Drug discovery for alopecia: gone today, hair tomorrow

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Abstract

Introduction—Hair loss or alopecia affects the majority of the population at some time in their life, and increasingly, sufferers are demanding treatment. Three main types of alopecia (androgenic [AGA], areata [AA] and chemotherapy-induced [CIA]) are very different, and have their own laboratory models and separate drug-discovery efforts.

Areas covered—In this article, the authors review the biology of hair, hair follicle (HF) cycling, stem cells and signaling pathways. AGA, due to dihydrotesterone, is treated by 5-α reductase inhibitors, androgen receptor blockers and ATP-sensitive potassium channel-openers. AA, which involves attack by CD8$^+$NK group 2D-positive (NKG2D$^+$) T cells, is treated with immunosuppressives, biologics and JAK inhibitors. Meanwhile, CIA is treated by apoptosis inhibitors, cytokines and topical immunotherapy.

Expert opinion—The desire to treat alopecia with an easy topical preparation is expected to grow with time, particularly with an increasing aging population. The discovery of epidermal stem cells in the HF has given new life to the search for a cure for baldness. Drug discovery efforts are being increasingly centered on these stem cells, boosting the hair cycle and reversing miniaturization of HF. Better understanding of the molecular mechanisms underlying the immune attack in AA will yield new drugs. New discoveries in HF neogenesis and low-level light therapy will undoubtedly have a role to play.

Keywords

5-α-reductase inhibitor; alopecia areata; androgenic alopecia; anti-androgen; baldness; chemotherapy-induced alopecia; hair follicle; low-level laser (light) therapy; stem cells; topical immunotherapy

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1. Introduction

Hair follicles (HFs) are an integral part of the mammalian skin where they produce filaments largely composed of the protein, keratin [1]. Hair can be either thick, long terminal hair or and fine, short vellus hair. Hair helps the epidermis to maintain the body's protective barrier against its external environment. In humans, however, the body hair has lost most of its importance as a physical protective barrier.

Several hypotheses have been put forward to explain how humans evolved to have radically different patterns of hair distribution compared to most other mammals [2]. These include the process of adaptation to provide a better thermoregulatory mechanism in the hot and dry climate conditions that arose about 3 million years ago when forests gave way to hot savanna grasslands. Another hypothesis is sexual selection based upon the dimorphism seen in the hair patterns of men and women. On average, men have more terminal body hair than women who have more vellus hair, which is less visible. A third hypothesis is adaptation to the advent of ectoparasites such as fleas and lice that could in theory have carried lethal diseases. The last hypothesis is that when humans began to walk upright, the need for hair by which young children could hold on to their mothers was lessened.

Nevertheless, hair still has great social significance for human beings. Healthy hair indicates health, youth and vigor. Male pattern baldness is taken to be a sign of age and loss of vigor, which may be concealed with a toupee, hats or simply by shaving the head. There is enormous demand for drugs and other treatments that can slow down or reverse hair loss; this has led to the creation of a multibillion-dollar industry [3]. In USA > 3.5 billion dollars is spent every year on treating hair loss. The American Hair Loss Association recognizes that hair loss is an extremely emotionally distressing disease that makes afflicted patients psychologically vulnerable [4]. This review aims to cover the biology of the HF, the different types of alopecia, laboratory models of hair loss and drug discovery efforts that are being undertaken for hair loss.

2. HFs, hair cycle, stem cells

Each strand of hair is made up of concentric regions, the medulla, cortex and cuticle (Figure 1). The innermost medulla is a variable space (sometimes present and sometimes not) and its function is still debated. The highly structured cortex is the primary source of the mechanical strength of the hair. The cortex contains melanin, which colors the fiber based on the number, distribution and types of melanin granules. The shape of the HF determines the shape of the cortex, so that hairs with circular cross-section are straight, and those with oval cross-section are curly. The cuticle is the outer covering of protein coated with a single molecular layer of lipid that makes the hair repel water. The diameter of the human hair varies from 17 to 180 μm [5].

The HF is a complex mini-organ [6] embedded in the skin and composed of the papilla, matrix, root sheath and bulge [7]. Structures closely associated with the HF are sebaceous glands, apocrine glands, the *arrector pili* muscle and mechanoreceptors that respond to touch [8]. Figure 2 shows the anatomy of the HF. There are between 250,000 and 500,000 HF on the human scalp and as many as 5,000,000 on the whole body.

Hair grows in cycles during which it moves sequentially from one phase to another (Figures 3 and 4) [9]. Anagen is the growth phase; catagen is the involuting or regressing phase; and telogen, the resting or quiescent phase. There is also a shedding phase, or exogen, that is independent of anagen and telogen, in which one out of several hairs in a single follicle is physically shed. Normally, up to 90% of the HF are in anagen phase, while 10 – 14% are in telogen and 1 – 2% in catagen [10]. The length of the hair cycle varies between HF in different parts of the body. For eyebrows, the cycle is completed in around 4 months, while it takes the scalp 3 – 4 years to complete the cycle. The physical length of the hair depends on the duration of the hair cycle, which is why eyebrows are relatively short and scalp hair is often long.

The signaling involved in the well-orchestrated process of hair growth and HF cycling is complex and incompletely understood [11]. The basic driving force is interaction between the mesenchymal and epithelial cell populations within the HF unit [12]. Figure 5 shows a schematic illustrating some of the different types of stem cells and the particular differentiated structures in the skin to which they contribute. The most important mesenchymal cells in the HF reside within the dermal papilla (DP). These cells produce signals to control sequential cycling of the follicular epithelium [13]. It is thought that epithelial stem cells, which reside in the bulge area of the HF, can respond to the signals from the DP [14]. This activation leads to production of progenitor cells from the stem cells in the bulge area, and then these progenitor cells become transiently amplifying cells that expand downward into the deep dermis, followed by differentiation into matrix cells that have the ability to produce the hair shaft, and its sheath. However, in both humans and especially in animals, the male or female genders have very different hair phenotypes, which are governed by the influence of sex hormones [15]. Several growth factor families are involved in HF cycling [13], namely fibroblast growth factor, EGF, hepatocyte growth factor, IGF-I, TGF-β families, among others. Signal transducer and activator of transcription 3 (stat3) is a latent cytoplasmic protein that conveys signals to the nucleus upon stimulation with cytokines/growth factors, leading to transcriptional activation of downstream genes that have the stat3 response element in their promoter region. Stat3 plays a critical role in HF cycling [16]. There is another, stat3 independent, pathway involving PKC, but both pathways eventually lead to activation of PI3K. It is thought the stat3-dependent pathway is involved in spontaneous HF cycling, while the stat3-independent pathway is involved in HF cycling after plucking for instance [16].

A series of signaling molecules is involved in each step of primary hair development and differentiation that have been elucidated in studies of embryogenesis [17]. Wingless type (Wnt) signaling is crucial for the initiation of HF development [18]. Wnt-protein is a ligand that binds to a cell-surface receptor 'Frizzled' family member, which then passes the biological signal to the intracellular protein 'Dishevelled' (Dsh). Dsh causes the accumulation of β-catenin in the cytoplasm (by protection from degradation) and its eventual translocation into the nucleus to act as a transcriptional co-activator of transcription factors that belong to the TCF/LEF family.

'Sonic hedgehog' (Shh) signaling plays an important role in both embryonic and adult HF development. Shh binds to and inhibits the extracellular domain 'Patched,' allowing the

intracellular domain 'Smoothened' to accumulate and inhibit the proteolytic cleavage of the Gli family of zinc-finger transcription factors [19]. Shh signaling (and in particular activation of Gli2 [20]) is obligatory for development of the epithelial hair germ, comprising epidermal placodes and associated dermal condensates [21]. Noggin [22], bone morphogenetic protein (BMP) [23] and ectodysplasin [24] signaling also play important roles at early stages of HF placode development.

Dlx homeobox transcription factors regulate epidermal, neural and osteogenic cellular differentiation [25]. It was found Dlx3 played a central role as a transcriptional regulator of hair formation and regeneration. DLX3 co-localized with phosphorylated Smad1/5/8 complex, and regulated BMP signaling during hair morphogenesis in animal models [26].

An interesting study by Hamanaka *et al.* [27] showed that mitochondrial-generated reactive oxygen species (ROS) were critical mediators of cellular differentiation and HF morphogenesis. They generated mice with a keratinocyte-specific deficiency in mitochondrial transcription factor A (TFAM), which is required for the transcription of mitochondrial genes encoding electron transport chain subunits. Ablation of TFAM in keratinocytes impaired epidermal differentiation and HF growth and resulted in death 2 weeks after birth. TFAM-deficient keratinocytes failed to generate mitochondria-derived ROS, a deficiency that prevented the transmission of Notch and -catenin signals essential for epidermal differentiation and HF development [28]. *In vitro* keratinocyte differentiation was inhibited in the presence of antioxidants, and the decreased differentiation marker abundance in TFAM-deficient keratinocytes was partly rescued by application of exogenous H_2O_2.

The DP remains associated with the overlying epithelial matrix cells, which differentiate to give rise to the different HF lineages such as the cells that make the medulla, cortex and cuticle of the hair shaft and the inner root sheath (IRS) [29]. The matrix is derived from epithelial stem cells located in the bulge region of the HF [30]. Several important pathways and transcription factors that initiate and promote differentiation of the matrix cells have been determined, including Gata3 and Cutl (which regulate IRS differentiation and BMP signaling [31]), and transcription factors such as Gdsma3 [32], Msx2 [33], Foxn1 [34] and Hoxc13 [35] that are required for complete HF development and optimal hair shaft structure.

Cells with stem cell properties have recently been described in many integumental appendages including feathers [36] and teeth [37] but the HF stands out as one of the best model systems for studying adult stem cells [7]. The identification, characterization and transplantation of adult stem cells is currently one of the most intensively investigated areas of biological and biomedical research. HF are accessible, well defined in terms of their developmental biology, and their stem cell populations are located in discrete compartments or niches. Since the landmark paper almost 25 years ago by Cotsarelis *et al.* [14] highlighted the bulge as an important anatomical niche for HF epithelial stem cells in mice, much work directed at refining the location of stem cell compartments has been ongoing.

Aside from the location of these stem cells, the focus has been on three different aspects of their biology. Stem cells have the ability to self-renew and to generate progeny capable of

differentiating into one or more cell types. The conventional view is that when stem cells divide, they give rise to one stem cell that remains in the niche and one transit-amplifying (TA) cell that can rapidly proliferate for a limited number of divisions, giving rise to the various differentiated cells of the tissue. It is thought that stem cells and TA cells are not interchangeable, and once the TA cell has left the niche, its progress toward differentiation is irreversible.

