the forgotten aetiology? *Exp Dermatol*. (2021) 31:102–9. doi: 10.1111/exd.14393
6. Whiting D. *The structure of the human hair follicle: light microscopy of vertical and horizontal sections of scalp biopsies*. Fairfield, NY: Canfield (2004).
7. Trüeb R. *Nutrition for healthy hair: guide to understanding and proper practice*. Berlin: Springer Nature (2020). doi: 10.1007/978-3-030-59920-1
8. Van Neste D. Exhaustive analysis of scalp hair regression: subjective and objective perception from initial hair loss to severe miniaturisation and drug-induced regrowth. *Plast Aesthet Res*. (2021) 8:16. doi: 10.20517/2347-9264.2020.220
9. Van Neste D. Placebo-controlled dose-effect studies with topical minoxidil 2% or 5% in male-patterned hair loss treated with oral finasteride employing an analytical and exhaustive study protocol. *Skin Res Technol*. (2020) 26:542–57. doi: 10.1111/srt.12827
10. Rushton D, Unger W, Cotterill P, Kingsley P, James K. Quantitative assessment of 2% topical minoxidil in the treatment of male pattern baldness. *Clin Exp Dermatol*. (1989) 14:40–6. doi: 10.1111/j.1365-2230.1989.tb00881.x
11. Kanti V, Messenger A, Dobos G, Reygagne P, Finner A, Blumeyer A, et al. Evidence-based (S3) guideline for the treatment of androgenetic alopecia in women and in men - short version. *J Eur Acad Dermatol Venereol*. (2018) 32:11–22. doi: 10.1111/jdv.14624
12. Feldman PR, Fiebig KM, Piwko C, Mints BM, Brown D, Cahan DJ, et al. Safety and efficacy of ALRV5XR in women with androgenetic alopecia or telogen effluvium: a randomised, double-blinded, placebo-controlled clinical trial. *EClinicalMedicine*. (2021) 37:100978. doi: 10.1016/j.eclinm.2021.100978
13. Feldman PR, Fiebig KM, Piwko C, Mints BM, Brown D, Cahan DJ, et al. Safety and efficacy of ALRV5XR in men with androgenetic alopecia: a randomised, double-blinded, placebo-controlled clinical trial. *EClinicalMedicine*. (2021) 40:101124. doi: 10.1016/j.eclinm.2021.101124
14. Adil A, Godwin M. The effectiveness of treatments for androgenetic alopecia: a systematic review and meta-analysis. *J Am Acad Dermatol*. (2017) 77:136–41.e5. doi: 10.1016/j.jaad.2017.02.054
15. Chen D, Yang X, Liu X, He Y, Fan W, Wang H, et al. Efficacy comparison of monotherapies and combination therapies for androgenetic alopecia: a bayesian network meta-analysis. *Dermatol Ther*. (2021) 35:e15262. doi: 10.1111/dth.15262
16. Chen L, Zhang J, Wang L, Wang H, Chen B. The efficacy and safety of finasteride combined with topical minoxidil for androgenetic alopecia: a systematic review and meta-analysis. *Aesthetic Plast Surg*. (2020) 44:962–70. doi: 10.1007/s00266-020-01621-5
17. Giordano S, Romeo M, di Summa P, Salval A, Lankinen PA. Meta-analysis on evidence of platelet-rich plasma for androgenetic alopecia. *Int J Trichol*. (2018) 10:1–10. doi: 10.4103/ijt.ijt_74_16
18. Giordano S, Romeo M, Lankinen P. Platelet-rich plasma for androgenetic alopecia: does it work? evidence from meta analysis. *J Cosmet Dermatol*. (2017) 16:374–81. doi: 10.1111/jocd.12331
19. Gupta A, Bamimore M, Foley K. Efficacy of non-surgical treatments for androgenetic alopecia in men and women: a systematic review with network meta-analyses, and an assessment of evidence quality. *J Dermatolog Treat*. (2020) 33:62–72. doi: 10.1080/09546634.2020.1749547
20. Gupta A, Carviel J. Meta-analysis of photobiomodulation for the treatment of androgenetic alopecia. *J Dermatolog Treat*. (2021) 32:643–7. doi: 10.1080/09546634.2019.1688755
21. Gupta A, Carviel J. Meta-analysis of efficacy of platelet-rich plasma therapy for androgenetic alopecia. *J Dermatolog Treat*. (2017) 28:55–8. doi: 10.1080/09546634.2016.1179712
22. Gupta A, Charrette A. Topical minoxidil: systematic review and meta-analysis of its efficacy in androgenetic alopecia. *Skinmed*. (2015) 13:185–9.
23. Gupta A, Charrette A. The efficacy and safety of 5alpha-reductase inhibitors in androgenetic alopecia: a network meta-analysis and benefit-risk assessment of finasteride and dutasteride. *J Dermatolog Treat*. (2014) 25:156–61. doi: 10.3109/09546634.2013.813011
24. Gupta A, Mays R, Dutzert M, Versteeg S, Shear N, Piguet V. Efficacy of non-surgical treatments for androgenetic alopecia: a systematic review and network meta-analysis. *J Eur Acad Dermatol Venereol*. (2018) 32:2112–25. doi: 10.1111/jdv.15081
25. Gupta A, Venkataraman M, Talukder M, Bamimore M. Relative efficacy of minoxidil and the 5-alpha reductase inhibitors in androgenetic alopecia treatment of male patients: a network meta-analysis. *JAMA Dermatol*. (2022) 158:266–74. doi: 10.1001/jamadermatol.2021.5743
26. You Q, Li L, Ma X, Gao T, Xiong S, Yan Y, et al. Meta-analysis on the efficacy and safety of traditional chinese medicine as adjuvant therapy for refractory androgenetic alopecia. *Evid Based Complement Alternat Med*. (2019) 2019:9274148. doi: 10.1155/2019/9274148
27. Zhou Y, Chen C, Qu Q, Zhang C, Wang J, Fan Z, et al. The effectiveness of combination therapies for androgenetic alopecia: a systematic review and meta-analysis. *Dermatol Ther*. (2020) 33:e13741. doi: 10.1111/dth.13741
28. Zhou Z, Song S, Gao Z, Wu J, Ma J, Cui Y. The efficacy and safety of dutasteride compared with finasteride in treating men with androgenetic alopecia: a systematic review and meta-analysis. *Clin Interv Aging*. (2019) 14:399–406. doi: 10.2147/CIA.S192435
29. Schünemann H, Brožek J, Guyatt G, Oxman A. *Handbook for grading the quality of evidence and the strength of recommendations using the GRADE approach*. (2013). Available online at: <https://gdt.gradepro.org/app/handbook/handbook.html> (accessed August 30, 2021).

30. Higgins JT, Chandler J, Cumpston M, Li T, Page M, Welch V editors. *Cochrane Handbook for Systematic Reviews of Interventions version 6.3 (updated February 2022)*. London: Cochrane (2022).
31. Schwarzer R, Leppin A. *Sozialer Rückhalt und Gesundheit: eine Meta-Analyse*. Göttingen: Verlag für Psychologie (1989).
32. Beliveau A, Boyne D, Slater J, Brenner D, Arora P. BUGSnet: an R package to facilitate the conduct and reporting of bayesian network meta-analyses. *BMC Med Res Methodol*. (2019) 19:196. doi: 10.1186/s12874-019-0829-2
33. Nikolakopoulou A, Higgins JPT, Papakonstantinou T, Chaimani A, Del Giovane C, Egger M, Salanti G, et al. CINeMA: an approach for assessing confidence in the results of a network meta-analysis. *PLoS Med*. (2020) 17:e1003082. doi: 10.1371/journal.pmed.1003082
34. Salanti G, Ades A, Ioannidis J. Graphical methods and numerical summaries for presenting results from multiple-treatment meta-analysis: an overview and tutorial. *J Clin Epidemiol*. (2011) 64:163–71. doi: 10.1016/j.jclinepi.2010.03.016
35. Papakonstantinou T, Nikolakopoulou A, Egger M, Salanti G. In network meta-analysis, most of the information comes from indirect evidence: empirical study. *J Clin Epidemiol*. (2020) 124:42–9. doi: 10.1016/j.jclinepi.2020.04.009
36. RStudio Team. *RStudio: integrated development for R*. Boston, MA: RStudio, Inc (2019).
37. Seo M, Furukawa TA, Veroniki AA, Pillinger T, Tomlinson A, Salanti G, et al. The Kilim plot: a tool for visualizing network meta-analysis results for multiple outcomes. *Res Synth Methods*. (2021) 12:86–95. doi: 10.1002/jrsm.1428
38. Papakonstantinou T, Nikolakopoulou A, Rücker G, Chaimani A, Schwarzer G, Egger M, et al. Estimating the contribution of studies in network meta-analysis: paths, flows and streams. *F1000Res*. (2018) 7:610. doi: 10.12688/f1000research.14770.3
39. Olsen E. Topical minoxidil in the treatment of androgenetic alopecia in women. *Cutis*. (1991) 48:243–6.
40. Whiting D, Jacobson C. Treatment of female androgenetic alopecia with minoxidil 2%. *Int J Dermatol*. (1992) 31:800–4. doi: 10.1111/j.1365-4362.1992.tb04251.x
41. Jacobs J, Szpunar C, Warner M. Use of topical minoxidil therapy for androgenetic alopecia in women. *Int J Dermatol*. (1993) 32:758–62. doi: 10.1111/j.1365-4362.1993.tb02755.x
42. DeVillez R, Jacobs J, Szpunar C, Warner M. Androgenetic alopecia in the female: treatment with 2% topical minoxidil solution. *Arch Dermatol*. (1994) 130:303–7. doi: 10.1001/archderm.130.3.303
43. Lucky AW, Piacquadro DJ, Ditre CM, Dunlap F, Kantor I, Pandya AG, et al. A randomized, placebo-controlled trial of 5% and 2% topical minoxidil solutions in the treatment of female pattern hair loss. *J Am Acad Dermatol*. (2004) 50:541–53. doi: 10.1016/j.jaad.2003.06.014
44. Bergfeld W, Washenik K, Callender V, Zhang P, Quiza C, Doshi U, et al. A phase III, multicenter, parallel-design clinical trial to compare the efficacy and safety of 5% minoxidil foam versus vehicle in women with female pattern hair loss. *J Drugs Dermatol*. (2016) 15:874–81.
45. Ablon G, Kogan S. A six-month, randomized, double-blind, placebo-controlled study evaluating the safety and efficacy of a nutraceutical supplement for promoting hair growth in women with self-perceived thinning hair. *J Drugs Dermatol*. (2018) 17:558–65.
46. Ablon G, Kogan S. A Randomized, double-blind, placebo-controlled study of a nutraceutical supplement for promoting hair growth in perimenopausal, menopausal, and postmenopausal women with thinning hair. *J Drugs Dermatol*. (2021) 20:55–61. doi: 10.36849/JDD.5701
47. Jimenez JJ, Wikramanayake TC, Bergfeld W, Hordinsky M, Hickman JG, Hamblin MR, et al. Efficacy and safety of a low-level laser device in the treatment of male and female pattern hair loss: a multicenter, randomized, sham device-controlled, double-blind study. *Am J Clin Dermatol*. (2014) 15:115–27. doi: 10.1007/s40257-013-0060-6
48. Gubelin Harcha W, Martínez JB, Tsai T, Katsuoka K, Kawashima M, Tsuboi R, et al. A randomized, active- and placebo-controlled study of the efficacy and safety of different doses of dutasteride versus placebo and finasteride in the treatment of male subjects with androgenetic alopecia. *J Am Acad Dermatol*. (2014) 70:489–98. doi: 10.1016/j.jaad.2013.10.049
49. Leavitt M, Charles G, Heyman E, Michaels D. HairMax lasercomb laser phototherapy device in the treatment of male androgenetic alopecia: a randomized, double-blind, sham device-controlled, multicentre trial. *Clin Drug Invest*. (2009) 29:283–92. doi: 10.2165/00044011-200929050-00001
50. Olsen EA, Dunlap FE, Funicella T, Koperski JA, Swinehart JM, Tschen EH, et al. A randomized clinical trial of 5% topical minoxidil versus 2% topical minoxidil and placebo in the treatment of androgenetic alopecia in men. *J Am Acad Dermatol*. (2002) 47:377–85. doi: 10.1067/mjd.2002.124088
51. Pumthong G, Asawanonda P, Varothai S, Jariyasethavong V, Triwongwanat D, Suthipinittharm P, et al. Curcuma aeruginosa, a novel botanically derived 5 α -reductase inhibitor in the treatment of male-pattern baldness: a multicenter, randomized, double-blind, placebo-controlled study. *J Dermatol Treat*. (2012) 23:385–92. doi: 10.3109/09546634.2011.568470
52. Hillmann K, Garcia Bartels N, Kottner J, Stroux A, Canfield D, Blume-Peytavi UA. Single-centre, randomized, double-blind, placebo-controlled clinical trial to investigate the efficacy and safety of minoxidil topical foam in frontotemporal and vertex androgenetic alopecia in men. *Skin Pharmacol Physiol*. (2015) 28:236–44. doi: 10.1159/000375320
53. Ablon G. A 6-month, randomized, double-blind, placebo-controlled study evaluating the ability of a marine complex supplement to promote hair growth in men with thinning hair. *J Cosmet Dermatol*. (2016) 15:358–66. doi: 10.1111/jocd.12265
54. Shea BJ, Reeves BC, Wells G, Thuku M, Hamel C, Moran J, et al. AMSTAR 2: a critical appraisal tool for systematic reviews that include randomised or non-randomised studies of healthcare interventions, or both. *BMJ*. (2017) 358:j4008. doi: 10.1136/bmj.j4008
55. Whiting P, Savovi JAE, Higgins JP, Caldwell DM, Reeves BC, Shea B, et al. ROBIS: a new tool to assess risk of bias in systematic reviews was developed. *J Clin Epidemiol*. (2016) 69:225–34. doi: 10.1016/j.jclinepi.2015.06.005
56. Bokhari L, Jones L, Sinclair R. Sublingual minoxidil for the treatment of male and female pattern hair loss: a randomized, double-blind, placebo-controlled, phase 1B clinical trial. *J Eur Acad Dermatol Venereol*. (2022) 36:e62–6. doi: 10.1111/jdv.17623
57. Abidin R, Zhang Y, Jimenez J. Treatment of androgenetic alopecia using PRP to target dysregulated mechanisms and pathways. *Front Med*. (2022) 9:843127. doi: 10.3389/fmed.2022.843127
58. Gentile P. The new regenerative and innovative strategies in hair loss. *EClinicalMedicine*. (2021) 37:100995. doi: 10.1016/j.eclinm.2021.100995
59. Feldman P, Feldman O, Guevara-Aguirre J, Fiebig K. Sex differences in clinical trials of ALRV5XR treatment of androgenetic alopecia and telogen effluvium. *Front Med*. (2022) 9:918058. doi: 10.3389/fmed.2022.918058
60. Ankawa R, Fuchs Y. May the best wound WIHN: the hallmarks of wound-induced hair neogenesis. *Curr Opin Genet Dev*. (2022) 72:53–60. doi: 10.1016/j.gde.2021.10.006

Supplementary Materials

Appendix 1 – Supplementary Tables and Figures

Hair Regrowth Treatment Efficacy and Resistance in Androgenetic Alopecia: A Systematic Review and Continuous Bayesian Network Meta-Analysis

Peter R. Feldman^{1,2*}, Pietro Gentile³, Charles Piwko⁴, Hendrik M. Motswaledi⁵, Samantha Gorun^{6,7}, Jacob Pesachov⁸, Michael Markel⁸, Maxwell I. Silver^{9,10}, Megan Brenkel¹¹, Oriel J. Feldman^{1,12}, Corey L. Kamen⁸, Elizabeth Uleryk¹³, Jaime Guevara-Aguirre^{14,15,16,17}, Klaus M. Fiebig¹

¹ Arbor Life Labs, Toronto, ON, Canada

² Norwich Medical School, University of East Anglia, Norwich, United Kingdom

³ Surgical Science Department, University of Rome Tor Vergata, Rome, Italy

⁴ CHP Pharma Inc., Thornhill, ON, Canada

⁵ Department of Dermatology, Sefako Makgatho Health Sciences University, South Africa

⁶ Faculty of Epidemiology & Biostatistics, Western University, ON, Canada

⁷ School of Mathematics and Statistics, University of Glasgow, Glasgow, United Kingdom

⁸ Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel

⁹ Faculty of Dentistry, University of Toronto, Toronto, ON, Canada

¹⁰ Faculty of Medicine and Dentistry, University of Alberta, Edmonton, AB, Canada

¹¹ Faculty of Medicine, University of Ottawa, Ottawa, ON, Canada

¹² Faculty of Science, Wilfrid Laurier University, Waterloo, ON, Canada

¹³ Uleryk Consulting, Mississauga, ON, Canada

¹⁴ Universidad San Francisco de Quito (USFQ), Quito, Ecuador

¹⁵ Faculty of Health, Medicine and Life Sciences, Maastricht University, Maastricht, Netherlands

¹⁶ Institute of Endocrinology, Metabolism, and Reproduction (IEMR), Quito, Ecuador

¹⁷ College of Medicine, University of Florida, Gainesville, FL, USA

* Correspondence:

Corresponding Author: Peter R Feldman

petefeldman@gmail.com

Keywords: hair loss, ALRV5XR, Dutasteride, Finasteride, LLLT, Minoxidil, Nutrafol, Viviscal

Submission Date: July 20, 2022

Revised: September 12, 2022

Accepted: December 7, 2022

Published: January 23, 2023

CITATION:

Feldman PR, Gentile P, Piwko C, Motswaledi H, Gorun S, Pesachov J, Markel M, Silver MI, Brenkel M, Feldman OJ, Kamen CL, Uleryk E, Guevara-Aguirre J and Fiebig KM (2023) Hair regrowth treatment efficacy and resistance in androgenetic alopecia: A systematic review and Bayesian network meta-analysis. *Front. Med.* 9:998623. doi: 10.3389/fmed.2022.998623

© 2023 Feldman, Gentile, Piwko, Motswaledi, Gorun, Pesachov, Markel, Silver, Brenkel, Feldman, Kamen, Uleryk, Guevara-Aguirre and Fiebig. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

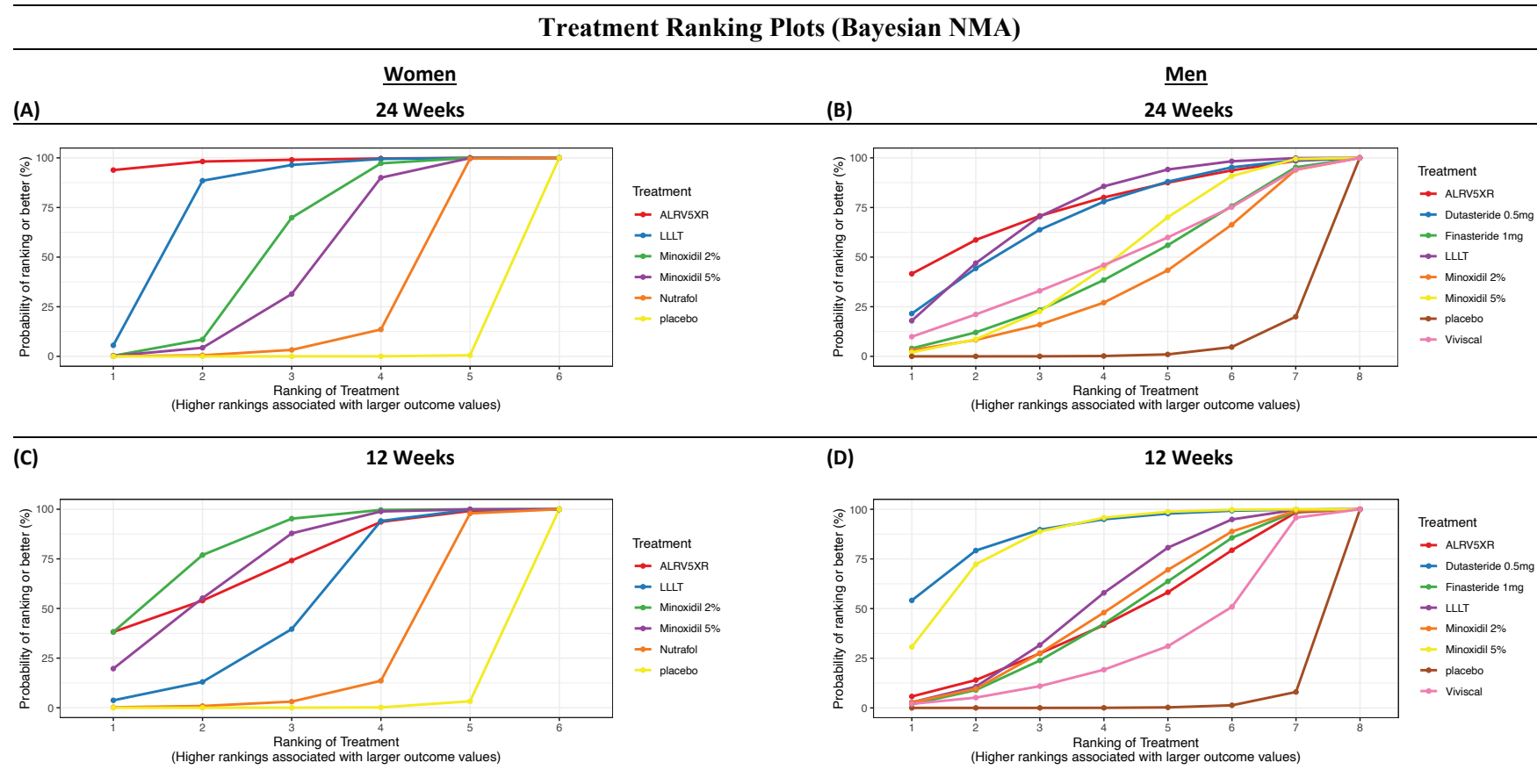
*Hair Regrowth Treatment Efficacy and Resistance in Androgenetic Alopecia:
A Systematic Review and Continuous Bayesian Network Meta-Analysis*

Table of Contents

	<u>Page</u>
Section 1: Additional Bayesian NMA Results	3
Figure S1-1: Treatment Ranking Plots (Frequentist NMA).....	3
Table S1-1: Bayesian NMA Sensitivity Test of Various Simulations in Men at 24 Weeks.....	4
Section 2: Additional Frequentist NMA Results	5
Figure S1-2: Frequentist Network Meta-Analysis Plots of Treatment Efficacy in Androgenetic Alopecia	5
Figure S1-3: Frequentist Relative Effects between Treatments	6
Figure S1-4: Treatment Ranking Plots (Frequentist NMA).....	8
Table S1-2: Statistical Comparison of differences in efficacy between the same treatment in Women and Men.....	9
Table S1-3: Comparison of Bayesian and Frequentist Credible and Confidence Intervals.....	9
Section 3: Confidence in Network Meta-Analysis and Risk of Bias Results	10
Figure S1-5: CiNeMa Network Plots and Risk of Bias between Treatments	10
Table S1-4: CiNeMA Results	11
Figure S1-6: Cochrane Risk of Bias.....	12
Table S1-5: Study Conflicts of Interest	13
Section 4: Continuous Outcomes Data and Additional Results.....	14
Table S1-6: Extracted Continuous Outcomes Data and Weekly imputed results in terminal hair regrowth in TH/ cm2 of AGA treatments in women by week	15
Table S1-7: Imputed results in terminal hair regrowth of AGA treatments in women by week in TH/ cm2	15
Table S1-8: Imputed results in terminal hair regrowth of AGA treatments in women by week in Percent (%).....	16
Figure S1-6: Continuous Outcomes of regrowth of AGA treatments in women by week	16
Figure S1-7: Continuous Outcomes in Women for Treatments with Multiple Studies	17
Table S1-9: Extracted Continuous Outcomes Data and Weekly imputed results in terminal hair regrowth per cm2 of AGA treatments in men by week	18
Table S1-10: Imputed results in terminal hair regrowth of AGA treatments in men by week per cm ²	19
Figure S1-8: Continuous Outcomes of regrowth of AGA treatments in men by week	20
Table S1-11: Imputed results in terminal hair regrowth of AGA treatments in men by week in Percent (%).....	20
Figure S1-9: Continuous in Men for Treatments with Multiple Studies.....	21
Section 5: Meta-Analysis Results.....	22
Figure S1-10: Meta-Analysis Forest Plot for Women after 24 weeks of treatment.....	22
Figure S1-11: Meta-Analysis Forest Plot for Women after 12 weeks of treatment.....	23
Figure S1-12: Meta-Analysis Forest Plot for Men after 24 weeks of treatment	24
Figure S1-13: Meta-Analysis Forest Plot for Men after 12 weeks of treatment	25
Figure S1-14: Funnel Plots.....	26
Section 6: Safety Results.....	28
Table S1-12: Summary of Adverse Events (AEs) from Eligible Studies	28

Section 1: Additional Bayesian NMA Results

Figure S1-1: Treatment Ranking Plots (Bayesian NMA)



*Hair Regrowth Treatment Efficacy and Resistance in Androgenetic Alopecia:
A Systematic Review and Continuous Bayesian Network Meta-Analysis*

Table S1-1. Bayesian NMA Sensitivity Test of Various Simulations in Men at 24 Weeks

Sensitivity tests of the Men's Bayesian NMA evaluated the effect of simulations on MD and CrIs to look for stability or other effects that might have caused Finasteride 1mg and Minoxidil 2% to lose significance. The results showed very low variability across a wide range of simulations. Treatments with a lower CrI less than that of placebo, were found not significantly different from placebo for the Bayesian NMA.

No. Simulations	Treatment	Comparator	MD	Lower CrI	Upper CrI	CrI Width
50k	ALRV5XR	placebo	20.99	1.13	40.85	39.72
150k	ALRV5XR	placebo	21.03	1.25	40.87	39.62
250k	ALRV5XR	placebo	21.03	1.15	40.94	39.79
300k	ALRV5XR	placebo	21.02	1.13	40.94	39.81
500k	ALRV5XR	placebo	21.00	1.14	40.90	39.76
50k	Dutasteride 0.5mg	placebo	18.40	0.89	35.83	34.94
150k	Dutasteride 0.5mg	placebo	18.37	0.86	35.85	34.99
250k	Dutasteride 0.5mg	placebo	18.37	0.85	35.84	34.99
300k	Dutasteride 0.5mg	placebo	18.37	0.81	35.88	35.07
500k	Dutasteride 0.5mg	placebo	18.38	0.83	35.86	35.04
50k	Finasteride 1mg	placebo	12.40	-5.35	29.99	35.34
150k	Finasteride 1mg	placebo	12.40	-5.14	29.87	35.00
250k	Finasteride 1mg	placebo	12.38	-5.18	29.79	34.98
300k	Finasteride 1mg	placebo	12.37	-5.25	29.83	35.08
500k	Finasteride 1mg	placebo	12.38	-5.19	29.86	35.05
50k	LLLT	placebo	18.78	8.14	28.83	20.70
150k	LLLT	placebo	18.75	8.19	28.80	20.62
250k	LLLT	placebo	18.75	8.16	28.83	20.67
300k	LLLT	placebo	18.75	8.15	28.87	20.72
500k	LLLT	placebo	18.76	8.18	28.87	20.69
50k	Minoxidil 2%	placebo	10.54	-5.77	26.38	32.15
150k	Minoxidil 2%	placebo	10.55	-5.81	26.29	32.10
250k	Minoxidil 2%	placebo	10.54	-5.81	26.32	32.12
300k	Minoxidil 2%	placebo	10.54	-5.82	26.35	32.17
500k	Minoxidil 2%	placebo	10.54	-5.81	26.31	32.12
50k	Minoxidil 5%	placebo	13.12	2.40	22.43	20.03
150k	Minoxidil 5%	placebo	13.14	2.40	22.42	20.02
250k	Minoxidil 5%	placebo	13.13	2.39	22.40	20.01
300k	Minoxidil 5%	placebo	13.13	2.38	22.39	20.01
500k	Minoxidil 5%	placebo	13.13	2.40	22.39	19.99
50k	placebo	placebo	0.00	0.00	0.00	0.00
150k	placebo	placebo	0.00	0.00	0.00	0.00
250k	placebo	placebo	0.00	0.00	0.00	0.00
300k	placebo	placebo	0.00	0.00	0.00	0.00
500k	placebo	placebo	0.00	0.00	0.00	0.00
50k	Viviscal	placebo	13.24	-5.87	32.28	38.15
150k	Viviscal	placebo	13.22	-5.84	32.27	38.11
250k	Viviscal	placebo	13.23	-5.90	32.29	38.19
300k	Viviscal	placebo	13.24	-5.91	32.32	38.24
500k	Viviscal	placebo	13.23	-5.87	32.31	38.18

Appendix 1 – Supplementary Tables and Figures

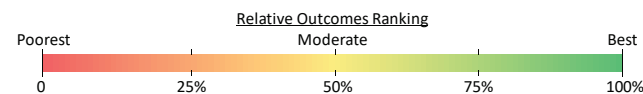
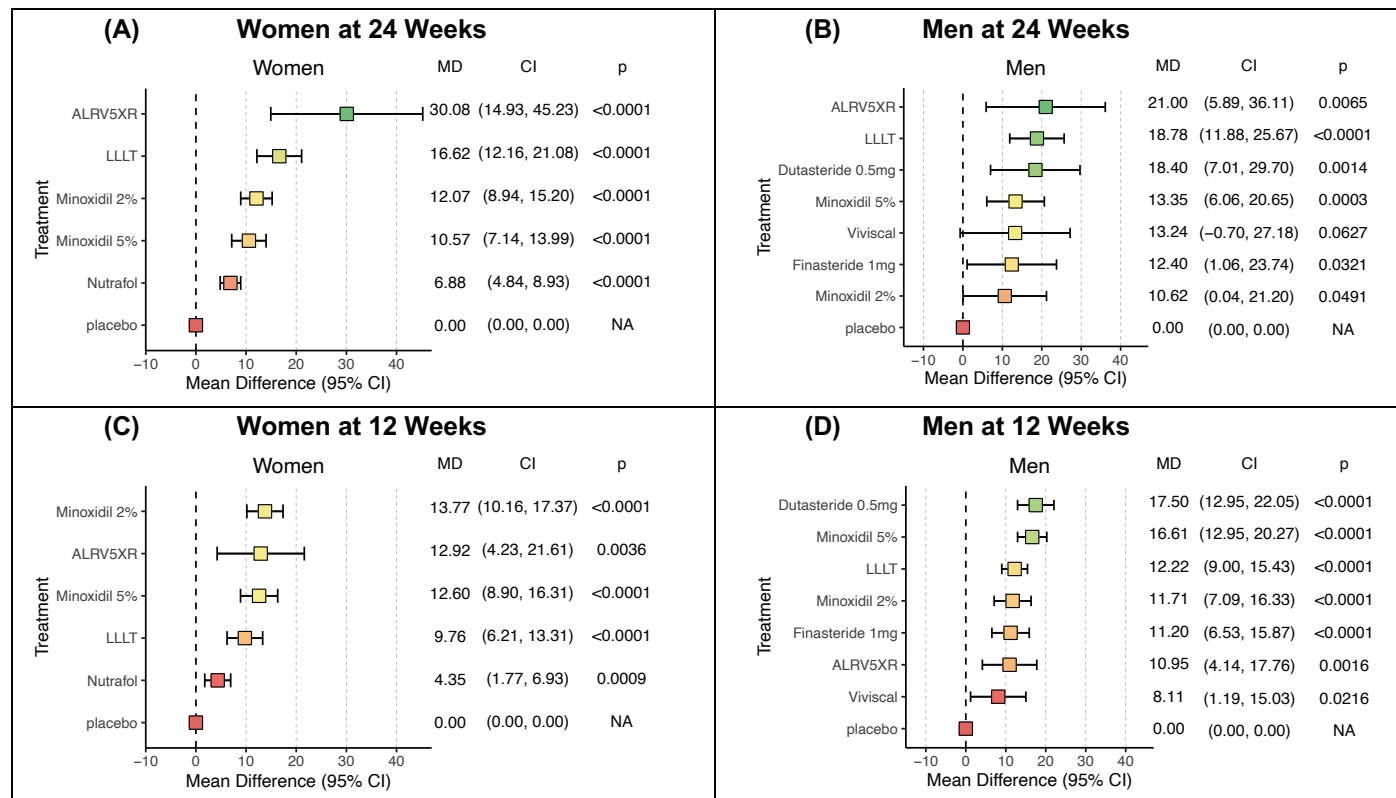
Front. Med. 9:998623. doi: 10.3389/fmed.2022.998623

4

Section 2: Additional Frequentist NMA Results

Figure S1-2. Frequentist Network Meta-Analysis Plots of Treatment Efficacy in Androgenetic Alopecia. Efficacy is measured as Mean Differences (MD) of changes from baseline of direct and indirect treatment comparisons vs. placebo in TH/cm². Plots for women are 24 weeks (A) and 12 weeks (C) and men are 24 weeks (B) and 12 weeks (D). Results are ranked in descending order of MD with 95% confidence intervals (CI). Statistical significance is $p < 0.0500$. The colors of treatment MD's correspond with the heat map colors of frequentist NMA results in Table 2.

Frequentist NMA Forest Plots of Treatment Efficacy in Androgenetic Alopecia



*Hair Regrowth Treatment Efficacy and Resistance in Androgenetic Alopecia:
A Systematic Review and Continuous Bayesian Network Meta-Analysis*

Figure S1-3. Frequentist Relative Effects between Treatments.

Frequentist League Tables Showing Relative Effects between Treatments after Network Meta-Analysis of changes in terminal hair per cm² in women at 24 weeks (A) and 12 weeks (B) and men 24 weeks (C) and 12 weeks (D) with AGA. These results show mean differences (MD) in regrowth of terminal hairs per cm² (95% Confidence Intervals) between each treatment at 12 and 24 weeks with significant differences underlined. League tables are ranked based on 24 week results with highest efficacy treatment from left – right and top to bottom. Differences between the treatments are rows vs. columns. At 12 and 24 weeks, all treatments were significantly greater than placebo with the exception of Viviscal at 24 weeks in men, and Minoxidil 2% in men at 24 weeks was borderline significant. At 24 weeks, In women at 24 weeks, ALRV5XR was significantly different from Minoxidil 2% and 5%, and Nutrafol. Notably, at 12 weeks in men, Dutasteride was significantly different to Finasteride and Minoxidil 5% was significantly different to Minoxidil 2%. There was no drug vs. drug significance after 12 weeks.

Frequentist Relative Effects between Treatments

Women

(A)

24 Weeks

ALRV5XR	LLLT	Minoxidil 2%	Minoxidil 5%	Nutrafol	placebo
ALRV5XR	13.46 (-2.33, 29.25) z = 1.67 p = 0.0948	18.01 (2.54, 33.48) z = 2.28 p = 0.0225	19.51 (3.99, 35.04) z = 2.46 p = 0.0138	23.20 (7.91, 38.48) z = 2.97 p = 0.0029	30.08 (14.93, 45.23) z = 3.89 p < 0.0001
	LLLT	4.55 (-0.90, 10.00) z = 1.64 p = 0.1016	6.05 (0.43, 11.68) z = 2.11 p = 0.0349	9.74 (4.83, 14.64) z = 3.89 p = 0.0001	16.62 (12.16, 21.08) z = 7.30 p < 0.0001
		Minoxidil 2%	1.51 (-2.61, 5.62) z = 0.72 p = 0.4735	5.19 (1.45, 8.92) z = 2.72 p = 0.0065	12.07 (8.94, 15.20) z = 7.57 p < 0.0001
			Minoxidil 5%	3.68 (-0.31, 7.67) z = 1.81 p = 0.0708	10.57 (7.14, 13.99) z = 6.04 p < 0.0001
				Nutrafol	6.88 (4.84, 8.93) z = 6.59 p < 0.0001

Legend:

MD: Mean Difference in TH/cm²

95% CI: 95% Confidence Interval

P-Value: Significance <0.05 (when MD underlined)

Z-Score: Significance >1.96 (when MD underlined)

(B)

12 Weeks

ALRV5XR	LLLT	Minoxidil 2%	Minoxidil 5%	Nutrafol	placebo
ALRV5XR	3.16 (-6.23, 12.55) z = 0.66 p = 0.5092	-0.85 (-10.25, 8.56) z = -0.18 p = 0.8601	0.32 (-9.13, 9.76) z = 0.07 p = 0.9476	8.57 (-0.40, 17.63) z = 1.85 p = 0.0639	12.92 (4.23, 21.61) z = 2.91 p = 0.0036
	LLLT	-4.01 (-9.07, 1.05) z = -1.55 p = 0.1207	-2.84 (-7.98, 2.29) z = -1.09 p = 0.2771	5.41 (1.02, 9.80) z = 2.41 p = 0.0158	9.76 (6.21, 13.31) z = 5.38 p < 0.0001
		Minoxidil 2%	1.16 (-3.29, 5.62) z = 0.51 p = 0.6089	9.41 (4.98, 13.85) z = 4.16 p < 0.0001	13.77 (10.16, 17.37) z = 7.48 p < 0.0001
			Minoxidil 5%	8.25 (3.74, 12.76) z = 3.59 p = 0.0003	12.60 (8.90, 16.31) z = 6.67 p < 0.0001
				Nutrafol	4.35 (1.77, 6.93) z = 3.31 p = 0.0009

*Hair Regrowth Treatment Efficacy and Resistance in Androgenetic Alopecia:
A Systematic Review and Continuous Bayesian Network Meta-Analysis*

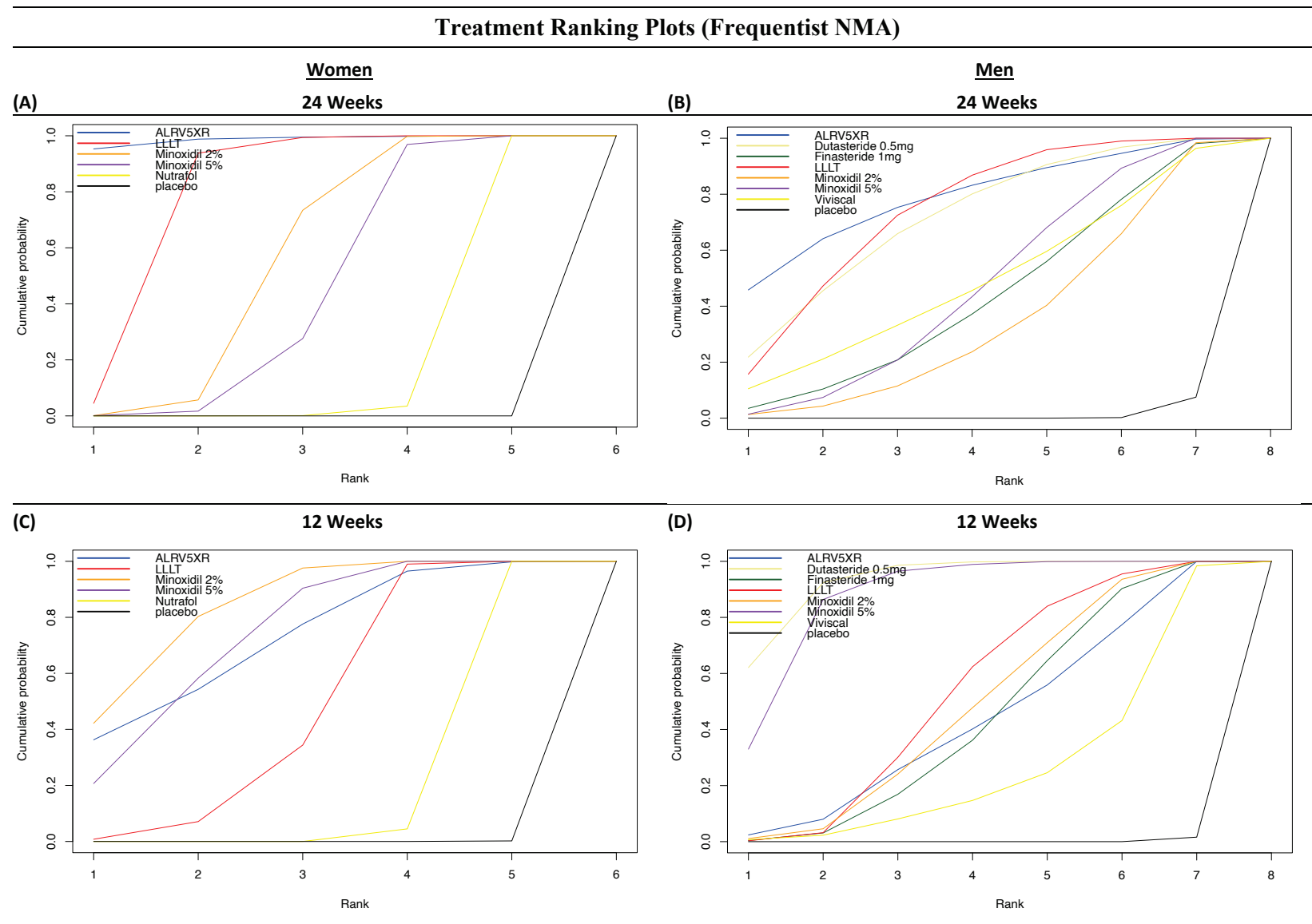
Figure S1-3. Frequentist Relative Effects between Treatments. (Cont.)

