# Chemotherapy-Induced Hair Loss: The Use of Biomarkers for Predicting Alopecic Severity and Treatment Efficacy

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Iain S Haslam[1] and Eleanor Smart[2]

[1]School of Applied Sciences, University of Huddersfield, Huddersfield, UK. [2]Centre for Dermatology Research, University of Manchester, Manchester, UK.

**ABSTRACT:** Damage to hair follicles following exposure to toxic chemotherapeutics can cause substantial hair loss, commonly known as chemotherapy-induced alopecia (CIA). Preventive therapies remain limited; however, recent advances in the use of scalp cooling technologies have proved successful in preventing or reducing hair loss in some patients. Further improvements in scalp cooling efficacy and/or development of novel treatments to prevent chemotherapy-induced hair loss are required. To achieve this, post-chemotherapy assessment of hair follicle damage markers, with and without scalp cooling, would provide invaluable mechanistic and prognostic information. At present, the availability of such data is extremely limited. This article describes the potential utility of a combination of biomarkers in assessing drug-induced alopecia and the protective potential of existing or new treatments. A greater understanding of the precise mechanisms of anti-CIA therapies through biomarker analysis would enhance the rationale, use, and development of such treatments.

**KEYWORDS:** chemotherapy-induced alopecia, hair loss, biomarkers, scalp cooling

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**CORRESPONDING AUTHOR:** Iain S Haslam, School of Applied Sciences, University of Huddersfield, Huddersfield HD1 3DH, UK. Email: i.haslam@hud.ac.uk

## Introduction

The hair follicle (HF) is a complex and remarkably dynamic skin appendage, responsible for the production of a filamentous hair shaft through rapid keratinocyte proliferation and terminal differentiation.[1] Hair plays a number of important roles, including physical protection, sensation, thermoregulation, sweat dispersion, production of sebum and pheromones, and social and sexual communication. As a result, hair loss (alopecia) presents a profound psychosocial burden[2,3] and pathological hair loss resulting from exposure to cytotoxic chemotherapy can be particularly distressing.[4–6]

Chemotherapy-induced alopecia (CIA) is a common and extremely visible side-effect of cancer treatment, although the likelihood of developing CIA and the severity of the hair loss is often drug or regimen dependent.[7] Preventive strategies for CIA are currently limited to scalp cooling, which has proven to be an effective anti-CIA therapy for some patients. This has led to the recent Food and Drug Administration (FDA) approval of the DigniCap and Paxman scalp cooling systems.[8] While providing a much-needed CIA-preventive treatment option, scalp cooling is not effective for all patients, with an approximately 50% to 71% success rate in substantially reducing hair loss.[9–12]

Furthermore, there remain notable lacunae in our understanding of both CIA pathobiology and the mechanism(s) by which cytotoxic damage can be prevented (ie, through scalp cooling). In addition, despite the introduction of more targeted treatments and immunotherapies, treatment-related hair loss can still occur (eg, with the hedgehog inhibitor Vismodegib),[13] albeit less frequently.[14–16] Currently, there are no available treatments and the efficacy of scalp cooling is yet to be determined in these cases.

The current state of knowledge relating to chemotherapy-induced hair follicular damage provides a number of potential read-outs that might serve as useful biomarkers of damage severity. What is more, biomarker analysis might also allow a real-time assessment of the efficacy of scalp cooling or other potential preventive treatments and provide vital insight into how these therapies might be further enhanced.

## Human HF Anatomy and CIA Pathophysiology

To describe the potential utility of human HF analysis in assessing CIA, familiarisation with the anatomy and physiology of this mini-organ is important, as is an understanding of the pathomechanisms of this hair loss.[7,17] Much of this detail can be found in Figure 1. In brief, HFs are composed of a series of concentric keratinocyte layers and ultimately function to produce a central hair shaft. Mature, terminal HFs can be divided into a permanent, non-cycling upper section and a lower section, which is continuously remodelled during the hair cycle.[19]

Throughout life, each HF undergoes continuous cycles of involution and regeneration. The hair cycle can be divided into 3 phases: (1) growth (anagen), (2) involution (catagen), and (3) rest (telogen).[19] Following chemotherapy, this normal hair cycle is disrupted, leading to 1 of 2 distinct pathways, dystrophic anagen or dystrophic catagen (Figure 1),[17] the latter pathway resulting in the most extensive hair loss. In a twist that provides a profound therapeutic conundrum, recovery from hair loss is considerably faster if dystrophic catagen is induced,