The role of tissue maintenance throughout the lifespan of an organism requires stem cells to preserve their genomic integrity. They do this by dividing infrequently, so as to reduce DNA replication errors, and are also commonly protected from environmental and chemical assault by the niches they inhabit. HF stem cells are suggested to be more quiescent than stem cells of other tissues such as the intestine. Using the accumulation of epigenetic errors as a measure of mitotic age, human scalp hairs from individuals of various ages were analyzed. Unexpectedly, no increase in epigenetic error frequency was observed relative to the age of individuals, suggesting a very slow cycling stem cell population that did not accumulate as many errors over time as stem cells seen in the colonic crypts [38].

3. Alopecia

Hair loss can be caused by any number of conditions, and can be classified as one out of a set of specific diagnoses, according to the 'American Society of Hair Loss Association.' Some diagnoses have alopecia in their title, such as androgenic alopecia (AGA), alopecia areata (AA) or chemotherapy-induced alopecia (CIA), while others do not. AGA is by far the commonest type of alopecia characterized by progressive hair loss caused by androgenic miniaturization of the HF, and has a gradual increase in incidence by age. Although AGA is often called 'male pattern baldness' it can affect up to 70% of men and also 40% of women at some point in their lifetime.

Probably the most common non-AGA alopecia a dermatologist will see are telogen effluvium (TE), AA and hair loss due to cosmetic over-processing. Other, more rare forms of hair loss may be difficult to diagnose, and some patients may wait months, even years for a correct diagnosis and undergo consultation with numerous dermatologists until they find one with knowledge of their condition. Plus, with rare diseases, there is little motivation for research to be conducted and for treatments to be developed. Often, even when a correct diagnosis is made, a dermatologist can offer no known treatment for the condition.

TE is a particularly alarming form of alopecia [39]. The typical patient is a woman claiming to have always had a 'full head of hair' and reporting her hair to come out suddenly 'by the handful'. Patients are usually in good health, with no anorexia or nutrient deficiencies, possibly anxious, and have occasionally felt a painful or burning sensation at the scalp (trichodynia). Usually, the course of the disorder is chronic but intermittent, with apparent remissions followed by relapses. TE can be classified into three main categories: the premature teloptosis, the collective teloptosis and the premature entry into telogen: (drug-induced TE, TE due to dietary deficiencies and 'autoimmune' TE). The majority of TE patients are the autoimmune type, with some analogies with AA, including the triggering role of emotional stress.

4. Androgenic alopecia

4.1 Pathogenesis of AGA

AGA is often called 'male pattern baldness,' but the condition can affect large numbers of both men and women. Men typically present with hairline recession at the temples and balding at the vertex, while women normally suffer from diffuse hair thinning over the top of their scalp. Both genetic and environmental factors play a role, and the etiology remain incompletely understood.

Classic androgenic hair loss in males begins above the temples and vertex, or crown, of the scalp. As it progresses, only a thin rim of hair at the sides and rear of the head may remain (sometimes referred to as a 'Hippocratic wreath'), but AGA rarely progresses to complete baldness. The Hamilton–Norwood scale has been developed to grade AGA in males [40].

Female AGA has been colloquially referred to as 'female pattern baldness,' although this pattern can occur in males as well. It more often causes diffuse thinning without hairline recession, and like its male counterpart rarely leads to total hair loss [41]. The Ludwig scale grades severity of AA in females [42].

It is accepted that AGA is caused by changes in the male steroid hormones known as androgens [43], and variants in the gene for the androgen receptor has been implicated in AGA [44]. Androgens are important in male sexual development around birth and at puberty. They regulate sebaceous glands, apocrine hair growth and libido. With increasing age, androgens stimulate hair growth on the face, but suppress it at the temples and scalp vertex, a condition that has been referred to as the 'androgen paradox.' Men with AGA typically have lower total testosterone, higher unbound/free testosterone and higher free androgens, including dihydrotestosterone (DHT) [45]. The enzyme that transforms testosterone into DHT is known as 5-α-reductase (5-α-R) (or 3-oxo-5α-steroid 4-dehydrogenase). There are three isoenzymes of 5-α-R: known as 5α-R1, 5α-R2 and 5α-R3. 5α-R1 is expressed about 50 times more in the scalp of adult males than in the scalp of male fetus. Males with AGA have more 5α-R1 in scalp HF than males without AGA [45]. Therefore, the prevailing hypothesis is that DHT is responsible for miniaturization of HF [46].

Adrenal steroids such as dehydroepiandrosterone can be converted to 5α-DHT by isolated HFs, which may provide an additional source of intrafollicular DHT. Elevated urinary dehydroepiandrosterone and serum dehydroepiandrosterone sulfate have been reported to be present in balding young men. These reports suggest that dehydroepiandrosterone sulfate may act as an important endocrine factor in the development of AGA [47].

A difference between AGA and CIA can be summarized as follows. Chemotherapy targets transiently amplifying progenitor cells but spares the mostly static stem cells. Therefore, the hairs regrow after the chemotherapy treatment is finished. In contrast, androgens inhibit Wnt signaling [48], which is required for the ability of the DP to induce hair cycling and regeneration [49]. This mechanism implies that targeting androgens as a therapy for AGA

gives only a transient effect, while CIA can completely recover and hair growth becomes normal again.

The growth and dormancy of HF have been related to the activity of IGF at the DP, which is affected by DHT [43]. Studies looking at serum levels of IGF-1 have shown it to be increased with vertex balding [50]. Earlier work looking at *in vitro* administration of IGF had no effect on HF when insulin was present, but when insulin was absent, IGF-1 caused follicle growth [51]. The effects on hair of IGF-I were found greater than IGF-II [52]. IGF-1 signaling controls the hair growth cycle and differentiation of hair shafts, possibly having an antiapoptotic effect during the catagen phase. Mutations of the gene encoding IGF-1 result in Laron syndrome shortened and morphologically bizarre hair growth and alopecia [53]. IGF-1 is modulated by IGF binding protein, which is produced in the DP [54].

DHT inhibits hair growth in mice by inhibiting IGF-1 at the DP [55]. The involvement of IGF signaling in the ongoing functioning of HF stem cells adds another angle to the effects of DHT on hair growth [56].

Extracellular histones inhibit hair shaft elongation and promote regression of HFs by decreasing IGF and alkaline phosphatase in transgenic mice [57]. Silencing P-cadherin, a HF protein at adherens junctions, decreases IGF-1, and increases TGF-β 2, although neutralizing TGF decreased catagenesis caused by loss of cadherin, suggesting additional molecular targets for therapy. P-cadherin mutants have short, sparse hair [58]. In addition, androgens enhance inducible nitric oxide synthase from occipital DP cells and stem cell factor for positive regulation of hair growth in beard and negative regulation of balding DP cells [59]. Other research suggests that the enzyme prostaglandin D2 (PGD2) synthase and its product, PGD2 in HF could contribute to hair loss in AGA [60].

Recently, a newly discovered class of tiny noncoding RNAs named microRNAs have been discovered to play a role in regulating gene expression, and have led to the new field of 'regulomics' [61]. These tiny bits of transcriptome fine-tune the expression of nearly one-third of the genes in mammals [62]. MicroRNAs affect skin development [63], and play a role in HF morphogenesis, maintenance and cycling (controlling proliferation, growth arrest and apoptosis) [64]. Micro- RNAs have also been shown to play a role in the pathogenesis of AGA, with higher expression in the DP of HF from AGA patients versus normal HF [65].

Reduced blood flow and oxygen pressure in the balding areas are also involved in male pattern baldness. It has been shown that decreased blood flow and lower partial pressure of oxygen occurs in the balding scalp compared with nonbalding areas and with controls [66]. The decreases found in partial pressure of oxygen and blood flow are ~ 40 and 62%, respectively. However, it is unlikely that these decreases in partial pressure of oxygen or blood flow are the root cause of hair loss. Only a fraction of the blood flow to the skin is used for metabolic needs and the rest is for temperature regulation. Furthermore, even for metabolically very active cells, the critical partial pressure of oxygen for impairment of mitochondrial function is < 5 mmHg and about 0.1 mmHg for hypoxic cell death [67].

4.2 Models for androgenic alopecia

The organ culture of entire HF isolated from the human scalp was first reported by Philpott *et al.* [68]. The HF organ culture model is commonly used to determine whether small molecules or biologics may have a hair growth-promoting effect [69]. The advantage is that the effect of a compound can be assessed simultaneously on the epithelial and mesenchymal compartment of the HF. However, obtaining sufficient human samples can be challenging and in such cases mouse or rat vibrissae follicles may serve as alternatives to human HF [70,71]. Another drawback of human HF is that, although they can be switched from anagen to catagen phase *in vitro*, via insulin/IGF-I starvation or culture with TGF-β, modeling of the human hair growth cycle *in vitro* is still not possible. Murine follicles may serve as excellent models for such cases [70]. A red deer mane follicle model described by Thornton *et al.* served a useful *in vitro* androgen-responsive model owing to their ability to produce a mane during the breeding season when plasma testosterone levels are high, which is replaced by the short neck hairs of the summer coat when testosterone levels are low [72].

A major challenge in developing new therapies for AGA is the lack of small animal models to support drug discovery research. Stump-tailed macaque (*Macaca arctoides*) that possesses hereditary balding characteristics similar in many respects to that of AGA remains the model of choice despite its high cost, difficulty of manipulation and low availability [73,74]. In an attempt to overcome this challenge, recently, Crabtree *et al.* developed the first transgenic mouse model of AGA overexpressing human androgen receptor (AR) in the skin under control of the keratin 5 promoter [45]. When the keratin 5-human AR transgenic mice was exposed to high levels of 5-αDHT, delayed hair regeneration was observed and upon administration of AR antagonist hydroxyflutamide (active metabolite of flutamide) DHT-induced baldness was prevented. This model enables investigation of both topically or systemically administered drugs for DHT-induced delay in hair regrowth. Furthermore, many studies of prostate cancer demonstrate that by binding to β-catenin, androgen-bound AR can inhibit Wnt signaling through sequesterization of β-catenin [75]. The same group has developed a double transgenic mouse model expressing both K5-hAR and tamoxifen-inducible β-catenin (S33Y-ER-ligand- binding domain) to directly assess the involvement of Wnt signaling in this mouse model. These models will also enable identification of specific genes and pathways regulated by AR and β-catenin within the context of AGA.