Relative Effects between Treatments							
Men							
24 Weeks							
ALRV5XR	LLLT	Dutasteride 0.5mg	Minoxidil 5%	Viviscal	Finasteride 1mg	Minoxidil 2%	placebo
ALRV5XR	2.22 (-14.38, 18.83) z = 0.26 p = 0.7930	2.60 (-16.27, 21.47) p = 0.7871 z = 0.27	7.65 (-9.13, 24.43) z = 0.89 p = 0.3717	7.76 (-12.80, 28.32) z = 0.74 p = 0.4594	8.60 (-10.29, 27.49) z = 0.89 p = 0.3723	10.38 (-8.07, 28.82) z = 1.10 p = 0.2701	21.00 (5.89, 36.11) z = 2.72 p = 0.0065
	LLLT	0.38 (-12.86, 13.62) z = 0.06 p = 0.9556	5.42 (-4.61, 15.46) z = 1.06 p = 0.2896	5.54 (-10.02, 21.09) z = 0.70 p = 0.4854	6.38 (-6.90, 19.65) z = 0.94 p = 0.3464	8.15 (-4.47, 20.78) z = 1.27 p = 0.2055	18.78 (11.88, 25.67) z = 5.34 p < 0.0001
		Dutasteride 0.5mg	5.05 (-8.41, 18.50) z = 0.74 p = 0.4621	5.16 (-12.79, 23.11) z = 0.56 p = 0.5731	6.00 (-5.34, 17.34) z = 1.04 p = 0.2997	7.78 (-7.70, 23.26) z = 0.98 p = 0.3247	18.40 (7.10, 29.70) z = 3.19 p = 0.0014
			Minoxidil 5%	0.11 (-15.62, 15.84) z = 0.01 p = 0.9889	0.95 (-12.53, 14.44) z = 0.14 p = 0.8899	2.73 (-7.66, 13.12) z = 0.52 p = 0.6063	13.35 (6.06, 20.65) z = 3.59 p = 0.0003
				Viviscal	0.84 (-17.13, 18.81) z = 0.09 p = 0.9270	2.62 (-14.88, 20.12) z = 0.29 p = 0.7692	13.24 (-0.70, 27.18) z = 1.86 p = 0.0627
					Finasteride 1mg	1.78 (-13.73, 17.29) z = 0.22 p = 0.8221	12.40 (1.06, 23.74) z = 2.14 p = 0.0321
						Minoxidil 2%	10.62 (0.04, 21.20) z = 1.97 p = 0.0491
Legend:							
MD: Mean Difference in TH/cm ²							
95% CI: 95% Confidence Interval							
P-Value: Significance <0.05 (when MD underlined)							
Z-Score: Significance >1.96 (when MD underlined)							

12 Weeks							
ALRV5XR	LLLT	Dutasteride 0.5mg	Minoxidil 5%	Viviscal	Finasteride 1mg	Minoxidil 2%	placebo
ALRV5XR	-1.27 (-8.80, 6.26) z = -0.33 p = 0.7413	-6.55 (-14.74, 1.64) z = -1.57 p = 0.1169	-5.66 (-13.39, 2.07) z = -1.43 p = 0.1514	2.84 (-6.87, 12.55) z = 0.57 p = 0.5665	-0.25 (-8.51, 8.01) z = -0.06 p = 0.9527	-0.76 (-8.99, 7.47) z = -0.18 p = 0.8562	10.95 (4.14, 17.76) z = 3.15 p = 0.0016
	LLLT	-5.28 (-10.85, 0.28) z = -1.86 p = 0.0629	-4.39 (-9.26, 0.48) z = -1.77 p = 0.0772	4.11 (-3.52, 11.74) z = 1.05 p = 0.2914	1.02 (-4.65, 6.69) z = 0.35 p = 0.7249	0.51 (-5.12, 6.14) z = 0.18 p = 0.8599	12.22 (9.00, 15.43) z = 7.45 p < 0.0001
		Dutasteride 0.5mg	0.89 (-4.94, 6.73) z = 0.30 p = 0.7645	9.39 (1.11, 17.67) z = 2.22 p = 0.0262	6.30 (1.60, 11.00) z = 2.63 p = 0.0086	5.79 (-0.69, 12.27) z = 1.75 p = 0.0801	17.50 (12.95, 22.05) z = 7.55 p < 0.0001
			Minoxidil 5%	8.50 (0.67, 16.33) z = 2.13 p = 0.0334	5.41 (-0.53, 11.34) z = 1.79 p = 0.0741	4.90 (0.77, 9.02) z = 2.33 p = 0.0200	16.61 (12.95, 20.27) z = 8.90 p < 0.0001
				Viviscal	-3.09 (-11.44, 5.26) z = -0.73 p = 0.4684	-3.60 (-11.92, 4.72) z = -0.85 p = 0.3965	8.11 (1.19, 15.03) z = 2.30 p = 0.0216
					Finasteride 1mg	-0.51 (-7.08, 6.06) z = -0.15 p = 0.8788	11.20 (6.53, 15.87) z = 4.70 p < 0.0001
						Minoxidil 2%	11.71 (7.09, 16.33) z = 4.97 p < 0.0001

*Hair Regrowth Treatment Efficacy and Resistance in Androgenetic Alopecia:
A Systematic Review and Continuous Bayesian Network Meta-Analysis*

Figure S1-4: Treatment Ranking Plots (Frequentist NMA)



*Hair Regrowth Treatment Efficacy and Resistance in Androgenetic Alopecia:
A Systematic Review and Continuous Bayesian Network Meta-Analysis*

Table S1-2: Statistical Comparison of differences in efficacy between the same treatment in Women and Men

Comparison	Sex Difference	p-value	95% CI
ALRV5XR - M vs. ALRV5XR - W	-9.08	0.3746	(-29.13, 10.97)
LLLT - M vs. LLLT - F	2.73	0.4619	(-4.55, 10.01)
Minoxidil 2% - M vs. Minoxidil 2% - F	-1.08	0.7898	(-8.98, 6.83)
Minoxidil 5% - M vs. Minoxidil 5% - F	3.87	0.2928	(-3.34, 11.08)

Table S1-3: Comparison of Bayesian and Frequentist MD's, Credible and Confidence Intervals**(A) Women**

Sex	Treatment	Week	Analysis	MD	Difference	CI low	CI high	CI size	CI size difference	Z	P	Significant	SUCRA
Women	ALRV5XR	24	Bayesian NMA	30.09	0.03%	14.14	45.96	31.82	4.78%				98.2
Women	ALRV5XR	24	Freq NMA	30.08		14.93	45.23	30.3		3.89	<0.0001	yes	98.7
Women	LLLT	24	Bayesian NMA	16.62	0.00%	10.92	22.3	11.38	21.62%				78
Women	LLLT	24	Freq NMA	16.62		12.16	21.08	8.92		7.3	<0.0001	yes	79.6
Women	Minoxidil 2%	24	Bayesian NMA	12.13	0.49%	8.17	16.19	8.02	21.95%				55.2
Women	Minoxidil 2%	24	Freq NMA	12.07		8.94	15.2	6.26		7.57	<0.0001	yes	56
Women	Minoxidil 5%	24	Bayesian NMA	10.82	2.31%	6.14	15.84	9.7	29.38%				45.2
Women	Minoxidil 5%	24	Freq NMA	10.57		7.14	13.99	6.85		6.04	<0.0001	yes	44.9
Women	Nutrafol	24	Bayesian NMA	7.32	6.01%	3.38	11.96	8.58	52.33%				23.4
Women	Nutrafol	24	Freq NMA	6.88		4.84	8.93	4.09		6.59	<0.0001	yes	20.7

(B) Men

Sex	Treatment	Week	Analysis	MD	Difference	CI low	CI high	CI size	CI size difference	Z	P	Significant	SUCRA
Men	ALRV5XR	24	Bayesian NMA	21.03	0.14%	1.15	40.94	39.79	24.05%				75.8
Men	ALRV5XR	24	Freq NMA	21		5.89	36.11	30.22		2.74	0.0065	yes	78.2
Men	Dutasteride 0.5mg	24	Bayesian NMA	18.37	0.16%	0.85	35.84	34.99	35.15%				69.9
Men	Dutasteride 0.5mg	24	Freq NMA	18.4		7.01	29.7	22.69		3.21	0.0014	yes	71.4
Men	Finasteride 1mg	24	Bayesian NMA	12.38	0.16%	-5.18	29.79	34.97	35.14%				43.5
Men	Finasteride 1mg	24	Freq NMA	12.4		1.06	23.74	22.68		2.16	0.0321	yes	43.5
Men	LLLT	24	Bayesian NMA	18.75	0.16%	8.16	28.83	20.67	33.28%				73.3
Men	LLLT	24	Freq NMA	18.78		11.88	25.67	13.79		5.78	<0.0001	yes	75.1
Men	Minoxidil 2%	24	Bayesian NMA	10.54	0.76%	-5.81	26.32	32.13	34.14%				36.9
Men	Minoxidil 2%	24	Freq NMA	10.62		0.04	21.2	21.16		2	0.0491	yes	35.6
Men	Minoxidil 5%	24	Bayesian NMA	13.13	1.68%	2.39	22.4	20.01	27.09%				48.3
Men	Minoxidil 5%	24	Freq NMA	13.35		6.06	20.65	14.59		3.73	0.0003	yes	46.8
Men	Viviscal	24	Bayesian NMA	13.23	0.08%	-5.9	32.29	38.19	27.00%				48.5
Men	Viviscal	24	Freq NMA	13.24		-0.7	27.18	27.88		1.23	0.0627	no	48.2

Appendix 1 – Supplementary Tables and Figures

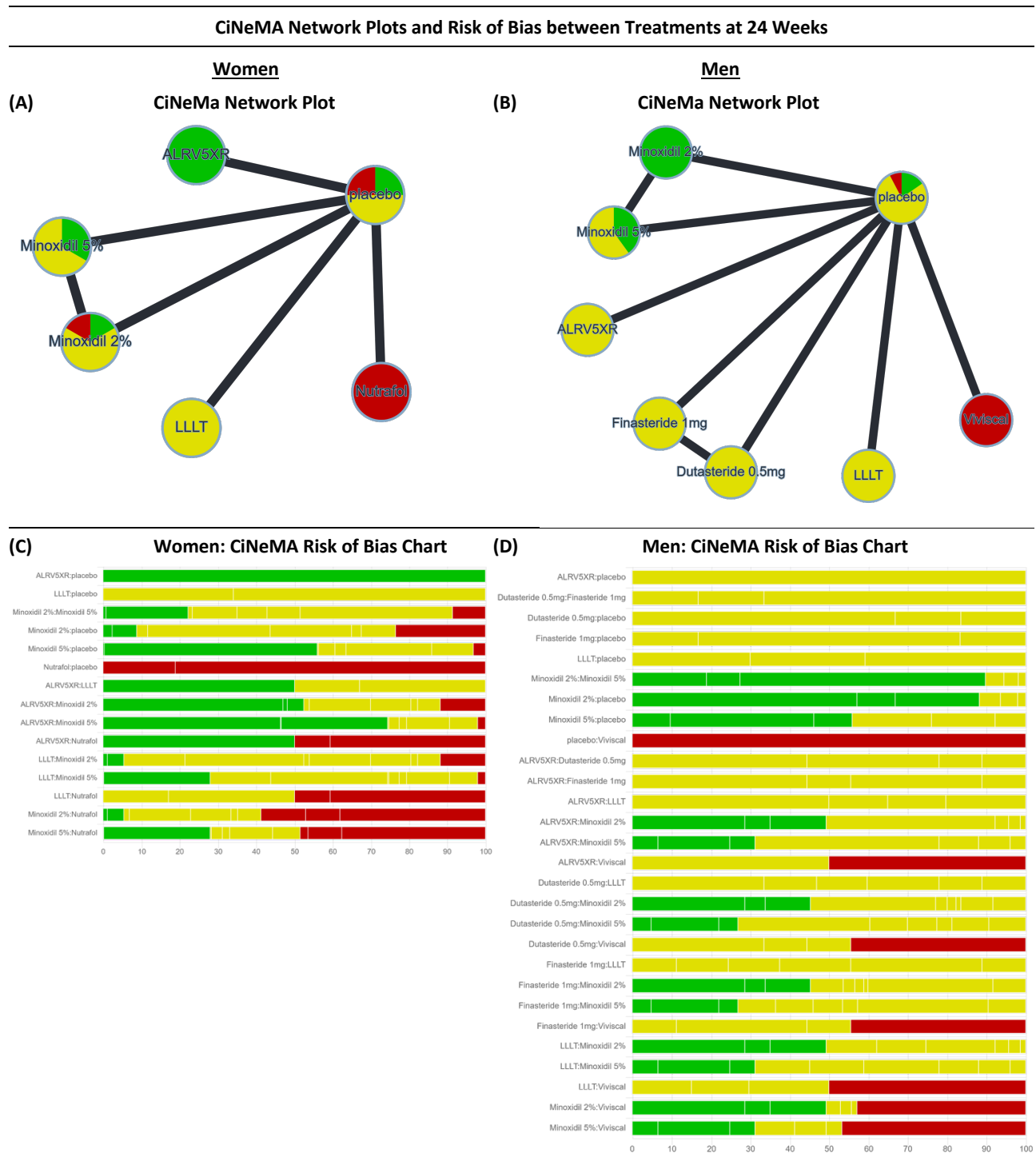
9

Front. Med. 9:998623. doi: 10.3389/fmed.2022.998623

*Hair Regrowth Treatment Efficacy and Resistance in Androgenetic Alopecia:
A Systematic Review and Continuous Bayesian Network Meta-Analysis*

Section 3: Confidence in Network Meta-Analysis and Risk of Bias Results

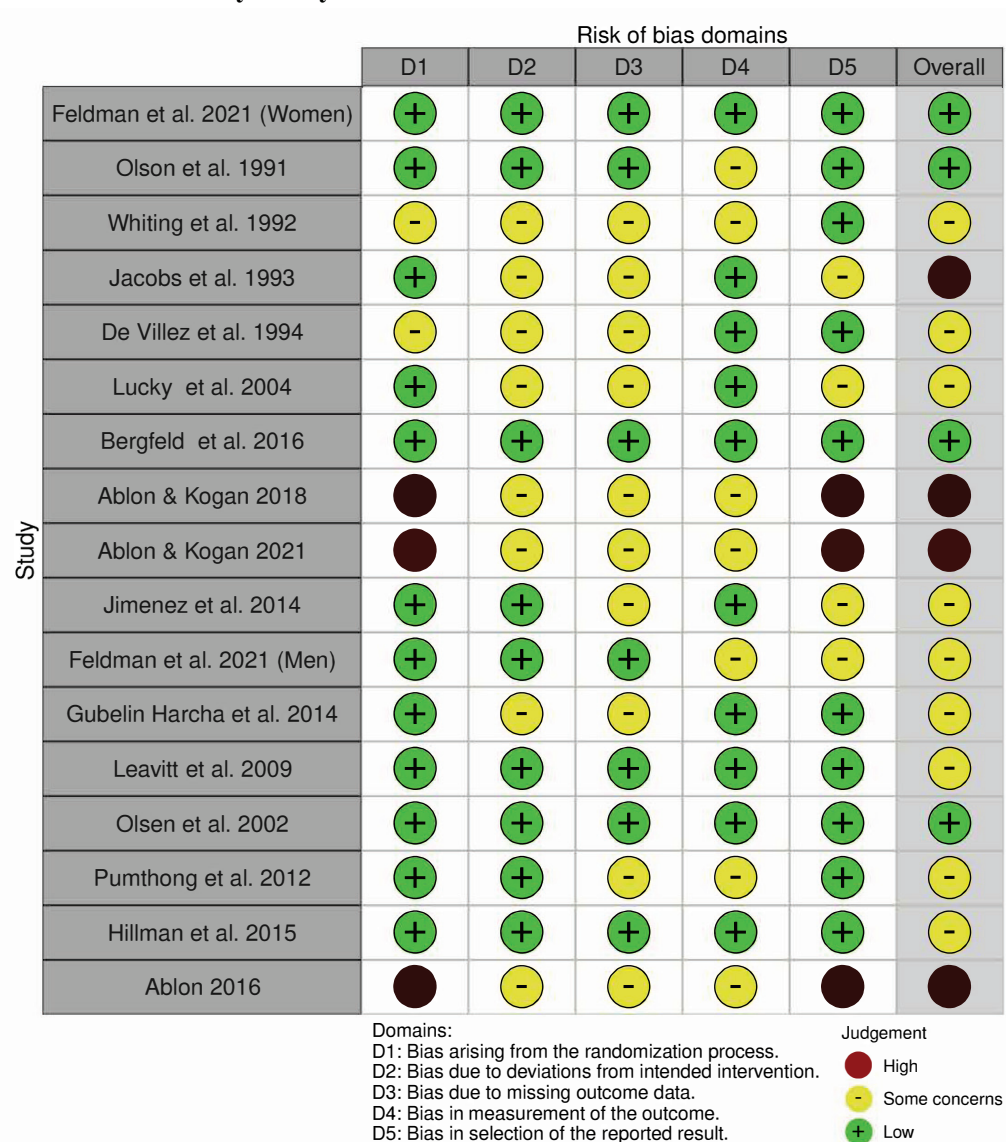
Figure S1-5: CiNeMa Network Plots and Risk of Bias between Treatments



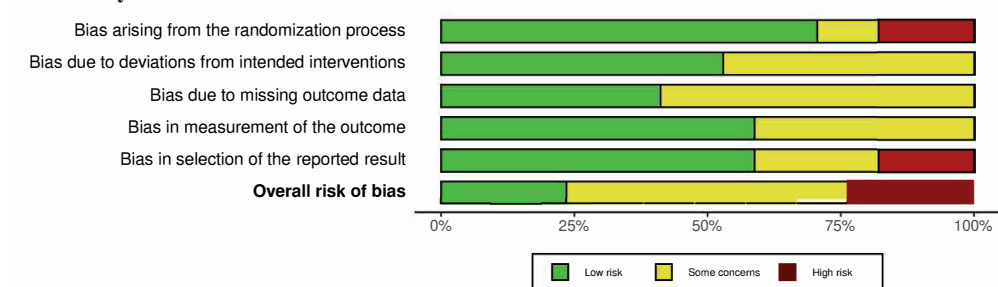
*Hair Regrowth Treatment Efficacy and Resistance in Androgenetic Alopecia:
A Systematic Review and Continuous Bayesian Network Meta-Analysis*

Figure S1-6: Cochrane Risk of Bias

(A) Cochrane Risk of Bias by Study



(B) Cochrane Summary Risk of Bias



*Hair Regrowth Treatment Efficacy and Resistance in Androgenetic Alopecia:
A Systematic Review and Continuous Bayesian Network Meta-Analysis*

Table S1-5: Study Conflicts of Interest

Author, Year	Sponsor / owner of investigated treatment	Treatment	Sponsor Brand	Sponsor Affiliated Authors	Sponsor Generated Data
Women					
Ablon et al, 2018 ⁴⁵	Unilever (Neutraceutical Wellness Inc.)	Nutrafol	Nutrafol	Yes	Unknown
Ablon et al, 2021 ⁴⁶	Unilever (Neutraceutical Wellness Inc.)	Nutrafol	Nutrafol	Yes	Unknown
Bergfeld et al, 2016 ⁴⁴	Johnson & Johnson Consumer Companies*	Minoxidil 5%	Rogaine	Yes	No
DeViliez et al, 1994 ⁴²	The Upjohn Company	Minoxidil 2%	Rogaine	Yes	Yes
Feldman et al, 2021 ¹²	Arbor Life Labs	ALRV5XR	Replenology	Yes	No
Jacobs et al, 1993 ⁴¹	The Upjohn Company	Minoxidil 2%	Rogaine	Yes	Yes
Jimenez et al, 2014 ^{49***}	Lexington International, LLC	LLLT (comb)	HairMax	No	No
Lucky et al, 2004 ⁴³	Pfizer Inc (formerly Pharmacia Corporation, formerly The Upjohn Company)	Minoxidil 2% & 5%	Rogaine	No	No
Olsen et al, 1991 ³⁹	The Upjohn Company	Minoxidil 2%	Rogaine	No	Unknown
Whiting et al, 1992 ⁴⁰	The Upjohn Company	Minoxidil 2%	Rogaine	No	Yes
Men					
Ablon, 2016 ⁵³	Church and Dwight (Lifes2good, Inc., Chicago, IL)	Viviscal	Viviscal	No	No
Feldman et al, 2021 ¹³	Arbor Life Labs	ALRV5XR	Replenology	Yes	No
Gubelin Harcha et al, 2014 ⁴⁸	GlaxoSmithKline	Dutasteride Finasteride	Avodart N/A (Generic)**	Yes	Yes
Hillmann et al, 2015 ⁵²	Johnson & Johnson Consumer Companies*	Minoxidil 5%	Rogaine	Yes	No
Jimenez et al, 2014 ^{47***}	Lexington International, LLC	LLLT (comb)	HairMax	No	No
Leavitt et al, 2009 ⁴⁹	Lexington International, LLC	LLLT (comb)	HairMax	Yes	Unknown
Olsen et al, 2002 ⁵⁰	Pharmacia Corporation (The Upjohn Corporation) *	Minoxidil 2% & 5%	Rogaine	Yes	Yes
Pumthong et al, 2012 ⁵¹	Unknown	Minoxidil 5%	Unknown	Unknown	Unknown

* Johnson & Johnson acquired the Rogaine brand in 2006.^{44, 52}

** Finasteride is also branded as Propecia and Proscar by Merck. Merck was not involved in this study.⁴⁸

*** This study is listed in both women and men groupings.⁴⁷

*Hair Regrowth Treatment Efficacy and Resistance in Androgenetic Alopecia:
A Systematic Review and Continuous Bayesian Network Meta-Analysis*

Section 4: Continuous Outcomes Data and Additional Results

Table S1-6: Extracted Continuous Outcomes Data and Weekly imputed results in terminal hair regrowth in TH/ cm² of AGA treatments in women by week

Extracted Data of Terminal Hair Regrowth from Eligible Studies											
Women				Placebo			Treatment			Efficacy	
#	Treatment	Study	Week	Population N	BL TH TH/cm ²	Change from BL TH/cm ²	Population N	BL TH TH/cm ²	Change from BL TH/cm ²	Absolute TH/cm ²	Relative %
1	ALRV5XR	Feldman et al. 2021	12	21	141.6	3.1	18	139.1	16.1	12.9	9.3%
2	ALRV5XR	Feldman et al. 2021	24	21	141.6	3.9	18	139.1	33.9	30.1	21.7%
3	LLLT	Jimenez et al. 2014 (12-beam)	16	18	168.4	-0.8	39	142.2	11.9	12.7	8.8%
4	LLLT	Jimenez et al. 2014 (12-beam)	26	18	168.4	3.0	39	142.2	20.6	17.6	12.7%
5	LLLT	Jimenez et al. 2014 (9-beam)	16	22	155.7	1.3	43	162.6	14.8	13.5	8.3%
6	LLLT	Jimenez et al. 2014 (9-beam)	26	21	155.7	2.8	42	162.6	20.2	17.4	10.6%
7	Minoxidil 2%	De Villez et al. 1994	4	122	138.6	3.8	122	140.4	3.6	-0.2	-0.2%
8	Minoxidil 2%	De Villez et al. 1994	8	124	138.6	5.9	125	140.4	16.3	10.4	7.4%
9	Minoxidil 2%	De Villez et al. 1994	12	121	138.6	9.2	121	140.4	25.5	16.3	11.5%
10	Minoxidil 2%	De Villez et al. 1994	16	126	138.6	8.4	126	140.4	26.4	18.0	12.7%
11	Minoxidil 2%	De Villez et al. 1994	20	123	138.6	11.8	120	140.4	25.3	13.5	9.5%
12	Minoxidil 2%	De Villez et al. 1994	24	123	138.6	9.9	125	140.4	22.2	12.3	8.7%
13	Minoxidil 2%	De Villez et al. 1994	28	121	138.6	15.7	119	140.4	26.6	10.9	7.6%
14	Minoxidil 2%	De Villez et al. 1994	32	128	138.6	10.1	128	140.4	22.7	12.6	8.9%
15	Minoxidil 2%	Jacobs et al. 1993	4	139	139.8	15.5	154	136.7	17.2	1.7	1.5%
16	Minoxidil 2%	Jacobs et al. 1993	8	138	139.8	8.9	154	136.7	20.6	11.7	8.7%
17	Minoxidil 2%	Jacobs et al. 1993	12	139	139.8	19.8	155	136.7	35.7	15.9	12.0%
18	Minoxidil 2%	Jacobs et al. 1993	16	139	139.8	10.2	153	136.7	28.4	18.2	13.5%
19	Minoxidil 2%	Jacobs et al. 1993	20	137	139.8	19.1	152	136.7	34.5	15.4	11.6%
20	Minoxidil 2%	Jacobs et al. 1993	24	137	139.8	14.0	147	136.7	26.1	12.1	9.1%
21	Minoxidil 2%	Jacobs et al. 1993	28	135	139.8	11.9	153	136.7	25.3	13.4	10.0%
22	Minoxidil 2%	Jacobs et al. 1993	32	139	139.8	19.1	155	136.7	33.1	14.0	10.6%
23	Minoxidil 2%	Olsen et al. 1991	32	14	154.2	20.6	14	160.1	50.1	29.5	17.9%
24	Minoxidil 2%	Whiting et al. 1992	8	16	161.0	7.0	16	169.0	20.0	13.0	7.5%
25	Minoxidil 2%	Whiting et al. 1992	16	16	161.0	10.0	16	169.0	21.0	11.0	6.2%
26	Minoxidil 2%	Whiting et al. 1992	24	20	161.0	17.0	14	169.0	21.0	4.0	1.9%
27	Minoxidil 2%	Whiting et al. 1992	32	13	161.0	20.0	15	169.0	28.0	8.0	4.1%
28	Minoxidil 2%	Lucky et al. 2004	8	51	138.4	11.1	108	150.4	18.3	7.2	4.1%
29	Minoxidil 2%	Lucky et al. 2004	16	51	138.4	20.0	108	150.4	35.9	15.9	9.4%
30	Minoxidil 2%	Lucky et al. 2004	32	51	138.4	15.2	108	150.4	26.7	11.5	6.8%
31	Minoxidil 2%	Lucky et al. 2004	48	51	138.4	9.4	108	150.4	20.7	11.3	7.0%
32	Minoxidil 5%	Lucky et al. 2004	8	51	138.4	11.1	101	141.3	21.7	10.6	7.3%
33	Minoxidil 5%	Lucky et al. 2004	16	51	138.4	20.0	101	141.3	36.0	16.0	11.0%
34	Minoxidil 5%	Lucky et al. 2004	32	51	138.4	15.2	101	141.3	27.1	11.9	8.2%
35	Minoxidil 5%	Lucky et al. 2004	48	51	138.4	9.4	101	141.3	24.5	15.1	10.5%
36	Minoxidil 5%	Bergfeld et al. 2016	12	168	152.7	5.3	172	158.6	16.4	11.2	6.9%
37	Minoxidil 5%	Bergfeld et al. 2016	24	165	152.7	4.3	159	158.6	13.4	9.1	5.6%
38	Nutrafol	Ablon & Kogan 2018	12.9	14	136.5	1.0	26	141.7	9.7	8.7	6.1%
39	Nutrafol	Ablon & Kogan 2018	25.7	14	136.5	4.8	26	141.7	14.7	9.9	6.9%
40	Nutrafol	Ablon & Kogan 2021	12.9	27	69.2	-0.3	33	68.1	2.9	3.2	4.7%
41	Nutrafol	Ablon & Kogan 2021	25.7	27	69.2	0.3	33	68.1	7.0	6.7	9.8%

*Hair Regrowth Treatment Efficacy and Resistance in Androgenetic Alopecia:
A Systematic Review and Continuous Bayesian Network Meta-Analysis*

Table S1-7: Imputed results in terminal hair regrowth of AGA treatments in women by week in TH/ cm²

WOMEN: Imputed Weighted Average Efficacy by Treatment by Week (TH/cm²)

	Week 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
ALRV5XR	0	1.1	2.2	3.2	4.3	5.4	6.5	7.5	8.6	9.7	10.8	11.8	12.9	14.4	15.8	17.2	18.6	20.1	21.5	22.9	24.4	25.8	27.2	28.7	30.1
LLLT	0	0.8	1.6	2.4	3.3	4.1	4.9	5.7	6.5	7.3	8.2	9.0	9.8	10.6	11.4	12.2	13.1	13.5	14.0	14.4	14.8	15.3	15.7	16.2	16.6
Minoxidil 2%	0	0.4	0.8	1.3	1.7	3.9	6.1	8.3	10.5	11.6	12.7	13.8	14.9	15.8	16.6	17.5	18.4	17.5	16.7	15.9	15.1	14.6	14.1	13.7	13.2
Minoxidil 5%	0	1.2	2.4	3.6	4.8	6.0	7.2	8.4	9.6	10.6	11.5	12.4	13.4	13.7	14.0	14.3	14.7	14.5	14.3	14.1	13.9	13.6	13.4	13.2	13.0
Nutrafol	0	0.4	0.9	1.3	1.8	2.2	2.7	3.1	3.6	4.0	4.5	4.9	5.4	5.8	6.0	6.2	6.4	6.7	6.9	7.1	7.3	7.5	7.7	8.0	8.2
	Week 25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	
ALRV5XR																									
LLLT		17.1	17.5																						
Minoxidil 2%		13.1	13.0	12.9	12.7	12.9	13.1	13.2	13.4																
Minoxidil 5%																									
Nutrafol		8.4																							

Notes: Imputed results are treatment efficacy at each week measured as difference between the weighted average by population of each group within each treatment cohort. Weeks are whole weeks (fractional weeks are rounded down to the nearest integer) and the full data point result for the fractional week is assigned to the rounded down week.

Hair Regrowth Treatment Efficacy and Resistance in Androgenetic Alopecia:
A Systematic Review and Continuous Bayesian Network Meta-Analysis

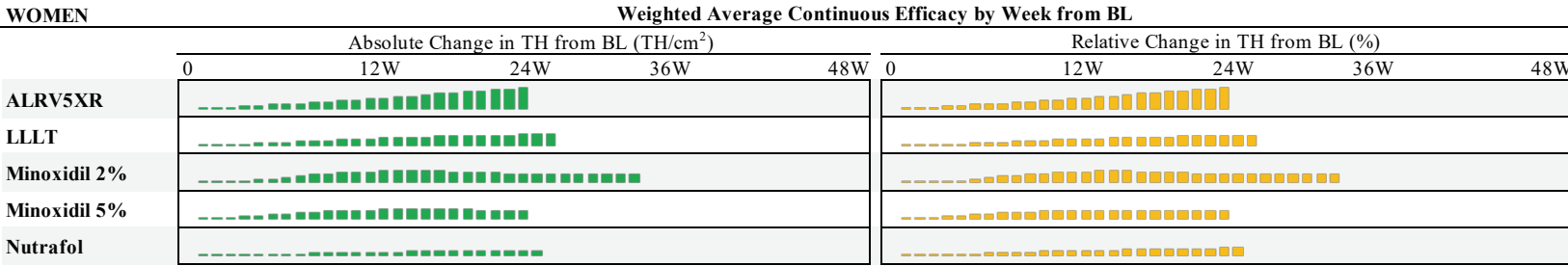
Table S1-8: Imputed results in terminal hair regrowth of AGA treatments in women by week in Percent (%)

WOMEN: Imputed Weighted Average Efficacy by Treatment by Week (%)																									
Week	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
ALRV5XR	0%	0.8%	1.6%	2.3%	3.1%	3.9%	4.7%	5.4%	6.2%	7.0%	7.8%	8.6%	9.3%	10.4%	11.4%	12.4%	13.4%	14.5%	15.5%	16.5%	17.6%	18.6%	19.6%	20.6%	21.7%
LLLT	0%	0.5%	1.1%	1.6%	2.1%	2.7%	3.2%	3.7%	4.3%	4.8%	5.3%	5.8%	6.4%	6.9%	7.4%	8.0%	8.5%	8.8%	9.1%	9.4%	9.8%	10.1%	10.4%	10.7%	11.0%
Minoxidil 2%	0%	0.3%	0.6%	0.8%	1.1%	2.7%	4.2%	5.7%	7.3%	8.1%	8.8%	9.6%	10.4%	11.0%	11.5%	12.1%	12.7%	12.1%	11.5%	10.9%	10.4%	10.0%	9.7%	9.4%	9.1%
Minoxidil 5%	0%	0.8%	1.6%	2.4%	3.2%	4.0%	4.8%	5.6%	6.4%	7.0%	7.6%	8.2%	8.8%	9.1%	9.3%	9.5%	9.8%	9.6%	9.5%	9.4%	9.2%	9.1%	9.0%	8.8%	8.7%
Nutrafol	0%	0.4%	0.8%	1.3%	1.7%	2.1%	2.5%	3.0%	3.4%	3.8%	4.2%	4.6%	5.1%	5.5%	5.7%	6.0%	6.3%	6.5%	6.8%	7.1%	7.3%	7.6%	7.9%	8.1%	8.4%

Week	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48
ALRV5XR																								
LLLT	11.3%	11.6%																						
Minoxidil 2%	9.0%	8.9%	8.8%	8.7%	8.8%	9.0%	9.1%	9.2%																
Minoxidil 5%																								
Nutrafol	8.6%																							

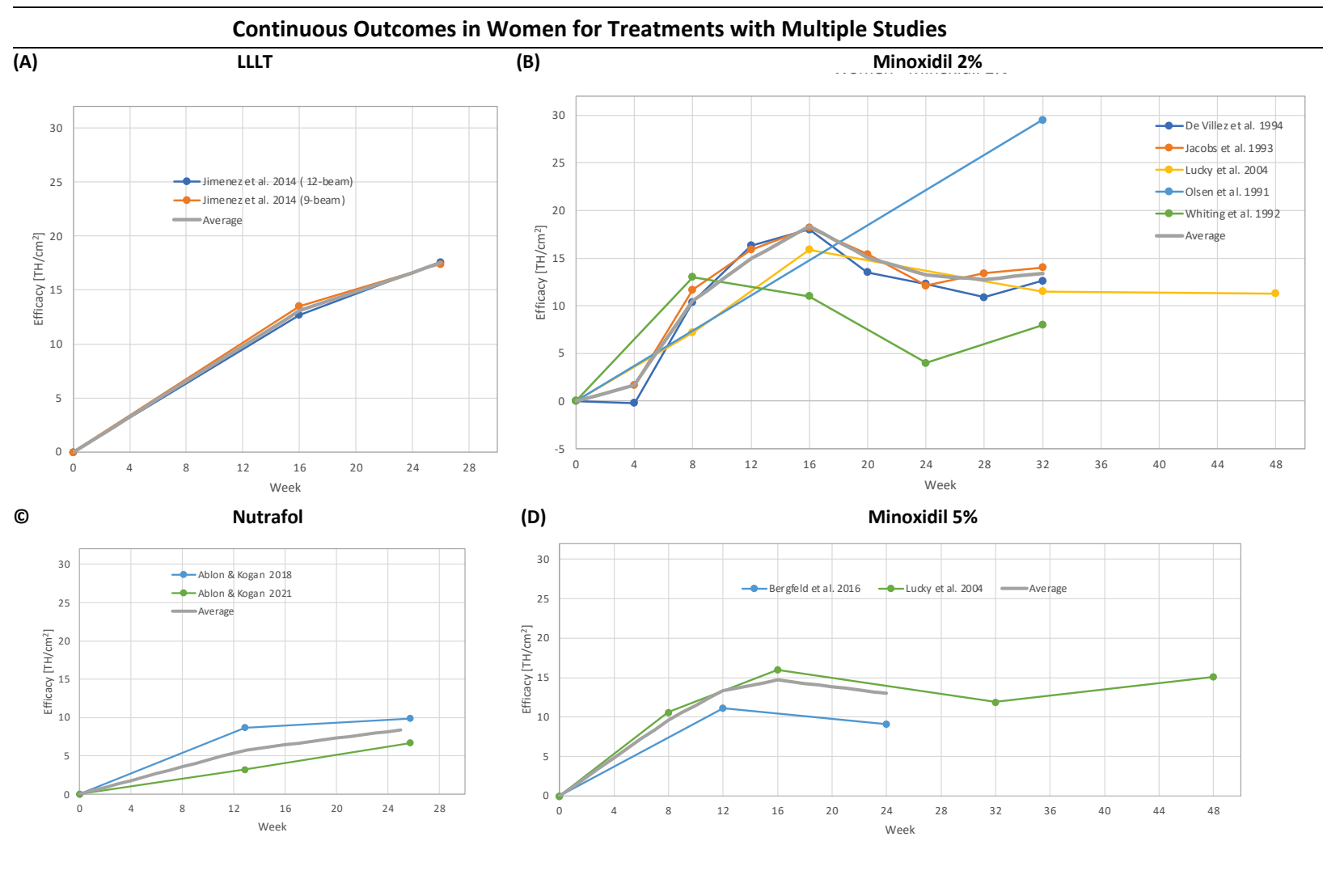
Notes: Results are treatment efficacy at each week measured as percent difference between the population weighted average of each group within each treatment cohort. Weeks are whole weeks (fractional weeks are rounded down to the nearest integer) and the full data point result for the fractional week is assigned to the rounded down week.

Figure S1-6 Continuous Outcomes of regrowth of AGA treatments in women by week



*Hair Regrowth Treatment Efficacy and Resistance in Androgenetic Alopecia:
A Systematic Review and Continuous Bayesian Network Meta-Analysis*

Figure S1-7: Continuous Outcomes in Women for Treatments with Multiple Studies



*Hair Regrowth Treatment Efficacy and Resistance in Androgenetic Alopecia:
A Systematic Review and Continuous Bayesian Network Meta-Analysis*

Table S1-9: Extracted Continuous Outcomes Data and Weekly imputed results in terminal hair regrowth per cm² of AGA treatments in men by week

Extracted Data of Terminal Hair Regrowth from Eligible Studies											
Men				Placebo			Treatment			Efficacy	
#	Treatment	Study	Week	Population N	BL TH TH/cm ²	Change from BL TH/cm ²	Population N	BL TH TH/cm ²	Change from BL TH/cm ²	Absolute TH/cm ²	Relative %
1	ALRV5XR	Feldman et al. 2021	12	11	141.6	-3.0	11	147.0	8.0	11.0	7.5%
2	ALRV5XR	Feldman et al. 2021	24	11	141.6	-5.8	11	147.0	15.2	21.0	14.4%
3	Dutasteride 0.5mg	Gubelin Harcha et al. 2014	12	157	148.0	-0.4	153	149.0	17.1	17.5	11.7%
4	Dutasteride 0.5mg	Gubelin Harcha et al. 2014	24	157	148.0	-0.3	153	149.0	18.1	18.4	12.4%
5	Finasteride 1mg	Gubelin Harcha et al. 2014	12	157	148.0	-0.4	141	148.0	10.8	11.2	7.6%
6	Finasteride 1mg	Gubelin Harcha et al. 2014	24	157	148.0	-0.3	141	148.0	12.1	12.4	8.4%
7	LLLT	Jimenez et al. 2014 (7-beam)	16	14	216.6	2.8	24	211.5	17.7	14.9	7.1%
8	LLLT	Jimenez et al. 2014 (7-beam)	26	14	216.6	1.6	24	211.5	18.4	16.8	8.0%
9	LLLT	Jimenez et al. 2014 (9-beam)	16	22	171.4	4.4	21	163.3	20.4	16.0	9.9%
10	LLLT	Jimenez et al. 2014 (9-beam)	26	21	171.4	9.4	21	163.3	20.9	11.5	7.3%
11	LLLT	Jimenez et al. 2014 (12-beam)	16	22	171.4	4.4	22	151.5	23.5	19.1	12.9%
12	LLLT	Jimenez et al. 2014 (12-beam)	26	21	171.4	9.4	19	151.5	25.7	16.3	11.5%
13	LLLT	Leavitt et al. 2009 (9-beam)	26	39	120.7	-8.9	71	122.9	17.3	26.2	21.5%
14	Minoxidil 2%	Olsen et al. 2002	8	71	152.4	14.5	141	143.6	25.0	10.5	7.9%
15	Minoxidil 2%	Olsen et al. 2002	16	71	152.4	15.3	141	143.6	29.8	14.5	10.7%
16	Minoxidil 2%	Olsen et al. 2002	32	71	152.4	7.7	141	143.6	22.5	14.8	10.6%
17	Minoxidil 2%	Olsen et al. 2002	48	71	152.4	3.9	139	143.6	12.7	8.8	6.3%
18	Minoxidil 5%	Olsen et al. 2002	8	71	152.4	14.5	139	151.1	29.7	15.2	10.1%
19	Minoxidil 5%	Olsen et al. 2002	16	71	152.4	15.3	139	151.1	35.7	20.4	13.6%
20	Minoxidil 5%	Olsen et al. 2002	32	71	152.4	7.7	139	151.1	29.0	21.3	14.1%
21	Minoxidil 5%	Olsen et al. 2002	48	71	152.4	3.9	141	151.1	18.6	14.7	9.8%
22	Minoxidil 5%	Hillmann et al. 2015 (Fron-temp)	16	34	217.2	4.5	30	208.3	27.7	23.2	11.2%
23	Minoxidil 5%	Hillmann et al. 2015 (Fron-temp)	24	34	217.2	-1.5	31	208.3	7.8	9.3	4.4%
24	Minoxidil 5%	Hillmann et al. 2015 (Vertex)	16	31	215.6	5.9	31	208.8	25.6	19.7	9.5%
25	Minoxidil 5%	Hillmann et al. 2015 (Vertex)	24	31	215.6	0.4	32	208.8	8.7	8.3	4.0%
26	Minoxidil 5%	Pumthong et al. 2012	4	22	178.6	6.9	22	173.3	8.2	1.3	0.9%
27	Minoxidil 5%	Pumthong et al. 2012	9	22	178.6	10.8	22	173.3	24.0	13.3	7.8%
28	Minoxidil 5%	Pumthong et al. 2012	13	22	178.6	15.0	22	173.3	23.7	8.8	5.3%
29	Minoxidil 5%	Pumthong et al. 2012	17	22	178.6	16.6	22	173.3	21.9	5.4	3.4%
30	Minoxidil 5%	Pumthong et al. 2012	22	22	178.6	24.0	22	173.3	27.2	3.3	2.3%
31	Minoxidil 5%	Pumthong et al. 2012	26	22	178.6	20.4	22	173.3	31.3	10.9	6.6%
32	Viviscal	Ablon 2016	12.9	30	104.7	-3.0	30	122.0	5.7	8.7	7.5%
33	Viviscal	Ablon 2016	25.7	30	104.7	-5.6	30	122.0	8.4	13.9	12.2%

*Hair Regrowth Treatment Efficacy and Resistance in Androgenetic Alopecia:
A Systematic Review and Continuous Bayesian Network Meta-Analysis*

Table S1-10: Imputed results in terminal hair regrowth of AGA treatments in men by week per cm²

MEN: Imputed Weighted Average Efficacy by Treatment by Week (TH/cm ²)																										
	Week	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
ALRV5XR	0	0.9	1.8	2.7	3.7	4.6	5.5	6.4	7.3	8.2	9.1	10.0	11.0	11.8	12.6	13.5	14.3	15.1	16.0	16.8	17.7	18.5	19.3	20.2	21.0	
Dutasteride 0.5mg	0	1.5	2.9	4.4	5.8	7.3	8.8	10.2	11.7	13.1	14.6	16.0	17.5	17.6	17.7	17.7	17.8	17.9	18.0	18.0	18.1	18.2	18.3	18.3	18.4	
Finasteride 1mg	0	0.9	1.9	2.8	3.7	4.7	5.6	6.5	7.5	8.4	9.3	10.3	11.2	11.3	11.4	11.5	11.6	11.7	11.8	11.9	12.0	12.1	12.2	12.3	12.4	
LLLT	0	1.0	1.9	2.9	3.8	4.8	5.7	6.7	7.6	8.6	9.5	10.5	11.4	12.4	13.3	14.3	15.2	15.5	15.8	16.2	16.5	16.8	17.2	17.5	17.8	
Minoxidil 2%	0	1.3	2.6	3.9	5.3	6.6	7.9	9.2	10.5	11.0	11.5	12.0	12.5	13.0	13.5	14.0	14.5	14.5	14.5	14.6	14.6	14.6	14.6	14.6	14.7	
Minoxidil 5%	0	1.8	3.6	5.4	7.2	9.2	11.2	13.3	15.4	16.3	16.9	17.5	18.1	18.7	19.4	20.0	20.7	20.2	19.7	19.2	18.6	18.1	17.7	17.5	17.3	
Viviscal	0	0.7	1.4	2.0	2.7	3.4	4.1	4.7	5.4	6.1	6.8	7.4	8.1	8.7	9.2	9.6	10.0	10.4	10.8	11.2	11.6	12.0	12.4	12.8	13.2	
	Week	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	
ALRV5XR																										
Dutasteride 0.5mg																										
Finasteride 1mg																										
LLLT		18.2	18.5																							
Minoxidil 2%		14.7	14.7	14.7	14.7	14.7	14.8	14.8	14.8	14.4	14.1	13.7	13.3	12.9	12.6	12.2	11.8	11.4	11.1	10.7	10.3	9.9	9.6	9.2	8.8	
Minoxidil 5%																										
Viviscal		13.6																								

Notes: Imputed results are treatment efficacy at each week measured as difference between the weighted average by population of each group within each treatment cohort. Weeks are whole weeks (fractional weeks are rounded down to the nearest integer) and the full data point result for the fractional week is assigned to the rounded down week.