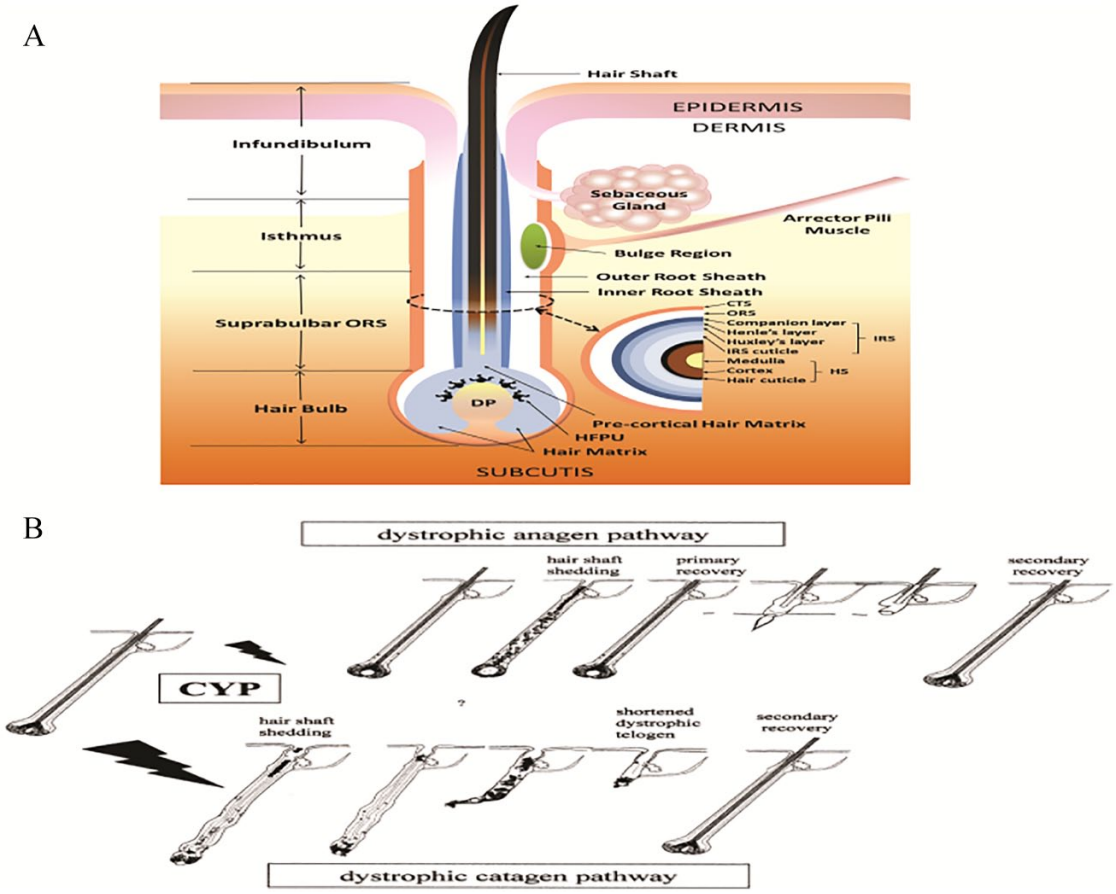


**Figure 1.** HF structure and the dystrophic pathways leading to chemotherapy-induced hair loss. (A) The upper region of the HF consists of the infundibulum and the isthmus, which meet at the insertion of the arrector pili muscle. The bulge region is located in the lower isthmus, at the origin of the arrector pili muscle, and contains epithelial and melanocyte stem cells. The lower region contains the hair bulb, housing the rapidly proliferating and chemotherapy-sensitive matrix keratinocytes. These matrix keratinocytes migrate and undergo terminal differentiation to produce the various cell lineages of the inner root sheath and the hair shaft. The outer root sheath is the external epithelial layer of the HF. The dermal papilla, which is derived from the mesenchyme, invaginates the hair bulb and is responsible for controlling the size of the HF, the length and diameter of the hair shaft, and the duration of the growth phase of hair cycling. Source: Reproduced from Haslam et al.[18] (B) On exposure to less severe chemotherapeutic insult, HFs undergo dystrophic anagen, whereby hair shaft production is paused but ultimately resumes, often with disturbed pigmentation and altered hair shaft structure. This leads to a poor-quality hair shaft being produced and a slow recovery as the hair follicles continue in this damaged anagen phase for a much longer period after cessation of treatment. Only on completion of catagen and telogen, at the onset of a new anagen phase, does new, healthy, fully pigmented hair shaft production resume (secondary recovery). In contrast, when the chemotherapeutic insult is more severe, HFs enter a dystrophic catagen phase in which regression is rapid and less well controlled, leading to sudden massive hair loss. Recovery is faster following this form of hair loss. Source: Modified after Hendrix et al.[19]
HF, hair follicle; HFPU, hair follicle pigmentary unit; IRS, inner root sheath; ORS, outer root sheath; CTS, connective tissue sheath; HS, hair shaft; DP, dermal papilla.

with a normal anagen phase initiated after cessation of chemotherapy.