The fuzzy rat model, a genetic mutant between hairless and hairy albino rat, shows androgen-dependent hypersecretion of sebum and hyperplastic sebaceous glands [76,77]. Another testosterone-inducible model of alopecia, called androchronogenetic alopecia, has been reported in B6CBAF1/j mice that were a hybrid cross between a C57BL/6 female and a CBA male [73,78]. Xenografts of human skin on immunodeficient mice, nude (Hfh11[nu]) or severe combined immunodeficiency (Prkdc[scid]) as well as xenografts to double mutant mice for severe combined immunodeficiency (Prkdc[scid]/Prkdc[scid]) and a number of hormone receptor null mutations offer new alternatives to testosterone-inducible models [73,79]. Nude mice are especially suitable to study AGA due to their lack of readily observable hair, making xenografts easy to identify and follow [79].

4.3 Drug discovery for androgenic alopecia

There are only two FDA-approved current therapies for AGA, namely topical minoxidil (Rogaine) and oral finasteride (Propecia). In 1988, the FDA approved 2% minoxidil topical solution (Rogaine®) for use in treating AGA in men. A 2% solution marketed to women became available in 1991, and a 5% solution became available over the counter for use in men in 1997. In 1997, finasteride (Propecia) was approved by the FDA for the treatment of male AGA at a dose of 1 mg/day. This medication is a competitive inhibitor of type II 5-α-R that inhibits the conversion of testosterone to DHT, which is involved in miniaturizing the HF in AGA [80].

Table 1 lists the approved and investigational drugs that have been used, tested or considered for AGA. Drug candidates largely fall into the classes of 5-α-R inhibitors, androgen receptor antagonists, ATP-sensitive potassium channel openers, topically growth factors, and a diverse selection of antioxidants and botanical extracts suggested by studies in ethnophamacology. It should be noted that the duration of the therapeutic effect does vary between different treatments. For instance hair loss can rapidly resume after cessation of minoxidil therapy. In fact, since AGA is an ongoing process, it is likely that even the most successful treatment will not lead to a permanent cure.

It is widely considered that topical application of active agents is superior to oral or parenteral administration of drugs for AGA [81]. The ideal therapeutic would be a cream or lotion that one could rub on one's head in the morning after one's shower. However this laudable desire comes up against the old problem of it being very difficult to get active drug molecules to cross the skin barrier. The problem may indeed be somewhat less daunting when the actual target of the drug is the HF itself [82] Lademann *et al.* at the Center for Experimental and Applied Cutaneous Physiology, Charité – Universitätsmedizin Berlin, Germany, suggested that HF are a highly relevant and efficient penetration pathway and reservoir for topically applied substances [83]. There have been efforts using specially designed nanoparticles to improve drug delivery via HF [84].

5. AA, alopecia totalis and alopecia universalis

5.1 Pathogenesis of AA

AA is a condition in which hair is lost from the scalp in patches sometimes called 'spot baldness'. The number of people with AA, who go on to develop alopecia totalis (loss of hair from the entire scalp) or alopecia universalis (loss of hair from the entire body) is not known, but estimates range from 7 to 30% [85]. AA is typically multifocal and the bald areas are commonly oval or circular in shape and smooth to the touch. Hair shaped like an exclamation mark can be present around the margins of the patch. Vitiligo and autoimmune thyroid disorders are sometimes associated with AA [86], and patchy AA often spares gray hairs [87].

AA is a common autoimmune disease resulting from damage caused to the HFs by T cells. Evidence of autoantibodies to anagen stage HF structures is found in affected humans and experimental mouse models [88]. Research currently points to a cell-mediated autoimmune mechanism as the underlying etiology of this disorder, although autoantibodies are

presumed to play an integral role in the mechanism of disease [89]. Biopsy specimens from affected individuals demonstrate a characteristic peri- and intrafollicular inflammatory infiltrate around anagen-stage HFs consisting of activated CD4 and CD8 T lymphocytes [90]. T lymphocytes cultivated from areas of affected scalp have also been shown to transfer AA to areas of nonaffected scalp in a severe combined immunodeficiency mouse model [91]. Recent studies found that transplantation of AA tissue to normal mice does not induce AA if the MoAb, anti-CD44v10, was injected into the normal mice shortly after transplant surgery [92]. CD44v10 is presumed to be involved in the activation mechanism of CD4 and CD8 lymphocyte and the migration into tissue and the subsequent initiation of the immune attack on HF. Similar investigations show that *in vivo* depletion of CD4^{+} cells with the CD4^{+} cell-depleting OX-35/OX-38 MoAb partially restores hair growth in AA-affected rats [93].

AA occurs more frequently in people who have affected family members, suggesting heredity may be a factor [94]. Strong evidence of genetic association with increased risk for AA was found by studying families with two or more affected members. This study identified at least four regions in the genome that are likely to contain these genes [95]. In addition, it is slightly more likely to occur in people who have relatives with autoimmune diseases.

Endogenous retinoids play a key role in the pathogenesis of AA [96]. Genes involved in retinoic acid (RA) synthesis were increased, whereas RA degradation genes were decreased both in AA mice, and in biopsies from AA patients. RA levels were also increased in C3H/HeJ mice with AA (see description of the model in next section). C3H/HeJ mice that were fed a purified diet containing high vitamin A showed accelerated development of AA.

AA has been linked with certain human leukocyte antigen (HLA) class II alleles, as have many autoimmune diseases. The HLA antigens DQB1*03 (DQ3) and DRB1*1104 (DR11) were strongly associated with a general susceptibility for AA [97]. Patients with alopecia totalis and alopecia universalis were found to express a significantly increased frequency of HLA alleles DQB1*0301 (DQ7), DRB1*0401 (DR4) and DRB1*1104 (DR11) [98].

In 2010, a genome-wide association study was completed that identified 129 single nucleotide polymorphisms that were associated with AA. The genes that were identified include those connected with regulatory T cells, cytotoxic T lymphocyte-associated antigen 4, IL-2, IL-2 receptor A, Eos, cytomegalovirus UL16-binding protein and the HLA region [89]. The study also identified two genes, PRDX5 and STX17 that were expressed in HF [99].

HF enjoy a relative degree of immune privilege that is characterized by downregulation of MHC class I, and local expression of immunosuppressants. Normally, NK cells attack cells with absent/low MHC class I expression, so healthy human anagen HF must somehow escape from NK cell attack. Ito *et al.* [100] found that immune avoidance happens via an active NK cell suppression. AA HF showed striking defects in NK cell inhibition/containment, with the NK cell inhibitor, macrophage migration inhibitory factor being strongly expressed by the HF epithelium, and very few CD56(+)/NK group 2D-positive

(NKG2D+) NK cells were observed in and around normal anagen HF. By flow cytometry, many fewer NK function-activating receptors (NKG2D, NKG2C) and significantly more killer cell Ig-like receptors- 2D2/2D3 were found to be expressed on peripheral blood CD56(+) NK cells of healthy controls than on those of AA patients.

Xing *et al.* [101] showed that cytotoxic CD8(+)NKG2D(+) T cells were both necessary and sufficient for the induction of AA in mouse models of disease. Global transcriptional profiling of mouse and human AA skin revealed gene expression signatures indicative of cytotoxic T-cell infiltration, an IFN-γ response and upregulation of several γ-chain cytokines known to promote the activation and survival of IFN-γ-producing CD8(+)NKG2D(+) effector T cells.

As AA is accepted to be an autoimmune disease, it can be concluded that certain host proteins can act as autoantigens. Leung *et al.* [102] isolated AA-reactive HF-specific antigens from normal human scalp anagen HF extracts by immunoprecipitation using serum antibodies from 10 AA patients. Samples were analyzed by LCMALDI-TOF/TOF mass spectrometry, which indicated strong reactivity to the hair growth phase-specific structural protein trichohyalin in all AA sera and to keratin 16 (K16) in some sera. A MoAb to trichohyalin revealed that AA sera contained immunoreactivity that co-localized with trichohyalin in the growth phase-specific IRS of HF, and AA serum reactivity with anti-K16 antibody was observed in the outer root sheath of the HF.

5.2 Models for AA

Since the pathology of AA involves the interplay between the immune cells of the host and the cells of the HF, it is less convenient to use *in vitro* or *ex vivo* models to study AA, than it is for AGA which more involves the biology of HF alone. Therefore, animal models are needed to study this cell-mediated, organ-specific autoimmune disease.

AA-like hair loss has been observed in several species, including monkeys, dogs, cats, horses, cattle, poultry and nonhuman primates [88,103,104]. However, the use of these species in AA research is restricted due to their limited numbers, genetic variability, scattered geographical distribution [104] therefore inbred rodent strains are likely to be considered better research models. Several rodent models with spontaneous and induced AA have been identified and of these, C3H/HeJ mice and Dundee experimental bald rat (DEBR) are most commonly used. The DEBR develops spontaneous AA at a higher frequency than mice, they are more expensive to use in drug studies owing to their larger size [105]. The low frequency of AA and not being able to predict the stage of AA as it evolves in the naturally occurring C3H/HeJ model of AA can be converted into a predictable system by grafting full thickness skin from AA-affected mice to normal haired mice of the same strain [105]. Human scalp explants grafted onto severe combined immunodeficient (SCID) mice is another experimental model reported by Kyoizumi *et al.* [106]. Recently, Gilhar *et al.* [107] developed a new humanized mouse model of AA, by transplantation of healthy human scalp skin obtained from normal volunteers on to SCID mice. This was followed by intradermal injection of either autologous or allogeneic peripheral blood mononuclear cells, which had been cultured with a high dose of IL-2 and were enriched for NK group 2D-positive (NKG2D+) and CD56+ cells. This protocol led to rapid and predictable development of focal

hair loss, with all the features of AA the Gu *et al.* [108] created a mouse model through repeated backcrossing/intercrossing between C57BL/6 and congenic AA(tj)mice (named B6.KM-AA). B6.KM-AA mice grew slower than B6 control mice and AA skin lesions developed by 4 weeks of age. The number of HF was reduced, but hair structures were normal. Loss of hair during disease progression was associated with CD4(+) and CD8(+) T lymphocytes infiltration peri- and intra-HF.