*Hair Regrowth Treatment Efficacy and Resistance in Androgenetic Alopecia:
A Systematic Review and Continuous Bayesian Network Meta-Analysis*

Table S1-11: Imputed results in terminal hair regrowth of AGA treatments in men by week in Percent (%)

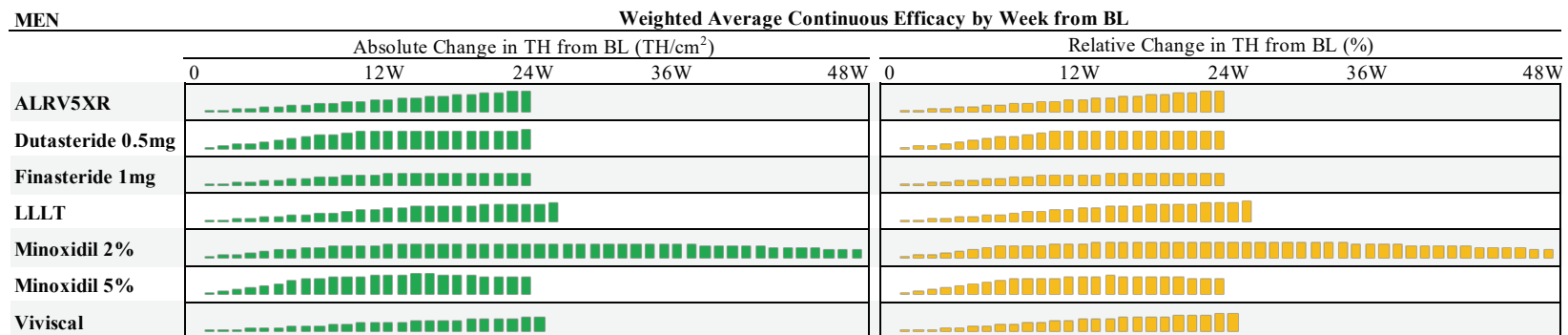
MEN: Imputed Weighted Average Efficacy by Week (%) for each Treatment

Week	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
ALRV5XR	0%	0.6%	1.3%	1.9%	2.5%	3.1%	3.8%	4.4%	5.0%	5.6%	6.3%	6.9%	7.5%	8.1%	8.7%	9.3%	9.8%	10.4%	11.0%	11.6%	12.1%	12.7%	13.3%	13.9%	14.4%
Dutasteride 0.5mg	0%	1.0%	2.0%	2.9%	3.9%	4.9%	5.9%	6.9%	7.8%	8.8%	9.8%	10.8%	11.7%	11.8%	11.8%	11.9%	11.9%	12.0%	12.0%	12.1%	12.1%	12.2%	12.2%	12.3%	12.4%
Finasteride 1mg	0%	0.6%	1.3%	1.9%	2.5%	3.2%	3.8%	4.4%	5.0%	5.7%	6.3%	6.9%	7.6%	7.6%	7.7%	7.8%	7.8%	7.9%	8.0%	8.0%	8.1%	8.2%	8.2%	8.3%	8.4%
LLLT	0%	0.7%	1.3%	2.0%	2.7%	3.4%	4.0%	4.7%	5.4%	6.1%	6.7%	7.4%	8.1%	8.7%	9.4%	10.1%	10.8%	11.1%	11.4%	11.7%	12.0%	12.3%	12.6%	12.9%	13.2%
Minoxidil 2%	0%	1.0%	2.0%	3.0%	3.9%	4.9%	5.9%	6.9%	7.9%	8.2%	8.6%	9.0%	9.3%	9.7%	10.0%	10.4%	10.7%	10.7%	10.7%	10.7%	10.7%	10.7%	10.7%	10.7%	10.7%
Minoxidil 5%	0%	1.1%	2.3%	3.4%	4.6%	5.8%	7.1%	8.4%	9.7%	10.2%	10.6%	11.0%	11.3%	11.7%	12.1%	12.5%	12.8%	12.6%	12.3%	12.0%	11.8%	11.5%	11.3%	11.2%	11.1%
Viviscal	0%	0.6%	1.2%	1.8%	2.3%	2.9%	3.5%	4.1%	4.7%	5.3%	5.9%	6.4%	7.0%	7.6%	7.9%	8.3%	8.7%	9.0%	9.4%	9.8%	10.1%	10.5%	10.8%	11.2%	11.6%

Week	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48
ALRV5XR																								
Dutasteride 0.5mg																								
Finasteride 1mg																								
LLLT	13.5%	13.8%																						
Minoxidil 2%	10.7%	10.7%	10.6%	10.6%	10.6%	10.6%	10.6%	10.6%	10.3%	10.1%	9.8%	9.5%	9.3%	9.0%	8.7%	8.5%	8.2%	7.9%	7.6%	7.4%	7.1%	6.8%	6.6%	6.3%
Minoxidil 5%																								
Viviscal	11.9%																							

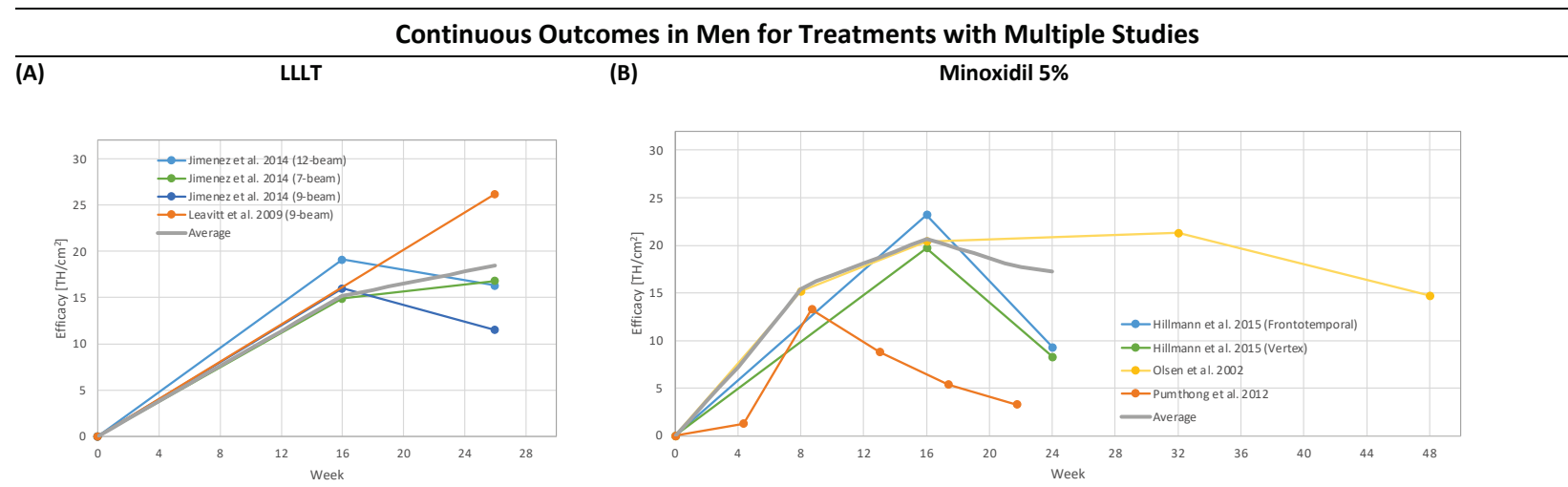
Notes: Results are treatment efficacy at each week measured as percent difference between the population weighted average of each group within each treatment cohort. Weeks are whole weeks (fractional weeks are rounded down to the nearest integer) and the full data point result for the fractional week is assigned to the rounded down week.

Figure S1-8 Continuous Outcomes of regrowth of AGA treatments in men by week



*Hair Regrowth Treatment Efficacy and Resistance in Androgenetic Alopecia:
A Systematic Review and Continuous Bayesian Network Meta-Analysis*

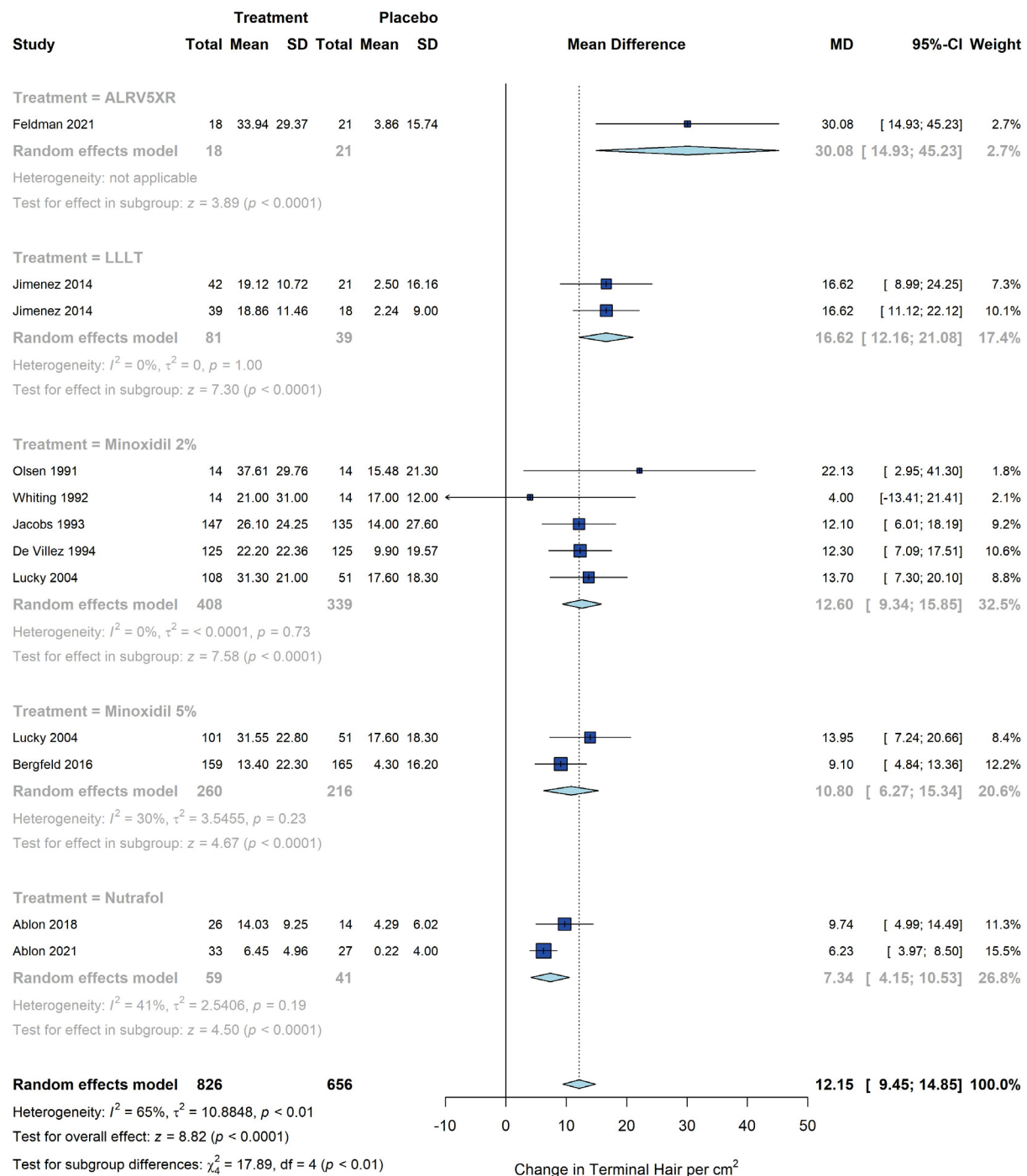
Figure S1-9: Continuous in Men for Treatments with Multiple Studies



*Hair Regrowth Treatment Efficacy and Resistance in Androgenetic Alopecia:
A Systematic Review and Continuous Bayesian Network Meta-Analysis*

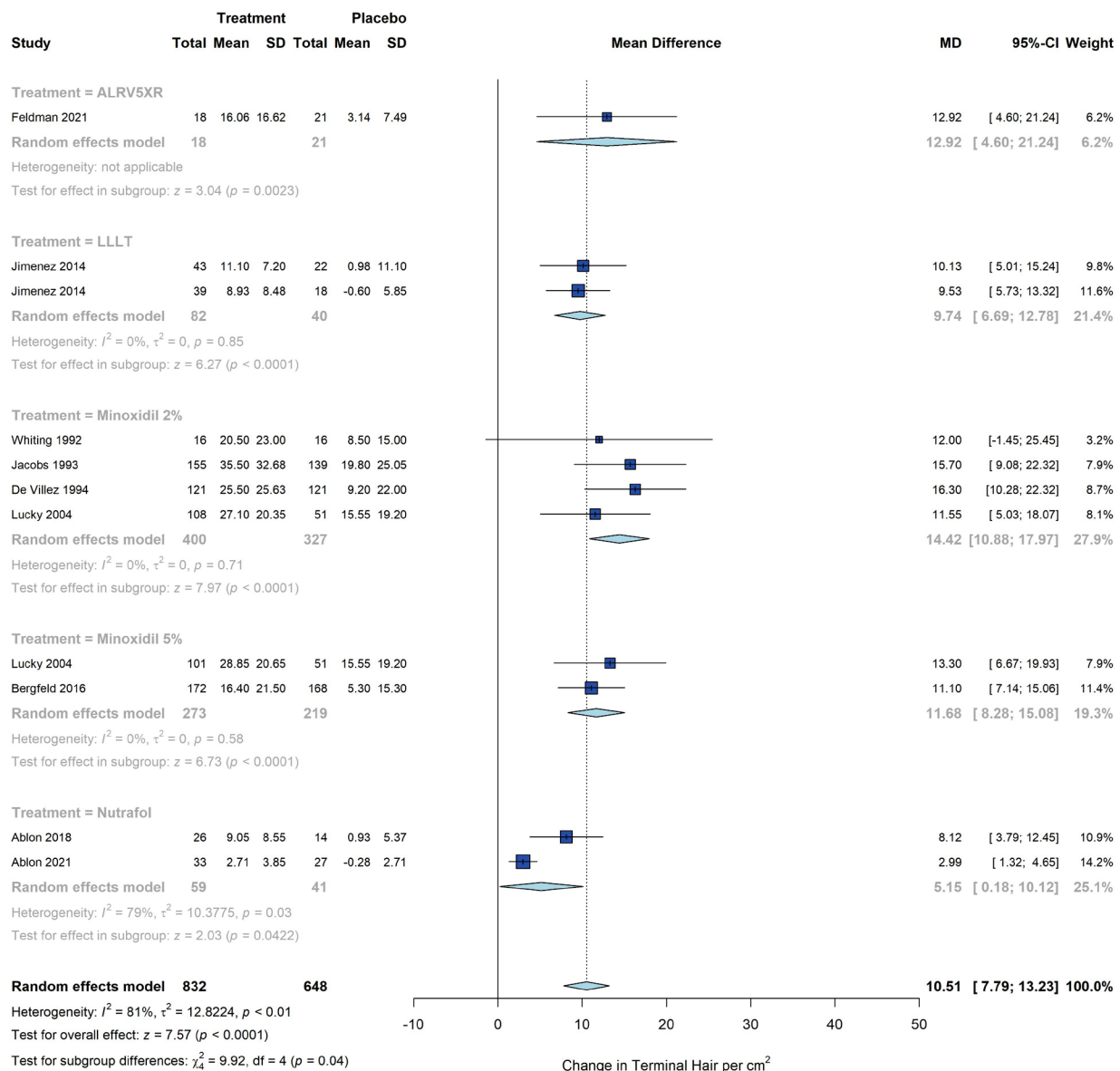
Section 5: Meta-Analysis Results

Figure S1-10: Meta-Analysis Forest Plot for Women after 24 weeks of treatment



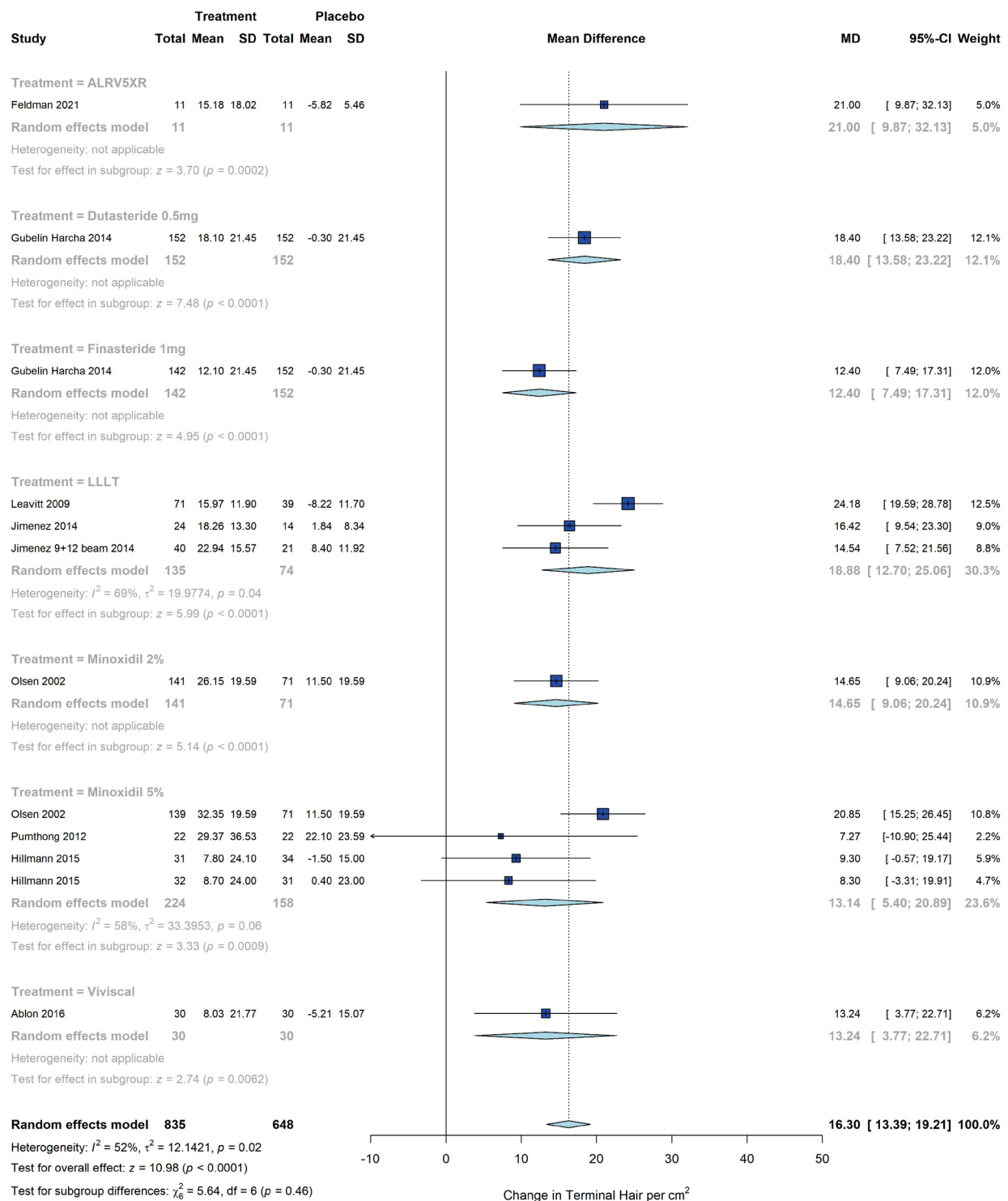
*Hair Regrowth Treatment Efficacy and Resistance in Androgenetic Alopecia:
A Systematic Review and Continuous Bayesian Network Meta-Analysis*

Figure S1-11: Meta-Analysis Forest Plot for Women after 12 weeks of treatment



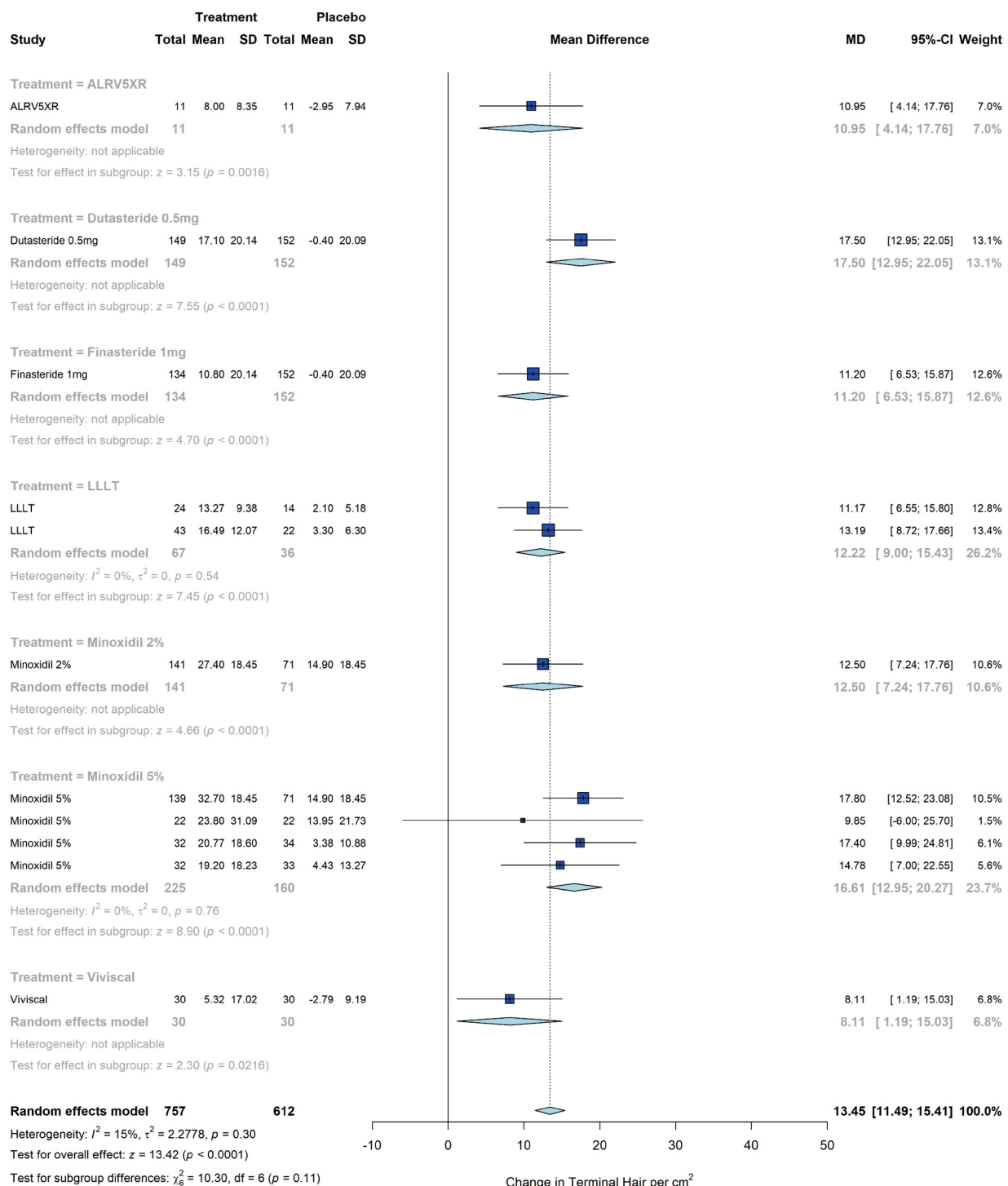
*Hair Regrowth Treatment Efficacy and Resistance in Androgenetic Alopecia:
A Systematic Review and Continuous Bayesian Network Meta-Analysis*

Figure S1-12: Meta-Analysis Forest Plot for Men after 24 weeks of treatment



*Hair Regrowth Treatment Efficacy and Resistance in Androgenetic Alopecia:
A Systematic Review and Continuous Bayesian Network Meta-Analysis*

Figure S1-13: Meta-Analysis Forest Plot for Men after 12 weeks of treatment



*Hair Regrowth Treatment Efficacy and Resistance in Androgenetic Alopecia:
A Systematic Review and Continuous Bayesian Network Meta-Analysis*

Figure S1-14: Meta-Analysis Funnel Plots

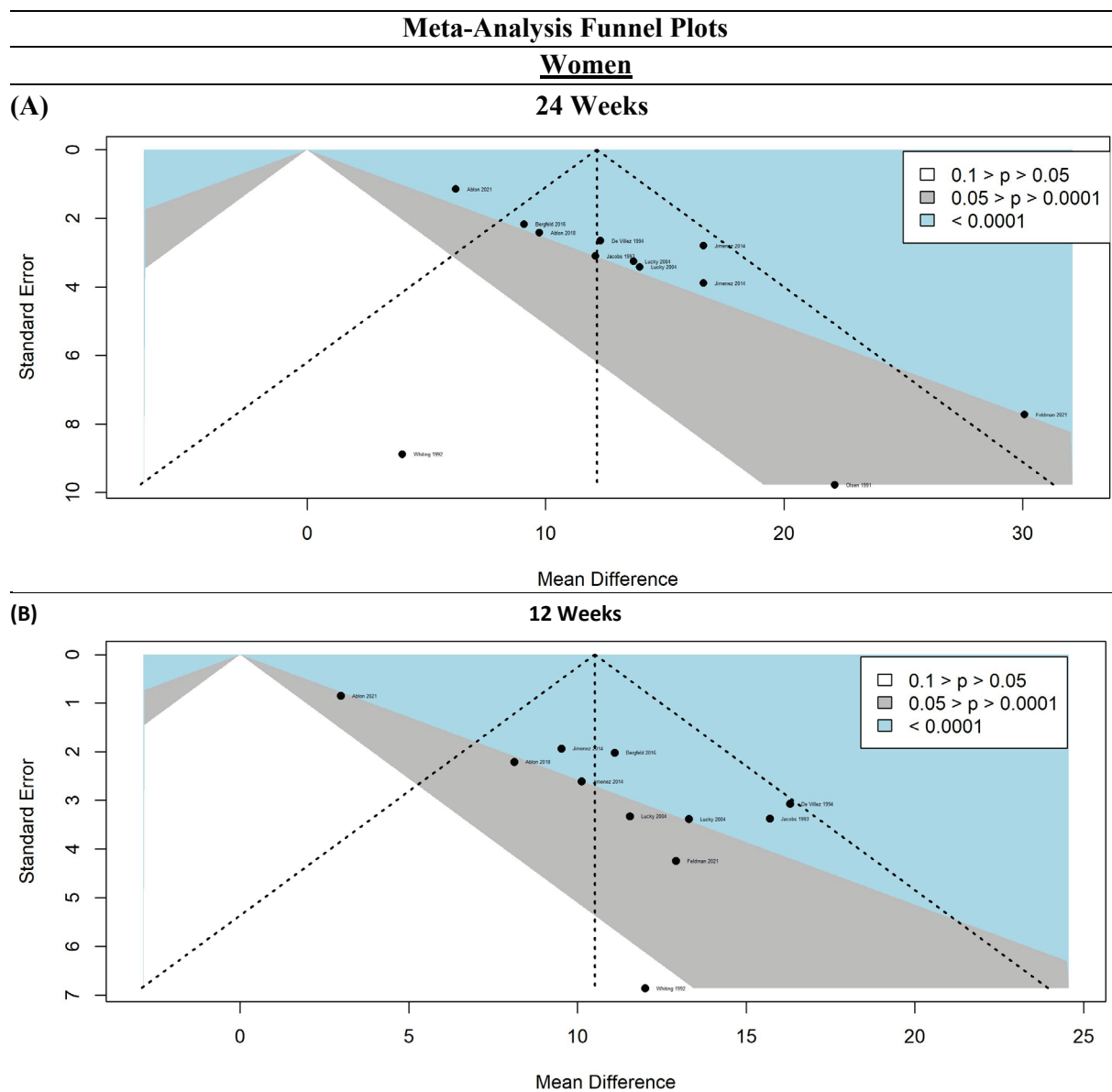
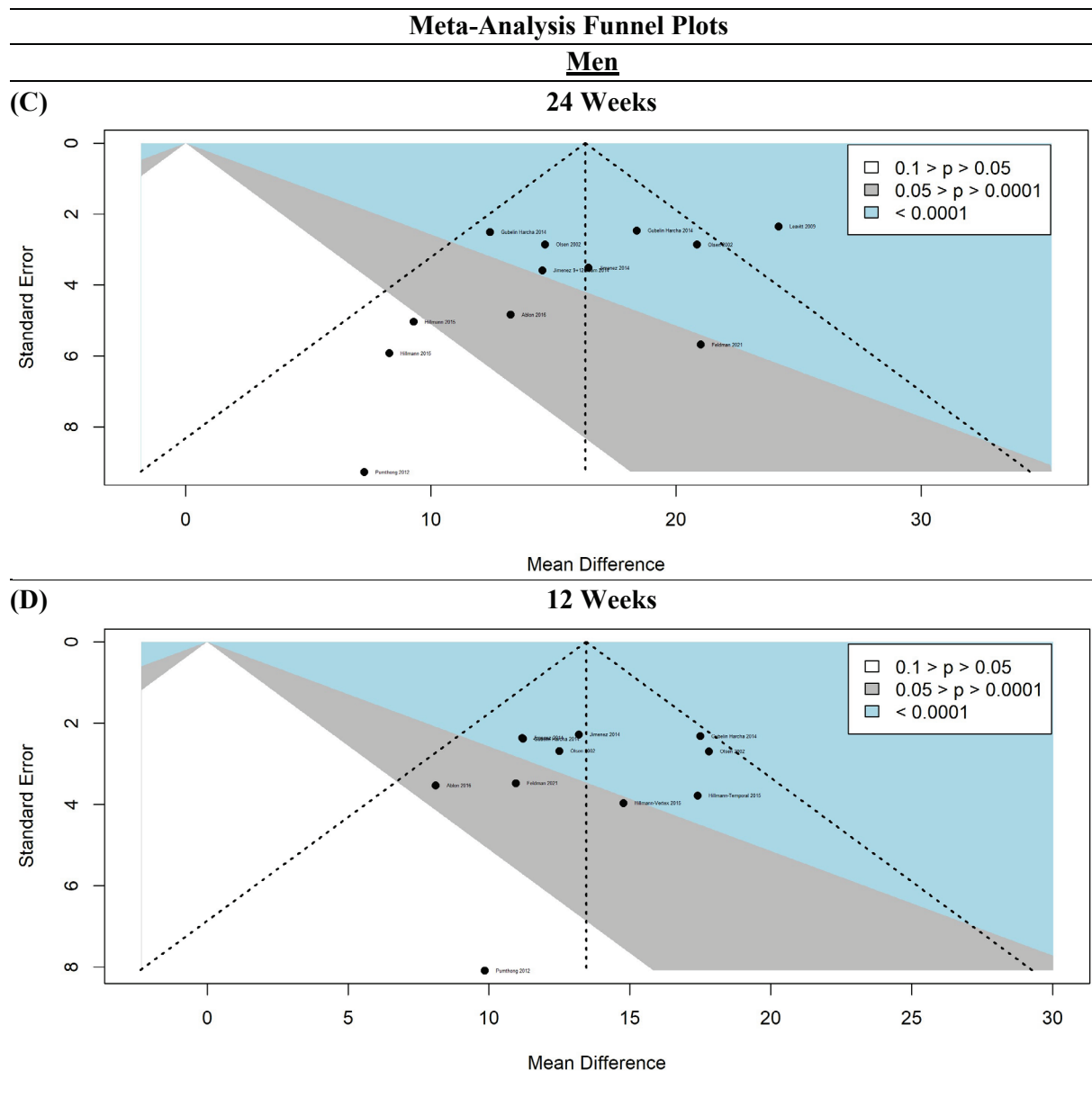


Figure S1-13: Meta-Analysis Funnel Plots (Continued)



*Hair Regrowth Treatment Efficacy and Resistance in Androgenetic Alopecia:
A Systematic Review and Continuous Bayesian Network Meta-Analysis*

Section 6: Safety Results

Table S1-12: Summary of Adverse Events (AEs) from Eligible Studies.

This table shows presence of Adverse Events by type reported from treatment groups of eligible studies. No analysis has been performed on frequency, severity or comparisons to placebo groups. In women, Minoxidil 5% reported the widest range of AEs followed by Minoxidil 2%. In men, Minoxidil 5% had the widest range of AEs followed by Dutasteride 0.5mg and Finasteride 1mg reporting the same types of AEs. LLLT and natural treatments had few to no AEs.

	Women					Men							
Adverse Events	ALRV5XR	LLLT	Minoxodil 2%	Minoxidil 5%	Nutrafol	ALRV5XR	Dutasteride 0.5mg	Finasteride 1mg	LLLT	Minoxidil 2%	Minoxidil 5%	Viviscal	
Cardiovascular			Y	Y									
Change in weight				Y		Y		Y					
Elevated Blood Pressure				Y						Y	Y		
Erythema			Y	Y									
Feeling of Skin Tension												Y	
Folliculitis			Y										
Gastrointestinal Complaints				Y	Y	Y		Y					
Hair Loss											Y		
Headache			Y	Y		Y		Y		Y	Y		
Hypertrichosis			Y	Y									
Malaise and Fatigue			Y		Y								
Nasopharyngitis						Y		Y					
Neurology-related Complaints									Y				
Palpitations												Y	
Papules				Y					Y				
Pruritus		Y	Y	Y						Y	Y		
Sexual Dysfunction						Y		Y					
Skin Disorders (dermatitis)		Y	Y	Y						Y	Y		
Total	0	2	8	10	2	0	5	5	2	4	7	0	

Supplementary Materials

Appendix 2 – Search Strategy, Articles Searched and Reviewed

Hair Regrowth Treatment Efficacy and Resistance in Androgenetic Alopecia: A Systematic Review and Continuous Bayesian Network Meta-Analysis

Peter R. Feldman^{1,2*}, Pietro Gentile³, Charles Piwko⁴, Hendrik M. Motswaledi⁵, Samantha Gorun^{6,7}, Jacob Pesachov⁸, Michael Markel⁸, Maxwell I. Silver^{9,10}, Megan Brenkel¹¹, Oriel J. Feldman^{1,12}, Corey L. Kamen⁸, Elizabeth Uleryk¹³, Jaime Guevara-Aguirre^{14,15,16,17}, Klaus M. Fiebig¹

¹ Arbor Life Labs, Toronto, ON, Canada

² Norwich Medical School, University of East Anglia, Norwich, United Kingdom

³ Surgical Science Department, University of Rome Tor Vergata, Rome, Italy

⁴ CHP Pharma Inc., Thornhill, ON, Canada

⁵ Department of Dermatology, Sefako Makgatho Health Sciences University, South Africa

⁶ Faculty of Epidemiology & Biostatistics, Western University, ON, Canada

⁷ School of Mathematics and Statistics, University of Glasgow, Glasgow, United Kingdom

⁸ Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel

⁹ Faculty of Dentistry, University of Toronto, Toronto, ON, Canada

¹⁰ Faculty of Medicine and Dentistry, University of Alberta, Edmonton, AB, Canada

¹¹ Faculty of Medicine, University of Ottawa, Ottawa, ON, Canada

¹² Faculty of Science, Wilfrid Laurier University, Waterloo, ON, Canada

¹³ Uleryk Consulting, Mississauga, ON, Canada

¹⁴ Universidad San Francisco de Quito (USFQ), Quito, Ecuador

¹⁵ Faculty of Health, Medicine and Life Sciences, Maastricht University, Maastricht, Netherlands

¹⁶ Institute of Endocrinology, Metabolism, and Reproduction (IEMR), Quito, Ecuador

¹⁷ College of Medicine, University of Florida, Gainesville, FL, USA

* Correspondence:

Corresponding Author: Peter R Feldman

petefeldman@gmail.com

Keywords: hair loss, ALRV5XR, Dutasteride, Finasteride, LLLT, Minoxidil, Nutrafol, Viviscal

Submission Date: July 20, 2022

Revised: September 12, 2022

Accepted: December 7, 2022

Published: January 23, 2023

CITATION:

Feldman PR, Gentile P, Piwko C, Motswaledi H, Gorun S, Pesachov J, Markel M, Silver MI, Brenkel M, Feldman OJ, Kamen CL, Uleryk E, Guevara-Aguirre J and Fiebig KM (2023) Hair regrowth treatment efficacy and resistance in androgenetic alopecia: A systematic review and Bayesian network meta-analysis. *Front. Med.* 9:998623. doi: 10.3389/fmed.2022.998623

© 2023 Feldman, Gentile, Piwko, Motswaledi, Gorun, Pesachov, Markel, Silver, Brenkel, Feldman, Kamen, Uleryk, Guevara-Aguirre and Fiebig. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

*Hair Regrowth Treatment Efficacy and Resistance in Androgenetic Alopecia:
A Systematic Review and Continuous Bayesian Network Meta-Analysis*

Supplementary Materials

Appendix 2 – Search Strategy, Articles Searched and Reviewed

Hair Regrowth Treatment Efficacy and Resistance in Androgenetic Alopecia: A Systematic Review and Continuous Bayesian Network Meta-Analysis

Table of Contents

Tables:	Page
Table S2-1. Tabulation of Eligible Studies	3
Table S2-2. Table of Inclusion/Exclusion Reason Codes	3
Table S2-3. Summary of Reasons for Inclusion/Exclusion by Treatment	3
Table S2-4. Eligible Articles Excluded from Study	4
Article Search Reports	
Article Database Search Methodology and Strategy Report	12

*Hair Regrowth Treatment Efficacy and Resistance in Androgenetic Alopecia:
A Systematic Review and Continuous Bayesian Network Meta-Analysis*

Table S2-1. Tabulation of Studies assessed, eligible and treatment cohorts analysed

	Articles Assessed	Eligible Articles			Study Cohorts			Treatments		
		Women	Men	Total	Women	Men	Total	Women	Men	Total
Sex	98 ^a	10	8	17	12	13	25	5	7	8
Women	48	10	-	10	12	-	12	5	-	5
Men	69	-	8	8	-	13	13	-	7	7

Notes: a: Articles add up to less than total of Men and Women due combined sex enrolments in some studies and common treatments.