The following sections provide an overview of potential molecular pathways, or HF characteristics, that could serve as biomarkers indicative of the severity of HF damage and how they might be used to assess current and prospective anti-CIA therapies.

## Molecular Pathways and Structural Hair Alterations Associated With CIA

The extent of hair loss following chemotherapy can vary, depending on the specific drug (or drug combinations) used, as well as drug dosages and regimen (such as the number of cycles undertaken).[7,17] Despite this, the associated disruption of hair growth and eventual hair loss involves conserved intrafollicular events.[7,17] Primarily, this includes the activation of distinct damage response pathways leading to reduced proliferation and abnormal levels of apoptosis in the hair matrix. Biomarkers associated with these events and the external appearance of damaged hair are discussed below.

### *Trichoscopic observations in chemotherapy-induced hair damage*

The structure of the hair shaft following cytotoxic damage was first described in the 1960s[20] and recently trichoscopy has proven to be a useful tool for the diagnosis of alopecia through non-invasive analysis of hair shaft formation.[21] This has now been extended to include an assessment of the damage occurring as a result of chemotherapy treatment.

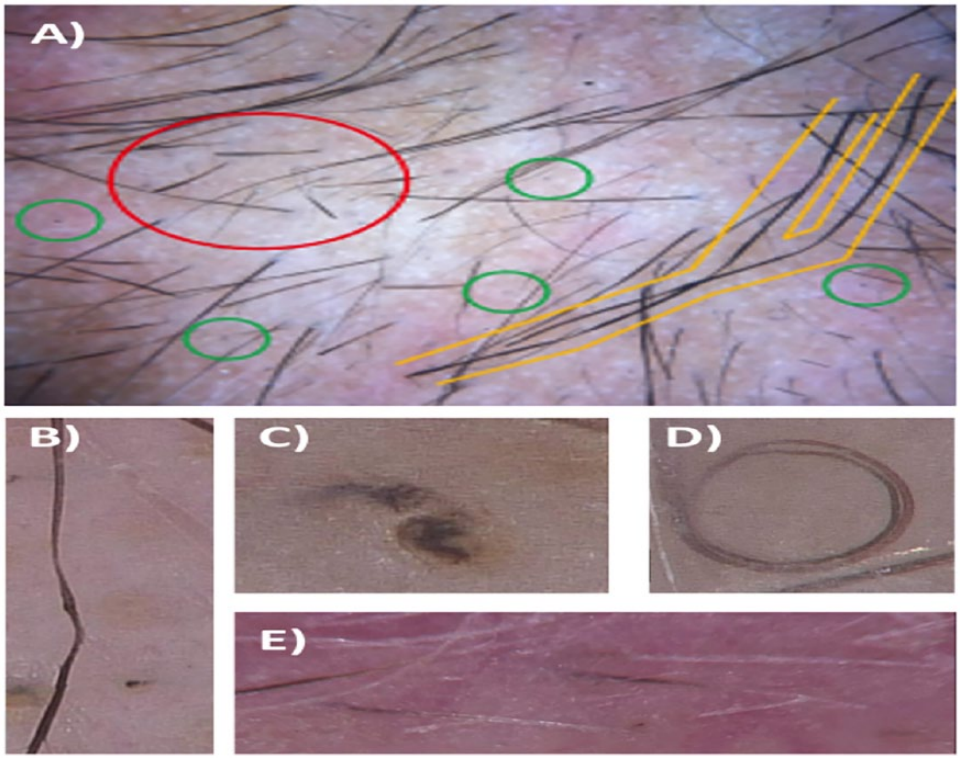


**Figure 2.** Trichoscopic features of chemotherapy-induced alopecia. Scalp of patient undergoing chemotherapy (A) showing Pohl-Pinkus constrictions (yellow lines), exclamation mark hairs (red circle), and black dots (green circles). Pohl-Pinkus constrictions are shown in more detail (B) with wave pattern of thickening and thinning within a single hair shaft. Examples of flame hairs (C) and circle hairs (D) are shown along with a black and white hair (E), where the hair shaft is depigmented except for the base where there is recovery of the damaged follicular melanocytes resulting in re-pigmentation. Source: Modified after Mill et al[23] and Brownell et al.[24]

Rossi et al[22] systematically examined patients undergoing treatment with FEC, which is a combination of fluorouracil (5FU), epirubicin, and cyclophosphamide. Patients were examined before, during, and after treatment, with the authors observing that, 3 weeks after treatment, 70% to 100% of HFs were in dystrophic anagen, as identified by commonly observed black dots, exclamation marks, broken hairs, flame hairs, and Pohl-Pinkus (Figure 2). These trichoscopic features are further detailed below.