5.3 Drug discovery for AA

For the patient with AA, there is currently no universally proven therapy that induces and sustains remission, nor is there an accepted cure for this disease. The unpredictable course of the disease with spontaneous remissions frequently occurring also makes it challenging to design and conduct trials. Ito [109] pointed out that since spontaneous remission occurs in 80% of patients within 1 year, and not all patients require intense therapy, that no therapy at all (watchful observation) could be an option. Indeed many different therapies are available for this disease, and treatment choices are frequently based on disease extent, activity, duration of disease, and age of the patient. The drugs that are clinically used, in clinical trials or under laboratory investigation for AA are listed in Table 2.

Because of its autoimmune character, topical corticosteroids remain the cornerstone of initial treatment, as they are for many other inflammatory skin disorders. There have been a number of topical 'sensitizing' agents such as diphenylcyclopropenone, employed that are known as 'topical immunotherapy.' These compounds have in common the ability to cause the induction and periodic elicitation of allergic contact dermatitis as they act as potent contact allergens [110]. There is a decrease in CD4 to CD8 T-cell ratio from 4:1 to 1:1 [111] together with a decrease in lymphocytes and Langerhans cells in the HF bulb. Happle proposed [112] the concept of 'antigenic competition,' where an allergic reaction generates suppressor T cells that nonspecifically inhibit the autoimmune reaction against an HF antigen.

6. Chemotherapy-induced alopecia

6.1 Pathogenesis of CIA

Cancer chemotherapy is associated with severe side effects due to the induction of apoptosis in rapidly dividing cells within sensitive tissues (such as the hematopoietic system, the epithelia of the digestive tract and other organs). This apoptosis largely depends on p53, known as 'the guardian of the genome,' which accumulates in sensitive cells after a variety of stresses, resulting in growth arrest or induction of programmed cell death [113]. Chemotherapy affects the proliferating matrix keratinocytes in the bulb in the anagen HF that are producing the hair shaft. Damage to proliferating cells causes the HF to enter a dystrophic catagen stage in which the integrity of the hair shaft is compromised and the hair falls out. A significant fraction of HF is in the anagen phase at any one time, and these hairs are rapidly lost during chemotherapy, with massive apoptosis seen in the proximal bulb region. Chemotherapy-induced DNA damage leads to the rapid accumulation of p53 protein in the affected cells, followed by upregulation of Fas, IGF-BP3 and Bax, all of which are encoded by the corresponding p53-responsive genes [114].

Moderate-to-severe CIA is produced by the anthracyclines (e.g., doxorubicin), taxanes (e.g., taxol), alkylating compounds (e.g., cyclophosphamide) and the topoisomerase inhibitor etoposide [115].

6.2 Models of CIA

The usual models of cultured HF have been used to study CIA, but one interesting addition is use of the feather follicle. Xie *et al.* [116] found that cyclophosphamide induced distinct defects in feather formation: feather branching was transiently and reversibly disrupted, whereas the rachis (feather axis) remained unperturbed. Similar defects were observed in feathers treated with 5-fluorouracil or taxol but not with doxorubicin or arabinofuranosyl cytidine. Selective blockade of cell proliferation was seen in the feather branching area, along with a downregulation of Shh transcription. Local delivery of the Shh inhibitor, cyclopamine, or Shh silencing both recapitulated this effect.

Mice, rats and rabbits have been studied as animal models of CIA. The main difference in hair growth on human versus rodent skin/scalp is the growth pattern, which can be either mosaic or wave pattern [117]. In wave pattern hair growth, the entry of HF into anagen begins from the head and moves toward the tail, which is a pattern seen in neonatal rats and humans with CIA [117] Animal models of CIA typically involve procedures that cause the HF to enter the anagen growth phase in order to be able to mimic the human scenario [117]. Two main approaches are used: i) neonatal rats that show spontaneous anagen hair growth; and ii) synchronizing the HF in adult mice by depilation [117].

Wikramanayake *et al.* [118] showed that neonatal pigmented Long-Evans rats developed CIA in response to etoposide and cyclophosphamide. The rat dorsal hair was clipped during the early telogen stage to synchronize the hair cycle, and starting 15 days later, the rats were treated with etoposide for 3 days. HF in the clipped areas had the typical CIA follicular dystrophy (dystrophic catagen). When the hair in the pigmented alopecic areas regrew, it had normal pigmentation.

6.3 Treatment of CIA

For CIA the overriding goal of therapy is to protect the HF from being killed by the chemotherapeutic drugs. This goal can be accomplished by two broad strategies; first, the toxic drugs can be prevented from reaching the HF, and second, the cells in the HF can be protected against dying, by intervening in the apoptotic signaling, or by inhibiting ROS, or by counteracting other mediators of cytotoxicity. Since most interventions are applied topically onto the scalp, the interference with the desired cytotoxic effects of the chemotherapy against the cancer cells is thought likely to be minimal.

Table 3 lists the clinically used and investigational drugs that have been used for CIA.

7. Nondrug therapies

A number of nondrug therapies for alopecia are listed in Table 4. Hair transplantation or surgical hair restoration is widely employed by men with AGA. The introduction of robotic systems for HF harvesting has improved the technical function of this procedure [119,120].

Research efforts are underway to allow reconstituted HF to be transplanted as an alternative to harvested HF. There has been a report of GMP-compliant culture of human HF cells for encapsulation and transplantation [121]. Stem cell therapies are being increasingly discussed as possible alternatives for alopecia, but the exact manner of application has not yet been entirely worked out. Fractional laser (Fraxel) treatment for alopecia works on the basic premise that initiation of the wound healing response in the scalp will cause new HF to be formed in the skin [122,123]. Fraxel is widely used for photorejuvenation of the facial skin, but when it is used on the scalp instead, new hair can be seen to grow [124,125]. Perhaps the most exciting nondrug therapy is the use of low levels of red or near-infrared light known as low-level laser (light) therapy (LLLT) or photobiomodulation. The reason that LLLT is so promising is that it can be applied to AGA, AA or CIA with an approximately equal chance of success [126–128], it works equally well for men and women [129], it is relatively inexpensive and it has no known side effects or adverse effects.

8. Conclusion

The three main forms of alopecia are AGA caused by miniaturization of HF due to male hormones, AA caused by immune system attack on HF, and CIA caused by toxicity to fast growing cells in HF. The laboratory models and drug discovery efforts are very different for all of these forms because of the diverse etiologies and pathways involved. The one common factor is that topical delivery routes are preferred over systemic routes. AGA is being mainly investigated using 5-α-R inhibitors, androgen receptor antagonists and drugs that affect stem cells. AA is being investigated using immunosuppressive drugs, topical immunotherapy with contact sensitizers and JAK inhibitors. CIA is being investigated using cytokines, antioxidants and inhibitors of apoptosis. Some nondrug therapies such as hair transplantation, follicular neogenesis and LLLT may be applied to all forms of alopecia.

9. Expert opinion

In recent years, there has been an explosion of interest in discovering new drugs and nondrug treatments for hair loss [130]. The commonly used topical minoxidil preparation (Rogaine) was originally discovered by accident as a side effect of an oral blood-pressure medication. The other commonly used medication, oral finasteride (Propecia) was originally an antiandrogen drug used for benign enlarged prostate. However, many men are conflicted when they have to choose between accepting progressive hair loss, and taking antiandrogen drugs with their associated possible side effects of sexual dysfunction and feminization. Therefore (in common with most biomedical disease areas), there is now a trend toward trying to discover more molecularly targeted therapeutics. This search is connected with the development of the concept of personalized medicine, that aims to understand the patterns of gene expression that occur in the particular patient and in the particular disease state the patient is suffering from, so that the drugs can be tailored for each set of circumstances. Gene expression analysis is expected to reveal difference between individuals relating to present and future risk of hair loss, and possible at-risk persons may be able to take preventive measures. These new developments have arisen in parallel with groundbreaking discoveries about stem cells, the biology of the HF and elucidation of the signaling pathways involved in the hair cycle. This explosion of stem cell science and cell biology is taking

place hand-in-hand with another explosion of interest in tissue engineering. Drug discovery efforts are now involved with finding compounds that may affect known signaling pathways involved in hair growth, HF cycling and stem cell mobilization, differentiation and proliferation.

The difference in the etiologic factors and pathophysiology that are involved with the three different types of alopecia, means that drug discovery efforts will likely take three divergent paths. Drugs that reverse the miniaturization of the HF seen in AGA and caused by 5-DHT will likely be ineffective against AA caused by attack against the HF by aberrantly activated NK2GD T cells. Likewise, CIA caused by cytotoxic chemotherapy killing rapidly proliferating cells in the HF is unlikely to respond well to either antiandrogens or to immunosuppressive drugs.

In the future, it is expected that completely new HF can be constructed in the laboratory using stem cell and tissue engineering technology to replace those that have been lost by age, disease or trauma. Since hair transplantation is already becoming popular using HF harvested from unaffected parts of the scalp, HF neogenesis could dramatically increase the supply of new HF. The topical delivery route is preferred for administering drugs to treat alopecia as the target tissue is well localized, application is easy for the patient to carry out and the risk of side- effects is much lower. However transdermal and trans-follicular drug delivery is another upcoming area of research, where further advances are expected to be made. LLLT (or photobiomodulation) mediated by red or near-infrared light that can be either LED or laser, is another rapidly growing treatment for hair loss. It is almost unique among alopecia therapies in that it can treat all three forms of alopecia (AGA, AA and CIA). Moreover, LLLT is also particularly effective in mobilizing stem cells, out of their hypoxic niches, and inducing them to differentiate and proliferate. The wide spectrum of activity, the noninvasive nature, lack of side effects, ease of application and relative affordability, suggests that LLLT will also play an increasing role in hair loss therapy.

Despite the otherwise rosy outlook for drug discovery for alopecia, it behooves us to sound a note of caution. The progressive loss of hair typical of AGA can most likely only be delayed and not completely reversed by drug treatment. Even supposedly long-lasting approaches, such as hair transplantation, may be defeated by the inevitable onslaught of DHT, and continuous androgen signaling. The newly transplanted HF may still go the way of their predecessors. The immunological mechanisms underlying AA might be thought to not suffer from the same long-term problems of recurrence as the hormonal mechanisms underlying AGA. However, autoimmune disease can often be progressive and chronic in nature (see, for example, multiple sclerosis and rheumatoid arthritis). Moreover, treatments that tackle autoimmune diseases by various immunosuppressive approaches are notoriously plagued by side effects. The form of alopecia with the most favorable prognosis is CIA. However in that case, for the eventual regrowth of hair to be meaningful for the patient, the chemotherapy for cancer has actually to be successful, which only applies to a minority of chemotherapy treatments. So for these reasons, treatments for alopecia, although highly desired by the general population, may not be as dramatically effective as hoped. 'Gone today, hair tomorrow' may only be a far-off goal to be striven after.