Table S2-2. Table of Inclusion/Exclusion Reason Codes

Reason Codes
1 Eligible
2 Not outcome of interest
3 Experimental formulation/treatment
4 Not randomized placebo-controlled trial
5 Insufficient subjects
6 Not Androgenetic Alopecia or patterned hair loss
7 No extractable data
8 No error data at 24 weeks
9 Data included in subsequent publication
10 Results or crossover < 24 weeks
11 Ineligible hair count methods
12 No sex specific data
13 Not English or German
14 Article not retrievable
15 Total Hair Only
16 Duplicate

Table S2-3. Summary of Primary Reasons for Inclusion/Exclusion by Treatment

Treatment	Eligible	Excluded by Reason Code														Total
	1	2	3	4	5	6	7	9	10	11	12	14	15	16		
ALRV5XR	2															2
Caffeine												1				1
Dutasteride			3													3
Dutasteride & Finasteride	1	1														2
Electrostatic				1												1
Fenugreek			1													1
Finasteride			4								2		3			9
Ketoconazole			1													1
LLLT	2	2					1		3			1				9
Minoxidil				1												1
Minoxidil 2%	4	2			1						15				1	23
Minoxidil 2% & Minoxidil 5%	2															2
Minoxidil 5%	3		1						1	2					1	8
Niacin			1													1
Nourkrin			1													1
Nutrafol	2															2
Pantogar							1									1
PRP			5		1	2		2		3		1	2	1	1	18
Serenoa repens			1													1
Stem Cell Tx										1		2				3
Tocotrienol			1													1
Viviscal	1		2				1									4
Hairgain			1													1
Wnt Activator				2												2
Total	17	27	4	1	3	2	3	1	9	17	4	6	1	3		98

*Hair Regrowth Treatment Efficacy and Resistance in Androgenetic Alopecia:
A Systematic Review and Continuous Bayesian Network Meta-Analysis*

Table S2-4. Studies assessed

Article		Reason Codes			Comments	Treatment
1	Ablon G. Double-blind, placebo-controlled study evaluating the efficacy of an oral supplement in women with self-perceived thinning hair. <i>Journal of Clinical and Aesthetic Dermatology</i> . 2012;5(11):28-34.	6			AGA Excluded	Viviscal
2	Ablon G. A 6-month, randomized, double-blind, placebo-controlled study evaluating the ability of a marine complex supplement to promote hair growth in men with thinning hair. <i>Journal of Cosmetic Dermatology</i> . 2016;15(4):358-366.	1				Viviscal
3	Ablon G, Dayan S. A randomized, double-blind, placebo-controlled, multi-center, extension trial evaluating the efficacy of a new oral supplement in women with self-perceived thinning hair. <i>Journal of Clinical and Aesthetic Dermatology</i> . 2015;8(12):15-21.	2			Outcome in %	Viviscal
4	Ablon G, Kogan S. A six-month, randomized, double-blind, placebo-controlled study evaluating the safety and efficacy of a nutraceutical supplement for promoting hair growth in women with self-perceived thinning hair. <i>Journal of Drugs in Dermatology</i> . 2018;17(5):558-565.	1				Nutrafol
5	Ablon G, Kogan S. A Randomized, Double-Blind, Placebo-Controlled Study of a Nutraceutical Supplement for Promoting Hair Growth in Perimenopausal, Menopausal, and Postmenopausal Women With Thinning Hair. <i>J Drugs Dermatol</i> . 2021;20(1):55-61.	1				Nutrafol
6	Alanis A, Barbara F, Meurehg C, Montes de Oca F, Ramirez L. Double-blind comparison of 2% topical minoxidil and placebo in early male pattern baldness. <i>Current Therapeutic Research - Clinical and Experimental</i> . 1991;49(5):723-730.	11			Manual	Minoxidil 2%
7	Alves R, Grimalt R. Randomized placebo-controlled, double-blind, half-head study to assess the efficacy of platelet-rich plasma on the treatment of androgenetic alopecia. <i>Dermatologic Surgery</i> . 2016;42(4):491-497.	12				PRP
8	Anderson CD, Hansted B, Abdallah MA, et al. Topical minoxidil in androgenetic alopecia. Scandinavian and Middle East experience. <i>International journal of dermatology</i> . 1988;27(6 SUPPL.):447-451.	11			Manual	Minoxidil 2%
9	Barikbin B, Khodamrzi Z, Kholoosi L, et al. Comparison of the effects of 665 nm low level diode Laser Hat versus and a combination of 665 nm and 808nm low level diode Laser Scanner of hair growth in androgenic alopecia. <i>J Cosmet Laser Ther</i> . 2017;17:17.	12				LLLT
10	Beoy LA, Woei WJ, Hay YK. Effects of tocotrienol supplementation on hair growth in human volunteers. <i>Trop Life Sci Res</i> . 2010;21(2):91-99.	2	12	15	Patchy hair loss	Tocotrienol
11	Bergfeld W, Washenik K, Callender V, et al. A phase III, multicenter, parallel-design clinical trial to compare the efficacy and safety of 5% minoxidil foam versus vehicle in women with female pattern hair loss. <i>Journal of Drugs in Dermatology</i> . 2016;15(7):874-881.	1				Minoxidil 5%

*Hair Regrowth Treatment Efficacy and Resistance in Androgenetic Alopecia:
A Systematic Review and Continuous Bayesian Network Meta-Analysis*

12	Blume-Peytavi U, Issiakhem Z, Gautier S, et al. Efficacy and safety of a new 5% minoxidil formulation in male androgenetic alopecia: A randomized, placebo-controlled, double-blind, noninferiority study. <i>Journal of Cosmetic Dermatology</i> . 2019;18(1):215-220.	10		16 Weeks	Minoxidil 5%
13	Cervelli V, Garcovich S, Bielli A, et al. The effect of autologous activated platelet rich plasma (AA-PRP) injection on pattern hair loss: Clinical and histomorphometric evaluation. <i>BioMed Research International</i> . 2014;2014 (no pagination)(760709).	5		PRP	PRP
14	Civatte J, Degreef H, Dockx P, et al. Topical 2% minoxidil solution in male pattern alopecia: The initial european experience. <i>International Journal of Dermatology</i> . 1988;27(6 SUPPL.):424-429.	11		Manual	Minoxidil 2%
15	Civatte J, Laux B, Simpson NB, Vickers CF. 2% topical minoxidil solution in male-pattern baldness: preliminary European results. <i>Dermatologica</i> . 1987;175 Suppl 2:42-49.	11		Manual	Minoxidil 2%
16	Connors TJ, Cooke DE, De Launey WE, et al. Australian trial of topical minoxidil and placebo in early male pattern baldness. <i>The Australasian journal of dermatology</i> . 1990;31(1):17-25.	11		Manual	Minoxidil 2%
17	De Villev RL. Androgenetic alopecia treated with topical minoxidil. <i>Journal of the American Academy of Dermatology</i> . 1987;16(3 II SUPPL.):669-672.	11		Manual	Minoxidil 2%
18	DeVillev RL, Jacobs JP, Szpunar CA, Warner ML. Androgenetic alopecia in the female: Treatment with 2% topical minoxidil solution. <i>Archives of Dermatology</i> . 1994;130(3):303-307.	1			Minoxidil 2%
19	Dicle O, Bilgic Temel A, Gulkesen KH. Platelet-rich plasma injections in the treatment of male androgenetic alopecia: A randomized placebo-controlled crossover study. <i>Journal of Cosmetic Dermatology</i> . 2020;19(5):1071-1077.	10		PRP	PRP
20	Draeos ZD, Jacobson EL, Kim H, Kim M, Jacobson MK. A pilot study evaluating the efficacy of topically applied niacin derivatives for treatment of female pattern alopecia. <i>Journal of Cosmetic Dermatology</i> . 2005;4(4):258-261.	2		Hair Fullness	Niacin
21	Dubin DP, Lin MJ, Leight HM, et al. The effect of platelet-rich plasma on female androgenetic alopecia: A randomized controlled trial. <i>Journal of the American Academy of Dermatology</i> . 2020;83(5):1294-1297.	15		PRP	PRP
22	Dutree-Meulenberg R. O. G. M. MD, C. Nieboer, M.D., PH.D., F. H. j ., Koedijk, M.D., and E. Stolz, M.D., PH.D. Treatment of Male Pattern Alopecia Using Topical Minoxidil in The Netherlands. <i>INTERNATIONAL JOURNAL OF DERMATOLOGY</i> . 1988;27(6):435-440.	11		Manual	Minoxidil 2%
23	Eun HC, Kwon OS, Yeon JH, et al. Efficacy, safety, and tolerability of dutasteride 0.5 mg once daily in male patients with male pattern hair loss: A randomized, double-blind, placebo-controlled, phase III study. <i>Journal of the American Academy of Dermatology</i> . 2010;63(2):252-258.	2	15		Dutasteride
24	Feldman PR, Fiebig KM, Piwko C, et al. Safety and efficacy of ALRV5XR in women with androgenetic alopecia or telogen effluvium: A randomised, double-blinded, placebo-controlled clinical trial. <i>EClinicalMedicine</i> . 2021;37 (no pagination)(100978).	1			ALRV5XR

*Hair Regrowth Treatment Efficacy and Resistance in Androgenetic Alopecia:
A Systematic Review and Continuous Bayesian Network Meta-Analysis*

25	Feldman PR, Fiebig KM, Piwko C, et al. Safety and efficacy of ALRV5XR in men with androgenetic alopecia: A randomised, double-blinded, placebo-controlled clinical trial. <i>EClinicalMedicine</i> . 2021;40:101124.	1				ALRV5XR
26	Finasteride Male Pattern Hair Loss Study Group. Long-term (5-year) multinational experience with finasteride 1 mg in the treatment of men with androgenetic alopecia. <i>European Journal of Dermatology</i> . 2002;12(1):38-49.	14			Article not retrievable	Finasteride
27	Friedman S, Schnoor P. Novel Approach to Treating Androgenetic Alopecia in Females with Photobiomodulation (Low-Level Laser Therapy). <i>Dermatologic Surgery</i> . 2017;43(6):856-867.	10			17 Weeks	LLLT
28	Gentile P. The effect of autologous activated platelet rich plasma (AA-PRP) injection on pattern hair loss: clinical and histomorphometric evaluation. <i>Biomed research international</i> . 2014;2014:760709.	16			Same as Cervelli, 2014	PRP
29	Gentile P. Autologous cellular method using micrografts of human adipose tissue derived follicle stem cells in androgenic alopecia. <i>International journal of molecular sciences</i> . 2019;20(14).	12				Stem Cell Tx
30	Gentile P, Cole JP, Cole MA, et al. Evaluation of not-activated and activated PRP in hair loss treatment: Role of growth factor and cytokine concentrations obtained by different collection systems. <i>International Journal of Molecular Sciences</i> . 2017;18 (2) (no pagination)(408).	2	15			PRP
31	Gentile P, Garcovich S, Bielli A, Scioli MG, Orlandi A, Cervelli V. The effect of platelet-rich plasma in hair regrowth: A randomized placebo-controlled trial. <i>Stem Cells Translational Medicine</i> . 2015;4(11):1317-1323.	10				PRP
32	Gentile P, Garcovich S, Scioli MG, Bielli A, Orlandi A, Cervelli V. Mechanical and Controlled PRP Injections in Patients Affected by Androgenetic Alopecia. <i>Journal of visualized experiments : JoVE</i> . 2018(pagination).	2	15			PRP
33	Ghonemy S, Alarawi A, Bessar H. Efficacy and safety of a new 10% topical minoxidil versus 5% topical minoxidil and placebo in the treatment of male androgenetic alopecia: a trichoscopic evaluation. <i>Journal of Dermatological Treatment</i> . 2021;32(2):236-241.	2			Outcomes Grade, Ratios	Minoxidil 5%
34	Ghonemy S, Bessar H, Alarawi A. Efficacy and safety of a new 10% topical minoxidil versus 5% topical minoxidil and placebo in the treatment of male androgenetic alopecia: a trichoscopic evaluation. <i>Journal of dermatological treatment</i> . 2019:1-24.	16			Author Sequence changed	Minoxidil 5%
35	Gressenberger P, Pregartner G, Gary T, Wolf P, Kopera D. Platelet-rich Plasma for Androgenetic Alopecia Treatment: A Randomized Placebo-controlled Pilot Study. <i>Acta Derm Venereol</i> . 2020;100(15):adv00247.	2	15			PRP
36	Gubelin Harcha W, Barboza Martinez J, Tsai TF, et al. A randomized, active- and placebo-controlled study of the efficacy and safety of different doses of dutasteride versus placebo and finasteride in the treatment of male subjects with androgenetic alopecia. <i>Journal of the American Academy of Dermatology</i> . 2014;70(3):489-498.	1				Dutasteride, Finasteride
37	Gupta T. Efficacy of platelet rich plasma in treating androgenetic alopecia. <i>Journal of the Dermatology Nurses' Association Conference: 24th World Congress of Dermatology Milan Italy</i> . 2020;12(2).	2	15			PRP
38	Hansted B, Abdallah MA, Oumeish OY, et al. Topical minoxidil in androgenetic alopecia. <i>Scandinavian and Middle East experience</i> . <i>International Journal of Dermatology</i> . 1988;27(6 SUPPL.):447-451.	16			Same as Anderson, 1988	Minoxidil 2%

*Hair Regrowth Treatment Efficacy and Resistance in Androgenetic Alopecia:
A Systematic Review and Continuous Bayesian Network Meta-Analysis*

39	Hillmann K, Garcia Bartels N, Kottner J, Stroux A, Canfield D, Blume-Peytavi U. A Single-Centre, Randomized, Double-Blind, Placebo-Controlled Clinical Trial to Investigate the Efficacy and Safety of Minoxidil Topical Foam in Frontotemporal and Vertex Androgenetic Alopecia in Men. <i>Skin Pharmacology and Physiology</i> . 2015;28(5):236-244.	1					Minoxidil 5%
40	Hillmann K, Garcia Bartels N, Stroux A, Canfield D, Blume-Peytavi U. Investigator-initiated double-blind, two-armed, placebo-controlled, randomized clinical trial with an open-label extension phase, to investigate the efficacy of 5% Minoxidil topical foam twice daily in men with androgenetic alopecia in the fronto-temporal and vertex regions regarding hair volume over 24/52 weeks. <i>Journal of Investigative Dermatology</i> . 2013;133(5):1400.	9					Minoxidil 5%
41	Jacobs JP, Szpunar CA, Warner ML. Use of topical minoxidil therapy for androgenetic alopecia in women. <i>International Journal of Dermatology</i> . 1993;32(10):758-762.	1					Minoxidil 2%
42	Jimenez JJ, Wikramanayake TC, Bergfeld W, et al. Efficacy and safety of a low-level laser device in the treatment of male and female pattern hair loss: A multicenter, randomized, sham device-controlled, double-blind study. <i>American Journal of Clinical Dermatology</i> . 2014;15(2):115-127.	1					LLLT
43	Kachhawa D, Vats G, Sonare D, Rao P, Khuraiya S, Kataiya R. A split head study of efficacy of placebo versus platelet-rich plasma injections in the treatment of androgenic alopecia. <i>Journal of Cutaneous and Aesthetic Surgery</i> . 2017;10(2):86-89.	14					PRP
44	Kang JS, Zheng Z, Choi MJ, Lee SH, Kim DY, Cho SB. The effect of CD34+ cell-containing autologous platelet-rich plasma injection on pattern hair loss: A preliminary study. <i>Journal of the European Academy of Dermatology and Venereology</i> . 2014;28(1):72-79.	4	15				PRP
45	Katz HI, Hien NT, Prawer SE, Goldman SJ. Long-term efficacy of topical minoxidil in male pattern baldness. <i>Journal of the American Academy of Dermatology</i> . 1987;16(3 II SUPPL.):711-718.	11			4 and 12 Months		Minoxidil 2%
46	Kaufman KD, Olsen EA, Whiting D, et al. Finasteride in the treatment of men with androgenetic alopecia. Finasteride Male Pattern Hair Loss Study Group. <i>Journal of the American Academy of Dermatology</i> . 1998;39(4 Pt 1):578-589.	2	15				Finasteride
47	Kawashima M, Hayashi N, Igarashi A, et al. Finasteride in the treatment of Japanese men with male pattern hair loss. <i>European Journal of Dermatology</i> . 2004;14(4):247-254.	14					Finasteride
48	Kim H, Choi JW, Kim JY, Shin JW, Lee SJ, Huh CH. Low-level light therapy for androgenetic alopecia: A 24-week, randomized, double-blind, sham device-controlled multicenter trial. <i>Dermatologic Surgery</i> . 2013;39(8):1177-1183.	2	12	15			LLLT
49	Koperski JA, Orenberg EK, Wilkinson DI. Topical minoxidil therapy for androgenetic alopecia. A 30-month study. <i>Archives of Dermatology</i> . 1987;123(11):1483- 1487.	11	10	14	4 and 12 Months		Minoxidil 2%

*Hair Regrowth Treatment Efficacy and Resistance in Androgenetic Alopecia:
A Systematic Review and Continuous Bayesian Network Meta-Analysis*

50	Lanzafame RJ, Blanche RR, Bodian AB, Chiacchierini RP, Fernandez-Obregon A, Kazmirek ER. The growth of human scalp hair mediated by visible red light laser and LED sources in males. <i>Lasers in Surgery and Medicine</i> . 2013;45(8):487-495.	10			16 Weeks	LLLT
51	Lanzafame RJ, Blanche RR, Chiacchierini RP, Kazmirek ER, Sklar JA. The growth of human scalp hair in females using visible red light laser and LED sources. <i>Lasers in Surgery and Medicine</i> . 2014;46(8):601-607.	10			16 Weeks	LLLT
52	Leavitt M, Charles G, Heyman E, Michaels D. HairMax LaserComb laser phototherapy device in the treatment of male androgenetic alopecia: A randomized, double-blind, sham device-controlled, multicentre trial. <i>Clin Drug Invest</i> . 2009;29(5):283-292.	1				LLLT
53	Lee WS, Chul Eun H, Sang Kwon O, In Ro B, Young Sim W. Effect of dutasteride on male pattern baldness: Phase III clinical trial: Hormones, hair growth and pattern hair loss. <i>Experimental Dermatology</i> . 2010;19 (6):572.	2	15			Dutasteride
54	Lengg N, Heidecker B, Seifert B, Trueb RM. Dietary supplement increases anagen hair rate in women with telogen effluvium: Results of a double-blind, placebo-controlled trial. <i>Therapy</i> . 2007;4(1):59-65.	6	15			Pantogar
55	Leyden J, Dunlap F, Miller B, et al. Finasteride in the treatment of men with frontal male pattern hair loss. <i>Journal of the American Academy of Dermatology</i> . 1999;40(6 I):930-937.	2	15			Finasteride
56	Lopez-Bran E, Robledo A, Aspiolea F, et al. Multicenter comparative study of the efficacy of topical 2% minoxidil (Regaine) versus placebo in the treatment of male baldness. <i>Advances in Therapy</i> . 1990;7(3):159-168.	11				Minoxidil 2%
57	Lucky AW, Piacquadio DJ, Ditre CM, et al. A randomized, placebo-controlled trial of 5% and 2% topical minoxidil solutions in the treatment of female pattern hair loss. <i>Journal of the American Academy of Dermatology</i> . 2004;50(4):541-553.	1				Minoxidil 2%, Minoxidil 5%
58	Maddin WS, Bell PW, James JH. The biological effects of a pulsed electrostatic field with specific reference to hair. <i>Electrotrichogenesis</i> . <i>International Journal of Dermatology</i> . 1990;29(6):446-450.	3				Electrostatic
59	Mapar MA, Shahriari S, Haghighizadeh MH. Efficacy of platelet-rich plasma in the treatment of androgenetic (male-patterned) alopecia: A pilot randomized controlled trial. <i>Journal of Cosmetic and Laser Therapy</i> . 2016;18(8):452-455.	7			Split Head	PRP
60	Morganti P, Fabrizi G, James B, Bruno C. Effect of gelatin-cystine and serenoa repens extract on free radicals level and hair growth. <i>Journal of Applied Cosmetology</i> . 1998;16(3):57-64.	2			Outcome in %	Serenoa Repens
61	Olsen EA. Topical minoxidil in the treatment of androgenetic alopecia in women. <i>Cutis</i> . 1991;48(3):243-246+248.	1				Minoxidil 2%
62	Olsen EA, DeLong ER, Weiner MS. Dose-response study of topical minoxidil in male pattern baldness. <i>Journal of the American Academy of Dermatology</i> . 1986;15(1):30-37.	11				Minoxidil 2%
63	Olsen EA, Dunlap FE, Funicella T, et al. A randomized clinical trial of 5% topical minoxidil versus 2% topical minoxidil and placebo in the treatment of androgenetic alopecia in men. <i>Journal of the American Academy of Dermatology</i> . 2002;47(3):377-385.	1				Minoxidil 2%, Minoxidil 5%

*Hair Regrowth Treatment Efficacy and Resistance in Androgenetic Alopecia:
A Systematic Review and Continuous Bayesian Network Meta-Analysis*

64	Olsen EA, Hordinsky M, Whiting D, et al. The importance of dual 5alpha-reductase inhibition in the treatment of male pattern hair loss: results of a randomized placebo-controlled study of dutasteride versus finasteride. <i>Journal of the American Academy of Dermatology</i> . 2006;55(6):1014-1023.	2	15			Dutasteride, Finasteride
65	Olsen EA, Weiner MS, Delong ER, Pinnell SR. Topical minoxidil in early male pattern baldness. <i>Journal of the American Academy of Dermatology</i> . 1985;13(2 I):185-192.	2	15			Minoxidil 2%
66	Olsen EA, Whiting D, Bergfeld W, et al. A multicenter, randomized, placebo-controlled, double-blind clinical trial of a novel formulation of 5% minoxidil topical foam versus placebo in the treatment of androgenetic alopecia in men. <i>Journal of the American Academy of Dermatology</i> . 2007;57(5):767-774.	10			16 Weeks	Minoxidil 5%
67	Petzoldt D, Borelli S, Braun-Falco O, et al. The German double-blind placebo-controlled evaluation of topical minoxidil solution in the treatment of early male pattern baldness. <i>International Journal of Dermatology</i> . 1988;27(6 SUPPL.):430-434.	11				Minoxidil 2%
68	Pierard-Franchimont C, De Doncker P, Cauwenbergh G, Pierard GE. Ketoconazole shampoo: effect of long-term use in androgenic alopecia. <i>Dermatology</i> . 1998;196(4):474-477.	2				Ketoconazole
69	Prasad HKNR. A randomized double blind study of the effect of finasteride on hair growth in male patients of androgenetic alopecia. <i>Indian journal of dermatology</i> . 2005;50(3):139-145.	11				Finasteride
70	Price VH, Menefee E. Quantitative estimation of hair growth I. Androgenetic alopecia in women: Effect of minoxidil. <i>Journal of Investigative Dermatology</i> . 1990;95(6):693-687.	2				Minoxidil 2%
71	Price VH, Roberts JL, Hordinsky M, et al. Lack of efficacy of finasteride in postmenopausal women with androgenetic alopecia. <i>Journal of the American Academy of Dermatology</i> . 2000;43(5):768-776.	2	15			Finasteride
72	Puig CJ, Reese R, Peters M. Double-blind, placebo-controlled pilot study on the use of platelet-rich plasma in women with female androgenetic alopecia. <i>Dermatologic Surgery</i> . 2016;42(11):1243-1247.	7				PRP
73	Pumthong G, Asawanonda P, Varothai S, et al. Curcuma aeruginosa, a novel botanically derived 5alpha-reductase inhibitor in the treatment of male-pattern baldness: a multicenter, randomized, double-blind, placebo-controlled study. <i>Journal of Dermatological Treatment</i> . 2012;23(5):385-392.	1			Curcumin not of interest	Minoxidil 5%
74	Rietschel RL, Duncan SH. Safety and efficacy of topical minoxidil in the management of androgenetic alopecia. <i>Journal of the American Academy of Dermatology</i> . 1987;16(3 II SUPPL.):677-685.	11				Minoxidil 2%
75	Rizer RL, Stephens TJ, Herndon JH, Sperber BR, Murphy J, Ablon GR. A Marine Protein-based Dietary Supplement for Subclinical Hair Thinning/Loss: Results of a Multisite, Double-blind, Placebo-controlled Clinical Trial. <i>Int J Trichology</i> . 2015;7(4):156-166.	2	6		AGA Excluded	Viviscal
76	Roberts JL. Androgenetic alopecia: Treatment results with topical minoxidil. <i>Journal of the American Academy of Dermatology</i> . 1987;16(3 II SUPPL.):705-710.	11				Minoxidil 2%
77	Rushton DH, Unger WP, Cotterill PC, Kingsley P, James KC. Quantitative assessment of 2% topical minoxidil in the treatment of male pattern baldness. <i>Clinical and Experimental Dermatology</i> . 1989;14(1):40-46.	5				Minoxidil 2%

*Hair Regrowth Treatment Efficacy and Resistance in Androgenetic Alopecia:
A Systematic Review and Continuous Bayesian Network Meta-Analysis*

78	Sasaki GH. The Effects of Lower Versus Higher Cell Number of Platelet-Rich Plasma (PRP) in Hair Density and Diameter in Androgenetic Alopecia (AGA): A Randomized, Double-Blinded, Placebo, Paralleled Group Half-Scalp IRB Study. <i>Aesthet.</i> 2021;29:29.	5			Split Head	PRP
79	Schulz C, Bielfeldt S, Reimann J. Fenugreek + micronutrients: Efficacy of a food supplement against hair loss. <i>Kosmetische Medizin.</i> 2006;27(4):176-179.	2	12			Fenugreek
80	Shapiro J, Ho A, Sukhdeo K, Yin L, Lo Sicco K. Evaluation of platelet-rich plasma as a treatment for androgenetic alopecia: A randomized controlled trial. <i>Journal of the American Academy of Dermatology.</i> 2020;83(5):1298-1303.	10	15		Split Head	PRP
81	Shupack JL, Kassimir JJ, Thirumoorthy T, Reed ML, Jondreau L. Dose-response study of topical minoxidil in male pattern alopecia. <i>Journal of the American Academy of Dermatology.</i> 1987;16(3 Pt 2):673-676.	11				Minoxidil 2%
82	Sisto T, Bussoletti C, Celleno L. Efficacy of a cosmetic caffeine shampoo in androgenetic alopecia management. II Note. <i>Journal of Applied Cosmetology.</i> 2013;31(1):57-66.	14				Caffeine
83	Stough D. Dutasteride improves male pattern hair loss in a randomized study in identical twins. <i>Journal of Cosmetic Dermatology.</i> 2007;6(1):9-13.	2	15			Dutasteride
84	Stough DB, Rao NA, Kaufman KD, Mitchell C. Finasteride improves male pattern hair loss in a randomized study in identical twins. <i>European Journal of Dermatology.</i> 2002;12(1):32-37.	14				Finasteride
85	Suchonwanit P, Chalermroj N, Khunkhet S. Low-level laser therapy for the treatment of androgenetic alopecia in Thai men and women: a 24-week, randomized, double-blind, sham device-controlled trial. <i>Lasers in Medical Science.</i> 2019;34(6):1107-1114.	2	15			LLLT
86	Tak YJ, Lee SY, Cho AR, Kim YS. A randomized, double-blind, vehicle-controlled clinical study of hair regeneration using adipose-derived stem cell constituent extract in androgenetic alopecia. <i>Stem Cells Translational Medicine.</i> 2020.	10	12			Stem Cell Tx
87	Tan CH, Lee JSS, Tan KT, Wang ECE, Chan RKW, Chuah SY. A randomized double-blind, split-scalp, placebo-controlled study to evaluate the efficacy of platelet-rich plasma for the treatment of androgenetic alopecia. <i>British Journal of Dermatology.</i> 2019;181 (Supplement 1):51.	14				PRP
88	Tawfik AA, Osman MAR. The effect of autologous activated platelet-rich plasma injection on female pattern hair loss: A randomized placebo-controlled study. <i>Journal of Cosmetic Dermatology.</i> 2018;17(1):47-53.	2	15			PRP
89	Thom E. Efficacy and tolerability of hairgain in individuals with hair loss: A placebo-controlled, double-blind study. <i>Journal of International Medical Research.</i> 2001;29(1):2-6.	2	12			Viviscal/Hairgain
90	Thom E. Nourkrin: Objective and subjective effects and tolerability in persons with hair loss. <i>Journal of International Medical Research.</i> 2006;34(5):514-519.	2	12			Nourkrin

*Hair Regrowth Treatment Efficacy and Resistance in Androgenetic Alopecia:
A Systematic Review and Continuous Bayesian Network Meta-Analysis*

91	Tsuboi R, Niiyama S, Irisawa R, Harada K, Nakazawa Y, Kishimoto J. Autologous cell-based therapy for male and female pattern hair loss using dermal sheath cup cells: A randomized placebo-controlled double-blinded dose-finding clinical study. <i>Journal of the American Academy of Dermatology</i> . 2020.	12	15			Stem Cell Tx
92	Uzel BPC, Takano GHS, Chartuni JCN, et al. Intradermal injections with 0.5% minoxidil for the treatment of female androgenetic alopecia: A randomized, placebo-controlled trial. <i>Dermatol Ther</i> . 2021;34(1):e14622.	3				Minoxidil
93	Van Neste D, Fuh V, Sanchez-Pedreno P, et al. Finasteride increases anagen hair in men with androgenetic alopecia. <i>British Journal of Dermatology</i> . 2000;143(4):804-810.	2				Finasteride
94	Whiting DA, Jacobson C. Treatment of female androgenetic alopecia with minoxidil 2%. <i>International Journal of Dermatology</i> . 1992;31(11):800-804.	1				Minoxidil 2%
95	Whiting DA, Waldstreicher J, Sanchez M, Kaufman KD. Measuring reversal of hair miniaturization in androgenetic alopecia by follicular counts in horizontal sections of serial scalp biopsies: Results of finasteride 1 mg treatment of men and postmenopausal women. <i>Journal of Investigative Dermatology Symposium Proceedings</i> . 1999;4(3):282-284.	11			Biopsy	Finasteride
96	Yazici Y, Smith S, Swearingen C, Simsek I, Di Francesco A, Hood J. Safety and efficacy of a topical treatment (SM04554) for androgenetic alopecia (AGA): Results from a phase 1 Trial. <i>Journal of the American Academy of Dermatology</i> . 2016;1):AB138.	3				Wnt Activator
97	Zanardo L. Effects of a Low Level Laser Therapy on Hormonal, Age-Related Hair Loss in Women after Treatment of 6 Months. [German]. <i>Aktuelle Dermatologie</i> . 2016;42(3):90-95.	7				LLLT
98	Zimber MP, Ziering C, Zeigler F, et al. Hair regrowth following a Wnt- and follistatin containing treatment: safety and efficacy in a first-in-man phase 1 clinical trial. <i>J Drugs Dermatol</i> . 2011;10(11):1308-1312.	3				Wnt Activator

*Hair Regrowth Treatment Efficacy and Resistance in Androgenetic Alopecia:
A Systematic Review and Continuous Bayesian Network Meta-Analysis*

Article Database Search Methodology and Strategy Report

Search Methodology:

Alopecia standard of care treatments safety and efficacy - RCTs

Methods section (suggested text for journal article)

A professional librarian (EU) ran a search in the MEDLINE, Medline-in-Process, Medline ePub Ahead of Print and EMBASE databases(OvidSP); and Cochrane (Wiley) on August 11, 2021. We used both subject headings and textword terms to search for articles on Alopecia AND current standard of care replacement therapies (e.g.minoxidil or finasteride or duasteride or biotin or low level laser light therapy or supplements or or transplant, etc.) AND Randomized Clinical Trials. The search results were not limited to publication date or language.

We retrieved a total of **3153** references. All references were saved in an EndNote library used to identify the **843** duplicates. The remaining **2310 + 3** hand searches + **1** grey search unique references were reviewed against our inclusion criteria. The complete search strategies are listed in below.

Database	Dates	Total	Unique	Duplicates
Cochrane	Issue 8 of 12, August 2021	829	422	407
Embase	1980 to 2021 Week 31	957	847	110
HandSearches	Scholarly research	3	3	0
Grey Literature Search	On file	1	1	0
MEDLINE(R)	1946 to July Week 5 2021	1219	953	266
Medline Epub Ahead of Print	to August 10, 2021	18	16	2
Medline -in-Process	1946 to August 10, 2021	130	72	58
Total		3157	2314	843

*Hair Regrowth Treatment Efficacy and Resistance in Androgenetic Alopecia:
A Systematic Review and Continuous Bayesian Network Meta-Analysis*

Search Strategy:

#	Searches	Results	Type
1	Alopecia/	11636	
2	((androgenetica or androgenetic or androgenic or male or female) adj2 Alopecia*).ti,ab,kf.	1869	
3	(telogen adj2 effluvium).ti,ab,kf.	261	
4	((("Non scarring" or "non-scarring") adj2 alopecia*).ti,ab,kf.	103	
5	(hair adj2 (loss or thinning or plug or plugs)).ti,ab,kf.	6481	
6	(Baldness or Balding).ti,ab,kf.	1288	
7	("self-reported" adj2 thinning).ti,ab,kf.	0	
8	Hair Follicle/in [Injuries]	38	
9	hair follicle/ and (injury or injuries).ti,ab,kf.	213	
10	or/1-9	16524	Alopecia terms
11	minoxidil/ or (minoxidil or aloctan or alopey or alopeyxl or alostil or aloxidil or "apo-gain" or crecisan or growell or "hair-treat" or hairgain or hairgrow or headway or hebald or kapodin or kenacin or lacovin or locemix or "locion epc" or loniten or lonnoten or lonolox or lonoten or manoxidil or rogain or minocutan or minodyl or minona or minorga or minotricon or minovital or "minoxi 5" or minoxicutan or minoxidil or minoximen or minoxitrim or minoxyl or moxidil or multigain or neocapil or neoxidil or nuhair or prexidil or regaine or regroe or regrou or regrowth or rehair or theroxidil or tiazolin or trefostil or unipexil or ylox).mp. or 38304-91-5.rn.	12123	
12	finasteride/ or (finasteride or alocare or andozac or "chibro proscar" or "chibro-proscar" or finastar or finastid or fincar or finired or finpro or fistrin or folians or frosst or genaprost or harifin or "l 652931" or l652931 or "mk 0906" or "mk 906" or mk0906 or mk906 or nasterol or pilus or "pro-cure" or prohair or propesia or propeshia or proscar or prosh or prostacare or prostacom or prostide or prostop or reprostom or symasteride or tensen or uromedin or "ym 152" or m152).mp. or 98319-26-7.rn.	6617	
13	Dutasteride/ or (dutasteride or advodart or avidart or avodart or avolve or duagen or "gg 745" or gg745 or "gi 198745" or "gi 198745x" or gi198745 or gi198745x).mp. or 164656-23-9.rn.	665	
14	Low-Level Light Therapy/ or (low adj2 (level or light or intensity or energy or laser or power) adj2 (therap* or treat*)).ti,ab,kf.	8107	
15	Viviscal.mp.	3	
16	exp Dietary Supplements/ or (nutraceutical* or supplement*).mp.	342827	
17	Nutrafol.mp.	2	
18	biotin/ or (biotin or "bio h tin" or bioepiderm or "bios ii" or biotine or "coenzyme r" or qizenday or "vitamin bw" or "vitamin h").mp. or 58-85-5.rn.	31986	
19	biocytin/ or (biocytin or biotinyllysine or "n biotinyl l lysine").mp. or 576-19-2.rn.	2294	
20	"2 iminobiotin".mp.	61	
21	("2 iminobiotin" or iminobiotin or guanidinobiotin).mp. or 13395-35-2.rn.	105	
22	exp Collagen/ and (administration, oral/ or administration, buccal/ or administration, sublingual/ or administration, topical/ or administration, cutaneous/)	1254	
23	seafood/ or fish products/ or exp shellfish/	16158	
24	((Marine or fish) adj2 extract).mp.	293	
25	exp sharks/	5606	
26	(samumed or SM04554).mp.	0	
27	Replicel.mp.	0	
28	Electric Stimulation Therapy/ or Electric Stimulation/	134849	

Appendix 2 – Search Strategy, Articles Searched and Reviewed
Front. Med. 9:998623. doi: 10.3389/fmed.2022.998623

*Hair Regrowth Treatment Efficacy and Resistance in Androgenetic Alopecia:
A Systematic Review and Continuous Bayesian Network Meta-Analysis*

29	(Haircell or electrostimulation).mp.	3098	
30	(follicle or (hair adj2 follicle adj2 Neogenesis) or "FOL-004" or wounding).mp.	7682	
31	platelet-rich plasma/ or platelet-rich fibrin/ or (platelet adj2 rich adj2 plasma*).ti,ab,kf. or ("platelet-rich" adj2 plasma*).ti,ab,kf.	10503	
32	hair/tr or hair follicle/tr or (hair adj2 (autograft* or graft* or transplant*).ti,ab,kf.	1379	
33	or/11-32	577691	Hair loss medications, supplements, transplants
34	10 and 33	2735	Base clinical set
35	controlled clinical trial.pt.	94293	
36	randomized controlled trial.pt.	538640	
37	randomized.ab.	456987	
38	placebo.ab.	199567	
39	drug therapy.fs.	2354230	
40	randomly.ab.	307217	
41	trial.ab.	483405	
42	groups.ab.	1897753	
43	or/35-42	4634227	
44	exp animals/ not humans.sh.	4867787	
45	43 not 44	3972091	Cochrane Therapy filter box 3.c
46	34 and 45	1209	Cochrane therapy filter results
47	limit 34 to multicenter study	60	Multicenter studies
48	46 or 47	1219	Final results

Medline Epub Ahead of Print

#	Searches	Results	Comment
1	((androgenetica or androgenetic or androgenic or male or female) adj2 Alopecia*).ti,ab,kf.	77	
2	(telogen adj2 effluvium).ti,ab,kf.	12	
3	((("Non scarring" or "non-scarring") adj2 alopecia*).ti,ab,kf.	3	
4	(hair adj2 (loss or thinning or plug or plugs)).ti,ab,kf.	168	
5	(Baldness or Balding).ti,ab,kf.	24	
6	("self-reported" adj2 thinning).ti,ab,kf.	0	
7	(hair adj2 follicle adj2 (injury or injuries)).ti,ab,kf.	1	
8	or/1-7	233	Alopecia terms
9	(minoxidil or aloctan or alopey or alopeyxl or alostil or aloxidil or "apogain" or crecisan or growell or "hair-treat" or hairgain or hairgrow or headway or hebal or kapodin or kenacin or lacovin or locemix or "locion epc" or loniten or lonnoten or lonolox or lonoten or manoxidil or rogaine or minocutan or minodil or minona or minorga or minotrican or minovital or "minoxi 5" or minoxicutan or minoxidil or minoximen or minoxitrim or minoxyl or moxidil or multigain or neocapil or neoxidil or nuhair or prexidil or regaine or regroe or regrou or regrowth or rehair or theroxidil or tiazolin or trefostil or unipexil or ylox).mp.	199	
10	(finasteride or alozare or andozac or "chibro proscar" or "chibro-proscar" or finastar or finastid or fincar or finired or finpro or fistrin or folians or frosst or genaprost or harifin or "l 652931" or l652931 or "mk 0906" or "mk 906"	58	

Appendix 2 – Search Strategy, Articles Searched and Reviewed

Front. Med. 9:998623. doi: 10.3389/fmed.2022.998623

*Hair Regrowth Treatment Efficacy and Resistance in Androgenetic Alopecia:
A Systematic Review and Continuous Bayesian Network Meta-Analysis*

	or mk0906 or mk906 or nasterol or pilus or "pro-cure" or prohair or propecia or propeshia or proscar or prosh or prostacare or prostacom or prostide or prostop or reprostom or symasteride or tensen or uromedin or "ym 152" or m152).mp.		
11	(dutaseride or advodart or avidart or avodart or avolve or duagen or "gg 745" or gg745 or "gi 198745" or "gi 198745x" or gi198745 or gi198745x).mp.	3	
12	(low adj2 (level or light or intensity or energy or laser or power) adj2 (therap* or treat*)).ti,ab,kf.	115	
13	Viviscal.mp.	0	
14	(nutraceutical* or supplement*).mp.	7367	
15	Nutrafol.mp.	0	
16	(biotin or "bio h tin" or bioepiderm or "bios ii" or biotine or "coenzyme r" or qizenday or "vitamin bw" or "vitamin h").mp.	191	
17	(biocytin or biotinyllysine or "n biotinyl l lysine").mp. or 576-19-2.rn.	6	
18	("2 iminobiotin" or iminobiotin or guanidinobiotin).mp. or 13395-35-2.rn.	1	
19	(Collagen adj2 (oral or orally or topical* or transdermal* or cutaneous*)).ti,ab,kf.	9	
20	(seafood or fish or shellfish).ti,ab,kf.	2246	
21	((Marine or fish) adj2 extract).mp.	3	
22	(shark or sharks).ti,ab,kf.	86	
23	(samumed or SM04554).mp.	0	
24	Replicel.mp.	0	
25	(Electric adj2 Stimulation).ti,ab,kf.	63	
26	(Haircell or electrostimulation).mp.	25	
27	(follicle or (hair adj2 follicle adj2 Neogenesis) or "FOL-004" or wounding).mp.	102	
28	((platelet adj2 rich adj2 (plasma* or fibrin*)) or ("platelet-rich" adj2 (fibrin or plasma*))).ti,ab,kf.	300	
29	(hair adj2 (autograft* or graft* or transplant*)).ti,ab,kf.	40	
30	or/9-29	10569	Hair loss medications, supplements, transplants
31	8 and 30	67	Base clinical set
32	((randomized or controlled or multicentre or multicenter) adj (trial or trials)).ti,ab,kf.	8734	
33	randomized.ab.	11247	
34	placebo.ab.	3012	
35	randomly.ab.	5447	
36	trial.ab.	11797	
37	groups.ab.	38625	
38	or/32-37	55599	Therapy filter
39	31 and 38	18	Therapy filter results

Medline-in-Process & In-Data-Review Citations

#	Searches	Results	Comment
1	((androgenetica or androgenetic or androgenic or male or female) adj2 Alopecia*).ti,ab,kf.	53	
2	(telogen adj2 effluvium).ti,ab,kf.	10	
3	((("Non scarring" or "non-scarring") adj2 alopecia*).ti,ab,kf.	9	
4	(hair adj2 (loss or thinning or plug or plugs)).ti,ab,kf.	173	
5	(Baldness or Balding).ti,ab,kf.	10	

Appendix 2 – Search Strategy, Articles Searched and Reviewed

Front. Med. 9:998623. doi: 10.3389/fmed.2022.998623

*Hair Regrowth Treatment Efficacy and Resistance in Androgenetic Alopecia:
A Systematic Review and Continuous Bayesian Network Meta-Analysis*

6	("self-reported" adj2 thinning).ti,ab,kf.	0	
7	(hair adj2 follicle adj2 (injury or injuries)).ti,ab,kf.	0	
8	or/1-7	227	Alopecia terms
9	(minoxidil or alocutan or alopey or alopexyl or alostil or aloxidil or "apo-gain" or crecisan or growell or "hair-treat" or hairgaine or hairgrow or headway or hebald or kapodin or kenacin or lacovin or locemix or "locion epc" or loniten or lonnoten or lonolox or lonoten or manoxidil or rogain or minocutan or minodyl or minona or minorga or minotricon or minovital or "minoxi 5" or minoxicutan or minoxidil or minoximen or minoxitrim or minoxyl or moxidil or multigain or neocapil or neoxidil or nuhair or prexidil or regaine or regroe or regrou or regrowth or rehair or theroxidil or tiazolin or trefostil or unipexil or ylox).mp.	204	
10	(finasteride or alocare or andozac or "chibro proscar" or "chibro-proscar" or finastar or finastid or fincar or finired or finpro or fistrin or folians or frosst or genaprost or harifin or "l 652931" or l652931 or "mk 0906" or "mk 906" or mk0906 or mk906 or nasterol or pilus or "pro-cure" or prohair or propecia or propeshia or proscar or prosh or prostacare or prostacom or proside or prostop or reprostop or symasteride or tensen or uromedin or "ym 152" or m152).mp.	101	
11	(dutaseride or advodart or avidart or avodart or avolve or duagen or "gg 745" or gg745 or "gi 198745" or "gi 198745x" or gi198745 or gi198745x).mp.	0	
12	(low adj2 (level or light or intensity or energy or laser or power) adj2 (therap* or treat*)).ti,ab,kf.	97	
13	Viviscal.mp.	0	
14	(nutraceutical* or supplement*).mp.	7015	
15	Nutrafol.mp.	0	
16	(biotin or "bio h tin" or bioepiderm or "bios ii" or biotine or "coenzyme r" or qizenday or "vitamin bw" or "vitamin h").mp.	254	
17	(biocytin or biotinyllysine or "n biotinyl l lysine").mp. or 576-19-2.rn.	1	
18	("2 iminobiotin" or iminobiotin or guanidinobiotin).mp. or 13395-35-2.rn.	1	
19	(Collagen adj2 (oral or orally or topical* or transdermal* or cutaneous*)).ti,ab,kf.	4	
20	(seafood or fish or shellfish).ti,ab,kf.	2862	
21	((Marine or fish) adj2 extract).mp.	11	
22	(shark or sharks).ti,ab,kf.	107	
23	(samumed or SM04554).mp.	0	
24	Replicel.mp.	0	
25	(Electric adj2 Stimulation).ti,ab,kf.	87	
26	(Haircell or electrostimulation).mp.	35	
27	(follica or (hair adj2 follicle adj2 Neogenesis) or "FOL-004" or wounding).mp.	92	
28	((platelet adj2 rich adj2 (plasma* or fibrin*)) or ("platelet-rich" adj2 (fibrin or plasma*))).ti,ab,kf.	301	
29	(hair adj2 (autograft* or graft* or transplant*)).ti,ab,kf.	12	
30	or/9-29	10906	Hair loss medications, supplements, transplants
31	8 and 30	51	Base clinical set
32	((randomized or controlled or multicentre or multicenter) adj (trial or trials)).ti,ab,kf.	8874	
33	randomized.ab.	12443	
34	placebo.ab.	3502	
35	randomly.ab.	6098	

Appendix 2 – Search Strategy, Articles Searched and Reviewed

Front. Med. 9:998623. doi: 10.3389/fmed.2022.998623

*Hair Regrowth Treatment Efficacy and Resistance in Androgenetic Alopecia:
A Systematic Review and Continuous Bayesian Network Meta-Analysis*

36	trial.ab.	14205	
37	groups.ab.	40718	
38	or/32-37	59679	Therapy filter
39	31 and 38	130	Therapy filter results

EMBASE

#	Searches	Results	Comment
1	alopecia/ or male type alopecia/	45106	
2	((androgenetica or androgenetic or androgenic or male or female) adj2 Alopecia*).ti,ab,kw.	3335	
3	hair loss/	13572	
4	(telogen adj2 effluvium).ti,ab,kw.	567	
5	((("Non scarring" or "non-scarring") adj2 alopecia*).ti,ab,kw.	276	
6	(hair adj2 (loss or thinning or plug or plugs)).ti,ab,kw.	11572	
7	(Baldness or Balding).ti,ab,kw.	1844	
8	("self-reported" adj2 thinning).ti,ab,kw.	1	
9	exp hair follicle/ and (injury or injuries).ti,ab,kw.	620	
10	or/1-9	60420	Alopecia terms
11	minoxidil/ or (minoxidil or alocutan or alopey or alopeyyl or alostil or aloxil or "apo-gain" or crecisan or growell or "hair-treat" or hairgain or hairgrow or headway or hebald or kapodin or kenacin or lacovin or locemix or "locion epc" or loniten or lonnoten or lonolox or lonoten or manoxidil or rogaie or minocutan or minodil or minona or minorga or minotricon or minovital or "minoxi 5" or minoxicutan or minoxidil or minoximen or minoxitrim or minoxyl or moxidil or multigain or neocapil or neoxidil or nuhair or prexidil or regaine or regroe or regrou or regrowth or rehair or theroxidil or tiazolin or trefostil or unipexil or ylox).mp. or 38304-91-5.rn.	21142	
12	finasteride/ or (finasteride or alocare or andozac or "chibro proscar" or "chibro-proscar" or finastar or finastid or fincar or finired or finpro or fistrin or folians or frosst or genaprost or harifin or "l 652931" or l652931 or "mk 0906" or "mk 906" or mk0906 or mk906 or nasterol or pilus or "pro-cure" or prohair or propecia or propeshia or proscar or prosh or prostacare or prostacom or prostide or prostop or reprostom or symasteride or tensen or uromedin or "ym 152" or m152).mp. or 98319-26-7.rn.	15696	
13	Dutasteride/ or (dutasteride or advodart or avidart or avodart or avolve or duagen or "gg 745" or gg745 or "gi 198745" or "gi 198745x" or gi198745 or gi198745x).mp. or 164656-23-9.rn.	3031	
14	dutasteride plus tamsulosin/ or (combodart or duodart or jalyn or jelnu or juteo).mp.	128	
15	dutasteride plus testosterone/	5	
16	Low level light laser therapy/ or (low adj2 (level or light or intensity or energy or laser or power) adj2 (therap* or treat*)).ti,ab,kw.	6413	
17	Viviscal.mp.	13	
18	nutraceutical/	5469	
19	nutraceutical*.mp.	12453	
20	exp supplementation/	263577	
21	Nutrafol.mp.	6	
22	biotin/ or (biotin or "bio h tin" or bioepiderm or "bios ii" or biotine or "coenzyme r" or qizenday or "vitamin bw" or "vitamin h").mp. or 58-85-5.rn.	38481	
23	biotin derivative/	734	
24	biocytin/ or (biocytin or biotinyllysine or "n biotinyl l lysine").mp. or 576-19-2.rn.	1939	

Appendix 2 – Search Strategy, Articles Searched and Reviewed

Front. Med. 9:998623. doi: 10.3389/fmed.2022.998623

*Hair Regrowth Treatment Efficacy and Resistance in Androgenetic Alopecia:
A Systematic Review and Continuous Bayesian Network Meta-Analysis*