Pohl-Pinkus are constrictions of the hair shaft that occur in a wave-like pattern due to the cyclic nature of chemotherapeutic regimens (Figure 2). When chemotherapy is administered, hair matrix cells are damaged, shutting off proliferation and causing thinning of the hair shaft. On cessation of chemotherapy, hair shaft production resumes before being prevented once more during the next round of therapy. This creates a hair shaft displaying a series of constrictions, as shown in Figure 2A and B.[25]

A more severe response to injury results in exclamation marks, which have been observed in a number of studies in which fracturing of hair appears as a common sign of chemotherapeutic damage.[26,27] These fractures occur as a result of hair shaft thinning, a direct result of decreased matrix proliferation, followed by premature telogen entry.[27] Black dots represent the most severe cessation of proliferation with total breakage of the hair shaft[27,28] and circle hairs were often present following prolonged chemotherapy (Figure 2D).[22]

Flame hairs on the other hand result from melanocyte damage, rather than a loss of keratinocyte proliferation. These have been observed previously in 6 cases of acute CIA,[29] caused by ectopic melanin accumulation around the damaged hair shaft (Figure 2C). HF miniaturisation is also a common feature of chemotherapy damage, although it was present to some degree in 30% of patients even before treatment, suggesting that this is a common observation in otherwise healthy scalps.

Following the cessation of treatment, Rossi et al[22] observed an increase in the number of HFs in anagen; however, these were frequently depigmented as a result of chemotherapy-induced melanocyte loss. Normal hair shaft production had usually resumed 1 year following treatment and some hair was detectable as being pigmented at the base and white at the tips, indicating melanocyte repopulation of the hair bulb (Figure 2E).[22,27] It has been suggested that the presence of previous disease may affect regrowth with permanent alopecia being a common feature of patients with early-stage androgenic alopecia or latent female pattern hair loss.[22]

Given the non-invasive nature of trichoscopy, it provides a clinically useful and pragmatic tool in the assessment of HF damage in response to chemotherapy. Although the routine use of trichoscopy to inform patients of the extent of HF damage they are subject to is not likely to provide direct benefit, the primary use of this technology is anticipated to centre on the development of new treatments. Adoption of trichoscopy for

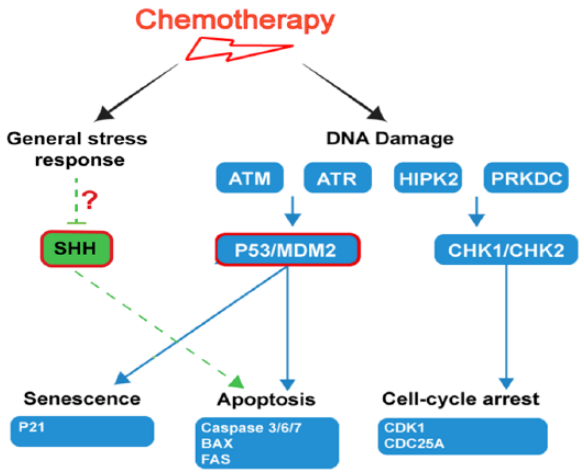


**Figure 3.** Damage response pathways associated with chemotherapy exposure in hair follicles. Exposure of HFs to chemotherapeutic insult can cause DNA damage, with transducer molecules (ie, ATM, ATR) triggering cell cycle arrest (via CHK1/CHK2), P53-mediated apoptosis, or senescence (p21). In addition to these established pathways, induction of general or metabolic stress might also inhibit Shh signalling, leading to further apoptosis. Solid lines represent established connections, with dotted lines representing putative interactions.[35]
Shh, sonic hedgehog.

assessment of HF damage would aid in our understanding of how different chemotherapeutics, dosages, and regimens impact on hair damage as well as providing valuable information on the effectiveness of current or new treatments in preventing this.