Acknowledgments

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Article highlights

- There is an increasing demand for alopecia treatments due to the aging population, more awareness of body image and higher disposable incomes.

- Hair follicle biology, role of stem cells and *in vitro/in vivo* models for drug discovery are active areas of research.

- Androgenic alopecia (AGA), alopecia areata (AA) and chemotherapy-induced alopecia (CIA) have very different pathophysiology and treatments.

- 5-α-reductase inhibitors, androgen receptor antagonists and modulators of stem cells are being studied for AGA.

- Immunosuppressive drugs, topical immunotherapy with contact sensitizers and JAK inhibitors are being investigated for AA.

- Cytokines, antioxidants and inhibitors of apoptosis are being investigated for CIA.

- Topical drug application is the preferred route.

This box summarizes key points contained in the article.
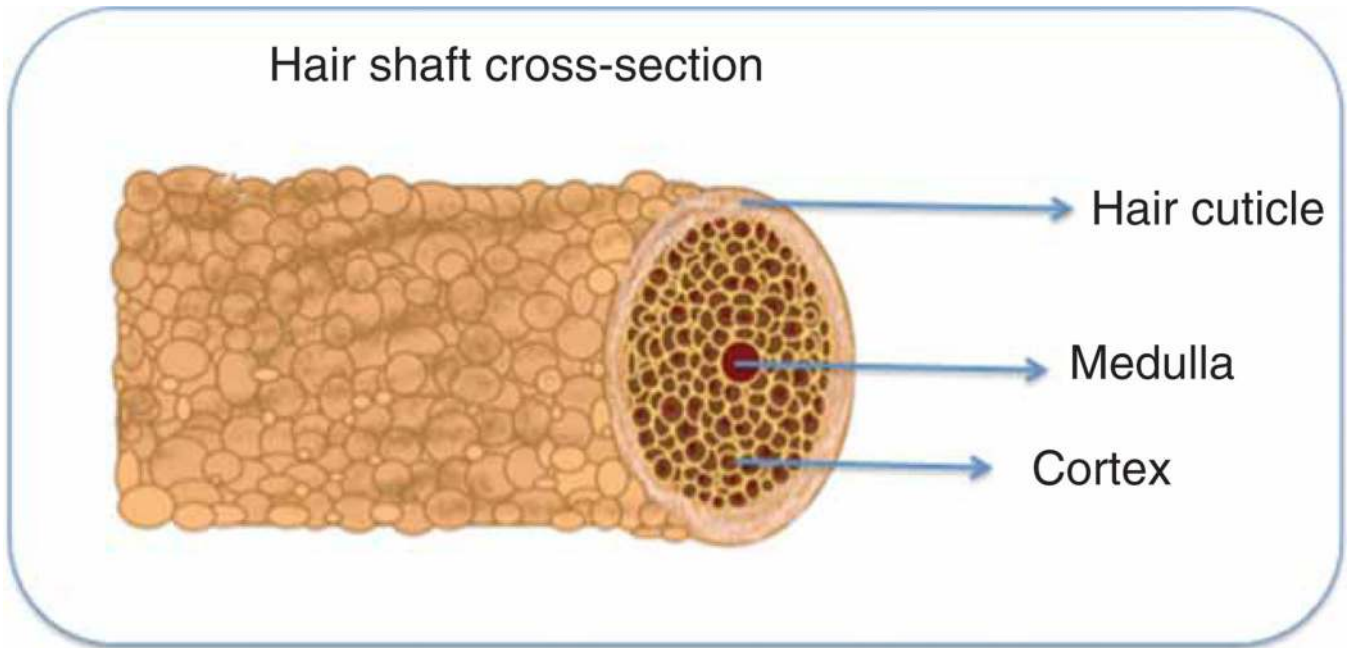
Figure 1.
Cross-section of a normal hair showing central medulla, intermediate cortex, and outer cuticle.
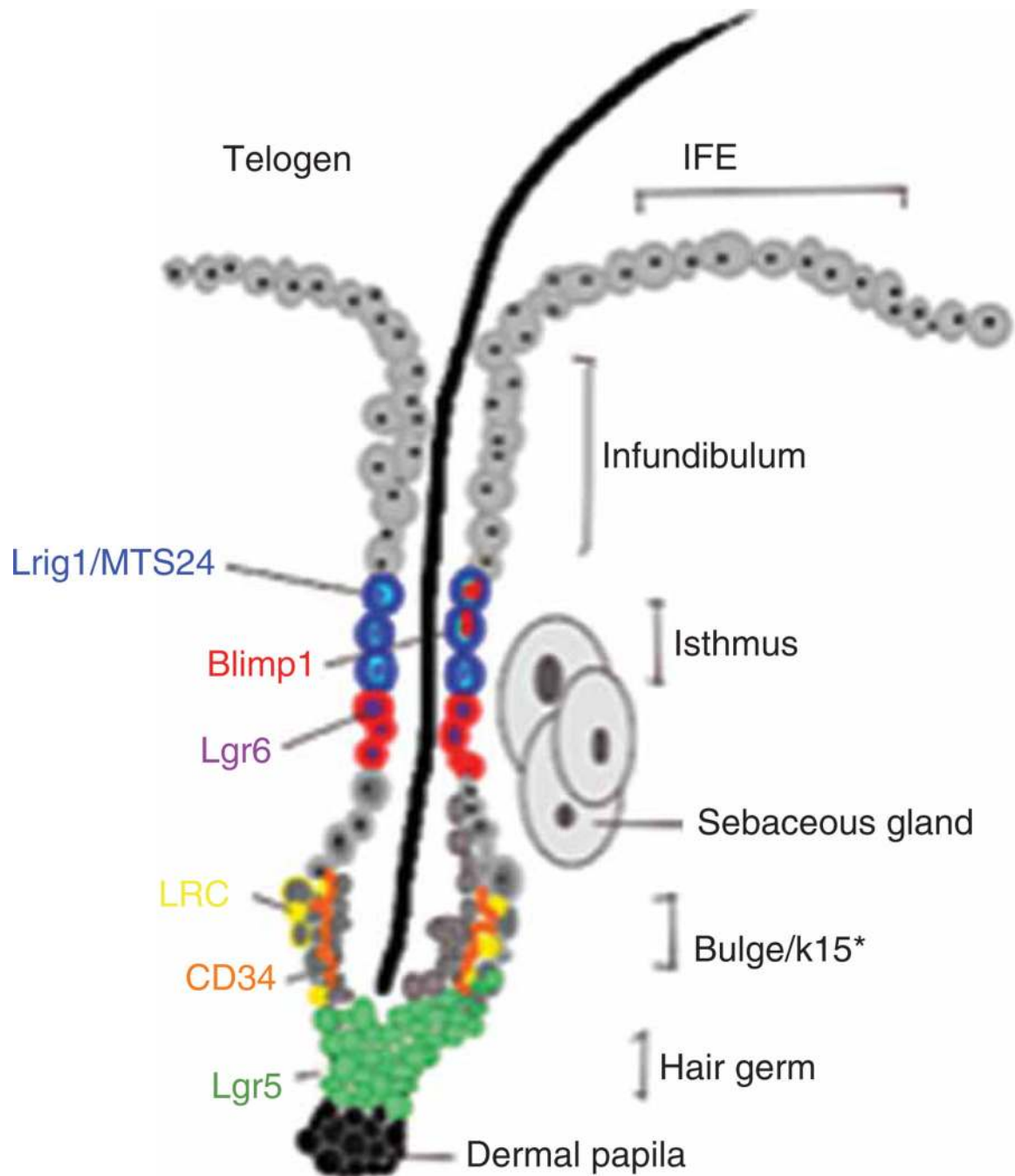
Figure 2.
Schematic organization of the telogen-phase adult HF showing location of the stem cells. The stem cell populations are represented by their well-marked gene/protein-expression or promoter-activity: Lgr5 (hair germ and bulge), CD34 (bulge), LRC (bulge), Lgr6 (lower isthmus), Lrig1/MTS24 (isthmus), Blimp1 (sebaceous gland) and K15* (K15 promoter, bulge area).
HF: Hair follicle.
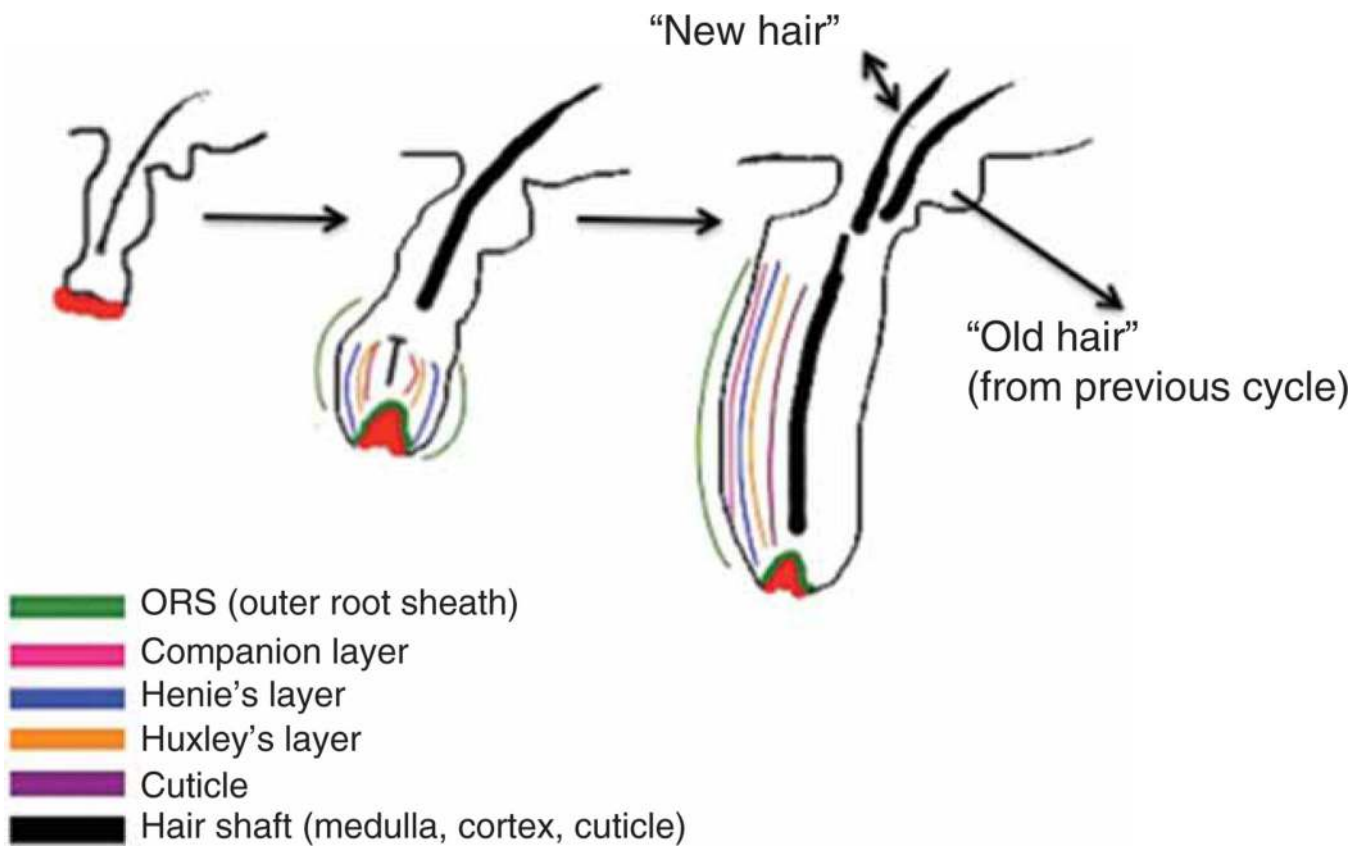
Figure 3.