25	"2 iminobiotin"/ or (iminobiotin or guanidinobiotin).mp. or 13395-35-2.rn. [****EMBASE since 2015****]	132	
26	exp collagen/po, tp, td [Oral Drug Administration, Topical Drug Administration, Transdermal Drug Administration]	236	
27	exp fish product/	2216	
28	((Marine or fish) adj2 extract).mp.	497	
29	shark/	4187	
30	shellfish/ or shellfish protein/	5617	
31	(samumed or SM04554).mp.	31	
32	Replicel.mp.	1	
33	electrostimulation/	67299	
34	(Haircell or electrostimulation).mp.	77705	
35	(follicle or (hair adj2 follicle adj2 Neogenesis) or "FOL-004" or wounding).mp.	10155	
36	thrombocyte rich plasma/ or platelet-rich fibrin/ or (platelet adj2 rich adj2 plasma*).ti,ab,kw. or ("platelet-rich" adj2 plasma*).ti,ab,kw.	18894	
37	hair transplantation/ or (hair adj2 (autograft* or graft* or transplant*).ti,ab,kw.	1781	
38	or/11-37	475887	Hair loss medications, supplements, transplants
39	10 and 38	6085	Base clinical set
40	randomized controlled trial/	665763	
41	Controlled clinical study/	463547	
42	random*.ti,ab.	1676923	
43	Randomization/	91335	
44	Intermethod comparison/	273774	
45	placebo*.ti,ab.	323213	
46	(compare or compared or comparison).ti.	522507	
47	((evaluated or evaluate or evaluating or assessed or assess) and (compare or compared or comparing or comparison)).ab.	2333287	
48	(open adj label).ti,ab.	89443	
49	((double or single or doubly or singly) adj (blind or blinded or blindly)).ti,ab.	239450	
50	double-blind procedure/	183270	
51	Parallel group\$1.ti,ab.	27828	
52	(Crossover or cross over).ti,ab.	109304	
53	((assign* or match or matched or allocation) adj5 (alternate or group\$1 or intervention\$1 or patient\$1 or subject\$1 or participant\$1)).ti,ab.	356604	
54	(assigned or allocated).ti,ab.	420570	
55	(controlled adj7 (study or design or trial)).ti,ab.	381039	
56	(volunteer or volunteers).ti,ab.	252598	
57	Human experiment/	548561	
58	Trial.ti.	329159	
59	or/40-58	5403014	
60	(Random\$ adj sample\$ adj7 ("cross section\$" or questionnaire\$1 or survey\$1 or database\$1)).ti,ab. not (comparative study/ or controlled study/ or randomi\$ed controlled.ti,ab. or randomly assigned.ti,ab.)	6032	
61	Cross-sectional study/ not (randomized controlled study/ or controlled clinical study/ or controlled study/ or randomi?ed controlled.ti,ab. or control groups\$1.ti,ab.)	280451	
62	((((case adj control\$) and random\$) not randomi?ed controlled).ti,ab.	18732	
63	(Systematic review not (trial or study)).ti.	182121	

Appendix 2 – Search Strategy, Articles Searched and Reviewed

Front. Med. 9:998623. doi: 10.3389/fmed.2022.998623

*Hair Regrowth Treatment Efficacy and Resistance in Androgenetic Alopecia:
A Systematic Review and Continuous Bayesian Network Meta-Analysis*

64	(nonrandom\$ not random\$).ti.ab.	16894	
65	"random field\$".ti.ab.	2533	
66	(random cluster adj3 sampl\$).ti.ab.	1372	
67	(review.ab. and review.pt.) not trial.ti.	909888	
68	("we searched".ab. and review.ti.) or review.pt.	2744712	
69	"update review".ab.	119	
70	(databases adj4 searched).ab.	44360	
71	(rat or rats or mouse or mice or swine or porcine or murine or sheep or lambs or pigs or piglets or rabbit or rabbits or cat or cats or dog or dogs or cattle or bovine or monkey or monkeys or trout or marmoset\$1).ti. and animal experiment/	1066398	
72	Animal experiment/ not (human experiment/ or human/)	2211214	
73	or/60-72	5398301	
74	59 not 73	4672820	Cochrane Therapy filter terms Box 3.e
75	39 and 74	901	Cochrane Therapy filter results
76	limit 39 to (randomized controlled trial or controlled clinical trial or multicenter study)	425	General Trials limit
77	75 or 76	957	Final results

Cochrane

ID	Search	Hits	Comment
#1	[mh alopecia]	682	
#2	((androgenetica or androgenetic or androgenic or male or female) NEAR/2 Alopecia*)	605	
#3	(telogen NEAR/2 effluvium)	35	
#4	((("Non scarring" or "non-scarring") NEAR/2 alopecia*))	8	
#5	(hair NEAR/2 (loss or thinning or plug or plugs))	1365	
#6	(balding or baldness)	225	
#7	("self-reported" NEAR/2 thinning)	2	
#8	MeSH descriptor: [Hair Follicle] this term only and with qualifier(s): [injuries - IN]	0	
#9	[mh ^"hair follicle"] and (injury or injuries)	3	
#10	{or #1-#9}	2147	Alopecia terms
#11	[mh minoxidil] or (minoxidil or aloctan or alopey or alopeyyl or alostil or aloxidl or "apo-gain" or crecisan or growell or "hair-treat" or hairgaine or hairgrow or headway or hebald or kapodin or kenacin or lacovin or locemix or "locion epc" or loniten or lonnoten or lonolox or lonoten or manoxidil or rogaie or minocutan or minodyl or minona or minorga or minotricon or minovital or "minoxi 5" or minoxicutan or minoxidil or minoximen or minoxitrim or minoxyl or moxidil or multigain or neocapil or neoxidil or nuhair or prexidil or regaine or regroe or regrou or regrowth or rehair or theroxidil or tiazolin or trefostil or unipexil or ylox)	1329	
#12	[mh finasteride] or (finasteride or aloicare or andozac or "chibro proscar" or "chibro-proscar" or finastar or finastid or fincar or finired or finpro or fistrin or folians or frosst or genaprost or harifin or "l 652931" or l652931 or "mk 0906" or "mk 906" or mk0906 or mk906 or nasterol or pilus or "pro-cure" or prohair or propecia or propeshia or proscar or prosh or prostacare or prostacom or prostide or prostop	863	

Appendix 2 – Search Strategy, Articles Searched and Reviewed

Front. Med. 9:998623. doi: 10.3389/fmed.2022.998623

*Hair Regrowth Treatment Efficacy and Resistance in Androgenetic Alopecia:
A Systematic Review and Continuous Bayesian Network Meta-Analysis*

	or reprostom or symasteride or tensen or uromedin or "ym 152" or m152)		
#13	[mh Dutasteride] or (dutasteride or advodart or avidart or avodart or avolve or duagen or "gg 745" or gg745 or "gi 198745" or "gi 198745x" or gi198745 or gi198745x)	255	
#14	[mh ^"Low-Level Light Therapy"]	1058	
#15	(low NEAR/2 (level or light or intensity or energy or laser or power) NEAR/2 (therap* or treat*)):ti,ab	2013	
#16	Viviscal	7	
#17	MeSH descriptor: [Dietary Supplements] explode all trees	13343	
#18	(nutraceutical* or supplement* or nutrafol)	95181	
#19	[mh biotin] or (biotin or "bio h tin" or bioepiderm or "bios ii" or biotine or "coenzyme r" or qizenday or "vitamin bw" or "vitamin h")	356	
#20	[mh biocytin] or (biocytin or biotinyllysine or "n biotinyl l lysine")	0	
#21	("2 iminobiotin" or iminobiotin or guanidinobiotin)	6	
#22	[mh Collagen] and ([mh "administration, oral"] or [mh "administration, buccal"] or [mh "administration, sublingual"] or [MH "administration, topical"] or [mh "administration, cutaneous"])	208	
#23	[mh ^seafood] or [mh ^"fish products"] or [mh shellfish]	193	
#24	((Marine or fish) NEAR/2 extract)	25	
#25	[mh sharks]	5	
#26	(samumed or SM04554)	39	
#27	replicel	0	
#28	[mh ^"Electric Stimulation Therapy"] or [mh ^"Electric Stimulation"]	3694	
#29	(Haircell or electrostimulation)	2117	
#30	(follica or (hair NEAR/2 follicle NEAR/2 Neogenesis) or "FOL-004" or wounding)	103	
#31	[mh ^"platelet-rich plasma"] or [mh ^"platelet-rich fibrin"] or (platelet NEAR/2 rich NEAR/2 plasma*) or ("platelet-rich" NEAR/2 plasma*)	2401	
#32	MeSH descriptor: [Hair] explode all trees and with qualifier(s): [transplantation - TR]	16	
#33	(hair NEAR/2 (autograft* or graft* or transplant*))	64	
#34	{or #11-#33}	109586	Hair loss medications, supplements, transplants
#35	#10 and #34	829	Base clinical set

6.2 Summary of Key Findings of Paper 4

This study compared the continuous efficacy of treatments claiming to promote hair regrowth. The literature search identified 2,314 unique articles. Ninety-eight were included for full-text review, and 17 articles met the inclusion criteria for data extraction and analyses with five different therapies in women and seven in men. Eligible treatments included (a) drugs: Dutasteride 0.5 mg/day, Finasteride 1 mg/day, Minoxidil 2% and 5%; (b) low-level laser comb treatment devices (LLLT); and (c) natural products: ALRV5XR, Nutrafol, and Viviscal.

At 24 weeks, the Bayesian network meta-analysis regrowth efficacy in TH/cm² and significance (**) in women were ALRV5XR: 30.09**, LLLT: 16.62**, Minoxidil 2%: 12.13**, Minoxidil 5%: 10.82**, and Nutrafol: 7.32**, and in men; ALRV5XR: 21.03**, LLLT: 18.75**, Dutasteride: 18.37**, Viviscal: 13.23, Minoxidil 5%: 13.13**, Finasteride: 12.38, and Minoxidil 2%: 10.54. There were no statistical differences for the same treatment between women and men. The Bayesian network meta-analysis provided more accurate, statistically relevant, and conservative results than the frequentist network meta-analysis.

Two distinct TH regrowth response profiles were found; Continuous: ALRV5XR regrowth rates were linear in men and accelerated in women; Resistant: after 12 weeks, LLLT, Nutrafol, and Viviscal regrowth rates attenuated while Dutasteride and Finasteride plateaued; Minoxidil 2% and 5% lost some regrowth.

Some TH regrowth can be expected from most AGA treatments, with less variability in women than men. Responses to drug treatments were rapid, showing strong early efficacy followed by the greatest resistance effects from flatlining to loss of regrowth after 12–16 weeks. Finasteride, Minoxidil 2%, and Viviscal in men were not statistically different from Placebo. LLLT

appeared more efficacious than pharmaceuticals. Nutrafol did not attain clinically relevant regrowth, and most treatments were significantly better. Notwithstanding the smaller sample sizes, the BVM treatment, ALRV5XR, showed better efficacy in all tested parameters without signs of treatment resistance.

7 Critical Analysis

The four publications included in this thesis explore a multi-molecular targeting approach to hair regeneration. Of these publications, Chapters 3 and 4 consisted of two articles of randomised placebo-controlled clinical trials (RCTs) of ALRV5XR, a multi-molecular targeting formulation of botanicals, vitamins and minerals (BVMs), that evaluated the safety and efficacy of terminal hair regeneration on the scalps of women and men diagnosed with androgenetic alopecia (AGA), some of whom also had telogen effluvium (TE).^{213,214} Chapter 5 compared sex differences in the RCT's primary results and new secondary trichometric outcomes, including vellus to terminal hair transitions, and analysed the dynamics of hair regeneration.²¹⁵ Whilst Chapter 6 was an exhaustive systematic review (SR) and Bayesian network meta-analysis (MA) that compared these RCT terminal hair (TH) regrowth outcomes to all RCTs of commercially available interventions in the literature.²¹⁶

The objective of these studies and publications was to test a multi-targeting BVM formulation designed to induce homeostasis in hair follicles by targeting the regulation of a broad spectrum of molecular pathways in such a way as to influence a favourable choreography of cell signalling, thereby restoring normal hair regrowth, follicle cycling and anagen prolongation. These novel effects were intended to maintain a normal scalp hair phenotype (SHPh) of the early post pubertal period and potentially lead to the safe regeneration of the individual's SHPh for AGA scalps with impaired hair follicles. Several multi-targeting approaches, combination treatments, expert opinions on restoring the AGA SHPh, and interesting recent hair biology discoveries in the literature will be discussed below. However, until the present time, this AGA SHPh restoration has only been cosmetically achievable by redistributing terminal follicles or

follicular units that are harvested from the occipital region of the scalp using surgical hair transplantation procedures.

The findings in these RCTs for the multi-molecular targeting BVM formulation, as per Chapters 3, 4 and 5, present statistical significance in clinically meaningful terminal hair regrowth outcomes. Despite the small populations in the multi-molecular targeting BVM RCTs, the SR/MA in Chapter 6, showed Bayesian and frequentist statistical superiority in terminal hair regrowth outcomes compared to all eligible commercially available treatments over a 24-week treatment period. The SR/MA also found that all of the eligible commercially available treatments experienced resistance effects to terminal hair regrowth after 12 weeks of treatment. The BVM formulation showed continuous linear regrowth in men and accelerated regrowth in women.

As compelling as these multi-molecular targeting findings are statistically when compared to other treatments, even with male or female population homogeneity as represented by similar populations, age ranges and hair loss patterns, they do present various limitations to generalisable interpretations - firstly, the multi-targeting RCTs in Chapters 3, 4 and 5 are small in populations, and global photographs, quality of life instruments and subject assessment were not evaluable; secondly, input quantification in the SR/MA of Chapter 6 are small in number of studies and some population sizes for different treatments; thirdly, although there appears to be broad acceptance that maintenance of a productive hair follicle is dependent on dermal papilla stem cells and Wnt/ β -catenin signalling, the overall complexity and influences of multiple supporting and opposing molecular actions in the hair follicle are challenging to observe contemporaneously; and fourthly, these actions, as in the example of androgen receptor sensitivity, also appear to have different emphasis in different locations of the scalp, and this

androgen sensitivity also has different effects in women and men, as observed by hair loss patterns, anagen/telogen ratios, terminal/vellus ratios and anisotrichosis scores.

In order to synthesise and develop integrated conclusions from the four studies, and with the abovementioned limitations in mind, this critical analysis will focus on the integration of the results of the studies relating to the clinical outcome's effect of regenerating terminal hair by exploring:

- (1) comparisons of multi-molecular targeting approaches to hair regeneration;
- (2) challenges in the literature and the interpretation of the reported clinical outcomes effects in both RCTs and SR/MAs;
- (3) expert perspectives on regenerating hair;
- (4) novel research that might lead to more effective therapeutics in hair regeneration;
- (5) implications for the broader field of hair loss disorders;
- (6) implications for the wider field of skin disorders; and
- (7) implications for other ageing and degenerative conditions.

7.1 Comparisons to Multi-Targeting Approaches to Hair Regeneration in the Literature

The literature is replete with many hair loss treatment studies using drugs, vitamins, minerals, botanicals, light and electrostimulation devices, and autologous stem cell, platelet-rich plasma and follicular unit transplants. In addition, ethnobotanical treatments such as traditional Chinese medicine (TCM) and Ayurveda have a long history of using various botanicals to treat diseases, including alopecia.^{217,218} TCM used for alopecia dates back at least two thousand years to the Qin Dynasty. There are a large number of TCM botanical formulations with rich experience in treating AGA, although evidence by contemporary standards is very limited.¹⁰

Recently, many BVM ingredients have been studied, showing evidence of both molecular signalling and hair regrowth effects, as illustrated in Table 2.1. Several BVMs have been developed using a multi-targeting approach to treating hair loss, and many ancient traditional medicine formulations have been retrospectively evaluated for pharmacological effects. In many cases, there was corroboration between ethnopharmacological and scientific findings, and experiments demonstrated herbal medicines have been responsible for inducing various required biological effects for hair growth.^{10,219-221} In the context of multi-targeting treatments, there are several studies of combination drug treatments and BVM formulations that are relevant to this analysis.

7.1.1 Combination drug treatments

A recent SR/MA of combination treatments of topical Minoxidil and oral or topical Finasteride vs monotherapy found changes in hair density favoured the monotherapy control groups.²²² In contrast, the same study found that global photographic assessments favoured combination treatments vs monotherapy. Although the study concluded that combination treatments were significantly better than monotherapy by global assessment, the heterogeneity between the studies was very high due to varying study durations, dosages, methods of administration, and the inclusion of open-label studies. Further combination Minoxidil and Finasteride studies with less heterogeneity, will lead to improved evidence for these options.

7.1.2 Mesotherapy

Mesotherapy is a non-surgical, minimally invasive procedure through intradermal or subcutaneous injection so that the drugs can step over the stratum corneum barrier and directly affect tissues rapidly, enhancing pharmacological efficacy.²²³ Drugs, stem cells and platelet-rich plasma (PRP) are commonly used in this procedure, and they are often formulated into a

cocktail with activators and vitamin adjuvants for added effect. Besides the cocktail, wounding is also thought to have a follicular regenerative effect. Mesotherapy RCTs have been performed with Minoxidil, Finasteride, Dutasteride, dexpanthenol, biotin, pyridoxine, amino acids, minerals, hyaluronic acid, vitamins, ginkgo biloba extract, and vitamins.^{224,225} PRP is discussed separately in section 7.3. Efficacy has been reported with adverse events similar to the conventional methods of drug administration. Treatment requirements are frequent, and post-treatment pain and itching are often reported. It is considered a potential treatment for hair loss and recommended as a procedure in aesthetic practices when approved treatments fail to present an ideal outcome.^{223,226}

7.1.3 BVM formulations

Two BVM oral supplement formulations, Nutrafol and Viviscal, include collagen and have been studied in several clinical trials. These two multi-targeting BVMs had three studies eligible for the SR/MA in Chapter 6.²²⁷⁻²²⁹

Nutrafol reports its multi-targeting approach to treating hair loss by using *Curcuma longa* for its anti-inflammatory and immunomodulatory properties, specifically claiming to regulate COX-2, iNOS, NF- κ B, TNF- α and IL-1, Nrf-2, TGF- β , Substance P and has anti-androgen effects by inhibiting 5 α -R. It also reports using *Withania somnifera* to regulate NF- κ B and cortisol, and *Serenoa repens* to inhibit DHT and regulate iNOS. Tocotrienols of the vitamin E family reduce antioxidants GSH and TBARS and Piperine to activate cAMP and PKA. Collagen hydrosylates are thought to improve hair growth in patients with AGA and TE.²¹⁹

Viviscal does not explicitly report a multi-targeting strategy; however, its BVM formulation comprises various peptides and micro-nutrients from shark extract in a form of collagen. In

addition, the botanicals *Equisetum arvense* is high in silica and *Malpighia glabra* provides vitamin C. Biotin, and zinc are included as a vitamin and mineral.

The SR/MA in Chapter 6 showed Nutrafol after 24 weeks of treatment to have an efficacy of 7.32 TH/cm² in women compared to 30.09 TH/cm² of ALRV5XR. The relative efficacy between ALRV5XR and Nutrafol was 22.77 TH/cm², and between LLLT and Nutrafol was 9.29 TH/cm², both statistically significant. Topical Minoxidil 2% and 5% vs Nutrafol were not statistically significant. Viviscal in men was found to have a non-statistically significant efficacy of 13.23 TH/cm² and was also not statistically significantly different to any of the SR/MA eligible treatments in men. Nutrafol in women and Viviscal in men, both found regrowth resistance after 12 weeks of treatment. Interestingly, a 6-month Viviscal RCT in women reported efficacy of -0.98 TH/cm², however it was not eligible for the SR/MA.²²⁷

Recently, an increasing number of AGA patients in East Asia have resorted to seeking safe and effective complementary therapies for AGA from traditional Chinese medicine (TCM). TCM herbal formulas with set ingredients, recorded in ancient Chinese classic medical books, have been shown in the treatment of some chronic conditions such as AGA.^{10,217,218} TCM used for alopecia dates back at least two thousand years to the Qin Dynasty. After thousands of years of development, traditional Chinese medicine has accumulated a large number of effective herbal formulas and rich experience in treating AGA. Interestingly, many of the commonly used TCM botanicals for AGA do not have a direct hair-related use, yet since they combined with other herbs in a formulation to be part of a broader treatment strategy, it would appear that these TCM formulations might have an inherent multi-targeting model.¹⁰

An SR/MA of TCM formulations recently reported efficacy and safety of 30 eligible RCTs.¹⁰ Many of the treatments included Minoxidil or Finasteride together with a TCM formulation. The treatments were mostly topical and oral combinations. Controls of these studies included Minoxidil, Finasteride, ketoconazole and various vitamins and amino acids. Although absolute efficacies in hair densities were not analysed in all cases, the odds ratio effect favoured the TCM or the drug combined with the TCM formulations, and many were statistically significant. The study identified the top 15 herbs used in the eligible TCM formulations, three of which are included in the ALRV5XR formulation from Table 2.1, i.e., *Eclipta prostrata*, *Polygonum multiflorum* and *Angelica sinensis*. The less frequent herbs used in these TCM formulations were *Scutellaria baicalensis*, *Sophora flavescens* and *Astragalus membranaceus*, which can also be found in Table 2.1. The TCM formulations ranged from 1 – 22 herbs. The study concluded that the efficacy was significantly improved when Minoxidil and Finasteride were complemented with TCM and recommended TCM as a safe and effective complementary therapy for AGA.¹⁰

7.1.4 Implications of comparisons of combination and multi-targeting hair loss treatments

The documented evolution of traditional medicine formulations going back at least 2,000 years suggests that a medicinal art with effective treatments has evolved. Plants, especially those with ethnopharmacological uses, have been the primary sources of medicine for early drug discovery. Almost 50% of the approved drugs during the 1980s were derived from natural products. By the end of the 20th century, 11% of the 252 drugs considered essential medicines by the World Health Organization were exclusively of flowering plant origin.²³⁰ Acceleration of scientific research in recent decades has opened up these traditional medicines to explore their potential.

In many cases, novel compounds have been isolated for effective treatments in many diseases. Nevertheless, the two approved drugs for AGA have different mechanisms of action. They are not known to be contraindicated, yet they do not appear to have a potentiated effect on hair regrowth when used in combination. TCM treatments, and all other traditional formulations of multiple botanicals, including Nutrafol, Viviscal and ALRV5XR, likely have both potentiation and inhibition effects within these formulations. However, one exciting and novel feature of the ALRV5XR formulation was its ability to seemingly escape the treatment resistance effects due to its continuous regrowth when compared to all other treatments.

One of the most interesting observations of all the combination drugs, multi-targeting and TCM treatments is that the most effective treatments targeted various molecular signalling pathways associated with stem cell activation-related functions, as per Table 2.1. These herbs in the effective TCM hair loss formulations included *Eclipta prostrata*, *Polygonum multiflorum*, *Scutellaria baicalensis*, *Sophora flavescens* and *Astragalus membranaceus* that are used in ALRV5XR, and all have well-documented stem cell signalling effects for Wnt/ β -catenin, Shh, BMP, Notch, TERT and mTORC1. Therefore, these stem cell pathway regulators may be an important differentiator in the effectiveness of ALRV5XR in hair regrowth.

7.2 Challenges in the Literature and the Interpretation of the Reported Clinical Outcomes Effects in Clinical Trials and SR/MAs

This section discusses the seminal clinical studies and outcomes in the vast body of literature on the treatment of AGA. The primary outcome measured in Chapters 3– 6 was a change in terminal hair density and is the contextual focus of hair regrowth outcomes in this critical analysis discussion.

7.2.1 Comparisons to clinical trials and SR/MAs in the literature

This section emphasises the SR/MAs of AGA treatments that have included most of the seminal hair loss RCTs in the literature. A SR/MA with low heterogeneity of RCTs is considered the highest level of evidence in medicine.²³¹ The proposed objective was to compare the terminal hair outcomes of the RCTs in Chapters 3 and 4 to AGA RCTs in the literature using an SR/MA methodology. Fifteen prior hair regrowth SR/MA studies were found in the literature that included most of the seminal clinical trials.^{10,222,232-244} By chance, only one previous SR/MA compared terminal hair outcomes that were limited to studying pharmaceuticals in men. This left the door open for a wider analysis of terminal hair outcomes, including all non-surgical hair loss treatments in both men and women.

In order to ensure the data synthesis of comparable outcomes, inclusion criteria for the studies used in the SR/MA in Chapter 6 would require eligible studies to be at least 24 weeks in duration, measure changes in non-vellus or terminal hair using reliable and comparable methods in an RCT that had a placebo as the control group. Eligible studies included all commercially available interventions with the eligible evidence. This included stem cell transplants, platelet-rich plasma (PRP) injections, on- and off-label pharmaceuticals, medical devices, natural health products, and cosmetics. The systematic search screening yielded 98 studies eligible for full-text review. The final 17 eligible RCTs for synthesis could not include many common treatments, such as off-label drugs, including bimatoprost, latanoprost, oral Minoxidil, spironolactone, tretinoin, or PRP. A few RCTs of dietary supplements were found, and none for shampoos or cosmetics. Many of the seminal RCTs of Minoxidil, Finasteride and PRP were excluded. The most frequent reasons for exclusion were: no outcome of interest (33%), ineligible hair count methods (21%), results or crossover < 24 weeks (11%). More details and reasons for exclusion can be found in Appendix 2 of Chapter 6.

This section will now compare the SR/MA in Chapter 6 to two of the most influential SR/MAs in the field that also use many seminal RCTs in the literature. These two SR/MAs were published in top dermatology journals with impact factors > 11 (as of current ratings listed for 2021).^{232,242} At the time of writing this thesis, Adil et al. 2018²³² had 169 citations, and a 7.46 Field-Weighted Citation Impact (FWCI) reported in Scopus and Gupta et al. 2022²⁴² had 14 citations and a 12.64 FWCI. These impact variables suggest that these two SR/MAs have become seminal publications in the field and foundational in the literature with strong influence in clinical practice. All articles included in these three SR/MAs were from English language sources; however, usage of the same articles in these SR/MAs was limited. The 17 RCTs used in the Chapter 6 SR/MA included all five of the Adil et al. 2018²³² studies in women and only two of its 15 studies in men. Only two Chapter 6 studies were common, with the 23 all-male studies included in Gupta et al. 2022²⁴². Adil et al. 2018²³² and Gupta et al. 2022²⁴² shared seven studies. The top-line efficacy results of the treatments in these three SR/MAs can be seen in Table 7.1.

On initial review of the 22 treatment results in Table 7.1, 15 (64%) were in the 10 – 20 hairs/cm² range with five (23%) outliers > 20 hairs/cm² included 5mg Minoxidil (40.5), 1mg Finasteride (30.0) and 1% Finasteride (29.2) for men in Gupta et al. 2022²⁴², and ALRV5XR (21.03 for men and 30.09 for women) in Feldman et al. 2023²¹⁶. Three (14%) outliers at the low end are Minoxidil 2% (8.11 for women) in Adil et al. 2018²³², Nutrafol (7.32 for women) in Feldman et al. 2023²¹⁶ and 0.25mg Minoxidil (-3.2 for men) in Gupta et al. 2022²⁴². Eighteen of the results were reported with statistically significant differences to placebo, and four of the results for men were not statistically significant; Feldman et al. 2023²¹⁶: 1mg Finasteride (12.38), Minoxidil 2% (10.54) and Viviscal (13.2) and Gupta et al. 2022²⁴²: 0.25mg Minoxidil (-3.2).

Table 7.1: Top-line efficacy comparisons to seminal SR/MAs

Table 7.1 shows the top-line mean difference between each treatment[†] and placebo results in hairs[‡]/cm² from seminal SR/MAs.

Comparison of Top Line Efficacy Results of Treatments in Androgenetic Alopecia from Seminal Systematic Reviews and Meta-Analyses											
	Treatments										
	ALRV5XR	0.5mg Dutasteride	1mg Finasteride	Finasteride 1%	LLLT	Minoxidil 5%	Minoxidil 2%	0.25mg Minoxidil	5mg Minoxidil	Nutrafol	Visiscal
Women											
Adil et al. 2018 ²³²	-	-	-	-	-	-	12.41*	-	-	-	-
Feldman et al. 2023 ²¹⁶	30.09**	-	-	-	16.62**	10.82**	12.13**	-	-	7.32**	-
Men											
Adil et al. 2018 ²³²	-	-	18.37*	-	17.66*	14.90*	8.11*	-	-	-	-
Gupta et al. 2022 ²⁴²	-	-	30.0**	29.2**	-	10.7**	11.3**	-3.2	40.5**	-	-
Feldman et al. 2023 ²¹⁶	21.03**	18.37**	12.38	-	18.75**	13.13**	10.54	-	-		13.2

[†] Topical drug treatments use %, Oral drug treatments use mg, ALRV5XR is a multi-targeting topical and oral, LLLT is a Low-Level Laser Treatment device, Visiscal and Nutrafol are multi-targeting orals.

[‡] Adil et al. 2018²³²: Frequentist MA (random effects model), Mixed terminal and/or total hair changes over mixed treatment durations, * Statistically significant;
 Gupta et al. 2022²⁴²: Bayesian NMA (200,000 simulations), Terminal hair changes over 24 weeks of treatment, ** Statistically significant;
 Feldman et al. 2023²¹⁶: Bayesian NMA (250,000 simulations), Terminal hair changes over 24 weeks of treatment, ** Statistically significant;

The results of Table 7.1 are generally close for the same treatment in the different studies, except in men for 1mg Finasteride which has three very different results (12.38, 18.37 and 30.0), Minoxidil 5% has some notable differences (10.7, 13.13, 14.90), and Minoxidil 2% also has some differences (8.11, 10.54, 11.3). Minoxidil 2% in women were remarkably close, with minor differences, likely due to the meta-analysis techniques used since the same studies were analysed for this treatment. LLLT results in men were similar, although there was limited study overlap.

Although the meta-analysis statistical techniques differed, i.e. Adil et al. 2018²³² used a standard frequentist random model meta-analysis technique. In contrast, Gupta et al. 2022²⁴² and Feldman et al. 2023²¹⁶ used the more conservative Bayesian network meta-analysis technique with 200,000 and 250,000 Markov Chain Monte Carlo simulations, respectively. In general, the mean difference results of all these techniques for the same treatment should be within a hair if the same studies and extracted data were used. The major differences would be in the confidence or credible intervals, which impact statistical significance. For example, Bayesian 95% credible intervals are generally wider than frequentist 95% confidence intervals. This is why Bayesian statistics are considered more real-world and conservative. When using the same clinical trial data, it is harder to reach Bayesian statistical significance than achieving a null hypothesis P-value of less than 0.05 with frequentist statistics. Interestingly, Gupta et al. 2022²⁴² used a frequentist $P < 0.05$ to determine statistical significance for the Bayesian results. However, they did correspond with the Bayesian significance displayed by the 95% credible interval results, which is not always the case, as seen in Feldman et al. 2023²¹⁶. To better understand how these outliers were determined and the similarities and differences between these SR/MA results, the SR/MAs and their included studies were investigated. This yielded

various non-comparable methods, extraction or data allocation errors, and confounding factors in forming the two seminal SR/MA data syntheses. These phenomena are noted in Table 7.2.

Table 7.2: Representative examples of non-comparable and confounding factors in SR/MAs

This Table shows two seminal SR/MAs with many seminal RCTs that have synthesised common outcomes of hair/cm² using uncommon variables such as hair types, counting methods, study durations and other confounding factors, including errors in data extraction.

Seminal SR/MAs & RCTs	Treatment	Non-Comparable & Confounding Factors
Adil et al. 2018²³²		
Olsen et al. 2002 ⁴⁷ *	Minoxidil 5% (Men)	- Non-vellus counts over 48 weeks.
Berger et al. 2003 ²⁴⁵	Minoxidil 5% (Men)	- Total hair counts over 6 months.
		- No placebo control group for Minoxidil 5%
		- Dosage BID vs once daily for other studies.
Olsen et al. 2007 ²⁴⁶	Minoxidil 5% (Men)	- Non-vellus hair counts over 16 weeks.
Olsen et al. 1985 ²⁴⁷	Minoxidil 2% (Men)	- Manual non-vellus hair counts.
		- Placebo crossover to Minoxidil at 4 months.
Petzoldt et al 1988 ²⁴⁸	Minoxidil 2% (Men)	- Manual non-vellus hair counts over 24 weeks.
		- Extracted as reported (per 5cm ²).
Roberts et al. 1987 ²⁴⁹	Minoxidil 2% (Men)	- Manual non-vellus hair counts.
		- Placebo crossover to Minoxidil at 4 months.
Shupack et al. 1987 ²⁵⁰	Minoxidil 2% (Men)	- Manual non-vellus hair counts over 6 months.
Olsen et al. 2002 ⁴⁷	Minoxidil 2% (Men)	- Non-vellus counts over 48 weeks.
Whiting 1999 ²⁵¹	1mg Finasteride (Men)	- 4mm punch biopsy over 48 weeks.
		- Extracted total hair as reported (per 0.125cm ²).
Leyden et al. 1999 ⁴⁸	1mg Finasteride (Men)	- Total Hair counts over 12 months.
		- Placebo reported -2.0, extracted as -20.6/cm ² .
		- Other studies over 48 weeks/12 months. ^{252,253}
Kim et al. 2013 ²⁵⁴	LLLT (Men)	- Total hair counts over 26 weeks.
Lanzafame, 2013 ²⁵⁵	LLLT (Men)	- Terminal hair counts over 16 weeks.
		- Error in density conversion to 1cm ² .
Leavitt et al. 2009 ²⁵⁶	LLLT (Men)	- Terminal hair counts over 26 weeks.
Lucky et al. 2004 ²⁵⁷	Minoxidil 2% (Women)	- Non-vellus hair counts over 48 weeks.
		- Other women studies, non-vellus, 32 weeks. ²⁵⁸⁻²⁶¹
Gupta et al. 2022²⁴²		
	(Men only)	
Olsen et al. 1985 ²⁴⁷	Minoxidil 2%, 3%	- Manual hair counts.
Olsen et al. 1986 ²⁶²	Minoxidil 0.01, 0.1, 1, 2%	- Manual hair counts.
Civatte et al. 1987 ²⁶³	Minoxidil 2%	- Manual hair counts.
		- Interim report of complete study Civatte et al. 1988 ²⁶⁴
Koperski et al. 1987 ²⁶⁵	Minoxidil 2, 3%	- Manual hair counts.
Shupack et al. 1987 ²⁵⁰	Minoxidil 0.1, 1, 2%	- Manual hair counts.
Anderson 1988 ²⁶⁶	Minoxidil 2%	- Manual hair counts.
Dutrée-Meulenberg et al. 1988 ²⁶⁷	Minoxidil 2%	- Manual hair counts.
Petzoldt 1988 ²⁴⁸	Minoxidil 2%	- Manual hair counts.
Rushton et al. 1989 ²⁶⁸	Minoxidil 2%	- Manual hair counts and unit area trichogram.
Gubelin Harcha et al. 2014 ²⁶⁹	0.02, 0.1, 0.5mg Dutasteride	- Non-vellus hair data in total hair analysis.
	1mg Finasteride	(Clinicaltrials.gov NCT0123160).
Saraswat et al. 2003 ²⁷⁰	Minoxidil 2%	- Open-label, count method unclear, results and figures do not correspond.
	1mg Finasteride	
Hajheydari et al. 2009 ²⁷¹	1mg Finasteride	- Count method unclear. Total & terminal hair counts.
	1% Finasteride	- Eligibility <20 hairs/cm ² , counts/10cm ² .
Shanshanwal et al. 2017 ²⁷²	1mg Finasteride	- Open Label, Total, thick, thin hair counts.
	0.5mg Dutasteride	- Unclear which hair type included.
Panchaprateep et al. 2020 ²⁷³	5mg oral Minoxidil	- Open Label, No control, total and terminal hair.
		- Phototrichoscopy of unclipped/undyed hair.
Pirmez et al. 2020 ²⁷⁴	0.25mg oral Minoxidil	- Retrospective Phototrichoscopy of unclipped/undyed hair. No Placebo Group.

When reviewing the manually counted topical Minoxidil 2% studies in men from the 1980s, the baseline terminal hair counts had a density less than half of the later phototrichogram baseline densities for subjects with similar age, race and range of Hamilton Norwood scales. For example, three of these studies in Table 7.2 have baseline densities between the treatment and placebo groups of approximately 25²⁴⁷, 20²⁵⁰ and 33²⁴⁸ non-vellus hairs per cm² from studies in 1985, 1987, and 1988. Many of the target areas for hair counting from the 1980s were 1 inch or 2.5 cm in diameter. Often, the results did not report a unit area for the density; therefore, the target area size should be assumed for the reported hair count. In Chapter 6, the range of baseline terminal hair counts of similar populations in men ranged from 104 – 217 terminal hairs per cm².

Rushton et al. in 1989²⁶⁸ found the results using these manual hair counting methods to be unreliable and inaccurate and attributed this to the operator learning curve effect. In the early 1990s, imaging technology evolved. The contrast-enhanced phototrichogram with clipped and dyed hair emerged as the gold standard in hair loss or regrowth studies within a few years.^{275,276} High-resolution handheld electronic image-capturing devices and computer analysis tools have further improved hair measurement accuracy.

Rushton et al. 1987²⁶⁸ proved that manually counting hairs was inaccurate. However, nine of the eleven topical Minoxidil 2% RCTs included in Gupta et al. 2022²⁴², and four of the five in Adil et al. 2018²³² used the manual hair counting method (see Table 7.2). The Gupta et al. 2022²⁴² study included Rushton et al. 1987²⁶⁸ that included both a manually counted and unit area trichogram counted studies, yet Gupta et al. 2022²⁴² did not disclose the extracted data as part of the study characteristics as required by the standards from Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)²⁷⁷. In Adil et al. 2018²³², the manual

hair counts of Petzoldt 1988²⁴⁸ were extracted as reported per 5cm² and was not reduced to 1cm² as per the compared RCTs. Nevertheless, the MA model assigned a 0.3% weighting to this outlier, therefore not significantly influencing the final result. Manually counted studies were not eligible for the study in Chapter 6 due to their abnormally low baseline densities that distorted the relative efficacy and homogeneity of the pooled Minoxidil 2% RCTs, and the findings of Rushton et al. 1987²⁶⁸.

The Finasteride data extraction errors referenced in Table 7.2 for Adil et al. 2018²³² came from two separate RCTs. Whiting et al. 1999²⁵¹ reported total hair density data from 4mm punch biopsies, with the Finasteride group regrowing 2.3 and the placebo group growing -0.1 hairs/0.125cm². Adil et al. 2018²³² was reporting regrowth per cm², however did not adjust the density and SDs by a factor of 8 to 18.4 and -0.8 hairs 1cm² for Finasteride and placebo respectively, before inclusion in the data synthesis. Adil et al. 2018²³² extracted the Leyden et al. 1999⁴⁸ placebo group as -20.6 hairs/cm² when it was reported as -2.0 hairs/cm² in the RCT. These extraction errors from Leyden et al. 1999⁴⁸ resulted in a distorted efficacy of 30.2 hairs/cm² in Adil et al. 2018²³² rather than the actual 11.66 hairs/cm², as reported in the RCT. The correct extractions and associated SDs would have likely adjusted the meta-analysis study weightings significantly more to the Leyden study resulting in a mean difference between 14 and 16 total hairs/cm² for Finasteride efficacy in Adil et al. 2018²³².

In one excellent study of oral Dutasteride and Finasteride, Gubelin Harcha et al. 2014²⁶⁹, reported total hair density outcomes in the published manuscript and true terminal hair density (>60 µm in diameter). However, this study reported a baseline total hair density that appeared too low for the enrolled population's HN (IIIv-V) range. For example, a HN IIIv-V would have a terminal-to-vellus ratio from 4:1 to 1:1. As a result, a mean total hair count could be

approximately 200 hairs/cm², when it was in fact, reported as 140-149 hairs/cm² (standard deviation 37.7 – 45.7) and relative standard deviation (25 – 33%). These baseline results were well within the norm of terminal hair baseline data for men with this HN pattern range in Figure 2 of Chapter 6. On further examination, more information was available on the trial registration record on clinicaltrials.gov than was published in the study. Total hair was recorded per the protocol with a diameter >30 µm and matched the total hair results published in the manuscript, allowing this study to be included in Chapter 6. Without this confirmation, there would have been no other eligible terminal hair studies of Dutasteride or Finasteride for the SR/MA in Chapter 6 since most of the seminal Finasteride clinical trials reported counts of total hair without diameter differentiation.^{48,251-253,278} Gupta et al. 2022²⁴² very likely took the language in the study at face value, allocating its data to a total hair density analysis. By allocating this study to the terminal hair category, the weighting in the meta-analysis might have led to a similar result as determined in Chapter 6 for 1mg Finasteride while also providing terminal hair results for 0.02, 0.1 and 0.5mg Dutasteride.

Gupta et al. 2022²⁴² reported terminal hair efficacy at 24 weeks of 1mg Finasteride at 30.0 TH/cm² and topical Finasteride 1% at 29.2 TH/cm². It is unclear which studies led to this result; however, of the three other included Finasteride studies, two were open-label Saraswat et al. 2003²⁷⁰ and Shanshanwal et al. 2017²⁷², and one, Hajheydari et al. 2009²⁷¹, required eligible subjects to have <20 hairs/cm² and hair count results appeared to have a density area of 10cm². Nevertheless, investigations of these RCTs could not find results to support these very high outcomes. Therefore, Shanshanwal et al. 2017²⁷² and Hajheydari et al. 2009²⁷¹ were both assigned a high risk of bias in Gupta et al. 2022²⁴².

In a 1998 review article on male AGA, the author wrote that oral Minoxidil provides no added benefit over topical Minoxidil and due to its potential side effects, it should not be used.²⁷⁹ Over time and with changes in dosage, these perspectives have changed. In 2020, the same author wrote that growing evidence supports the off-label use of low-dose oral Minoxidil.²⁸⁰ Furthermore, documenting this evidence of oral Minoxidil in an SR/MA is presently of high interest. The 5mg oral Minoxidil result of 40.5 TH/cm² reported in Gupta et al. 2022²⁴² is a spectacularly high outcome for a cohort study. This data was extracted from Panchaprateep et al. 2020²⁷³, an open-label single cohort study of 30 subjects in Thailand using phototrichoscopies of unclipped and undyed hair. A 24-week terminal hair regrowth of 35.9 TH/cm² was reported. Panchaprateep et al. 2020²⁷³ was assessed for quality of evidence vs an assigned placebo from a separate study, using the Confidence in Network Meta-Analysis (CiNeMA), as moderate due to within-study bias as a major concern by Gupta et al. 2022²⁴². While the results are not disputed, including this lower level of evidence study type in a meta-analysis alongside seminal RCTs is an unusual approach to show a breakout result compared to the field. Interestingly, the lead author of Gupta et al. 2022²⁴² wrote in a follow-up article comparing oral Minoxidil, Finasteride, and Dutasteride in treating AGA that the likely efficacy ranking, in declining order, was 0.5 mg Dutasteride, 5 mg Finasteride, 5 mg Minoxidil, 1mg Finasteride, followed by 0.25mg Minoxidil. The article further reported that oral Minoxidil predominantly causes hypertrichosis and cardiovascular system signs and symptoms in a dose-dependent manner. In contrast, oral Finasteride and Dutasteride have been associated with sexual dysfunction and neuropsychiatric side effects although the frequency of these adverse events need further study.²⁸¹

One further observation about Gupta et al. 2022²⁴² is a basic feasibility test comparing the results of the same treatment's total hair vs terminal hair result. One would expect total hair

results to be equal, if not greater, than terminal hair results. For example, 1mg Finasteride reported 11.6 total hairs and 30 terminal hairs at 24 weeks, Minoxidil 2% reported 10.2 total and 11.3 terminal, Minoxidil 5% reported 15.3 total and 10.7 terminal, and 5mg Minoxidil reported 3.7 total and 40.5 terminal hairs at 24 weeks. Small tests like these help validate the inclusion criteria and other possible errors in a greater context.

The novelty of Gupta et al. 2022²⁴² was twofold, i.e. the separation of total and terminal hair results in men, analysing outcomes at different time points. Although Adil et al. 2018²³² mixed terminal with total hair and study durations, this was the first SR/MA in the field of AGA treatment outcomes for men and women. Feldman et al. 2023²¹⁶ of Chapter 6 was the first to use an SR/MA to evaluate standardised continuous outcomes of terminal hair from the same RCTs in men and women that included drugs, devices and dietary supplements. Feldman et al. 2023²¹⁶ was also the first to use the SR/MA to identify relative treatment resistance effects in AGA and compare Bayesian to frequentist efficacy results.

RCTs in hair loss are challenging, and hair-related variables have fungibility that, without adequate definition in methods and reporting, can lead to many interpretations or possible errors, as illustrated in this section. Understanding the norms and nuances of this hair data fungibility is a challenge. As an example, some data anomalies were identified from three RCTs of two multi-targeting treatments that were included in Chapter 6.^{228,229,282} The results of these RCTs were included as reported in Figures 1 and 2 of Chapter 6; however, two of these studies reported abnormally low baseline SDs, i.e. >3SDs less than the mean of the baselines from all other included studies, and had an RSD of <10% compared to the 20 – 40% range of the other RCTs.^{228,229} On further investigation, a test of these reported SDs was considered as SEMs. These SEMs were converted to an SD, where $SD = \sqrt[2]{N} * SEM$. The resulting RSDs

were well within the range of the other included RCTs. These errors are easily made; however, both RCTs would have lost statistical significance in these cases. Nevertheless, it is easy to speculate without the raw data.

The remaining 13 SR/MAs^{10,222,233-241,243,244} prior to Chapter 6 were not investigated in this detail; however, similar phenomena in varying degrees can be found.

The RCTs of Chapters 3 and 4 were designed with a high degree of specificity in mind. Chapter 4 regrettably had only half of its subject phototrichograms evaluable due to operator error. Nevertheless, the evaluable populations in Chapter 4 retained statistical power and is of a similar size to some of the early Minoxidil and Finasteride studies. The 17 RCTs that were eligible for Chapter 6 could be considered the seminal RCTs in the androgenetic alopecia literature for terminal hair regrowth. Minor differences in an SR/MA analysing the same treatments and hair types should be expected, particularly when there are differences in study inclusion criteria and analytical methods. However, except for differences by sex, these variances should be within a few hairs.

7.2.2 Comparisons to long-term clinical trials in the literature

The SR/MA in Chapter 6 included a few RCTs that reported results beyond 24 weeks that only included the treatments of Minoxidil 2% and 5%. Long-term data from these studies were reported in Section 4 of Appendix 1 in Chapter 6, but were not analyzed in the main SR/MA due to insufficient studies beyond 24 weeks. These longer-term studies were analysed using weighted moving averages. They included five Minoxidil 2% cohorts in women and one in men extended to 32 weeks^{47,257-261} and one of these cohorts in women and men extended as far as 48 weeks.^{47,257} One Minoxidil 5% cohort in women and one in men extended to 32 and 48

weeks.^{47,257} These longer-term Minoxidil RCTs showed stability in the weighted average terminal hair efficacy in women from 24 to 48 weeks and a slight decline in men.

Although Finasteride and Dutasteride RCTs included in the SR/MA did not extend beyond 24 weeks, other Finasteride RCTs in men reporting total hair counts show very slight progression in regrowth from 24 to 48 weeks.^{48,252,283} A study showing 24 months of Finasteride use in male AGA found continued improvements in efficacy of total hair counts, however from 12 – 24 months the Finasteride group showed a slight decline in hair count, while the placebo group lost hair at a faster rate than the treatment group. This same study reported a partial placebo 12 month crossover group to Finasteride that regrew the same amount of hair in the 12 – 24 months as the original Finasteride group regrew from BL – 12 months. A partial Finasteride 12 month crossover group to placebo showed a decline of the 12 month regrowth to less than BL at 24 months.^{283,284}

A 48 week Finasteride RCT in men with AGA, extending to 192 weeks, reported a progressive increase in total hair count efficacy beyond 24 weeks although the treatment group reported no meaningful increase in hair count from 24 -72, weeks which was followed by a decline from 72 – 96 weeks.^{285,286} This RCT did not provide hair count results at 12 weeks, however it did provide hair weight results that showed continuous improvement in efficacy, although after 24 weeks, the treatment group showed increase in hair weight, yet after 48 weeks, an oscillating pattern appeared in each year thereafter with the weights reducing to below the 48 week weight after 144 weeks. The study reported that Finasteride treatment produced greater increases in hair weight than in hair count at all time points, and concluded that increased growth rate or hair thickness, or both, contributed to the increase in hair weight beyond that expected because

of the increase in the number of hairs.^{285,286} This RCT had a progressive participant attrition rate, and the results of only men who completed 96 weeks showed a more conservative result.

Interestingly, this hair thickening concept was also reported in a 1999 RCT of Finasteride vs placebo over 48 weeks, where it found a non-statistically significant increase in the terminal to vellus ratio and suggested that treatment with Finasteride can reverse hair miniaturisation in men.²⁵¹ This study used a biopsy method to measure hair diameters at baseline and 48 weeks. A cross-section biopsy will give the most accurate diameter measurement; however, unlike a phototrichogram using a tattoo for repeat measures of the same follicle hairs, a follow-up biopsy cannot repeat the trichoscopic measurements of the same hairs or follicles due to the permanent removal of the tissue including the follicles at the biopsy site. Nevertheless, this proposed miniaturisation reversal effect of Finasteride was later studied over a two-year period using phototrichogram methods following the same miniaturised hairs. In 2019, this study reported that these same miniaturised hairs did not thicken, and in-fact regressed further; however, new terminal hairs from deficient terminal follicles increased productivity, leading to moderate improvements in hair density.²¹⁰ The study concluded that its data suggested the rejection of the miniaturisation reversal hypothesis with Finasteride unless proven otherwise with duly validated methods.²¹⁰ Interestingly, the terminal hair regrowth resistance effects of treatments after 12 weeks as reported in Chapter 6 suggest that the greater the resistance effect, the lower the chance of miniaturisation reversal. Further investigation into changes in anisotrichosis and the same miniaturised hairs from the phototrichograms of the RCTs in Chapters 3 and 4 should provide a conclusive result to any miniaturisation reversal effects.

Similarly to the Finasteride hair weight studies, a 96 week study of Minoxidil 2% and 5% in female AGA reporting changes in hair weight, showed similar efficacy profiles over the first

24 weeks as was reported in Chapter 6. This same study showed general stability in efficacy from 24 – 96 weeks, however, as in the 24 month Finasteride study, there was a general decline in hair weight in the Minoxidil group, while there was also a continued decline in the placebo group. The Minoxidil hair weight studies showed a rapid decline in hair weight after withdrawal of treatment at 96 weeks and by 120 weeks, the Minoxidil group had a lower hair weight than the placebo group.^{284,287} Even though these two 24 month studies provide longer term efficacy results measuring changes in total hair count and hair weight, the initial 24 week regrowth profiles are similar to the SR/MA of Chapter 6, however, the inclusion of vellus and vellus like hair in these measurements does not yield direct comparison to the SR/MA of Chapter 6.

While longer term RCTs are important for efficacy and safety, most studies are designed to crossover from placebo to treatment anywhere from 3 – 12 months. Maintaining a subject on placebo beyond 6 months and potentially as long as 12 months can become an ethical consideration, due to the potential for non-recoverable hair loss. The continuous regrowth profiles of ALRV5XR over 24 weeks, would benefit from longer-term studies to evaluate the full regrowth potential and safety profile of the treatment.

7.2.3 Implications for Outcomes Measures of Hair Loss Treatments

There is an unmet need for effective treatment in AGA. The field of AGA treatment is filled with fungible variables and studies that can show, mask and easily distort the meaningful outcomes and relevance to clinicians and patients. The findings in Chapter 6 suggest that finding benchmark standards that should be included in hair loss studies is feasible. For example, minimum study durations should be at least 24 weeks with a 12-week midpoint to show any initial treatment effects and subsequent resistance, as was commonly seen in Chapter

6. However, RCTs measuring terminal hair regrowth of at least one year or longer with midpoints every three months would provide for more robust evidence in efficacy and safety. In RCTs, hair clipped to at least 1mm and dyed should be measured at the same scalp position by Phototrichogram to ensure changes in hair follicle productivity can be derived from changes in hair diameters and growth rates.

Similarly, since there might be variations in computer-assisted phototrichogram image analysis, these images used for measurement should be included as part of the published data set. In addition, since hair loss studies tend to use hair measurement techniques such as the contrast enhanced phototrichogram that is not often used in clinical practice, different levels of quality could be assigned to other hair measurement methods, such as the unit area trichogram, hair weight measurements, global assessments or hair shedding allowing for lower-cost studies. This could help establish global scalp scoring and other meaningful surrogate hair productivity scoring methods. The emergence of practical artificial intelligence tools might soon assist in reducing the cost and improving the accuracy of hair measurements. Finally, standards should be established for journals that can eliminate or disqualify many potential p-hacks that might offer misleading findings in the literature. Many treatments with claims and data do not give a clinician or patient an easy way to compare outcomes, particularly of the primary marker of the scalp hair phenotype – terminal hair. Tools being developed like scalp coverage scoring methods might be useful in this endeavour.^{207,288}

The hair loss industry is often accused of “selling snake oil”, “overpromising and underdelivering”, “P-hacking”, or “selective reporting”. At the time of the commercial launch of Minoxidil 2%, journals received many letters-to-the-editor, of which one appeared in The Lancet in 1989 titled “*Baldness in Minoxidil Advertising*”²⁸⁹ and a few years later, in 1992,

another one appeared in the New England Journal of Medicine titled “*Advertising baldly to the public*”.²⁹⁰ In April 2023, a USA consumer advocacy group filed a letter of complaint to the USA Food and Drug Administration and the Federal Trade Commission, accusing 25 hair loss supplement companies of using deceptive marketing methods and unsubstantiated scientific evidence for product marketing claims.²⁹¹

Therefore, it appears that a better-defined set of outcomes standards and hierarchies could be determined, adopted as extensions to CONSORT²⁹² and PRISMA²⁷⁷, and promoted by medical societies. In addition, adherence to the peer review process would support unambiguous RCT and SR/MA findings and conclusions and, ultimately, improve the practitioner and patient confidence in treatment options supported by credible product or procedure marketing claims in the field of hair loss.

7.3 Expert Perspectives on Regenerating Hair

In early 2016, Nature Biotechnology published a news article, “*Biotechs target stagnant baldness market*”, listing seven of the most promising hair loss treatments in development.²⁹³ Of the seven treatments listed, Propecia (Finasteride – oral 5 α reductase inhibitor) was the only approved treatment, and of the other six treatments. Avodart (Dutasteride – oral 5 α reductase inhibitor), as reported in Chapter 6, had efficacy in men; however, it has been approved for an alopecia indication only in Japan and South Korea. Latisse (bimatoprost – topical solution) has not shown efficacy on the scalp. The last four: CB-03-01 (topical cortexolone 17 α -Propionate 5%), SM04554 (topical Wnt activator), Setipiprant (ACT-129968), and SHAPE (suberohydroxamic acid phenyl ester) have been abandoned.

In parallel to these treatments playing out their clinical development process, several published expert opinions proposed novel, diverse, yet relevant approaches to treating androgenetic alopecia (AGA). Ten expert perspectives published since 2016 have been selected to illustrate that the hair and hair loss research field is highly active. However, its rich body of knowledge appears far from maturity or consensus on how to treat hair loss. Some of these articles support single targets, some support a multi-targeting approach, and others support anatomical, injury or biome-controlling effects; however, very few propose a specific intervention or procedure, and few authors have followed up with implementations of their opinions. Nevertheless, each expert opinion is compelling and has a strong scientific basis.

Table 7.3: Expert perspectives on regenerating hair

This Table reports ten diverse perspectives on approaches to regenerating hair.

I. Glycans are key players in hair growth control.

Bernard, 2016³⁵ opines that the fine-tuning of the influences on hair growth control cannot solely rely on the timely and spatially controlled gene expressions but also glycans, “the third revolution in evolution”. The author further states that glycans are endowed with such a broad molecular diversity that they can be considered the third language of life, after DNA and proteins. Glycans are made up of oligosaccharides, glycosaminoglycans and proteoglycans. Syndecans modulate Wnt signalling cascades, the glycosaminoglycan chains of proteoglycans shape Hedgehog gradients and signal transduction, and O-linked glycosylation controls Notch1 interaction with its cognate Delta-like 4 receptor. Decorin, a small leucine-rich proteoglycan, directly modulates TGF- β , epidermal growth factor (EGF), IGF-1 and hepatocyte growth factor (HGF) signalling, all known as actors of hair follicle cycling and appear to mostly act as anagen inducers. These glycans are key players in hair growth control, and enzymes modulate their biological activity. Bernard BA, 2016³⁵ proposes that fine-tuning the activities of growth factors and morphogens by the modulating effects of glycans and their enzymes should be considered for therapeutic opportunities.

II. Topically applied botanicals sustain hair growth mechanisms of action.

Herman & Herman, 2016²⁰² discuss the mechanisms of action of various topically applied botanicals and the effects of their active compounds on the hair growth process. Many botanicals and their active compounds can achieve a therapeutic effect by penetrating the epidermal barrier and reaching specific sites of the hair follicles. This study reported that a two-minute contact between botanical extract-based shampoo and the scalp was sufficient for compound penetration deep into the hair follicles and detectable for up to 48 hours. This demonstrated the long-term reservoir function of the hair follicles for topically applied substances. In addition, various botanicals have been shown to influence hair growth via stimulation or inhibition activity of growth factors, cytokines, hormones, and enzymes, as well as modulation of signalling pathways. Several botanicals were found to stimulate mechanisms of hair growth, such as IGF-1, VEGF, EGF, FGF-2, endothelial nitric oxide synthase, Wnt/ β -catenin, and prostaglandins E and F. Other botanicals were found to inhibit mechanisms causing hair loss such as 5- α R and DHT, TGF- β , FGF-5, prostaglandin D2. (See Figure 7.1.) Herman & Herman, 2016²⁰² note that the therapeutic role of botanicals lies in the synergistic interaction of their constituent compounds, and often, the most active constituents from herbs are not necessarily responsible for the major part of the effect. Furthermore, the fractionation of a botanical extract will not necessarily produce active compounds. Due to their sustained actions, the authors concluded that botanical formulations could be considered therapeutic replacements for synthetic pharmaceuticals in treating hair loss.

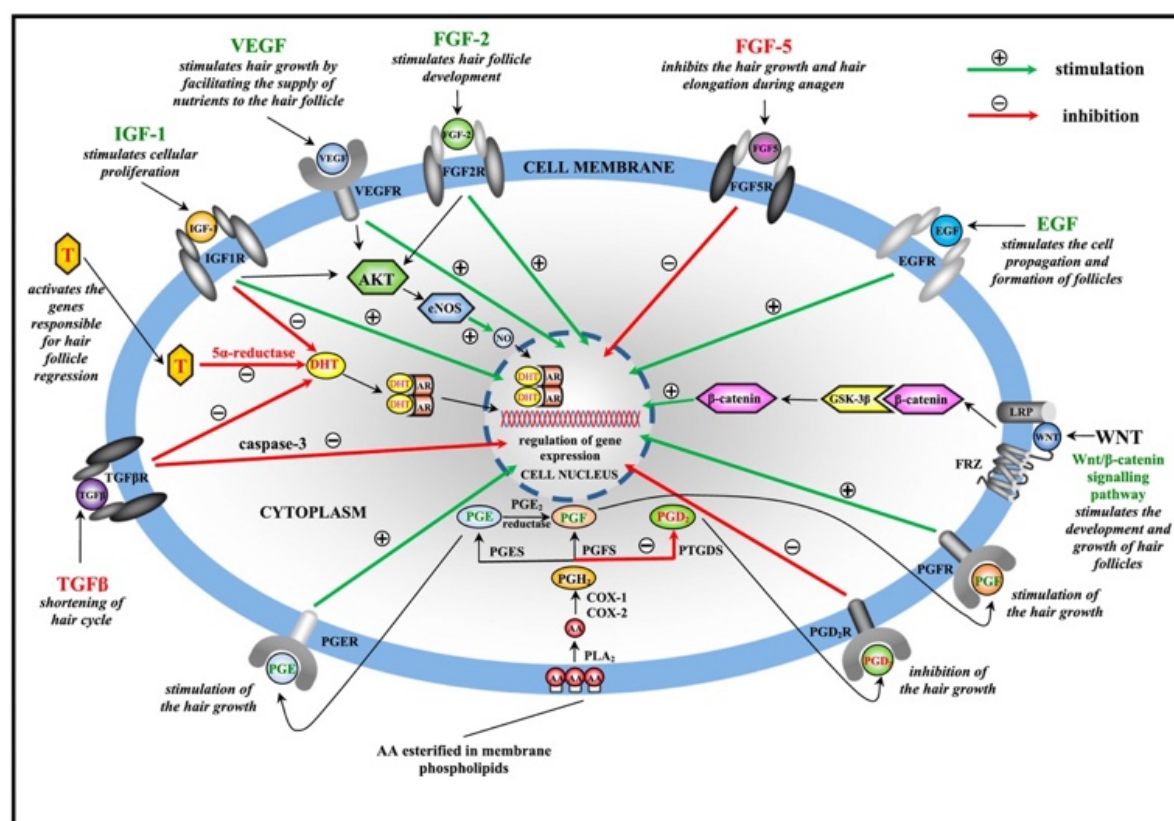


Figure 7.1 Different mechanisms of action of botanicals and their active constituents for hair growth.²⁰²

Legend: IGF-1 - insulin-like growth factor-1; IGF1R - IGF-1 receptor; DHT - dihydrotestosterone; AR - androgen receptor; AKT - serine/threonine protein kinase; eNOS - endothelial nitric oxide synthase; NO - nitric oxide; T - testosterone; TGF- β - transforming growth factor beta; TGF β R - TGF- β receptor; PGE - prostaglandin E; PGE₂ - prostaglandin E₂; PGER - prostaglandin E receptor; PGES - prostaglandin E synthase; PGF - prostaglandin F; PGFR - prostaglandin F receptor; PGFS - prostaglandin F synthase; PGD₂ - prostaglandin D₂; PGD₂R - prostaglandin D₂ receptor; PTGDS - prostaglandin D₂ synthase; AA - arachidonic acid; PLA₂ - phospholipase A₂; COX-1 - cyclooxygenase 1; COX-2 - cyclooxygenase 2; PGH₂ - prostaglandin H₂; WNT - WNT proteins; LRP - lipoprotein receptor related proteins; FRZ - Frizzled receptor; GSK-3 β - glycogen synthase kinase 3 β ; EGF - epidermal growth factor; EGFR - EGF receptor; FGF-5 - fibroblast growth factor 5; FGF5R - FGF-5 receptor; FGF-2 - fibroblast growth factor 2; FGF2R - FGF-2 receptor; VEGF - vascular endothelial growth factor; VEGFR - VEGF receptor.

III. Enlarging the dermal papilla grows larger hair fibre.

Pantelireis & Higgins, 2018²⁹⁴ write that the influx and efflux of cells into and out from the dermal papilla (DP) can modulate DP size during the hair cycle. As DP size is positively correlated with the size of the hair fibre produced by a follicle, Pantelireis & Higgins, 2018²⁹⁴ propose that therapies for treating AGA should be developed to alter DP size rather than just promote hair growth.

IV. The paradoxical role of dihydrotestosterone in hair loss and hair growth.

English, 2018¹⁴¹ writes that there is consensus that AGA is polygenic and androgen-mediated by DHT. English, 2018¹⁴¹ reports that (1) chronic scalp tension transmitted from the galea aponeurotica induces an inflammatory response (increased ROS, COX- 2 signalling, IL-1, TNF- α) in AGA-prone tissues; (2) this inflammatory response induces TGF- β 1 alongside DHT, 5- α R, and androgen receptor increases in AGA-prone tissues as part of this inflammatory response, which furthers TGF- β 1 expression in already-inflamed AGA-prone tissues; and (3) DHT does not miniaturise hair follicles. Rather, DHT and TGF- β 1 co-mediate three chronic, progressive conditions concomitant with AGA progression – tissue dermal sheath thickening, calcification, and perifollicular fibrosis. These conditions remodel AGA-prone tissues – restricting follicle oxygen, nutrient supply, and growth space – leading to slow, persistent hair follicle miniaturisation as characterised in AGA. This model explains the mechanisms by which miniaturises AGA-prone hair follicles, describes a rationale for AGA progression and patterning, and confirms DHT's paradoxical role in hair growth and loss. English, 2018¹⁴¹ proposes fibrosis, calcification, and chronic scalp tension as targets to improve AGA recovery rates. Notwithstanding the interesting tension hypothesis, English, 2018¹⁴¹ does not explain the survival of transplanted hair follicles into a bald scalp, where this tension would remain.

V. Morphogenic pathways and wound healing induces embryogenic hair follicle neogenesis in barren skin.

Vasserot et al. 2019⁵⁹ write that the discovery of promising new targets may soon enable treatment options that modulate the hair cycle to preserve or extend the growth phase of the hair follicle. In addition, these new targets could also be leveraged to stimulate progenitor cells and morphogenic pathways to reactivate miniaturised follicles in the bald scalp or to harness the potential of wound healing and embryogenic development as an emerging paradigm to generate new hair follicles in barren skin.

VI. Regulating macrophages and T cell treatment of immune-mediated alopecia.

Rahmani et al. 2020²⁹⁵ wrote that the immune niche is a key regulator of stem cell behaviour and hair follicle regeneration. Hair follicles attract macrophages and T cells so that they are in range to regulate epithelial stem cell proliferation, quiescence and differentiation during physiologic and injured states. Disrupting this dynamic relationship leads to clinical forms of hair loss, such as scarring and non-scarring alopecia. Rahmani et al. 2020²⁹⁵ propose

exploiting this relationship for treating various immune-mediated alopeciae has therapeutic potential.

VII. Inhibiting TGF- β in catagen could prevent fibrosing deposits in AGA follicles.

Rushton et al. 2021¹⁵ write that a ladder-like array of elastin deposits form in the streamer beneath and the regressed telogen dermal papilla following subsequent anagen/telogen transitions in male AGA. TGF- β is increased in catagen and is thought to mediate androgens that shorten the hair cycle. In addition, TGF- β has been shown to act transcriptionally to increase elastin synthesis. This progressive fibrosing elastin architecture may prevent follicular extension into the dermis, contributing to cycle-dependent miniaturisation. Inhibiting the formation or dissipating of these fibrosing elastin bodies may indicate a new area for therapeutic intervention to prevent and reverse follicle miniaturisation.

VIII. Platelet-rich plasma with stem cell therapy stimulates hair regeneration.

Gentile et al. 2021²⁹⁶ wrote that there is convincing evidence of positive outcomes in human hair regrowth with autologous stem cell-based therapy (ASC-BT) using hair follicle stem cells and adipose-derived stem cells (ASC-BT). The evidence highlights the positive effects of platelet-rich plasma (PRP) and ASC-BT in AGA patients. Appropriate PRP preparation methods should be selected only after carefully considering their biomolecular specifications and intended patient-use indications. The upregulation of FGF-7/ β -catenin signalling pathways, produced by autologous not-activated PRP plasma (A-N-A-PRP) injection, stimulates hair regrowth by inducing hair follicle stem cell differentiation, prolonging the anagen phase of the hair growth cycle, improving the hair density better than autologous activated PRP (A-A-PRP). Regarding wound healing, both A-N-A-PRP and autologous activated PRP (A-A-PRP) may improve the neo-angiogenesis, stimulate cell growth, morphogenesis, and recruitment, constituting a three-dimensional matrix that allows cellular arrangement into a correct three-dimension organisation promoting tissue regeneration and repair, without any significant difference, between PRP-leukocyte-poor and/or PRP-leukocyte-rich in soft tissue defects. Gentile et al. 2021²⁹⁶ believe the future will depend on regenerative-based therapies.

IX. Wnt pathway activation for the treatment of AGA opens new opportunities.

Goswami & Patel, 2021²⁹⁷ wrote reported that the scientific community is working extensively to bring a first-in-the-class molecule that targets Wnt pathway activation for treating AGA and other conditions. Ongoing preclinical and clinical trials will define the role of the Wnt pathway in different therapeutic areas and open new treatment opportunities.

X. Characterisation of the hair follicle microbiome is still in its infancy.

Lousada, MB, et al. 2021²⁹⁸ note that the microbiome is altered in several hair loss disorders. In AGA, an increase in *Propionibacterium acnes*, *Malassezia restricta*, *M. globosa*, and the role of Demodex have been hypothesised. In alopecia areata (AA) increase in *P. acnes*, a decrease in *Staphylococcus epidermidis* and the presence of *Alternaria*. Scarring alopecia have an increase in *S. aureus* and *P. acnes*. Hair shafts inhibit the growth of *S. aureus* and *S. epidermidis*. At the same time, *Pseudomonas aeruginosa* produces a biofilm on the hair shaft surfaces, and *Escherichia coli* inhabits the edges of the cuticle scales. Neither affects the hair shaft's surface morphology. Demodex is a saprophytic mite in humans commonly present in the hair follicles and pilosebaceous units. Demodex is associated with folliculitis and is clinically characterised by hair loss, erythema, scales, pustules, and neutrophilic and/or mononuclear-cell folliculitis with occasional granulomas. Lousada et al. 2021²⁹⁸ conclude that how and how much changes in the microbiome contribute to the development of diseases and hair loss remain a topic of great debate and is still in its infancy.

7.3.1 Implications of expert perspectives in regenerating hair

These ten expert opinions in Table 7.3 can generally be classified into four groupings (Group 1) Multi-targeting approaches^{15,35,59,141,202,294,297}, (Group 2) physical interventions including wounding, PRP, Stem Cell Transplantation, anti-fibrosis, anti-calcification, and chronic scalp tension^{59,141,296}, (Group 3) Immune Modulation²⁹⁵, and (Group 4) Microbiome²⁹⁸.

These opinions suggest that AGA has more than one cause, resulting from micro-inflammatory disturbances that downregulate growth promoters that often exacerbate the inflammatory responses through multiple cytokines. The growth promoters most commonly identified were

Wnt and TGF- β - both are also glycoproteins; however, TGF- β has a pleiotropic effect and can also be an inflammatory cytokine. TGF- β is known to induce catagen and the miniaturising follicle effect of laddered fibrosing. A study in mice upregulating Wnt/ β -catenin signalling showed the regeneration of anagen hair follicles that grew larger, and the hair bulb, dermal papilla and hair shaft were enlarged.²⁹⁹ Three counterpoints to the inflammatory-induced effects are TGF- β that also induces stem cell activation, micro-wounding, where inflammatory responses can induce growth factor responses and neogenesis of new hair follicles, and androgens which can not only induce hair loss but are also required to promote hair growth

The independent cycling of each follicle and molecular expressions that both promote and inhibit growth depending on the microenvironment regulating the follicle's cycle seem to create an irreconcilable enigma of expression timing that make conditions for a scalp-wide or systemic treatment challenging. This enigma is further confounded by the degree of progression of hair loss, whereby interventions appear more likely to prevent further hair loss rather than promote hair regrowth.

The most daring interventions proposed are wounding, stem cell transplant and mechanotransduction. Interestingly, a recent study of platelet-rich plasma (PRP) found no statistical difference between placebo and plasma groups, although both groups regrew hair. The study concluded that PRP might have benefits in increasing hair density; however, it also stated in its limitations that the microinjections caused microinjury to the scalp.³⁰⁰ In contrast to this finding, a recent small study comparing a 1.5 mm depth wound by roller micro-needling to a tattoo gun in males with AGA showed no improvement in total hair count after four monthly sessions of scalp micro-needling.³⁰¹ Mechanotransduction studies demonstrated that mechanical offloading of scalp tension in AGA subjects by administering botulinum toxin

injections in galea aponeurotica-connected muscles or scalp tension relaxer devices could stop hair loss and result in visible hair regrowth.^{302,303} Mechanotransduction has also been shown to prevent or even partially reverse fibrosis in a cutaneous wound; hence it might be a potential tool to reverse one of AGA's rate-limiting recovery factors - fibrosis.¹⁴¹

The activation of the Wnt/ β -catenin pathway, as suggested by a number of the ten expert opinions, is consistent with a broad consensus in the literature that it is a requirement for initiating and maintaining anagen. However, few experts have proposed specifically Wnt promotion in hair growth interventions. A recent drug development program of a topically applied Wnt activation molecule, SM04554, conducted a phase 3 study of 625 male subjects from 2018 to 2021 (Registration: [NCT03742518](https://clinicaltrials.gov/ct2/show/study/NCT03742518)). The results of early phase 1 and 2 studies looked promising; however, no results have been published from the phase 3 study, and the molecule has been withdrawn from further clinical development.³⁰⁴ This Wnt activation clinical trial of SM04554 could suggest that targeting Wnt in the hair follicle is a tried and failed intervention; however, other than the sponsor-driven claims that SM04554 upregulated Wnt, it is not clear which Wnt ligand was activated, nor have there been any published studies of SM04554 and its effect on Wnt in general. Nevertheless, even if SM04554 does activate Wnt, the multi-targeting hypothesis of this thesis proposes and studies Wnt activators as part of a broad range of targets requiring regulation to induce self-renewal and the related regenerative effects.³⁰⁴

Another interesting aspect of Wnt/ β -catenin signalling is the interest in inhibiting aberrant Wnt/ β -catenin signalling as a cancer therapeutic. Many therapies targeting Wnt/ β -catenin signalling inhibition in cancers have been in development. Considering the ubiquitous and extensive involvements of Wnt/ β -catenin signalling in regulating various normal tissue

functions, the clinical translation of these therapies has not been successful in clinical trials. There have been many unintended and off-target adverse events of Wnt inhibition, most significantly, impairment of bone mass where Wnt upregulation is necessary for osteoblast differentiation and inducing many osteoblast differentiation genes.^{305,306}

Notwithstanding these cancer risks in regulating Wnt/ β -catenin, the botanicals in Table 2.1: Eclipta, Sophora, Scutellaria, Polygonum and Vitis have all been found to have various anti-cancer effects, including inhibition of the efflux transporter p-glycoprotein (P-GP) and multi-drug resistance (MDR), and are expected to be promising alternative or complementary medical treatments for cancer as well as MDR reversal.^{198,307-321}

The approved drugs for treating hair loss as androgenetic alopecia (topical Minoxidil 2% and 5% and oral Finasteride 1mg) and alopecia areata (JAK inhibitors) have limited efficacy and complex safety profiles, and new treatments with high hopes in clinical development are failing to meet efficacy endpoints. The mere idea that these ten diverse expert opinions of novel approaches to treating hair loss have been recently published in reputable journals, not only confirm that an unmet need exists, but also suggest that research is still in the early stages.³²²

Although seven of the ten expert opinions in this section 7.3 suggest various forms of multi-targeting approaches to treat hair loss, there are three different expert consensus opinions in the field:

- (1) There is an unmet medical need for effective hair loss treatments,
- (2) There is no consensus on how to approach this unmet medical need,
- (3) Wnt/ β -catenin signalling is essential for hair follicle anagen induction and maintenance.

7.4 Novel Research that Might Lead to More Effective Therapeutics in Hair Regeneration

Although research efforts in the field of hair loss and hair regeneration are vast and varied, there are some emerging novelties focussing on signalling within dermal papilla stem cells, of which two important novelties are highlighted relating to Wnt and Col17A1.

7.4.1 Wnt signalling is a major driver of hair follicle tissue homeostasis.

Xin et al. 2018 published a hair follicle tissue homeostasis study evaluating stem cell self-renewal and differentiation in mice. Compared to control, the flexibility of homeostatic stem cell differentiation was found after reducing the population to only one. The study demonstrated that a single hair germ stem cell could flexibly establish all the differentiation lineages in the hair follicle. Furthermore, previously thought to be unipotent, stem cell-derived hair progenitors in the matrix flexibly change differentiation outcomes due to dynamic relocation. When stem cells were spatially primed, they were flexible in establishing a diverse set of differentiation lineages and actively maintaining a normal rate of hair follicle growth during homeostasis. Finally, the flexible cell fate determination mechanism maintained normal differentiation and tissue architecture induced by Wnt activation.³²³ (See Figure 7.2).

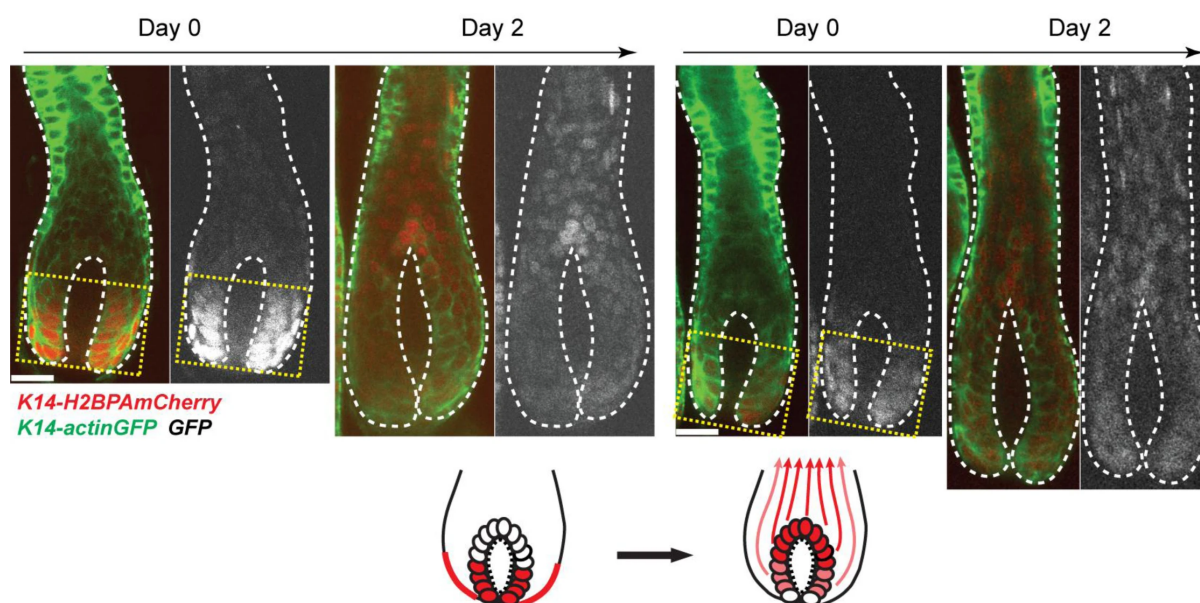


Figure 7.2 Photo-inducible labelling and tracing show upward lineage shifting of the matrix progenitors ³²³

Representative images showing lower matrix progenitors labelled by photo-activated H2BmCherry (K14-H2BPAmCherry, red) took over the upper matrix over time through relocation or expansion. Epithelial membranes were marked by K14-actinGFP (green). White dashed lines outline hair follicle epithelium. Yellow dashed boxes indicate photo-activated areas. H2BmCherry signal was diluted through cell proliferation after photo-activation. Images representative of 7 mice. Scale bar, 20 μ m.

7.4.2 Novel opportunities and targets in hair follicle stem cells.

Matsumura et al. 2021³²⁴ reported that hair follicle stem cells (HFSCs) appear to lose their regenerative capabilities during ageing due to adopting an atypical cell division program. Cell fate tracing and cell division analyses showed that HFSCs in young mice have typical symmetric and asymmetric cell divisions to regenerate hair follicles; when ageing or under stress, they adopt an atypical ‘stress-responsive’ type of asymmetric cell division. This type of cell division is accompanied by the destabilisation of hemidesmosomal protein COL17A1 and cell polarity protein atypical protein kinase C- λ (aPKC λ). It generates terminally differentiating epidermal cells rather than regenerating the hair destabilisation. Repeating these atypical divisions, HFSCs separate from the basal membrane, causing exhaustion, elimination, and organ ageing. The experimentally induced stabilisation of COL17A1 rescued organ homeostasis through aPKC λ stabilisation. This study demonstrated that distinct stem cell

division programs might govern tissue and organ ageing.³²⁴ (See Figure 7.3). While the relationship between Wnt and COL17A1 is unclear, their signalling declines with age. Their normal expression is essential for normal hair growth. Also, Wnt/ β -catenin signalling has been shown to regenerate hair and other high-turnover tissues.^{57,323}

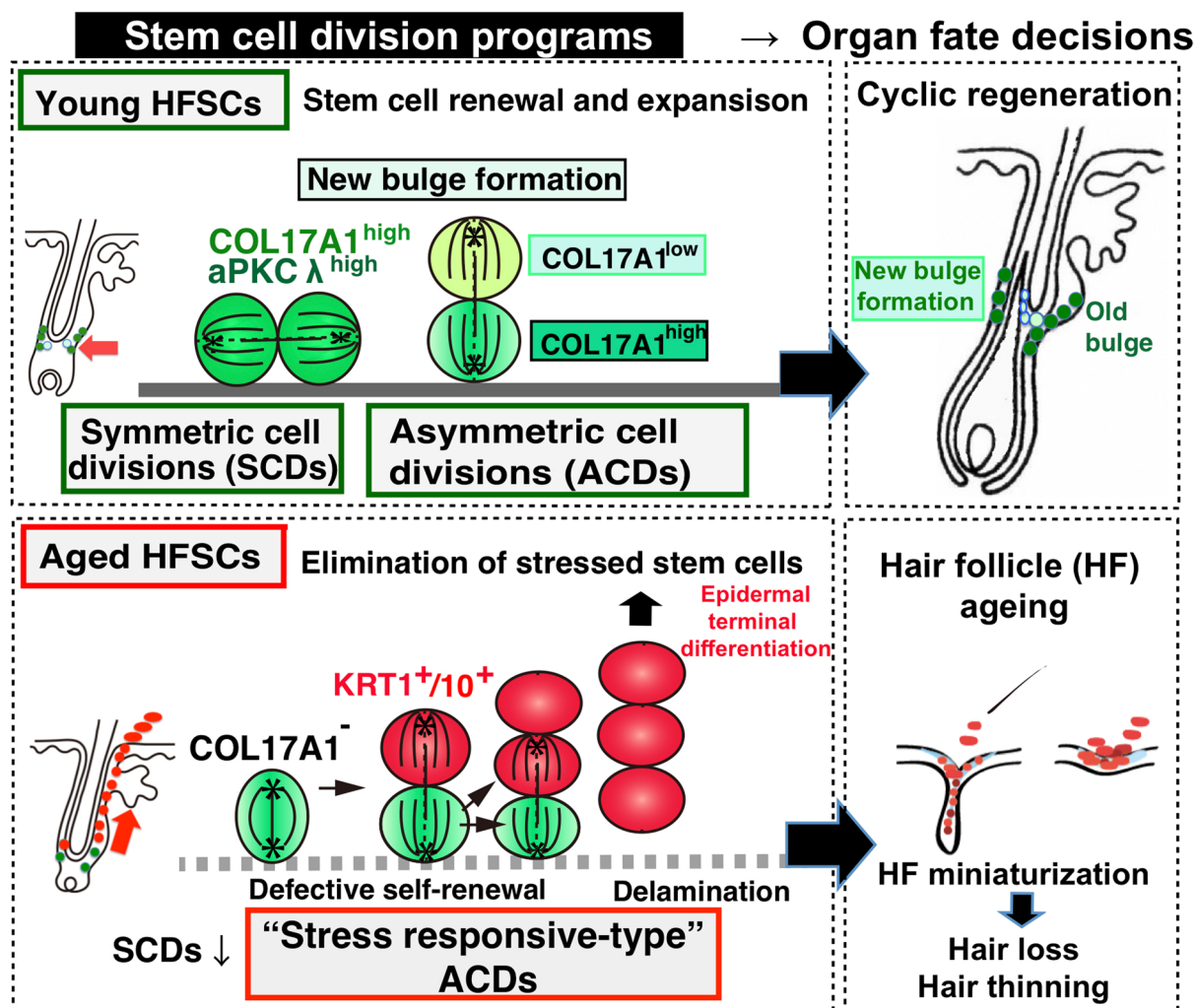


Figure 7.3 Schematic model showing distinct types of stem cell divisions determine HF homeostasis and ageing³²⁴

Young HFSCs undergo symmetric cell divisions (SCDs), and asymmetric cell divisions (ACDs) generate new bulge cells for self-renewal and expansion. Whereas aged HFSCs provoke hemidesmosomal instability, including COL17A1 and undergo stress response type ACDs to induce epidermal differentiation triggering their delamination, causing stepwise miniaturisation of HFs, hair thinning and loss.

7.5 Synthesis of Findings: A Multi-Targeting Approach to Hair Regeneration

Hair loss and its treatments have been recorded for thousands of years, with many effective multi-botanical formulations evolving as traditional medicines. However, hair loss treatment remains an unmet medical need, and there is no consensus on the approach to a solution. Existing treatments (topical Minoxidil 2% and 5%, oral Finasteride) are approved for treating androgenetic alopecia, yet have limited efficacy and various adverse effects. A class of JAK inhibitors have been approved for alopecia areata; however, they have black box safety warnings. Other novel treatments in clinical development have either failed to gain regulatory approval, did not meet efficacy targets or were withdrawn.

Prior formulations of multi-targeting treatments as dietary supplements have low efficacy, and similar to drugs and medical devices, they meet hair regrowth resistance effects after a few months. A multi-targeting botanical, vitamin and mineral formulation to regenerate hair was conceived from literature research and codenamed ALRV5XR. It was studied using oral and topical administration in two randomised placebo-controlled clinical trials to determine safety and efficacy in hair regrowth in androgenetic alopecia, and telogen effluvium. Terminal hair was determined as the principal marker of the scalp hair phenotype and measurement of its changes in density was the primary outcome in Chapters 3 – 6.

7.5.1 Implications for the broader field of hair loss disorders

When evaluating the totality of the molecular targets implicated in a broad range of hair disorders, like the orchestration of molecular signalling in normal hair cycling, it appears from the literature that hair disorders might be associated with alternative signalling harmonics with different amplifications or attenuations of molecular signals, and different modes of discord might give rise to a specific hair disorder phenotype. This speculative idea is already being

played out to some degree by combination and off-label use of drug treatments, PRP, the traditional medicine approaches as well as the ALRV5XR multi-targeting model, however, specific correlative effects of the interventions have limited characterisation in human biology studies. Nevertheless, given that single molecule targeting treatments in hair disorders have found low efficacy with treatment resistance effects as per Chapter 6 and various adverse effects, multi-targeting BVM treatments, such as ALRV5XR, have shown early efficacy in treating AGA and TE and might have a wide range of benefits in multiple hair and scalp disorders.

Some of the key botanicals in ALRV5XR, *Eclipta prostrata*, *Polygonum multiflorum* and *Angelica sinensis*, were found amongst the most frequently used herbs in traditional Chinese medicine for hair loss. In addition, *Scutellaria baicalensis*, *Sophora flavescens* and *Astragalus membranaceus* are herbs also commonly used in TCM formulations for hair loss. These six botanicals have been identified as regulators of 23 of the 25 molecular pathways in Table 2.1 including the key Wnt/ β -catenin stem cell activation pathway.

7.5.2 Implications for the wider field of skin disorders

The considerable overlap of molecular pathway regulation between hair and skin, particularly the Wnt/ β -catenin pathway suggests that evaluating and optimising ALRV5XR for inducing homeostasis in skin disorders such as psoriasis, atopic dermatitis and weeping eczema might yield a novel range of safe and effective therapeutics.^{122,325,326}

7.5.3 Implications for other ageing and degenerative conditions

The multi-targeting studies using ALRV5XR suggest, as a proof of concept, that such interventions could be of significant benefit in slowing the ageing process and treating diseases.