Hair follicle IRS structure. This features three distinct layers of epithelial cells, which are known as Henle's layer, Huxley's layer, and the IRS cuticle.
IRS: Inner root sheath.
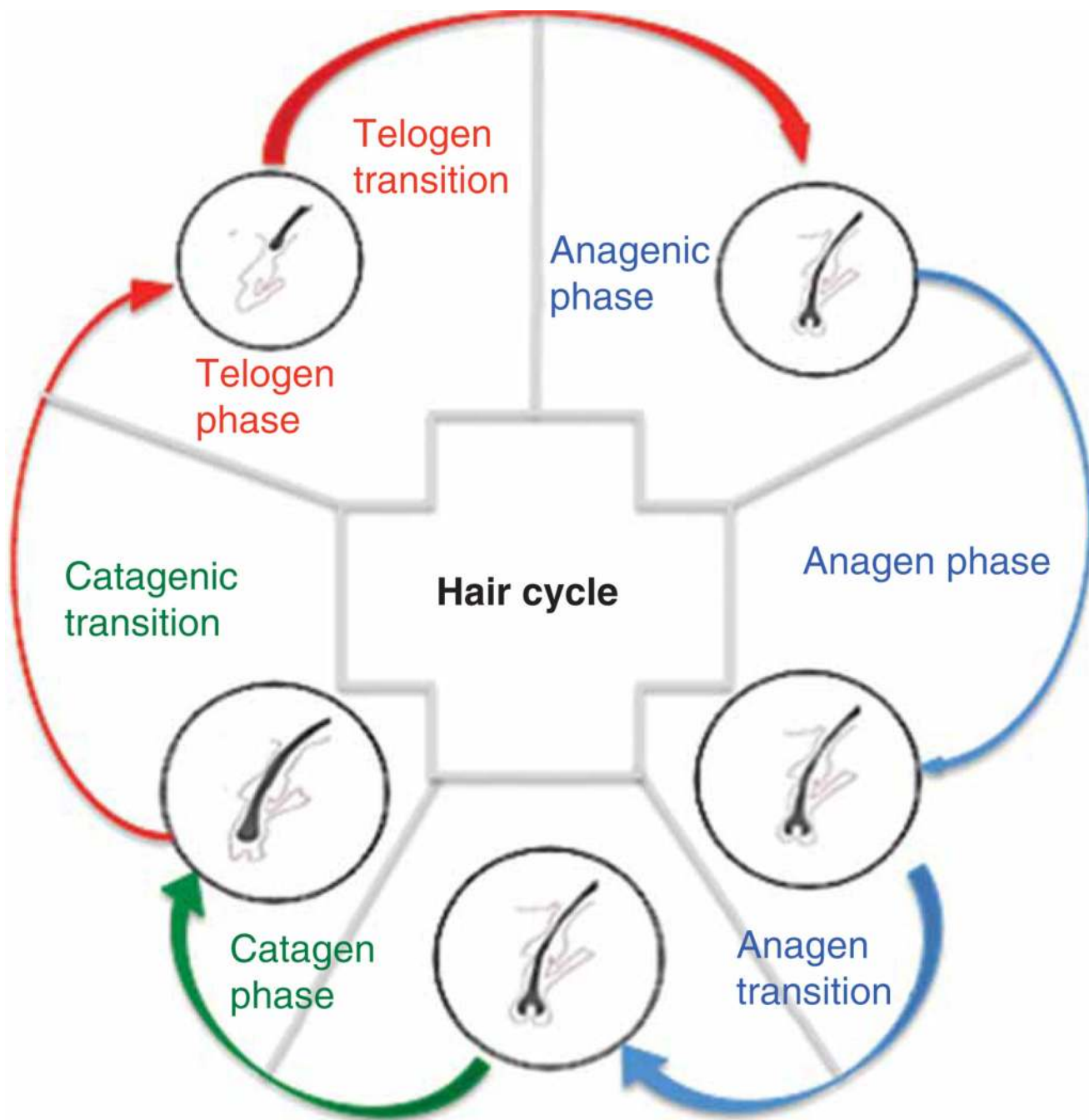
Figure 4.
Hair cycle and its transitions. There are three main phases of the hair growth cycle; anagen, catagen and telogen with anagen further subdivided into proanagen, mesanagen and metanagen.
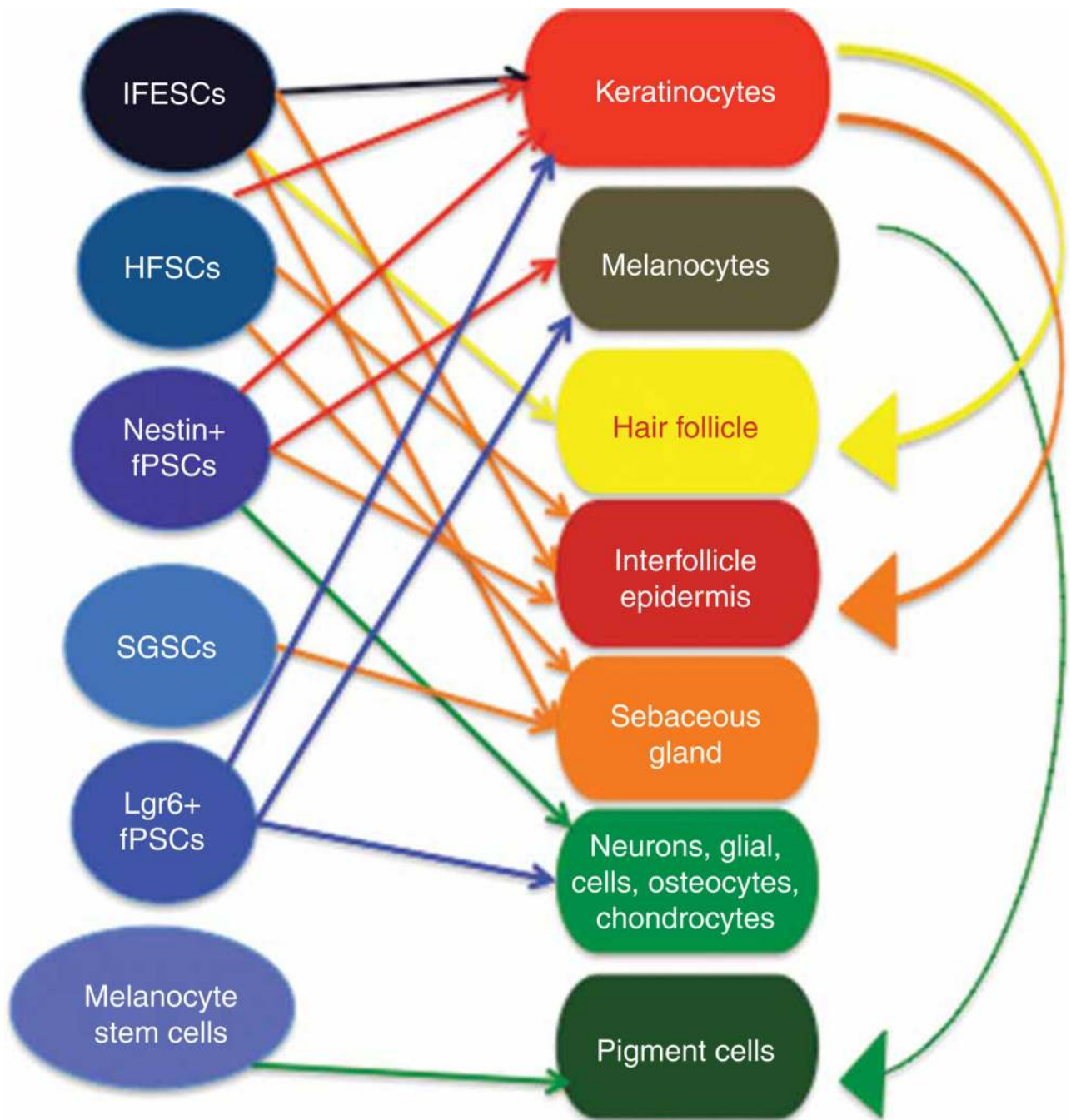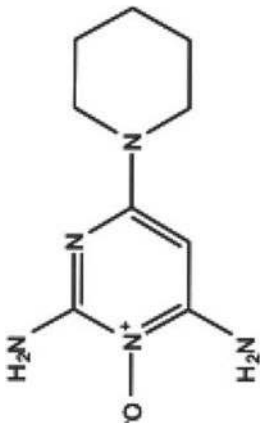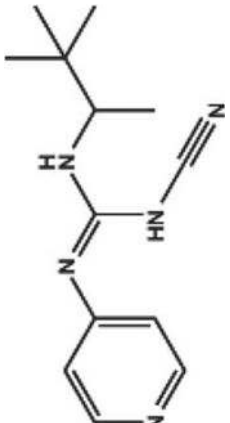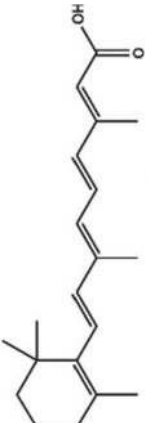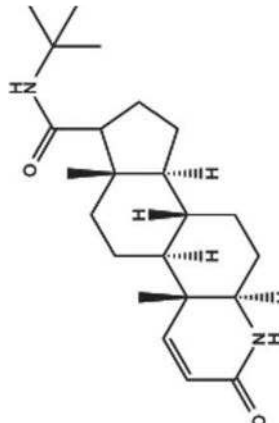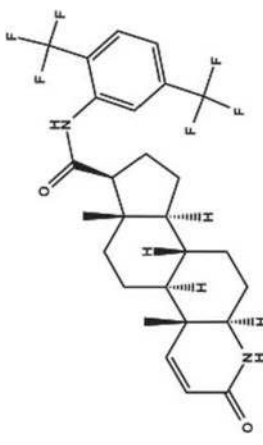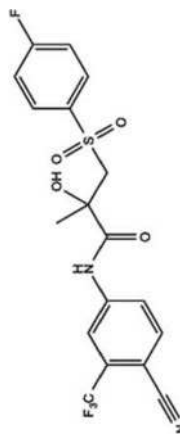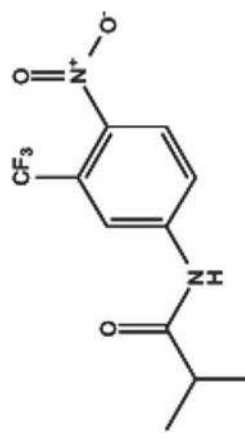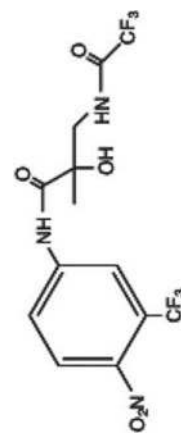
Figure 5.
Interactions between stem cells, progenitor cells, and cells in and related to the skin.
IFESCs: Interfollicle epidermal stem cells; HFSCs: Hair follicle stem cells; SGSCs:
Sebaceous gland stem cells; fPSCs: follicle nestin + pluripotent stem cells; Lgr6 + fPSCs,
could be identical to fPSCs.
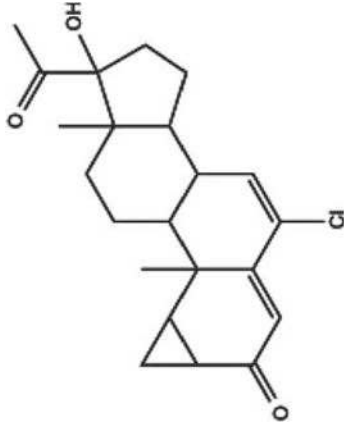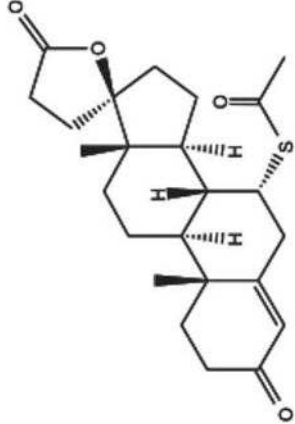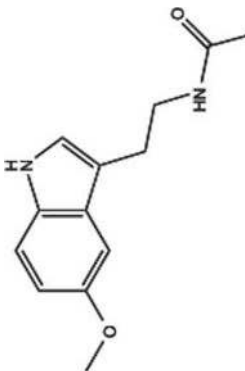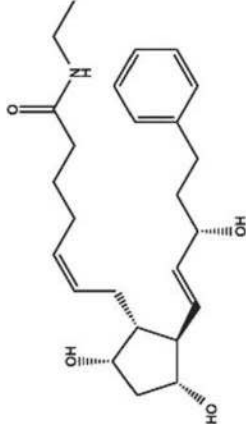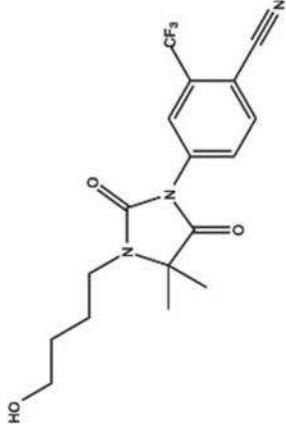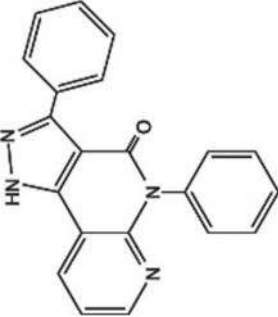
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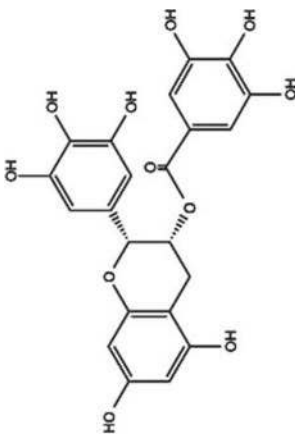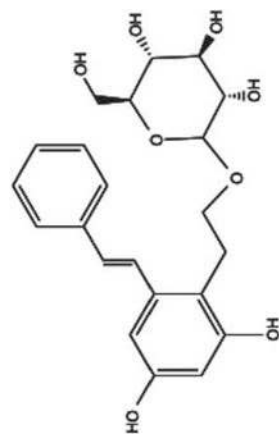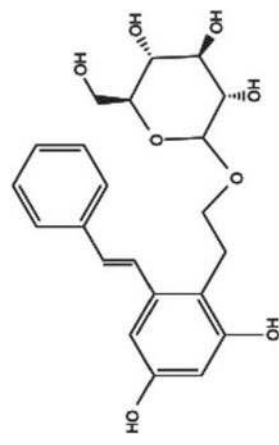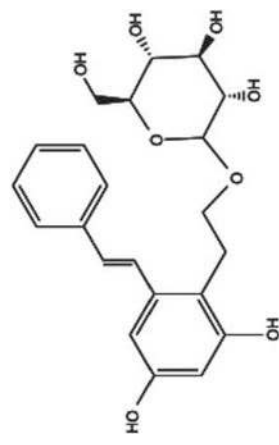
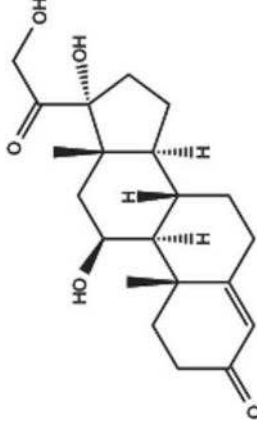Approved and Investigational drug therapies for androgenetic alopecia