The initial clinical effects of ALRV5XR in hair regeneration suggest that there might be a meaningful mild stimulation of Wnt/ β -catenin and co-regulation of supporting molecular pathways known to induce self-renewal in many tissues besides hair including bone, skin, adipocytes, muscle, heart and the immune system.³⁰⁶ Furthermore, studies of some of the ALRV5XR botanicals employed as therapies and co-therapies in cancer suggest a potential reparative effect that should be investigated for the prevention and treatment of various life-threatening conditions.^{307,308,310,311,315,318,327}

A pharmacopoeia of herbs used in traditional Chinese medicine³²⁸ indicates *Angelica Sinensis* for promoting blood circulation, modulating immunity, constipation and menstrual disorders, and it has anti-atherosclerotic, anti-hypertensive, antioxidant and anti-inflammatory pharmacological properties.³²⁹ *Eclipta prostrata* is indicated for strengthening bones and blackening hair.³³⁰ *Polygonum multiflorum* is indicated for promoting hair growth, and reducing lipids.³¹⁵

The potential for multi-targeting botanical formulations appears to have a wide range of therapeutic potential. Therefore, a diligent therapeutic target and effect evaluation process can potentially lead to multiple novel and safe treatments.

7.5.4 Limitations of the research

This research shows a high degree of efficacy of the multi-targeting formulation, ALRV5XR, for hair regrowth in limited studies with small populations with AGA in Chapters 3 and 4. In addition, the continuous terminal hair regrowth effect without resistance or adverse events in women and men indicates the potential of a novel treatment that might restore a scalp hair phenotype.

Notwithstanding the limited scope of the clinical trials in Chapters 3 and 4, the concept of a multi-targeting approach to hair regeneration using botanicals, vitamins and minerals, as formulated into ALRV5XR, can be said to encompass the majority of the ten recent expert opinions on approaches to treat hair loss. In addition, its initial evidence has found a legitimate footing in the field of hair loss treatments by appearing to induce homeostasis and self-renewal in the hair follicles. However, further studies are required to elucidate the specific mechanisms of action to refine the formulations and to verify the initial findings of miniaturisation reversal and superior terminal hair regrowth.

Although these clinical trials in Chapters 3 and 4 were adequately powered for statistical purposes, they should be repeated and conducted with more extensive and diverse populations and geographies over more than 24 weeks.

Chapter 5 shows interesting hair regrowth dynamics over time and suggests a reversal of hair miniaturisation as measured by mean changes in hair diameter and terminal-to-vellus ratios. These phenomena of regrowth dynamics and miniaturisation reversal should be parsed out more granularly to confirm these findings.

Chapter 6 was limited by eligible studies in the literature. Ideally, each treatment should have at least three studies, and smaller studies like ALRV5XR also need larger populations. Although some eligible studies had treatment durations longer than 24 weeks, insufficient data were available for statistical comparisons after this period. Searching for studies in multiple languages might also yield a greater data set.

7.5.5 Proposed follow-up research

Follow-up research of multi-targeting BVM formulations for the treatment of AGA to:

- (i) Re-evaluate ALRV5XR for redundancies, missed targets, compound isolations, optimisations and improving the outcomes in men;
- (ii) Expand the body of clinical evidence in AGA with larger populations in more extended studies and compare outcomes of topical versus oral administration, global scalp scoring and quality of evaluations;
- (iii) The phenomena of regrowth dynamics and miniaturisation reversal found in Chapter 5 should be parsed more granularly to confirm these findings. For example, terminal hair could be subdivided into at least three diameter groups such as thin (40-60 μ m), regular (60-80 μ m) and thick (>80 μ m).
- (iv) A study characterising the changes in active follicular unit counts and characteristics of hairs per follicular unit to more fully evaluate the extent of reactivating follicular units and follicles, the effects on hair cycling, and also evaluating the empirical effect of miniaturisation reversal at the follicle level.

7.5.6 Considerations for multi-targeting formulation refinement

The BVM ingredients selected for ALRV5XR have shown initial effectiveness; however, further aspects will be required to consider commercial applications: long-term safety, regulatory, aesthetic, and sensory considerations. Most importantly, the active constituents of the botanicals should be isolated and studied for targeted signalling effects. In particular, finding the ideal compounds from the botanicals that activate, for example, Wnt/ β -catenin signalling, in a dose-measured manner to specifically treat to maintain a safe and effective targeted level of signalling. This should be applied similarly to other targets in the follicle.

7.6 Conclusions

The hypothesis that a multi-targeting treatment formulated from botanicals, vitamins and minerals regenerates hair in subjects with androgenetic alopecia as demonstrated using the gold standard of randomised placebo-controlled clinical trials in women and men in Chapters 3 – 5, is viable and effective. Furthermore, in comparing the primary outcomes of terminal hair regrowth to all commercially available treatments in Chapter 6, the multi-targeting treatment, ALRV5XR, showed promising early outcomes when compared to other treatments, however, more RCTs with larger cohorts are required to develop a more robust evidence profile. It also has the promising characteristic of experiencing continuous regrowth over the study period, which will also have to be validated in additional RCTs. Therefore, the potential of restoring a normal scalp hair phenotype with a multi-targeting treatment of ALRV5XR could be feasible. This outcome can be anticipated in a shorter time in women than in men. Furthermore, this multi-targeting approach to treatment also identified opportunities for broader applications in hair and skin disorders.

References

1. Dhouailly D. A new scenario for the evolutionary origin of hair, feather, and avian scales. *J Anat.* 2009;214(4):587-606.
2. McMichael AJ, Hordinsky MK. Hair and scalp disorders : medical, surgical, and cosmetic treatments. In: Second edition. ed. Boca Raton: CRC Press,; 2018.
3. Schneider MR, Schmidt-Ullrich R, Paus R. The hair follicle as a dynamic miniorgan. *Curr Biol.* 2009;19(3):R132-142.
4. Lemasters JJ, Ramshesh VK, Lovelace GL, et al. Compartmentation of Mitochondrial and Oxidative Metabolism in Growing Hair Follicles: A Ring of Fire. *J Invest Dermatol.* 2017;137(7):1434-1444.
5. Chueh SC, Lin SJ, Chen CC, et al. Therapeutic strategy for hair regeneration: hair cycle activation, niche environment modulation, wound-induced follicle neogenesis, and stem cell engineering. *Expert Opin Biol Ther.* 2013;13(3):377-391.
6. 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):568-575.
7. Aukerman EL, Jafferany M. The psychological consequences of androgenetic alopecia: A systematic review. *J.* 2022;11:11.
8. Su LH, Chen LS, Lin SC, Chen HH. Association of androgenetic alopecia with mortality from diabetes mellitus and heart disease. *JAMA Dermatol.* 2013;149(5):601-606.
9. Cash TF. The psychosocial consequences of androgenetic alopecia: a review of the research literature. *Br J Dermatol.* 1999;141(3):398-405.

10. You Q, Li L, Ma X, et al. Meta-Analysis on the Efficacy and Safety of Traditional Chinese Medicine as Adjuvant Therapy for Refractory Androgenetic Alopecia. *Evid Based Complement Alternat Med*. 2019;2019:9274148.
11. DISEASES OF THE HAIRS AND HAIR-FOLLICLES. *The Lancet*. 1843;40(1047):885-888.
12. Kanti V, Messenger A, Dobos G, et al. Evidence-based (S3) guideline for the treatment of androgenetic alopecia in women and in men - short version. *J Eur Acad Dermatol Venereol*. 2018;32(1):11-22.
13. Myers RJ, Hamilton JB. Regeneration and rate of growth of hairs in man. *Ann N Y Acad Sci*. 1951;53(3):562-568.
14. Cruz CF, Costa C, Gomes AC, Matamá T, Cavaco-Paulo A. Human Hair and the Impact of Cosmetic Procedures: A Review on Cleansing and Shape-Modulating Cosmetics. *Cosmetics*. 2016;3(3):26.
15. Rushton DH, Westgate GE, Van Neste DJ. Following historical "tracks" of hair follicle miniaturisation in patterned hair loss: Are elastin bodies the forgotten aetiology? *Exp Dermatol*. 2021.
16. Whiting DA. The Structure of the Human Hair Follicle: Light Microscopy of Vertical and Horizontal Sections of Scalp Biopsies. 2004.
17. Olsen EA, Canfield D. SALT II: A new take on the Severity of Alopecia Tool (SALT) for determining percentage scalp hair loss. *J Am Acad Dermatol*. 2016;75(6):1268-1270.
18. Headington JT. Transverse microscopic anatomy of the human scalp. A basis for a morphometric approach to disorders of the hair follicle. *Arch Dermatol*. 1984;120(4):449-456.
19. Rook AJ, Dawber RPR. Diseases of the Hair and Scalp. 1991.

20. Cutrone M, Grimalt R. Transient neonatal hair loss: a common transient neonatal dermatosis. *Eur J Pediatr.* 2005;164(10):630-632.
21. Paus R, Cotsarelis G. The biology of hair follicles. *N Engl J Med.* 1999;341(7):491-497.
22. Pecoraro V, Astore I, Barman J, Ignacioaraujo C. THE NORMAL TRICHOGRAM IN THE CHILD BEFORE THE AGE OF PUBERTY. *J Invest Dermatol.* 1964;42:427-430.
23. Randall VA, Ebling FJ. Seasonal changes in human hair growth. *Br J Dermatol.* 1991;124(2):146-151.
24. Liu C, Yang J, Qu L, et al. Changes in Chinese hair growth along a full year. *Int J Cosmet Sci.* 2014;36(6):531-536.
25. Kunz M, Seifert B, Trueb RM. Seasonality of hair shedding in healthy women complaining of hair loss. *Dermatology.* 2009;219(2):105-110.
26. Dardente H, Wood S, Ebling F, Saenz de Miera C. An integrative view of mammalian seasonal neuroendocrinology. *J Neuroendocrinol.* 2019;31(5):e12729.
27. Loussouarn G, El Rawadi C, Genain G. Diversity of hair growth profiles. *Int J Dermatol.* 2005;44 Suppl 1:6-9.
28. Maymone MBC, Laughter M, Pollock S, et al. Hair Aging in Different Races and Ethnicities. *J Clin Aesthet Dermatol.* 2021;14(1):38-44.
29. Hamilton JB. Quantitative measurement of a secondary sex character, axillary hair. *Ann N Y Acad Sci.* 1951;53(3):585-599.
30. Messenger AG. Hair through the female life cycle. *Br J Dermatol.* 2011;165 Suppl 3:2-6.
31. Ieremia E, Stefanato CM. THE ROLE OF HAIR FOLLICLE COUNTS AND RATIOS IN THE HISTOPATHOLOGICAL ASSESSMENT OF ANDROGENIC

ALOPECIA, ALOPECIA AREATA AND TELOGEN EFFLUVIUM: DOES COUNTING 'COUNT'? *Hum Pathol.* 2023.

32. Cotsarelis G, Sun TT, Lavker RM. Label-retaining cells reside in the bulge area of pilosebaceous unit: implications for follicular stem cells, hair cycle, and skin carcinogenesis. *Cell.* 1990;61(7):1329-1337.
33. Rebora A, Guarrera M. Kenogen. A new phase of the hair cycle? *Dermatology.* 2002;205(2):108-110.
34. Courtois M, Loussouarn G, Hourseau C, Grollier JF. Ageing and hair cycles. *Br J Dermatol.* 1995;132(1):86-93.
35. Bernard BA. Advances in Understanding Hair Growth. *F1000Res.* 2016;5.
36. Elliott K, Stephenson TJ, Messenger AG. Differences in hair follicle dermal papilla volume are due to extracellular matrix volume and cell number: implications for the control of hair follicle size and androgen responses. *J Invest Dermatol.* 1999;113(6):873-877.
37. Botchkareva NV, Ahluwalia G, Shander D. Apoptosis in the hair follicle. *J Invest Dermatol.* 2006;126(2):258-264.
38. Shakshouk H, Tosti A. Trichoscopy beyond scalp. A narrative review. *Int J Dermatol.* 2023;62(3):416-427.
39. Vañó-Galván S, Saceda-Corralo D, Blume-Peytavi U, et al. Frequency of the Types of Alopecia at Twenty-Two Specialist Hair Clinics: A Multicenter Study. *Skin Appendage Disord.* 2019;5(5):309-315.
40. Mirmirani P. Age-related hair changes in men: mechanisms and management of alopecia and graying. *Maturitas.* 2015;80(1):58-62.
41. Mirmirani P, Fu J. Diagnosis and Treatment of Nonscarring Hair Loss in Primary Care in 2021. *Jama.* 2021;325(9):878-879.

42. Mostaghimi A, Gao W, Ray M, et al. Trends in Prevalence and Incidence of Alopecia Areata, Alopecia Totalis, and Alopecia Universalis Among Adults and Children in a US Employer-Sponsored Insured Population. *JAMA Dermatology*. 2023;159(4):411-418.
43. Varothai S, Bergfeld WF. Androgenetic alopecia: an evidence-based treatment update. *Am J Clin Dermatol*. 2014;15(3):217-230.
44. Tamashunas NL, Bergfeld WF. Male and female pattern hair loss: Treatable and worth treating. *Cleve Clin J Med*. 2021;88(3):173-182.
45. García-Hernández M-J, Camacho FM. Chronic Telogen Effluvium: Incidence, Clinical and Biochemical Features, and Treatment. *Archives of Dermatology*. 1999;135(9):1123-1124.
46. Daunton A, Harries M, Sinclair R, Paus R, Tosti A, Messenger A. Chronic Telogen Effluvium: Is it a Distinct Condition? A Systematic Review. *Am J Clin Dermatol*. 2023.
47. Olsen EA, Dunlap FE, Funicella T, et al. A randomized clinical trial of 5% topical minoxidil versus 2% topical minoxidil and placebo in the treatment of androgenetic alopecia in men. *J Am Acad Dermatol*. 2002;47(3):377-385.
48. Leyden J, Dunlap F, Miller B, et al. Finasteride in the treatment of men with frontal male pattern hair loss. *J Am Acad Dermatol*. 1999;40(6 Pt 1):930-937.
49. Drake L, Reyes-Hadsall S, Martinez J, Heinrich C, Huang K, Mostaghimi A. Evaluation of the Safety and Effectiveness of Nutritional Supplements for Treating Hair Loss: A Systematic Review. *JAMA Dermatology*. 2023;159(1):79-86.
50. Dina Y, Aguh C. Use of Platelet-Rich Plasma in Cicatricial Alopecia. *Dermatol Surg*. 2019;45(7):979-981.
51. Whiting DA. *Hair Growth and Disorders*. Springer-Verlag Berlin Heidelberg; 2008.

52. Poliner AD, Tosti A. Hair regrowth in cicatricial alopecia: A literature review. *J Dermatol.* 2021;48(8):1113-1128.
53. Trager MH, Lavian J, Lee EY, et al. Prevalence estimates for lichen planopilaris and frontal fibrosing alopecia in a New York City health care system. *J Am Acad Dermatol.* 2021;84(4):1166-1169.
54. Prevention CfDcA. ICD-10 Coordination and Maintenance Committee Meeting. March 7-8, 2023, 2023; Centers for Disease Control and Prevention.
55. Cummins DM, Chaudhry IH, Harries M. Scarring Alopecias: Pathology and an Update on Digital Developments. *Biomedicines.* 2021;9(12).
56. Ioannidis AD, Khan SG, Tamura D, et al. Trichothiodystrophy hair shafts display distinct ultrastructural features. *Experimental Dermatology.* 2022;31(8):1270-1275.
57. Matsumura H, Mohri Y, Binh NT, et al. Hair follicle aging is driven by transepidermal elimination of stem cells via COL17A1 proteolysis. *Science.* 2016;351(6273):aad4395.
58. Blanpain C, Fuchs E. Epidermal homeostasis: a balancing act of stem cells in the skin. *Nat Rev Mol Cell Biol.* 2009;10(3):207-217.
59. Vasserot AP, Geyfman M, Poloso NJ. Androgenetic alopecia: combing the hair follicle signaling pathways for new therapeutic targets and more effective treatment options. *Expert Opin Ther Targets.* 2019;23(9):755-771.
60. Lim X, Tan SH, Yu KL, Lim SB, Nusse R. Axin2 marks quiescent hair follicle bulge stem cells that are maintained by autocrine Wnt/ β -catenin signaling. *Proc Natl Acad Sci U S A.* 2016;113(11):E1498-1505.
61. Andl T, Reddy ST, Gaddapara T, Millar SE. WNT signals are required for the initiation of hair follicle development. *Dev Cell.* 2002;2(5):643-653.

62. Hashimoto K, Yamada Y, Sekiguchi K, Mori S, Matsumoto T. NLRP3 inflammasome activation contributes to development of alopecia areata in C3H/HeJ mice. *Experimental Dermatology*. 2022;31(2):133-142.
63. Suda T, Arai F. Wnt signaling in the niche. *Cell*. 2008;132(5):729-730.
64. Clevers H, Nusse R. Wnt/ β -catenin signaling and disease. *Cell*. 2012;149(6):1192-1205.
65. Huelsken J, Vogel R, Erdmann B, Cotsarelis G, Birchmeier W. beta-Catenin controls hair follicle morphogenesis and stem cell differentiation in the skin. *Cell*. 2001;105(4):533-545.
66. Huang P, Yan R, Zhang X, Wang L, Ke X, Qu Y. Activating Wnt/ β -catenin signaling pathway for disease therapy: Challenges and opportunities. *Pharmacol Ther*. 2019;196:79-90.
67. Lien WH, Fuchs E. Wnt some lose some: transcriptional governance of stem cells by Wnt/ β -catenin signaling. *Genes Dev*. 2014;28(14):1517-1532.
68. Reddy S, Andl T, Bagasra A, et al. Characterization of Wnt gene expression in developing and postnatal hair follicles and identification of Wnt5a as a target of Sonic hedgehog in hair follicle morphogenesis. *Mechanisms of Development*. 2001;107(1):69-82.
69. Rishikaysh P, Dev K, Diaz D, Qureshi WM, Filip S, Mokry J. Signaling involved in hair follicle morphogenesis and development. *Int J Mol Sci*. 2014;15(1):1647-1670.
70. Kim D, Garza LA. The Negative Regulator CXXC5: Making WNT Look a Little Less Dishevelled. *J Invest Dermatol*. 2017;137(11):2248-2250.
71. Lei MX, Chuong CM, Widelitz RB. Tuning Wnt signals for more or fewer hairs. *J Invest Dermatol*. 2013;133(1):7-9.

72. Lee J, Tumber T. Hairy tale of signaling in hair follicle development and cycling. *Semin Cell Dev Biol.* 2012;23(8):906-916.
73. Mahé YF, Buan B, Billoni N, et al. Pro-inflammatory cytokine cascade in human plucked hair. *Skin Pharmacol.* 1996;9(6):366-375.
74. Inui S, Itami S. Molecular basis of androgenetic alopecia: From androgen to paracrine mediators through dermal papilla. *J Dermatol Sci.* 2011;61(1):1-6.
75. Billoni N, Buan B, Gautier B, Gaillard O, Mahé YF, Bernard BA. Thyroid hormone receptor beta1 is expressed in the human hair follicle. *Br J Dermatol.* 2000;142(4):645-652.
76. Khidhir KG, Woodward DF, Farjo NP, et al. The prostamide-related glaucoma therapy, bimatoprost, offers a novel approach for treating scalp alopecias. *Faseb j.* 2013;27(2):557-567.
77. Imamura T. Physiological functions and underlying mechanisms of fibroblast growth factor (FGF) family members: recent findings and implications for their pharmacological application. *Biol Pharm Bull.* 2014;37(7):1081-1089.
78. Geyfman M, Plikus MV, Treffeisen E, Andersen B, Paus R. Resting no more: re-defining telogen, the maintenance stage of the hair growth cycle. *Biol Rev Camb Philos Soc.* 2015;90(4):1179-1196.
79. Xiong Y, Liu Y, Song Z, Hao F, Yang X. Identification of Wnt/beta-catenin signaling pathway in dermal papilla cells of human scalp hair follicles: TCF4 regulates the proliferation and secretory activity of dermal papilla cell. *J Dermatol.* 2014;41(1):84-91.
80. Tsuji H, Ishida-Yamamoto A, Takahashi H, Iizuka H. Nuclear localization of beta-catenin in the hair matrix cells and differentiated keratinocytes. *Journal of Dermatological Science.* 2001;27(3):170-177.

81. Shin H, Kwack MH, Shin SH, et al. Identification of transcriptional targets of Wnt/beta-catenin signaling in dermal papilla cells of human scalp hair follicles: EP2 is a novel transcriptional target of Wnt3a. *Journal of Dermatological Science*. 2010;58(2):91-96.
82. Philpott M. A new model of human telogen-anagen transformation helps decipher Wnt signalling in the human hair cycle. *British Journal of Dermatology*. 2020;182(5):1084.
83. Michel L, Reygagne P, Benech P, et al. Study of gene expression alteration in male androgenetic alopecia: evidence of predominant molecular signalling pathways. *British Journal of Dermatology*. 2017;177(5):1322-1336.
84. Lu GQ, Wu ZB, Chu XY, Bi ZG, Fan WX. An investigation of crosstalk between Wnt/beta-catenin and transforming growth factor-beta signaling in androgenetic alopecia. *Medicine*. 2016;95(30):e4297.
85. Leiros GJ, Ceruti JM, Castellanos ML, Kusinsky AG, Balana ME. Androgens modify Wnt agonists/antagonists expression balance in dermal papilla cells preventing hair follicle stem cell differentiation in androgenetic alopecia. *Molecular & Cellular Endocrinology*. 2017;439:26-34.
86. Leiros GJ, Attorresi AI, Balana ME. Hair follicle stem cell differentiation is inhibited through cross-talk between Wnt/beta-catenin and androgen signalling in dermal papilla cells from patients with androgenetic alopecia. *British Journal of Dermatology*. 2012;166(5):1035-1042.
87. Kishimoto J, Burgeson RE, Morgan BA. Wnt signaling maintains the hair-inducing activity of the dermal papilla. *Genes Dev*. 2000;14(10):1181-1185.
88. Jamerson TA, Conover Talbot C, Jr., Dina Y, Kwatra SG, Garza LA, Aguh C. Gene expression profiling suggests severe, extensive central centrifugal cicatricial alopecia

may be both clinically and biologically distinct from limited disease subtypes.

Experimental Dermatology. 2022;31(5):789-793.

89. Blanpain C. [Impact of beta-catenin signaling pathway on stem cell differentiation in the skin]. *M S-Medecine Sciences*. 2007;23(1):34-36.
90. Choi BY. Targeting Wnt/beta-Catenin Pathway for Developing Therapies for Hair Loss. *Int J Mol Sci*. 2020;21(14).
91. Hawkshaw NJ, Hardman JA, Haslam IS, 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 Biol*. 2018;16(5):e2003705.
92. Woo WM, Zhen HH, Oro AE. Shh maintains dermal papilla identity and hair morphogenesis via a Noggin-Shh regulatory loop. *Genes Dev*. 2012;26(11):1235-1246.
93. Oro AE, Higgins K. Hair cycle regulation of Hedgehog signal reception. *Dev Biol*. 2003;255(2):238-248.
94. Paladini RD, Saleh J, Qian C, Xu GX, Rubin LL. Modulation of hair growth with small molecule agonists of the hedgehog signaling pathway. *J Invest Dermatol*. 2005;125(4):638-646.
95. Botchkarev VA, Sharov AA. BMP signaling in the control of skin development and hair follicle growth. *Differentiation*. 2004;72(9-10):512-526.
96. Plikus MV. New activators and inhibitors in the hair cycle clock: targeting stem cells' state of competence. *J Invest Dermatol*. 2012;132(5):1321-1324.
97. Ceruti JM, Oppenheimer FM, Leiros GJ, Balana ME. Androgens downregulate BMP2 impairing the inductive role of dermal papilla cells on hair follicle stem cells differentiation. *Molecular & Cellular Endocrinology*. 2021;520:111096.

98. Botchkarev VA, Botchkareva NV, Sharov AA, Funa K, Huber O, Gilchrest BA. Modulation of BMP signaling by noggin is required for induction of the secondary (nontylotrich) hair follicles. *J Invest Dermatol.* 2002;118(1):3-10.
99. Botchkarev VA, Botchkareva NV, Nakamura M, et al. Noggin is required for induction of the hair follicle growth phase in postnatal skin. *Faseb j.* 2001;15(12):2205-2214.
100. Choi J, Southworth LK, Sarin KY, et al. TERT promotes epithelial proliferation through transcriptional control of a Myc- and Wnt-related developmental program. *PLoS Genet.* 2008;4(1):e10.
101. Sarin KY, Cheung P, Gilson D, et al. Conditional telomerase induction causes proliferation of hair follicle stem cells. *Nature.* 2005;436(7053):1048-1052.
102. Li W, Man XY, Li CM, et al. VEGF induces proliferation of human hair follicle dermal papilla cells through VEGFR-2-mediated activation of ERK. *Exp Cell Res.* 2012;318(14):1633-1640.
103. Andjelkov K, Eremin, II, Korac A. Different levels of EGF, VEGF, IL-6, MCP-1, MCP-3, IP-10, Eotaxin and MIP-1alpha in the adipose-derived stem cell secretome in androgenetic alopecia. *Experimental Dermatology.* 2022;31(6):936-942.
104. Shin JM, Lee YY, Kim KM, et al. The potential role of fibroblast-derived multi-peptide factors in activation of growth factors and beta-Catenin in hair follicle cells. *J.* 2022;28:28.
105. Yano K, Brown LF, Detmar M. Control of hair growth and follicle size by VEGF-mediated angiogenesis. *J Clin Invest.* 2001;107(4):409-417.
106. Plikus MV, Chuong CM. Macroenvironmental regulation of hair cycling and collective regenerative behavior. *Cold Spring Harb Perspect Med.* 2014;4(1):a015198.

107. Choi K, Park SH, Park SY, Yoon SK. The stem cell quiescence and niche signaling is disturbed in the hair follicle of the hairpoor mouse, an MUHH model mouse. *Stem Cell Res Ther.* 2022;13(1):211.
108. Qi Y, Li M, Xu L, Chang Z, Shu X, Zhou L. Therapeutic role of human hepatocyte growth factor (HGF) in treating hair loss. *PeerJ.* 2016;4:e2624.
109. Yamazaki M, Tsuboi R, Lee YR, Ishidoh K, Mitsui S, Ogawa H. Hair cycle-dependent expression of hepatocyte growth factor (HGF) activator, other proteinases, and proteinase inhibitors correlates with the expression of HGF in rat hair follicles. *J Invest Dermatol Symp Proc.* 1999;4(3):312-315.
110. Nicu C, O'Sullivan JDB, Ramos R, et al. Dermal Adipose Tissue Secretes HGF to Promote Human Hair Growth and Pigmentation. *Journal of Investigative Dermatology.* 2021;141(7):1633-1645.e1613.
111. Weger N, Schlake T. Igf-I signalling controls the hair growth cycle and the differentiation of hair shafts. *J Invest Dermatol.* 2005;125(5):873-882.
112. Ahn SY, Pi LQ, Hwang ST, Lee WS. Effect of IGF-I on Hair Growth Is Related to the Anti-Apoptotic Effect of IGF-I and Up-Regulation of PDGF-A and PDGF-B. *Ann Dermatol.* 2012;24(1):26-31.
113. Guevara-Aguirre J, Torres C, Pena G, et al. IGF-I deficiency and enhanced insulin sensitivity due to a mutated growth hormone receptor gene in humans. *Mol Cell Endocrinol.* 2021;519:111044.
114. Inui S, Itami S. Androgen actions on the human hair follicle: perspectives. *Exp Dermatol.* 2013;22(3):168-171.
115. Paus R, Ito N, Takigawa M, Ito T. The hair follicle and immune privilege. *J Invest Dermatol Symp Proc.* 2003;8(2):188-194.

116. Kellenberger AJ, Tauchi M. Mammalian target of rapamycin complex 1 (mTORC1) may modulate the timing of anagen entry in mouse hair follicles. *Exp Dermatol*. 2013;22(1):77-80.
117. Zeng H, Lu B, Zamponi R, et al. mTORC1 signaling suppresses Wnt/ β -catenin signaling through DVL-dependent regulation of Wnt receptor FZD level. *Proc Natl Acad Sci U S A*. 2018;115(44):E10362-e10369.
118. Di-Poï N, Michalik L, Desvergne B, Wahli W. Functions of peroxisome proliferator-activated receptors (PPAR) in skin homeostasis. *Lipids*. 2004;39(11):1093-1099.
119. Festa E, Fretz J, Berry R, et al. Adipocyte lineage cells contribute to the skin stem cell niche to drive hair cycling. *Cell*. 2011;146(5):761-771.
120. Pratt CH, King LE, Jr., Messenger AG, Christiano AM, Sundberg JP. Alopecia areata. *Nat Rev Dis Primers*. 2017;3:17011.
121. Xu Y, Yang L, Yang T, Xiang M, Huang E, Lian X. Expression pattern of cyclooxygenase-2 in normal rat epidermis and pilosebaceous unit during hair cycle. *Acta Histochem Cytochem*. 2008;41(6):157-163.
122. Suzuki T, Ito T, Gilhar A, Tokura Y, Reich K, Paus R. The hair follicle-psoriasis axis: Shared regulatory mechanisms and therapeutic targets. *Experimental Dermatology*. 2022;31(3):266-279.
123. Omar SI, Hamza AM, Eldabah N, Habiba DA. IFN-alpha and TNF-alpha serum levels and their association with disease severity in Egyptian children and adults with alopecia areata. *Int J Dermatol*. 2021;60(11):1397-1404.
124. Ito T, Ito N, Saathoff M, Bettermann A, Takigawa M, Paus R. Interferon-gamma is a potent inducer of catagen-like changes in cultured human anagen hair follicles. *Br J Dermatol*. 2005;152(4):623-631.

125. Gregoriou S, Papafragkaki D, Kontochristopoulos G, Rallis E, Kalogeromitros D, Rigopoulos D. Cytokines and other mediators in alopecia areata. *Mediators Inflamm.* 2010;2010:928030.
126. Lee P, Gund R, Dutta A, et al. Stimulation of hair follicle stem cell proliferation through an IL-1 dependent activation of gammadeltaT-cells. *elife.* 2017;6(12):04.
127. Kowdley KV, Gochanour EM, Sundaram V, Shah RA, Handa P. Hepcidin Signaling in Health and Disease: Ironing Out the Details. *Hepatol Commun.* 2021;5(5):723-735.
128. Lensing M, Jabbari A. An overview of JAK/STAT pathways and JAK inhibition in alopecia areata. *Front.* 2022;13.
129. Hassan AS, Al-Dhoun MQ, Shaker OG, AlOrbani AM. Expression of Signal Transducer and Activator of Transcription-3 in Androgenetic Alopecia: A case control study. *Skin Pharmacol Physiol.* 2022;16:16.
130. Seneschal J, Boniface K, Jacquemin C. Alopecia areata: Recent advances and emerging therapies. *Ann Dermatol Venereol.* 2022;22:22.
131. Kwon O, Senna MM, Sinclair R, et al. Efficacy and Safety of Baricitinib in Patients with Severe Alopecia Areata over 52 Weeks of Continuous Therapy in Two Phase III Trials (BRAVE-AA1 and BRAVE-AA2). *Am J Clin Dermatol.* 2023;24(3):443-451.
132. King B, Ohyama M, Kwon O, et al. Two Phase 3 Trials of Baricitinib for Alopecia Areata. *N Engl J Med.* 2022;386(18):1687-1699.
133. Liu T, Zhang L, Joo D, Sun S-C. NF- κ B signaling in inflammation. *Signal Transduct Target Ther.* 2017;2(1):17023.
134. Garza LA, Liu Y, Yang Z, et al. Prostaglandin D2 inhibits hair growth and is elevated in bald scalp of men with androgenetic alopecia. *Sci Transl Med.* 2012;4(126):126ra134.

135. Nieves A, Garza LA. Does prostaglandin D2 hold the cure to male pattern baldness? *Exp Dermatol*. 2014;23(4):224-227.
136. Namazi MR. Prostaglandin analogs for hair growth: greater expectations. *Dermatol Online J*. 2003;9(5):29.
137. Hibino T, Nishiyama T. Role of TGF-beta2 in the human hair cycle. *J Dermatol Sci*. 2004;35(1):9-18.
138. Chen J, Roop DR. Mimicking hair disorders by genetic manipulation of organ-cultured human hair follicles. *Journal of Investigative Dermatology*. 2012;132(10):2312-2314.
139. Deng Z, Chen M, Liu F, et al. Androgen Receptor-Mediated Paracrine Signaling Induces Regression of Blood Vessels in the Dermal Papilla in Androgenetic Alopecia. *Journal of Investigative Dermatology*. 2022;142(8):2088-2099.e2089.
140. Rokhlin OW, Taghiyev AF, Guseva NV, et al. Androgen regulates apoptosis induced by TNFR family ligands via multiple signaling pathways in LNCaP. *Oncogene*. 2005;24(45):6773-6784.
141. English RS, Jr. A hypothetical pathogenesis model for androgenic alopecia: clarifying the dihydrotestosterone paradox and rate-limiting recovery factors. *Med Hypotheses*. 2018;111:73-81.
142. Hamad M, Bajbouj K, Taneera J. The Case for an Estrogen-iron Axis in Health and Disease. *Exp Clin Endocrinol Diabetes*. 2020;128(4):270-277.
143. Steinbicker AU, Muckenthaler MU. Out of balance--systemic iron homeostasis in iron-related disorders. *Nutrients*. 2013;5(8):3034-3061.
144. Saad HKM, Abd Rahman AA, Ab Ghani AS, et al. Activation of STAT and SMAD Signaling Induces Heparin Re-Expression as a Therapeutic Target for β -Thalassemia Patients. *Biomedicines*. 2022;10(1).

145. Rauf A, Shariati MA, Khalil AA, et al. Hepcidin, an overview of biochemical and clinical properties. *Steroids*. 2020;160:108661.
146. Hou Y, Zhang S, Wang L, et al. Estrogen regulates iron homeostasis through governing hepatic hepcidin expression via an estrogen response element. *Gene*. 2012;511(2):398-403.
147. Karnik P, Shah S, Dvorkin-Wininger Y, Oshtory S, Mirmirani P. Microarray analysis of androgenetic and senescent alopecia: comparison of gene expression shows two distinct profiles. *J Dermatol Sci*. 2013;72(2):183-186.
148. Heilmann S, Kiefer AK, Fricker N, et al. Androgenetic alopecia: identification of four genetic risk loci and evidence for the contribution of WNT signaling to its etiology. *J Invest Dermatol*. 2013;133(6):1489-1496.
149. Hochfeld LM, Bertolini M, Broadley D, et al. Evidence for a functional interaction of WNT10A and EBF1 in male-pattern baldness. *PLoS ONE [Electronic Resource]*. 2021;16(9):e0256846.
150. Betz RC, Petukhova L, Ripke S, et al. Genome-wide meta-analysis in alopecia areata resolves HLA associations and reveals two new susceptibility loci. *Nat Commun*. 2015;6:5966.
151. Pombo A, Dillon N. Three-dimensional genome architecture: players and mechanisms. *Nat Rev Mol Cell Biol*. 2015;16(4):245-257.
152. Pospiech E, Karlowska-Pik J, Kukla-Bartoszek M, et al. Overlapping association signals in the genetics of hair-related phenotypes in humans and their relevance to predictive DNA analysis. *Forensic Sci Int Genet*. 2022;59:102693.
153. Patel S, Sharma V, Chauhan NS, Thakur M, Dixit VK. Hair Growth: Focus on Herbal Therapeutic Agent. *Curr Drug Discov Technol*. 2015;12(1):21-42.

154. Feldman PR, Marra, Luciano E., Brown, Dennis M., Cahan, Deborah J., Inventor. BOTANICALS AS WNT/ β -CATENIN ACTIVATORS, MOLECULAR PATHWAY REGULATORS, TISSUE REGENERATORS AND HEALTH BIOMARKER REGULATORS. US patent WO/2022/146435. 07.07.2022, https://patentscope.wipo.int/search/en/detail.jsf?docId=WO2022146435&_cid=P10-LF1L3Q-86517-1.
155. Gupta A, Kumar A, Kumar D, et al. Ecliptal, a promising natural lead isolated from *Eclipta alba* modulates adipocyte function and ameliorates metabolic syndrome. *Toxicol Appl Pharmacol*. 2018;338:134-147.
156. Park HJ, Zhang N, Park DK. Topical application of *Polygonum multiflorum* extract induces hair growth of resting hair follicles through upregulating Shh and β -catenin expression in C57BL/6 mice. *J Ethnopharmacol*. 2011;135(2):369-375.
157. Li Y, Han M, Lin P, He Y, Yu J, Zhao R. Hair Growth Promotion Activity and Its Mechanism of *Polygonum multiflorum*. *Evid Based Complement Alternat Med*. 2015;2015:517901.
158. Shin SH, Bak SS, Kim MK, Sung YK, Kim JC. Baicalin, a flavonoid, affects the activity of human dermal papilla cells and promotes anagen induction in mice. *Naunyn Schmiedebergs Arch Pharmacol*. 2015;388(5):583-586.
159. Takahashi T, Ishino A, Arai T, et al. Improvement of androgenetic alopecia with topical *Sophora flavescens* Aiton extract, and identification of the two active compounds in the extract that stimulate proliferation of human hair keratinocytes. *Clin Exp Dermatol*. 2016;41(3):302-307.
160. Takahashi T, Kamiya T, Yokoo Y. Proanthocyanidins from grape seeds promote proliferation of mouse hair follicle cells in vitro and convert hair cycle in vivo. *Acta Derm Venereol*. 1998;78(6):428-432.

161. Jian J, Sun L, Cheng X, Hu X, Liang J, Chen Y. Calycosin-7-O- β -d-glucopyranoside stimulates osteoblast differentiation through regulating the BMP/WNT signaling pathways. *Acta Pharm Sin B*. 2015;5(5):454-460.
162. Grothe T, Wandrey F, Schuerch C. Clinical evaluation of pea sprout extract in the treatment of hair loss. *Phytother Res*. 2020;34(2):428-431.
163. Schmid D, Belser E, Züllig F. The FGF7 and Noggin Genes are Key Targets to Treat Hair Loss. 09-2013, 2013.
164. Wang J, Zhang H, Liu T, et al. [Baicalin inhibits the activity of keratinocytes in psoriasis by activating Notch signaling pathway]. *Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi*. 2019;35(5):441-446.
165. Liu P, Zhao H, Luo Y. Anti-Aging Implications of Astragalus Membranaceus (Huangqi): A Well-Known Chinese Tonic. *Aging Dis*. 2017;8(6):868-886.
166. Zhang L, Yang Y, Wang Y, Gao X. Astragalus membranaceus extract promotes neovascularisation by VEGF pathway in rat model of ischemic injury. *Pharmazie*. 2011;66(2):144-150.
167. Lam HW, Lin HC, Lao SC, et al. The angiogenic effects of Angelica sinensis extract on HUVEC in vitro and zebrafish in vivo. *J Cell Biochem*. 2008;103(1):195-211.
168. Merighi S, Benini A, Mirandola P, et al. Caffeine inhibits adenosine-induced accumulation of hypoxia-inducible factor-1 α , vascular endothelial growth factor, and interleukin-8 expression in hypoxic human colon cancer cells. *Mol Pharmacol*. 2007;72(2):395-406.
169. Datta K, Singh AT, Mukherjee A, Bhat B, Ramesh B, Burman AC. Eclipta alba extract with potential for hair growth promoting activity. *J Ethnopharmacol*. 2009;124(3):450-456.

170. Roh SS, Kim CD, Lee MH, Hwang SL, Rang MJ, Yoon YK. The hair growth promoting effect of *Sophora flavescens* extract and its molecular regulation. *J Dermatol Sci*. 2002;30(1):43-49.
171. Toru Imamura MKM, Kawano Nozomi, Tsujino Akiko, Kuramochi Yuko, Oda Kaori, Motomura Masashi, Suzuki Masahiro, Asada Azusa, Kameyama Sumie, Togayachi Syuichi Oka Inventor; National Institute of Advanced Industrial Science and Technology AIST, assignee. Hair regrowth promoter. Aug.12,2010, 2009.
172. Zuo C, Xie XS, Qiu HY, Deng Y, Zhu D, Fan JM. Astragalus mongholicus ameliorates renal fibrosis by modulating HGF and TGF-beta in rats with unilateral ureteral obstruction. *J Zhejiang Univ Sci B*. 2009;10(5):380-390.
173. Ono T, Tsuji T, Sakai M, et al. Induction of hepatocyte growth factor production in human dermal fibroblasts and their proliferation by the extract of bitter melon pulp. *Cytokine*. 2009;46(1):119-126.
174. Rau O, Wurglics M, Paulke A, et al. Carnosic acid and carnosol, phenolic diterpene compounds of the labiate herbs rosemary and sage, are activators of the human peroxisome proliferator-activated receptor gamma. *Planta Med*. 2006;72(10):881-887.
175. Les F, Prieto JM, Arbonés-Mainar JM, Valero MS, López V. Bioactive properties of commercialised pomegranate (*Punica granatum*) juice: antioxidant, antiproliferative and enzyme inhibiting activities. *Food Funct*. 2015;6(6):2049-2057.
176. Kim EH, Shim B, Kang S, et al. Anti-inflammatory effects of *Scutellaria baicalensis* extract via suppression of immune modulators and MAP kinase signaling molecules. *J Ethnopharmacol*. 2009;126(2):320-331.

177. Wun ZY, Lin CF, Huang WC, et al. Anti-inflammatory effect of sophoraflavanone G isolated from *Sophora flavescens* in lipopolysaccharide-stimulated mouse macrophages. *Food Chem Toxicol.* 2013;62:255-261.
178. Habijan J, Berovic M, Boh B, Plankl M, Wraber B. Submerged cultivation of *Ganoderma lucidum* and the effects of its polysaccharides on the production of human cytokines TNF- α , IL-12, IFN- γ , IL-2, IL-4, IL-10 and IL-17. *N Biotechnol.* 2015;32(1):85-95.
179. Silva AM, Machado ID, Santin JR, et al. Aqueous extract of *Rosmarinus officinalis* L. inhibits neutrophil influx and cytokine secretion. *Phytother Res.* 2015;29(1):125-133.
180. Li BQ, Fu T, Gong WH, et al. The flavonoid baicalin exhibits anti-inflammatory activity by binding to chemokines. *Immunopharmacology.* 2000;49(3):295-306.
181. Xu J, Liu J, Yue G, et al. Therapeutic effect of the natural compounds baicalein and baicalin on autoimmune diseases. *Mol Med Rep.* 2018;18(1):1149-1154.
182. Ryu M, Kim EH, Chun M, et al. Astragali Radix elicits anti-inflammation via activation of MKP-1, concomitant with attenuation of p38 and Erk. *J Ethnopharmacol.* 2008;115(2):184-193.
183. Dudhgaonkar S, Thyagarajan A, Sliva D. Suppression of the inflammatory response by triterpenes isolated from the mushroom *Ganoderma lucidum*. *Int Immunopharmacol.* 2009;9(11):1272-1280.
184. Denis MC, Furtos A, Dudonné S, et al. Apple peel polyphenols and their beneficial actions on oxidative stress and inflammation. *PLoS One.* 2013;8(1):e53725.
185. Kuo CF, Su JD, Chiu CH, et al. Anti-inflammatory effects of supercritical carbon dioxide extract and its isolated carnosic acid from *Rosmarinus officinalis* leaves. *J Agric Food Chem.* 2011;59(8):3674-3685.

186. Hong GE, Kim JA, Nagappan A, et al. Flavonoids Identified from Korean *Scutellaria baicalensis* Georgi Inhibit Inflammatory Signaling by Suppressing Activation of NF- κ B and MAPK in RAW 264.7 Cells. *Evid Based Complement Alternat Med*. 2013;2013:912031.
187. Yu MH, Choi JH, Chae IG, et al. Suppression of LPS-induced inflammatory activities by *Rosmarinus officinalis* L. *Food Chem*. 2013;136(2):1047-1054.
188. Yang YZ, Tang YZ, Liu YH. Wogonoside displays anti-inflammatory effects through modulating inflammatory mediator expression using RAW264.7 cells. *J Ethnopharmacol*. 2013;148(1):271-276.
189. Du X, Chen X, Zhao B, et al. Astragalus polysaccharides enhance the humoral and cellular immune responses of hepatitis B surface antigen vaccination through inhibiting the expression of transforming growth factor β and the frequency of regulatory T cells. *FEMS Immunol Med Microbiol*. 2011;63(2):228-235.
190. Begum S, Lee MR, Gu LJ, Hossain J, Sung CK. Exogenous stimulation with *Eclipta alba* promotes hair matrix keratinocyte proliferation and downregulates TGF- β 1 expression in nude mice. *Int J Mol Med*. 2015;35(2):496-502.
191. Georgiev V, Ananga A, Tsoleva V. Recent advances and uses of grape flavonoids as nutraceuticals. *Nutrients*. 2014;6(1):391-415.
192. Fischer TW, Herczeg-Lisztes E, Funk W, Zillikens D, Bíró T, Paus R. Differential effects of caffeine on hair shaft elongation, matrix and outer root sheath keratinocyte proliferation, and transforming growth factor- β 2/insulin-like growth factor-1-mediated regulation of the hair cycle in male and female human hair follicles in vitro. *Br J Dermatol*. 2014;171(5):1031-1043.

193. Kumar T, Chaiyasut C, Rungseevijitprapa W, Suttajit M. Screening of steroid 5 α -reductase inhibitory activity and total phenolic content of Thai plants. *Journal of Medicinal Plants Research*. 2011;5:1265-1271.
194. Zhang B, Zhang RW, Yin XQ, et al. Inhibitory activities of some traditional Chinese herbs against testosterone 5 α -reductase and effects of *Cacumen platycladi* on hair re-growth in testosterone-treated mice. *J Ethnopharmacol*. 2016;177:1-9.
195. Panahi Y, Taghizadeh M, Marzony ET, Sahebkar A. Rosemary oil vs minoxidil 2% for the treatment of androgenetic alopecia: a randomized comparative trial. *Skinmed*. 2015;13(1):15-21.
196. Kim AR, Kim SN, Jung IK, Kim HH, Park YH, Park WS. The inhibitory effect of *Scutellaria baicalensis* extract and its active compound, baicalin, on the translocation of the androgen receptor with implications for preventing androgenetic alopecia. *Planta Med*. 2014;80(2-3):153-158.
197. Wang K, Wu J, Xu J, et al. Correction of Anemia in Chronic Kidney Disease With *Angelica sinensis* Polysaccharide via Restoring EPO Production and Improving Iron Availability. *Front Pharmacol*. 2018;9:803.
198. Husain I, Dale OR, Martin K, et al. Screening of medicinal plants for possible herb-drug interactions through modulating nuclear receptors, drug-metabolizing enzymes and transporters. *J Ethnopharmacol*. 2023;301:115822.
199. Food and Nutrition Board IoM, National Academies. Dietary Reference Intakes (DRIs): Recommended Dietary Allowances and Adequate Intakes, Vitamins. <https://www.ncbi.nlm.nih.gov/books/NBK56068/table/summarytables.t2/?report=objectonly>. Published 2011. Updated 2011. Accessed 12 May, 2023.
200. Health Canada DaNHPID. Listing of Monographs. <https://webprod.hc-sc.gc.ca/nhpiddipsn/monosReq.do?lang=eng>. Published 2023. Accessed 12 May, 2023.

201. Chen L, Cao H, Xiao J. 2 - Polyphenols: Absorption, bioavailability, and metabolomics. In: Galanakis CM, ed. *Polyphenols: Properties, Recovery, and Applications*. Woodhead Publishing; 2018:45-67.
202. Herman A, Herman AP. Mechanism of action of herbs and their active constituents used in hair loss treatment. *Fitoterapia*. 2016;114:18-25.
203. Otberg N, Patzelt A, Rasulev U, et al. The role of hair follicles in the percutaneous absorption of caffeine. *Br J Clin Pharmacol*. 2008;65(4):488-492.
204. Aberle H, Bauer A, Stappert J, Kispert A, Kemler R. β -catenin is a target for the ubiquitin–proteasome pathway. *The EMBO Journal*. 1997;16(13):3797-3804.
205. Norwood OT. Incidence of female androgenetic alopecia (female pattern alopecia). *Dermatol Surg*. 2001;27(1):53-54.
206. Trüeb RM. *Nutrition for Healthy Hair: Guide to Understanding and Proper Practice*. Switzerland: Springer Nature; 2020.
207. Van Neste D. Placebo-controlled dose-effect studies with topical minoxidil 2% or 5% in male-patterned hair loss treated with oral finasteride employing an analytical and exhaustive study protocol. *Skin Res Technol*. 2020;26(4):542-557.
208. D. VN. Exhaustive analysis of scalp hair regression: subjective and objective perception from initial hair loss to severe miniaturisation and drug-induced regrowth. *Plast Aesthet Res* 2021;8:16. 2021.
209. Hamilton JB. Male hormone stimulation is prerequisite and an incitant in common baldness. *American Journal of Anatomy*. 1942;71(3):451-480.
210. Van Neste D. Viable terminal scalp hair follicles constitute a necessary and sufficient biological end-organ that conditions clinical efficacy of finasteride in males with male pattern hair loss without implying reversal of miniaturized follicles. *Skin Res Technol*. 2019;25(5):701-711.

211. Nuwaihdy R, Redler S, Heilmann S, et al. Investigation of four novel male androgenetic alopecia susceptibility loci: no association with female pattern hair loss. *Archives of Dermatological Research*. 2014;306(4):413-418.
212. Redler S, Messenger AG, Betz RC. Genetics and other factors in the aetiology of female pattern hair loss. *Exp Dermatol*. 2017;26(6):510-517.
213. Feldman PR, Fiebig KM, Piwko C, et al. Safety and efficacy of ALRV5XR in women with androgenetic alopecia or telogen effluvium: A randomised, double-blinded, placebo-controlled clinical trial. *EClinicalMedicine*. 2021;37:100978.
214. Feldman PR, Fiebig KM, Piwko C, et al. Safety and efficacy of ALRV5XR in men with androgenetic alopecia: A randomised, double-blinded, placebo-controlled clinical trial. *EClinicalMedicine*. 2021;40:101124.
215. Feldman PR, Feldman OJ, Guevara-Aguirre J, Fiebig KM. Sex differences in clinical trials of ALRV5XR treatment of androgenetic alopecia and telogen effluvium. *Frontiers in Medicine*. 2022;9.
216. Feldman PR, Gentile P, Piwko C, et al. Hair regrowth treatment efficacy and resistance in androgenetic alopecia: A systematic review and continuous Bayesian network meta-analysis. *Frontiers in Medicine*. 2023;9.
217. Jiang M, Zhang C, Cao H, Chan K, Lu A. The role of Chinese medicine in the treatment of chronic diseases in China. *Planta Med*. 2011;77(9):873-881.
218. Yang CS, Chen G, Wu Q. Recent scientific studies of a traditional chinese medicine, tea, on prevention of chronic diseases. *J Tradit Complement Med*. 2014;4(1):17-23.
219. Farris PK, Rogers N, McMichael A, Kogan S. A Novel Multi-Targeting Approach to Treating Hair Loss, Using Standardized Nutraceuticals. *J Drugs Dermatol*. 2017;16(11):s141-s148.

220. Asnaashari S, Javadzadeh Y. Herbal Medicines For Treatment of Androgenic Alopecia. *Altern Ther Health Med*. 2020;26(4):27-35.
221. Lassus A, Eskelinen E. A comparative study of a new food supplement, ViviScal, with fish extract for the treatment of hereditary androgenic alopecia in young males. *J Int Med Res*. 1992;20(6):445-453.
222. Chen L, Zhang J, Wang L, Wang H, Chen B. The Efficacy and Safety of Finasteride Combined with Topical Minoxidil for Androgenetic Alopecia: A Systematic Review and Meta-analysis. *Aesthetic Plast Surg*. 2020;44(3):962-970.
223. Tang Z, Hu Y, Wang J, Fan Z, Qu Q, Miao Y. Current application of mesotherapy in pattern hair loss: A systematic review. *J*. 2022;06:06.
224. Tang Z, Hu Y, Wang J, Fan Z, Qu Q, Miao Y. Current application of mesotherapy in pattern hair loss: A systematic review. *J Cosmet Dermatol*. 2022;21(10):4184-4193.
225. Saceda-Corralo D, Moustafa F, Moreno-Arrones Ó, Jaén-Olasolo P, Vañó-Galván S, Camacho F. Mesotherapy With Dutasteride for Androgenetic Alopecia: A Retrospective Study in Real Clinical Practice. *J Drugs Dermatol*. 2022;21(7):742-747.
226. Melo DF, Cortez de Almeida RF, Frattini SC, Santos LDN, Ramos PM. Minimally invasive procedures for the management of female pattern hair loss. *J*. 2022;17:17.
227. Rizer RL, Stephens TJ, Herndon JH, Sperber BR, Murphy J, Ablon GR. A Marine Protein-based Dietary Supplement for Subclinical Hair Thinning/Loss: Results of a Multisite, Double-blind, Placebo-controlled Clinical Trial. *Int J Trichology*. 2015;7(4):156-166.
228. Ablon G, Kogan S. A Randomized, Double-Blind, Placebo-Controlled Study of a Nutraceutical Supplement for Promoting Hair Growth in Perimenopausal,

- Menopausal, and Postmenopausal Women With Thinning Hair. *J Drugs Dermatol*. 2021;20(1):55-61.
229. Ablon G, Kogan S. A Six-Month, Randomized, Double-Blind, Placebo-Controlled Study Evaluating the Safety and Efficacy of a Nutraceutical Supplement for Promoting Hair Growth in Women With Self-Perceived Thinning Hair. *J Drugs Dermatol*. 2018;17(5):558-565.
230. Veeresham C. Natural products derived from plants as a source of drugs. *J Adv Pharm Technol Res*. 2012;3(4):200-201.
231. Oxford Centre for Evidence-Based Medicine: Levels of Evidence (March 2009). Available at: <https://www.cebm.ox.ac.uk/resources/levels-of-evidence/oxford-centre-for-evidence-based-medicine-levels-of-evidence-march-2009>. Accessed May 8, 2023, 2023.
232. Adil A, Godwin M. The effectiveness of treatments for androgenetic alopecia: A systematic review and meta-analysis. *J Am Acad Dermatol*. 2017;77(1):136-141.e135.
233. Chen D, Yang X, Liu X, et al. Efficacy comparison of monotherapies and combination therapies for androgenetic alopecia: A Bayesian network meta-analysis. *Dermatol Ther*. 2021;35(3):e15262.
234. Giordano S, Romeo M, di Summa P, Salval A, Lankinen P. A Meta-analysis On Evidence Of Platelet-rich Plasma for Androgenetic Alopecia. *Int J Trichology*. 2018;10(1):1-10.
235. Giordano S, Romeo M, Lankinen P. Platelet-rich plasma for androgenetic alopecia: Does it work? Evidence from meta analysis. *J Cosmet Dermatol*. 2017;16(3):374-381.
236. Gupta AK, Bamimore MA, Foley KA. Efficacy of non-surgical treatments for androgenetic alopecia in men and women: a systematic review with network meta-analyses, and an assessment of evidence quality. *J Dermatolog Treat*. 2020:1-11.

237. Gupta AK, Carviel JL. Meta-analysis of photobiomodulation for the treatment of androgenetic alopecia. *J Dermatolog Treat.* 2021;32(6):643-647.
238. Gupta AK, Carviel JL. Meta-analysis of efficacy of platelet-rich plasma therapy for androgenetic alopecia. *J Dermatolog Treat.* 2017;28(1):55-58.
239. Gupta AK, Charrette A. Topical Minoxidil: Systematic Review and Meta-Analysis of Its Efficacy in Androgenetic Alopecia. *Skinmed.* 2015;13(3):185-189.
240. Gupta AK, Charrette A. The efficacy and safety of 5alpha-reductase inhibitors in androgenetic alopecia: a network meta-analysis and benefit-risk assessment of finasteride and dutasteride. *J Dermatolog Treat.* 2014;25(2):156-161.
241. Gupta AK, Mays RR, Dotzert MS, Versteeg SG, Shear NH, Piguet V. Efficacy of non-surgical treatments for androgenetic alopecia: a systematic review and network meta-analysis. *J Eur Acad Dermatol Venereol.* 2018;32(12):2112-2125.
242. Gupta AK, Venkataraman M, Talukder M, Bamimore MA. Relative Efficacy of Minoxidil and the 5-alpha Reductase Inhibitors in Androgenetic Alopecia Treatment of Male Patients: A Network Meta-analysis. *JAMA Dermatol.* 2022;158(3):266-274.
243. Zhou Y, Chen C, Qu Q, et al. The effectiveness of combination therapies for androgenetic alopecia: A systematic review and meta-analysis. *Dermatol Ther.* 2020;33(4):e13741.
244. Zhou Z, Song S, Gao Z, Wu J, Ma J, Cui Y. The efficacy and safety of dutasteride compared with finasteride in treating men with androgenetic alopecia: a systematic review and meta-analysis. *Clin Interv Aging.* 2019;14:399-406.
245. Berger RS, Fu JL, Smiles KA, et al. The effects of minoxidil, 1% pyrithione zinc and a combination of both on hair density: a randomized controlled trial. *Br J Dermatol.* 2003;149(2):354-362.

246. Olsen EA, Whiting D, Bergfeld W, et al. A multicenter, randomized, placebo-controlled, double-blind clinical trial of a novel formulation of 5% minoxidil topical foam versus placebo in the treatment of androgenetic alopecia in men. *J Am Acad Dermatol.* 2007;57(5):767-774.
247. Olsen EA, Weiner MS, DeLong ER, Pinnell SR. Topical minoxidil in early male pattern baldness. *J Am Acad Dermatol.* 1985;13(2 Pt 1):185-192.
248. Petzoldt D, Group tGTMS. The German Double-Blind Placebo-Controlled Evaluation of Topical Minoxidil Solution in the Treatment of Early Male Pattern Baldness. *Int J Dermatol.* 1988;27(s6):430-434.
249. Roberts JL. Androgenetic alopecia: treatment results with topical minoxidil. *J Am Acad Dermatol.* 1987;16(3 Pt 2):705-710.
250. Shupack JL, Kassimir JJ, Thirumoorthy T, Reed ML, Jondreau L. Dose-response study of topical minoxidil in male pattern alopecia. *J Am Acad Dermatol.* 1987;16(3 Pt 2):673-676.
251. Whiting DA, Waldstreicher J, Sanchez M, Kaufman KD. Measuring reversal of hair miniaturization in androgenetic alopecia by follicular counts in horizontal sections of serial scalp biopsies: results of finasteride 1 mg treatment of men and postmenopausal women. *J Investig Dermatol Symp Proc.* 1999;4(3):282-284.
252. Van Neste D, Fuh V, Sanchez-Pedreno P, et al. Finasteride increases anagen hair in men with androgenetic alopecia. *Br J Dermatol.* 2000;143(4):804-810.
253. Stough DB, Rao NA, Kaufman KD, Mitchell C. Finasteride improves male pattern hair loss in a randomized study in identical twins. *Eur J Dermatol.* 2002;12(1):32-37.
254. Kim H, Choi JW, Kim JY, Shin JW, Lee SJ, Huh CH. Low-level light therapy for androgenetic alopecia: a 24-week, randomized, double-blind, sham device-controlled multicenter trial. *Dermatol Surg.* 2013;39(8):1177-1183.

255. Lanza fame RJ, Blanche RR, Bodian AB, Chiacchierini RP, Fernandez-Obregon A, Kazmirek ER. The growth of human scalp hair mediated by visible red light laser and LED sources in males. *Lasers Surg Med.* 2013;45(8):487-495.
256. Leavitt M, Charles G, Heyman E, Michaels D. HairMax LaserComb laser phototherapy device in the treatment of male androgenetic alopecia: A randomized, double-blind, sham device-controlled, multicentre trial. *Clin Drug Investig.* 2009;29(5):283-292.
257. Lucky AW, Piacquadio DJ, Ditre CM, et al. A randomized, placebo-controlled trial of 5% and 2% topical minoxidil solutions in the treatment of female pattern hair loss. *J Am Acad Dermatol.* 2004;50(4):541-553.
258. Whiting DA, Jacobson C. Treatment of female androgenetic alopecia with minoxidil 2%. *Int J Dermatol.* 1992;31(11):800-804.
259. DeVillez RL, Jacobs JP, Szpunar CA, Warner ML. Androgenetic alopecia in the female. Treatment with 2% topical minoxidil solution. *Arch Dermatol.* 1994;130(3):303-307.
260. Jacobs JP, Szpunar CA, Warner ML. Use of topical minoxidil therapy for androgenetic alopecia in women. *Int J Dermatol.* 1993;32(10):758-762.
261. Olsen EA. Topical minoxidil in the treatment of androgenetic alopecia in women. *Cutis.* 1991;48(3):243-248.
262. Olsen EA, DeLong ER, Weiner MS. Dose-response study of topical minoxidil in male pattern baldness. *J Am Acad Dermatol.* 1986;15(1):30-37.
263. Civatte J, Laux B, Simpson NB, Vickers CF. 2% topical minoxidil solution in male-pattern baldness: preliminary European results. *Dermatologica.* 1987;175 Suppl 2:42-49.

264. Civatte J, Group tETMS. Topical 2% Minoxidil Solution in Male Pattern Alopecia. The Initial European Experience. *Int J Dermatol*. 1988;27(s6):424-429.
265. Koperski JA, Orenberg EK, Wilkinson DI. Topical minoxidil therapy for androgenetic alopecia. A 30-month study. *Arch Dermatol*. 1987;123(11):1483-1487.
266. Helman SL, Zhou J, Fuqua BK, et al. The biology of mammalian multi-copper ferroxidases. *Biometals*. 2022;15:15.
267. Dutrée-Meulenberg ROGM, Nieboer C, Koedijk FHJ, Stolz E. Treatment of Male Pattern Alopecia Using Topical Minoxidil in The Netherlands. *Int J Dermatol*. 1988;27(s6):435-440.
268. Rushton DH, Unger WP, Cotterill PC, Kingsley P, James KC. Quantitative assessment of 2% topical minoxidil in the treatment of male pattern baldness. *Clin Exp Dermatol*. 1989;14(1):40-46.
269. Gubelin Harcha W, Barboza Martínez J, Tsai TF, et al. A randomized, active- and placebo-controlled study of the efficacy and safety of different doses of dutasteride versus placebo and finasteride in the treatment of male subjects with androgenetic alopecia. *J Am Acad Dermatol*. 2014;70(3):489-498.e483.
270. Saraswat A, Kumar B. Minoxidil vs Finasteride in the Treatment of Men With Androgenetic Alopecia. *Archives of Dermatology*. 2003;139(9):1219-1221.
271. Hajheydari Z, Akbari J, Saeedi M, Shokoohi L. Comparing the therapeutic effects of finasteride gel and tablet in treatment of the androgenetic alopecia. *Indian J Dermatol Venereol Leprol*. 2009;75(1):47-51.
272. Shanshanwal SJ, Dhurat RS. Superiority of dutasteride over finasteride in hair regrowth and reversal of miniaturization in men with androgenetic alopecia: A randomized controlled open-label, evaluator-blinded study. *Indian J Dermatol Venereol Leprol*. 2017;83(1):47-54.

273. Panchaprateep R, Lueangarun S. Efficacy and Safety of Oral Minoxidil 5 mg Once Daily in the Treatment of Male Patients with Androgenetic Alopecia: An Open-Label and Global Photographic Assessment. *Dermatol Ther (Heidelb)*. 2020;10(6):1345-1357.
274. Pirmez R, Salas-Callo CI. Very-low-dose oral minoxidil in male androgenetic alopecia: A study with quantitative trichoscopic documentation. *J Am Acad Dermatol*. 2020;82(1):e21-e22.
275. Van Neste DJ, de Brouwer B, De Coster W. The phototrichogram: analysis of some technical factors of variation. *Skin Pharmacol*. 1994;7(1-2):67-72.
276. Rushton DH, de Brouwer B, de Coster W, van Neste DJ. Comparative evaluation of scalp hair by phototrichogram and unit area trichogram analysis within the same subjects. *Acta Derm Venereol*. 1993;73(2):150-153.
277. Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ*. 2021;372:n71.
278. Kawashima M, Hayashi N, Igarashi A, et al. Finasteride in the treatment of Japanese men with male pattern hair loss. *Eur J Dermatol*. 2004;14(4):247-254.
279. Sinclair R. Male pattern androgenetic alopecia. *Bmj*. 1998;317(7162):865-869.
280. John JM, Sinclair RD. Systemic minoxidil for hair disorders in pediatric patients: a safety and tolerability review. *Int J Dermatol*. 2023;62(2):257-259.
281. Gupta AK, Talukder M, Williams G. Comparison of oral minoxidil, finasteride, and dutasteride for treating androgenetic alopecia. *Journal of Dermatological Treatment*. 2022:1-17.
282. Ablon G. A 6-month, randomized, double-blind, placebo-controlled study evaluating the ability of a marine complex supplement to promote hair growth in men with thinning hair. *J Cosmet Dermatol*. 2016;15(4):358-366.

283. Kaufman KD, Olsen EA, Whiting D, et al. Finasteride in the treatment of men with androgenetic alopecia. Finasteride Male Pattern Hair Loss Study Group. *J Am Acad Dermatol.* 1998;39(4 Pt 1):578-589.
284. Price VH. Treatment of hair loss. *N Engl J Med.* 1999;341(13):964-973.
285. Price VH, Menefee E, Sanchez M, Ruane P, Kaufman KD. Changes in hair weight and hair count in men with androgenetic alopecia after treatment with finasteride, 1 mg, daily. *J Am Acad Dermatol.* 2002;46(4):517-523.
286. Price VH, Menefee E, Sanchez M, Kaufman KD. Changes in hair weight in men with androgenetic alopecia after treatment with finasteride (1 mg daily): three- and 4-year results. *J Am Acad Dermatol.* 2006;55(1):71-74.
287. Price VH, Menefee E. Quantitative estimation of hair growth. I. androgenetic alopecia in women: effect of minoxidil. *J Invest Dermatol.* 1990;95(6):683-687.
288. Van Neste D, Sandraps E, Herbaut D, Lelubre P, Leroy T. Validation of scalp coverage scoring methods for scalp hair loss in male pattern hair loss (androgenetic alopecia). *Skin Res Technol.* 2006;12(2):89-93.
289. Burton JL. BALDNESS AND MINOXIDIL ADVERTISING. *The Lancet.* 1989;333(8635):442-443.
290. Gandy W. Advertising baldly to the public. *N Engl J Med.* 1992;326(5):350-351.
291. TINA.org Reports Unilever's Nutrafol to the FTC and FDA [press release]. GlobeNewswire, 2023.
292. Schulz KF, Altman DG, Moher D, Group C. CONSORT 2010 statement: updated guidelines for reporting parallel group randomised trials. *BMJ.* 2010;340:c332.
293. Schmidt C. Biotechs target stagnant baldness market. *Nat Biotechnol.* 2016;34(2):120-121.

294. Pantelireis N, Higgins CA. A bald statement - Current approaches to manipulate miniaturisation focus only on promoting hair growth. *Exp Dermatol*. 2018;27(9):959-965.
295. Rahmani W, Sinha S, Biernaskie J. Immune modulation of hair follicle regeneration. *NPJ Regen Med*. 2020;5:9.
296. Gentile P, Alves R, Cole JP, et al. AIRMESS – Academy of International Regenerative Medicine & Surgery Societies: recommendations in the use of platelet-rich plasma (PRP), autologous stem cell-based therapy (ASC-BT) in androgenetic alopecia and wound healing. *Expert Opinion on Biological Therapy*. 2021:1-7.
297. Goswami VG, Patel BD. Recent updates on Wnt signaling modulators: a patent review (2014-2020). *Expert Opin Ther Pat*. 2021;31(11):1009-1043.
298. Lousada MB, Lachnit T, Edelkamp J, et al. Exploring the human hair follicle microbiome*. *British Journal of Dermatology*. 2021;184(5):802-815.
299. Lei M, Guo H, Qiu W, et al. Modulating hair follicle size with Wnt10b/DKK1 during hair regeneration. *Exp Dermatol*. 2014;23(6):407-413.
300. Shapiro J, Ho A, Sukhdeo K, Yin L, Lo Sicco K. Evaluation of platelet-rich plasma as a treatment for androgenetic alopecia: A randomized controlled trial. *J Am Acad Dermatol*. 2020;83(5):1298-1303.
301. Kakizaki P, Contin LA, Barletta M, et al. Efficacy and Safety of Scalp Microneedling in Male Pattern Hair Loss. *Skin Appendage Disord*. 2023;9(1):18-26.
302. Toshitani S, Nakayama J, Yahata T, Yasuda M, Urabe H. A new apparatus for hair regrowth in male-pattern baldness. *J Dermatol*. 1990;17(4):240-246.
303. Freund BJ, Schwartz M. Treatment of male pattern baldness with botulinum toxin: a pilot study. *Plast Reconstr Surg*. 2010;126(5):246e-248e.

304. Ismail Simsek AD, Christopher J. Swearingen, John Seykora, David Herman, Yusuf Yazici. A small-molecule Wnt pathway modulator (SM04554) as a potential topical treatment for androgenetic alopecia. Paper presented at: International Dermatology and Cosmetology Congress (INDERCOS); March 27 - 30, 2019, 2019.
305. Yu F, Yu C, Li F, et al. Wnt/ β -catenin signaling in cancers and targeted therapies. *Signal Transduct Target Ther.* 2021;6(1):307.
306. Perkins RS, Singh R, Abell AN, Krum SA, Miranda-Carboni GA. The role of WNT10B in physiology and disease: A 10-year update. *Frontiers in Cell and Developmental Biology.* 2023;11.
307. Chaudhary H, Jena PK, Seshadri S. In vivo evaluation of Eclipta alba extract as anticancer and multidrug resistance reversal agent. *Nutrition and cancer.* 2014;66(5):904-913.
308. Zou YX, Mu ZQ, Wang J, Tian S, Li Y, Liu Y. Wedelolactone, a Component from Eclipta prostrata (L.) L., Inhibits the Proliferation and Migration of Head and Neck Squamous Cancer Cells through the AhR Pathway. *Curr Pharm Biotechnol.* 2022;23(15):1883-1892.
309. Bhattacharyya S, Law S. Environmental pollutant ENU induced leukemic NF- κ B signaling amelioration by Eclipta alba in murine model. *Int J Environ Health Res.* 2022;32(11):2435-2449.
310. Feng L, Zhai YY, Xu J, et al. A review on traditional uses, phytochemistry and pharmacology of Eclipta prostrata (L.) L. *J Ethnopharmacol.* 2019;245:112109.
311. Gu Y, Zheng Q, Fan G, Liu R. Advances in Anti-Cancer Activities of Flavonoids in Scutellariae radix: Perspectives on Mechanism. *Int J Mol Sci.* 2022;23(19).

312. Sajeev A, Hegde M, Daimary UD, et al. Modulation of diverse oncogenic signaling pathways by oroxylin A: An important strategy for both cancer prevention and treatment. *Phytomedicine*. 2022;105:154369.
313. Hegedüs I, Andreidesz K, Szentpéteri JL, et al. The Utilization of Physiologically Active Molecular Components of Grape Seeds and Grape Marc. *Int J Mol Sci*. 2022;23(19).
314. Ferraz da Costa DC, Pereira Rangel L, Quarti J, Santos RA, Silva JL, Fialho E. Bioactive Compounds and Metabolites from Grapes and Red Wine in Breast Cancer Chemoprevention and Therapy. *Molecules*. 2020;25(15).
315. Wang D, Duan J, Chen XJ, et al. Pharmacokinetic characteristics of emodin in polygoni Multiflori Radix Praeparata. *J Ethnopharmacol*. 2023;303:115945.
316. Peng F, Wang L, Xiong L, Tang H, Du J, Peng C. Maackiain Modulates miR-374a/GADD45A Axis to Inhibit Triple-Negative Breast Cancer Initiation and Progression. *Front Pharmacol*. 2022;13:806869.
317. Zhang S, Morris ME. Effects of the flavonoids biochanin A, morin, phloretin, and silymarin on P-glycoprotein-mediated transport. *J Pharmacol Exp Ther*. 2003;304(3):1258-1267.
318. Enomoto R, Koshiba C, Suzuki C, Lee E. Wogonin potentiates the antitumor action of etoposide and ameliorates its adverse effects. *Cancer Chemother Pharmacol*. 2011;67(5):1063-1072.
319. Huang C, Xu D, Xia Q, Wang P, Rong C, Su Y. Reversal of P-glycoprotein-mediated multidrug resistance of human hepatic cancer cells by Astragaloside II. *J Pharm Pharmacol*. 2012;64(12):1741-1750.

320. Zhao BX, Sun YB, Wang SQ, et al. Grape seed procyanidin reversal of p-glycoprotein associated multi-drug resistance via down-regulation of NF- κ B and MAPK/ERK mediated YB-1 activity in A2780/T cells. *PLoS One*. 2013;8(8):e71071.
321. Dewanjee S, Dua TK, Bhattacharjee N, et al. Natural Products as Alternative Choices for P-Glycoprotein (P-gp) Inhibition. *Molecules*. 2017;22(6).
322. Huang KP, Senna MM. Hair Are the Rankings—5- α Reductase Inhibitors and Minoxidil in Male Androgenetic Alopecia. *JAMA Dermatology*. 2022;158(3):242-243.
323. Xin T, Gonzalez D, Rompolas P, Greco V. Flexible fate determination ensures robust differentiation in the hair follicle. *Nat Cell Biol*. 2018;20(12):1361-1369.
324. Matsumura H, Liu N, Nanba D, et al. Distinct types of stem cell divisions determine organ regeneration and aging in hair follicles. *Nature Aging*. 2021;1(2):190-204.
325. Diotallevi F, Campanati A, Martina E, et al. The Role of Nutrition in Immune-Mediated, Inflammatory Skin Disease: A Narrative Review. *Nutrients*. 2022;14(3):29.
326. Domaszewska-Szostek A, Puzianowska-Kuźnicka M, Kuryłowicz A. Flavonoids in Skin Senescence Prevention and Treatment. *International Journal of Molecular Sciences*. 2021;22(13):6814.
327. Yadav NK, Arya RK, Dev K, et al. Alcoholic Extract of Eclipta alba Shows In Vitro Antioxidant and Anticancer Activity without Exhibiting Toxicological Effects. *Oxid Med Cell Longev*. 2017;2017:9094641.
328. Wang J, Zhang B. Clinical Chinese Materia Medica. *People's Medical Publishing House, Beijing, China*. 2012.
329. Wu YC, Hsieh CL. Pharmacological effects of Radix Angelica Sinensis (Danggui) on cerebral infarction. *Chin Med*. 2011;6:32.

330. Liu YQ, Zhan LB, Liu T, Cheng MC, Liu XY, Xiao HB. Inhibitory effect of Ecliptae herba extract and its component wedelolactone on pre-osteoclastic proliferation and differentiation. *J Ethnopharmacol.* 2014;157:206-211.

Appendices

Appendix 1: List of Publications

Appendix 2: Statement regarding published material submitted in this thesis and nature of contribution.

Appendix 3: Copies of correspondence from co-authors confirming contribution.

Appendix 1: List of publications submitted for PhD by Publication

Paper 1 (Chapter 3): Feldman, Peter R., Klaus M. Fiebig, Charles Piwko, Boris M. Mints, Dennis Brown, Deborah J. Cahan, and Jaime Guevara-Aguirre. "Safety and Efficacy of ALRV5XR in Women with Androgenetic Alopecia or Telogen Effluvium: A Randomised, Double-Blinded, Placebo-Controlled Clinical Trial." *EClinicalMedicine* 37 (2021/07/01/2021): 100978. <https://doi.org/10.1016/j.eclinm.2021.100978>

Paper 2 (Chapter 4): Feldman, P. R., K. M. Fiebig, C. Piwko, B. M. Mints, D. Brown, D. J. Cahan, and J. Guevara-Aguirre. "Safety and Efficacy of ALRV5XR in Men with Androgenetic Alopecia: A Randomised, Double-Blinded, Placebo-Controlled Clinical Trial." *EClinicalMedicine* 40 (Oct 2021): 101124. <https://dx.doi.org/10.1016/j.eclinm.2021.101124>.

Paper 3 (Chapter 5): Feldman, Peter R., Oriel J. Feldman, Jaime Guevara-Aguirre, and Klaus M. Fiebig. "Sex Differences in Clinical Trials of ALRV5XR Treatment of Androgenetic Alopecia and Telogen Effluvium." Brief Research Report, *Frontiers in Medicine* 9 (2022-August-04 2022). <https://dx.doi.org/10.3389/fmed.2022.918058>.

Paper 4 (Chapter 6): Feldman, Peter R., Pietro Gentile, Charles Piwko, Hendrik M. Motswaledi, Samantha Gorun, Jacob Pesachov, Michael Markel, Maxwell I. Silver, Megan Brenkel, Oriel J. Feldman, Corey L. Kamen, Elizabeth Uleryk, Jaime Guevara-Aguirre, and Klaus M. Fiebig. "Hair Regrowth Treatment Efficacy and Resistance in Androgenetic Alopecia: A Systematic Review and Continuous Bayesian Network Meta-Analysis." Systematic Review, *Frontiers in Medicine* 9 (2023-January-23 2023). <https://dx.doi.org/10.3389/fmed.2022.998623>.

Appendix 2: Statement regarding published material submitted in this thesis and nature of contribution

I have previously submitted no part of the material presented in this PhD by Publication for a degree in this or any other University. Therefore, the Papers 1-4 (Chapters 3-6) presented in this thesis represent work undertaken primarily by myself with the collaboration of co-authors. Key co-authors confirm my contribution to each paper in Appendix 3.

Paper 1 (Chapter 3):

Feldman, Peter R., Klaus M. Fiebig, Charles Piwko, Boris M. Mints, Dennis Brown, Deborah J. Cahan, and Jaime Guevara-Aguirre. "Safety and Efficacy of ALRV5XR in Women with Androgenetic Alopecia or Telogen Effluvium: A Randomised, Double-Blinded, Placebo-Controlled Clinical Trial." *EClinicalMedicine* 37 (2021/07/01/ 2021): 100978. <https://dx.doi.org/https://doi.org/10.1016/j.eclinm.2021.100978>.

I designed the clinical trial protocol in conjunction with Dr Luciana Marra, raised funds for the study, acquired relevant institutional/ethics review board approvals, designed and supervised the manufacturing of the intervention agents and placebo, contracted the clinical research site and analytical laboratories, collated the dataset and performed the statistical analysis in conjunction with Dr Elizabeth Renouf a statistician and Dr Klaus Fiebig, a co-author. I selected the journal for submission, drafted the manuscript, coordinated the revisions, and submitted it for publication. In addition, I drafted responses to reviewer comments, submitted the final manuscript and reviewed the production typesetting.

Paper 2 (Chapter 4):

Feldman, P. R., K. M. Fiebig, C. Piwko, B. M. Mints, D. Brown, D. J. Cahan, and J. Guevara-Aguirre. "Safety and Efficacy of ALRV5XR in Men with Androgenetic Alopecia: A Randomised, Double-Blinded, Placebo-Controlled Clinical Trial." *EClinicalMedicine* 40 (Oct 2021): 101124. <https://dx.doi.org/10.1016/j.eclinm.2021.101124>.

I designed the clinical trial protocol in conjunction with Dr Luciana Marra, raised funds for the study, acquired relevant institutional/ethics review board approvals, designed and supervised the manufacturing of the intervention agents and placebo, contracted the clinical research site and analytical laboratories, collated the dataset and performed the statistical analysis in conjunction with Dr Elizabeth Renouf a statistician and Dr Klaus Fiebig, a co-author. I selected the journal for submission, drafted the manuscript, coordinated the revisions, and submitted it for publication. In addition, I drafted responses to reviewer comments, submitted the final manuscript and reviewed the production typesetting.

Paper 3 (Chapter 5):

Feldman, Peter R., Oriel J. Feldman, Jaime Guevara-Aguirre, and Klaus M. Fiebig. "Sex Differences in Clinical Trials of ALRV5XR Treatment of Androgenetic Alopecia and Telogen Effluvium." Brief Research Report, *Frontiers in Medicine* 9 (2022-August-04 2022). <https://dx.doi.org/10.3389/fmed.2022.918058>.

I designed the study in conjunction with Dr Klaus Fiebig, raised funds for the study, collated the dataset and performed the statistical and hair dynamics analysis with Dr Klaus Fiebig as a co-author. I selected the journal for submission, drafted the manuscript and submitted it for publication. After the initial submission was rejected, I revised the manuscript, added sample subject cases with case hair dynamic charts and resubmitted for publication. I drafted responses to reviewer comments, submitted the final manuscript and reviewed the production typesetting.

Paper 4 (Chapter 6):

Feldman, Peter R., Pietro Gentile, Charles Piwko, Hendrik M. Motswaledi, Samantha Gorun, Jacob Pesachov, Michael Markel, Maxwell I. Silver, Megan Brenkel, Oriel J. Feldman, Corey L. Kamen, Elizabeth Uleryk, Jaime Guevara-Aguirre, and Klaus M. Fiebig. "Hair Regrowth Treatment Efficacy and Resistance in Androgenetic Alopecia: A Systematic Review and Continuous Bayesian Network Meta-Analysis." Systematic Review, *Frontiers in Medicine* 9 (2023-January-23 2023). <https://dx.doi.org/10.3389/fmed.2022.998623>.

I wrote the grant applications, which co-funded the study. I wrote the protocol together with co-authors Drs Charles Piwko and Klaus Fiebig. I acted as chief investigator for the systematic review and meta-analysis, chairing research team meetings, soliciting expert opinions, making final decisions on the inclusion and exclusion of studies with Drs Piwko and Fiebig, and supervised members of the research team. I contributed to the statistical analyses with statistician Samantha Gorun and Dr Klaus Fiebig and designed the tables and graphs. I selected the journals for submission, drafted the manuscript, coordinated the revisions and submitted it for publication. I drafted responses to reviewer comments, submitted the final manuscript and reviewed the production typesetting.

Appendix 3: Copies of correspondence from co-authors confirming contribution.

Contribution of Work by Peter Feldman in the Papers included in his thesis for PhD by Publication

Klaus Fiebig <klausfiebig@gmail.com>

Thu 2023-03-02 9:54 AM

To: Peter Feldman (MED - Postgraduate Researcher) <P.Feldman@uea.ac.uk>

Warning: This email is from outside the UEA system. Do not click on links or attachments unless you expect them from the sender and know the content is safe.

Dear Peter,

Here is my statement regarding your leading role in the 4 papers we published on ALRV5XR.

To: Faculty of Medicine, University of East Anglia

Peter Feldman was the first author on the 4 peer-reviewed publications listed below. In all these papers Peter led the study design, data curation, analysis, writing of the manuscripts, and coordinated all the revisions. He was also the corresponding author for each journal submission. I was a co-author in and worked closely with Peter on all of these publications. I can attest that Peter's contribution was by far the majority for all these papers.

Sincerely,

Klaus Fiebig, PhD

Paper 1:

Feldman, Peter R., et al. "Safety and Efficacy of ALRV5XR in Women with Androgenetic Alopecia or Telogen Effluvium: A Randomised, Double-Blinded, Placebo-Controlled Clinical Trial." *EClinicalMedicine* 37 (2021-Jun-23): 100978.

Paper 2:

Feldman, P. R., "Safety and Efficacy of ALRV5XR in Men with Androgenetic Alopecia: A Randomised, Double-Blinded, Placebo-Controlled Clinical Trial." *EClinicalMedicine* 40 (2021-Sep-11): 101124.

Paper 3:

Feldman, Peter R., et al. "Sex Differences in Clinical Trials of ALRV5XR Treatment of Androgenetic Alopecia and Telogen Effluvium." Brief Research Report, *Frontiers in Medicine* 9 (2022-August-04): 918058.

Paper 4:

Feldman, Peter R., et al., "Hair Regrowth Treatment Efficacy and Resistance in Androgenetic Alopecia: A Systematic Review and Continuous Bayesian Network Meta-Analysis." Systematic Review, *Frontiers in Medicine* 9 (2023-Jan-23): 998623.

--

[KlausFiebig@gmail.com](mailto:klausfiebig@gmail.com)

Cell: +1-416-903-9757

[LinkedIn](#)

Quito, March the 2nd, 2023

To: Faculty of Medicine, University of East Anglia

Ref: Joint publications

Dear Sir:

I would like to categorically recognize that Mr. Peter Feldman deserved to be and indeed was, the 1st author on the 4 peer-reviewed publications listed below.

Mr. Feldman was the lead in study design, data curation, analysis, writing the manuscripts and coordination of all revisions; consequently, he was the corresponding author for each journal submission. While I was a co-author in all these publications; I must mention that Mr. Feldman's contribution was by far the most important of the entire group of coauthors. Furthermore, his knowledge of hair, hair biology, therapeutic interventions for hair loss, and the general body of literature on these matters is thorough.

Sincerely,

Professor Jaime Guevara-Aguirre, MD

Universidad San Francisco de Quito, Ecuador

Contribution of Work by Peter Feldman in the Papers included in his thesis for PhD by Publication

Charles Piwko <cpiwko@chppharma.com>

Thu 2023-03-02 12:01 PM

To: Peter Feldman (MED - Postgraduate Researcher) <P.Feldman@uea.ac.uk>

Warning: This email is from outside the UEA system. Do not click on links or attachments unless you expect them from the sender and know the content is safe.

To: Faculty of Medicine, University of East Anglia

Peter Feldman was the 1st author on the 4 peer-reviewed publications (listed below). Peter was the lead in study design, data curation, analysis, writing the manuscripts and coordinating all the revisions. He was also the corresponding author for each journal submission. I was a co-author in 3 of these publications.

Sincerely,

Charles Piwko, PhD

Paper 1:

Feldman, Peter R., et al. "Safety and Efficacy of ALRV5XR in Women with Androgenetic Alopecia or Telogen Effluvium: A Randomised, Double-Blinded, Placebo-Controlled Clinical Trial." *EClinicalMedicine* 37 (2021/07/01/ 2021): 100978.

Paper 2:

Feldman, P. R., "Safety and Efficacy of ALRV5XR in Men with Androgenetic Alopecia: A Randomised, Double-Blinded, Placebo-Controlled Clinical Trial." *EClinicalMedicine* 40 (Oct 2021): 101124.

Paper 3:

Feldman, Peter R., et al. "Sex Differences in Clinical Trials of ALRV5XR Treatment of Androgenetic Alopecia and Telogen Effluvium." Brief Research Report, *Frontiers in Medicine* 9 (2022-August-04 2022).

Paper 4:

Feldman, Peter R., et al., "Hair Regrowth Treatment Efficacy and Resistance in Androgenetic

Alopecia: A Systematic Review and Continuous Bayesian Network Meta-Analysis." Systematic Review, *Frontiers in Medicine* 9 (2023-January-23 2023).

--

Charles Piwko PhD, PharmD, MSc

President

(P) ++1- 416-433-5787

cpiwko@chppharma.com

www.chppharma.com