Drug (Trade name)	Structure	M, F, Both, animal	Class/route	Mechanism	Ref.
Minoxidil (Rogaine)		Both; approved	Vasodilator, ATP-sensitive potassium channel opener; topical	Increases blood flow to HF, stimulates VEGF and PGE2	[131]
PInacidil (Pindac)		Both; clinical trial	Vasodilator, ATP-sensitive potassium channel opener; topical	Increases blood flow to HF, stimulates VEGF and PGE2	[132]
Tretinoin (Retin–A)		Both; clinical trial	Retinoid; topical	Promotes proliferation and differentiation, may promote vascular proliferation.	[133]
Finasteride (Propecia)		Both, approved M	5-α-reductase (5-α-R) inhibitor; oral, topical, mesotherapy	Inhibits 5-α-R2	[134,135]

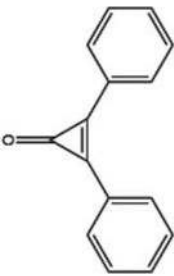| Drug (Trade name)	Structure	M, F, Both, animal	Class/route	Mechanism	Ref.
Dutasteride (Avodart)		Both, approved M	5-α-R inhibitor oral, topical, mesotherapy	Inhibits 5-α-R1 and 5-α-R2	[136,137]
Bicalutamide (Casodex)		F; clinical trial	Nonsteroidal antiandrogen; oral	Androgen receptor antagonist	[138]
Flutamide (Eulexin, Flutamin, Cytomid)		F; clinical trial	Nonsteroidal antiandrogen; Oral	Androgen receptor antagonist	[139]
Fluridil (Eucapil)		M; clinical trial	Nonsteroidal antiandrogen; topical	Androgen receptor antagonist	[140]

Drug (Trade name)	Structure	M, F, Both, animal	Class/route	Mechanism	Ref.
Cyproterone acetate (Androcur, Cyprostat)		W; clinical trial	Steroidal antiandrogen	Androgen receptor antagonist; progesterone receptor agonist	[141] Santos et al.
Spironolactone (Aldactone)		F; clinical trial	Steroidal potassium-sparing diuretic	Reduces adrenal androgen production and competitively blocks androgen receptors	[142]
Melatonin; N-acetyl-5-methoxytryptamine		F; clinical trial	Hormone produced to regulate photo-periods, topical	Responsible for seasonal hair growth in animals	[143]
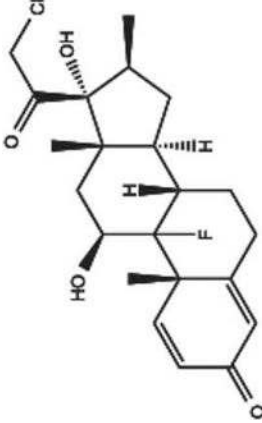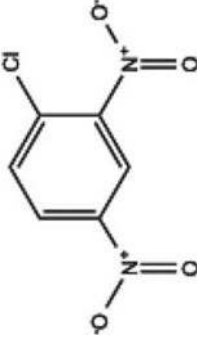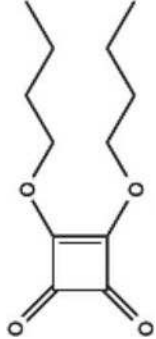
Drug (Trade name)	Structure	M, F, Both, animal	Class/route	Mechanism	Ref.
Bimatoprost (Lumigan, Latisse)		Both; approved for eyebrows	Prostaglandin analog prodrug; topical	Activates prostamide α F2 receptors	[144] Santos et al.
RU-58841 myristate;		Hamsters, macaques	Pro-drug for nonsteroidal antiandrogen; topical	Androgen receptor antagonist	[138]
SGF57 growth factors: bFGF, VEGF, IGF, SCF, KCF, SOD1, noggin		F; clinical trial	Growth factor cocktail; topical + microneedling	Stimulates HF	[145]
Liposomal IGF-1		Hamster	Growth factor, Topical	Stimulates HF	[146]
Thymosin β 4		Rats, mice	43-amino acid protein; topical	Stimulates HF stem cells	[147]
KFI9418		Mice	Condensed naphthyridine derivative; topical	Anti-inflammatory, bronchodilator	[148]

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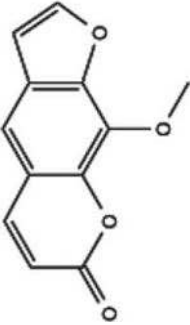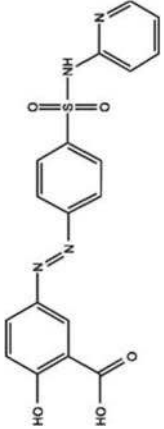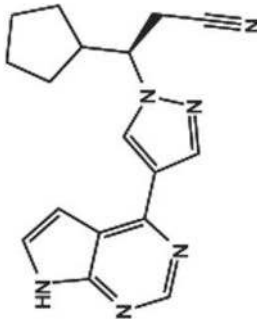
Drug (Trade name)	Structure	M, F, Both, animal	Class/route	Mechanism	Ref.
Epigallocatechin-3-gallate (EGCG)		Mice	Polyphenolic antioxidant; topical	Downregulated androgen receptor, reduced apoptosis	[149] Santos et al.
Polygonum multiflorum extract		Mice	Botanical extract in water containing 2,3,5,4-tetrahydroxystilbene-2-O-β-dglucoside; topical with added lactobacilli	Increases anagen-phase HF; traditional folk-medicine for baldness	[150]
Eclipta alba extract		Mice	Botanical extract in methanol containing coumestans, triterpenes, saponins, flavonoids; topical	Increases melanogenesis, anagen-phase HF; traditional folk-medicine for baldness	[151]
Thuja orientalis extract		Mice	Botanical extract in water containing flavonoids and diterpene; topical	Contains natural 5– reductase inhibitors; Traditional folk-medicine for baldness	[152]
Steroid sulfatase inhibitors		Investigational	Topical	Reduce additional source of DHT	[153]
Antisense oligonucleotides against AR		Investigational	Topical	Reduce expression of AR	[154]

bFGF: Basic fibroblast growth factor; DHT: Dihydrotestosterone; HF: Hair follicle.

Table 2

Approved/investigational drug therapies for alopecia areata or alopecia universalis.

Drug (Trade name)	Structure	M, F, Both, animal	Class/route	Mechanism	Ref
Hydrocortisone (Cortizone)		Both; approved	Corticosteroid; topical; intradermal injection, oral	Anti-inflammatory, immunosuppressive	[155]
Clobetasol propionate (Temovate)		Both; clinical trial	'Strong' corticosteroid; topical; intradermal injection	Anti-inflammatory, immunosuppressive	[155]
Dinitrochlorobenzene		Both	Local irritant; topical	Triggering sensitization counteracting autoreactive T cells	[110]
Squaric acid dibutylester		Both	Local irritant; topical	Triggering sensitization counteracting autoreactive T cells	[156]

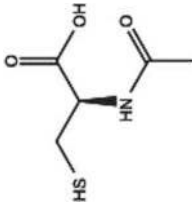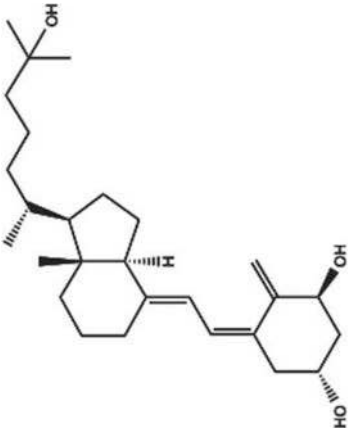| Drug (Trade name)	Structure	M, F, Both, animal	Class/route	Mechanism	Ref
Diphenylcyclopropenone (Diphencyprone)		Both	Local irritant; topical	Triggering sensitization counteracting autoreactive T cells	[157]
8-Methoxypsoralen and UVA (PUVA)		Both	Photochemotherapy, drug-light combination; topical	Kills autoreactive T cells; induces tolerogenic dendritic cells	[158]
Sulfasalazine (Azulfidine)		Both; clinical trial	Developed as antibiotic; oral	Anti-inflammatory immunomodulator	[159]
Adalimumab (Humira)		F; case report	Recombinant human IgG1 MoAb against TNFα; s.c. injection	Anti-inflammatory; inhibits T cells	[160]
Alefacept (Amevive)		F; case report	1-92-LFA-3 (human) fusion protein with Ig G1	Inhibits T cells	[161]
Ruxolitinib (Jakavi)		Case reports, mice	JAK inhibitor; topical, systemic	Affects downstream signaling of IFN-γ; and GC cytokine receptors	[101]
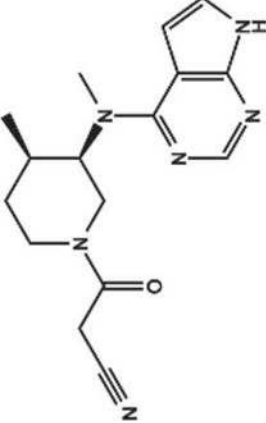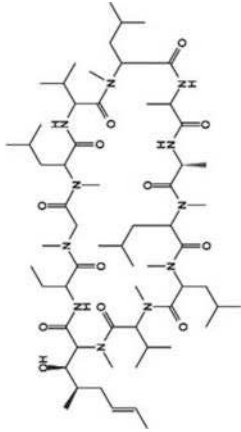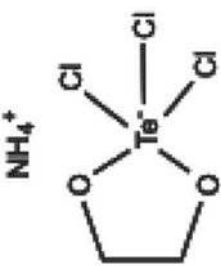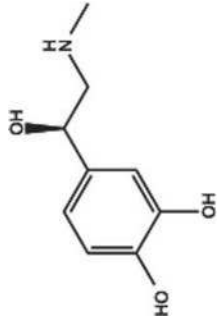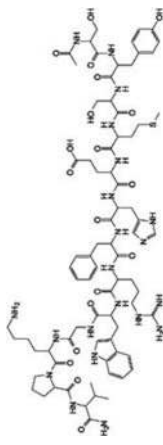
Drug (Trade name)	Structure	M, F, Both, animal	Class/route	Mechanism	Ref
Tofacitinib (Jakvinus)		Mice	JAK inhibitor; topical, systemic	Affects downstream signaling of IFN-γ and GC cytokine receptors	[101]

Table 3

Investigational drug therapies for chemotherapy-induced alopecia.

Drug (Trade name)	Structure	M, F, Both, animal	Class/route	Mechanism	Ref.
Cyclosporin A (Neoral)		Rats	Inhibitor of T cells; topical	Binds to cyclophilin and inhibits calcineurin; possibly increases P-glycoporotein	[162]
Immuvert/N-acetyl cysteine		Rats	Immunomodulator derived from *Serratia marscescens*/thiol-based antioxidant; topical	Activates NK cells, produces IL1β/inhibits ROS	[163]
IL-1		Rats	Pro-inflammatory cytokine, i.p. injection	Prevents HF cells undergoing mitosis	[164]
Calcitriol, 1,25-dihydroxyvitamin D-3 (Topitriol)		Rats	Active metabolite of vitamin D; topical	Stimulates differentiation in HF	[165]
PTH(1–34), PTH(7–34)		Mice	Parathyroid hormone receptor agonist or antagonist; systemic	Shifted HF towards 'mild dystrophic anagen'; reduced apoptosis	[166]

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Drug (Trade name)	Structure	M, F, Both, animal	Class/route	Mechanism	Ref.
AS101		Both; Clinical Trial; Rats	Tellurium containing redox modulator; topical/ IV injection	Immunomodulator Anti-inflammatory Antioxidant	[167]
liposomal MAD11		Rats	Topical liposomes containing mAb against doxorubicin	Neutralizes any chemotherapy that arrives at HF	[168]
Adrenalin (Epinephrine) or norepinephrine		Rats	Topical vasoconstrictors	Prevents chemotherapy accumulating in HF	[169]
EGF		Rats	Growth factor; topical	Prevents HF cells undergoing mitosis	[170]
Acidic Fibroblast growth factor		Rats	Growth factor; s.c. injection	Prevents HF cells undergoing mitosis	[170]
Cuscuta reflexa Roxb extract		Rats	Botanical extract in EtOH/PE containing coumestans, triterpenes, saponins, flavonoids; topical		[171]
α-Melanocyte-stimulating hormone		*ex vivo* HF	Endogenous peptide hormone of the melanocortin family	Cytoprotective to HF, upregulates heme oxidase-1	[172]
p53 inhibitors; Caspase-3 inhibitors		Investigational	Small molecule inhibitors; topical	Prevent apoptosis in HF	[173]
Anti-death rFNK protein-TAT peptide		Rats	Site-directed mutagenesis of rat Bcl-x(L); topical	Anti-apoptotic and cytoprotective activity	[174]

HF: Hair follicle; ROS: Reactive oxygen species.

Table 4

Nondrug therapies for alopecia.

Treatment	Mechanism	Application	Ref.
Hair transplantation	Replaces old with new HF	AGA	[175]
LLLT	Stimulates HF and stem cells, protects HF from dying	AGA, AA, CIA	[176]
Platelet rich plasma	Mixed growth factors in dalteparin and protamine microparticles	AGA	[177]
Follicular neogenesis	Dissociated epidermal and dermal cells in suspension reconstitute on a matrix	AGA	[178]
Fractional laser	Induction of thermal micro-wounds stimulates HF	AGA	[124]
Inductive cell therapy	Use spheroids of dermal papilla cells	AGA	[179]
Stem cells	Skin derived progenitor cells	AGA	[180]

AA: Alopecia areata; AGA: Androgenic alopecia; CIA: Chemotherapy-induced alopecia; HF: Hair follicle; LLLT: Low-level laser (light) therapy.