### *HF drug concentration*

Additional non-invasive procedures might also prove useful in assessing HF exposure to damaging chemotherapy. As mentioned, the ability of scalp cooling to prevent or reduce hair loss following chemotherapy is now well established,[9,11,15] yet the mechanism(s) by which cooling exerts its protective effect remains to be defined. The primary assumption is that cooling results in vasoconstriction, reducing blood flow in the scalp microvasculature and thereby reducing drug delivery to damage sensitive HFs.[7,17] Furthermore, the reduction in temperature is likely to reduce active drug uptake into the hair matrix keratinocytes.[7,30] Yet robust evidence for this theory is notably lacking. Understandably, most clinical trials have focussed on the efficacy of scalp cooling in preventing visible hair loss. There is a realisation, however, that direct measurement of drug concentrations in the HF or hair shaft could provide a key insight into the capacity for damage resistance in the face of chemotherapeutic insult.

To this end, preliminary data have been published suggesting that cyclophosphamide and doxorubicin concentrations are lower in plucked HFs from patients subjected to scalp cooling and this was associated with hair preservation.[31] Given the time-point for analysis in the study by Chae et al[31] (5 days post drug exposure), their results could indicate that drug incorporation into the hair shaft was the primary measurement. If sufficient levels of the drug and/or its metabolites are found to be present in the hair shaft, it could be anticipated that the analysis of cut hair (close to the scalp) might be used to assess HF drug exposure and also be correlated with hair loss. This would somewhat mimic forensic analysis of drug concentrations within cut hair samples[32] and prove even less invasive than hair plucking.

As such, analysis of drug concentrations within hair shafts or plucked HFs would likely provide an important biomarker for determining whether the accumulation of toxic drug concentrations could be prevented by cooling or other potential therapies. By adopting the measurement of drug concentrations and correlating this with levels of hair loss, as well as signs of damage indicated by trichoscopy, even greater insight into the protective potential of these treatments would be developed, enabling optimisation of treatments for the greatest efficacy.

### *Apoptotic pathways*

It is well established that the off-target effects of chemotherapy are often caused by stimulation of apoptosis in normal, highly proliferative cells. Hair loss is no exception, with excessive apoptosis in hair matrix keratinocytes responsible for the associated hair loss (chemotherapy-induced damage response pathways leading to HF apoptosis are summarised in Figure 3).[33,34] A central role of P53 in mediating chemotherapy-induced apoptosis in various tissues has been confirmed and this was also demonstrated in the HF. Indeed, deletion of P53 prevents chemotherapy-induced hair loss in mice and, importantly, also reduces the protein expression of direct P53 transcriptional targets, namely, Fas and IGFBP3, both of which stimulate apoptosis.[33]

Downstream of chemotherapy-induced P53 activation, Fas ligand interacts with Fas receptors to stimulate procaspase-8, which, on proteolytic cleavage, activates caspase-3 leading to apoptosis. Sharov et al[36] demonstrated that antibody neutralisation of Fas ligand significantly reduced matrix keratinocyte apoptosis and HF regression, as did genetic ablation of Fas. However, neither method completely prevented alopecia, but rather delayed the rate of onset.[36] This suggests that although P53-mediated apoptosis is an important causative mechanism in chemotherapy-induced hair loss, additional pathways likely exist.

More recent data examining doxorubicin-induced toxicity in human HFs suggest that other members of the tumour necrosis factor (TNF) family of death receptors, namely, TNF-related apoptosis-inducing ligand (TRAIL) receptors, also have prominent roles in associated apoptosis in the HF.[37] The authors demonstrated that α-TRAIL receptor antibodies significantly reduced doxorubicin-induced apoptosis (ie, lower number of TUNEL+ cells in the hair bulb). Whereas TRAILR1 is a direct P53 transcriptional target, P53-independent regulation has also been identified.[38] This supports the premise that apoptosis in the HF could be induced via multiple pathways.

Indeed, a new pathway has recently been identified that further enhances our understanding of the molecular controls of HF apoptosis in response to chemotherapy, namely, sonic hedgehog (Shh) signalling.[35,39] The Shh pathway is a vital element in both HF morphogenesis[23,40–44] and HF stem cell activation where it is critically involved in communication between the dermal papilla and matrix progenitor cells.[24] Stimulation of Shh has also been shown to accelerate hair regrowth after CIA in mouse models,[45] but until recently a direct link to chemotherapy-induced HF apoptosis had not been appreciated.

Initially and unconventionally, the avian feather follicle was used as a model to investigate chemotherapy-induced hair loss. Disruption of Shh signalling was found to be a critical event in the pathogenesis of chemotherapy damage, with cyclophosphamide treatment of feather follicles decreasing Shh levels and pharmacologic inhibition of Shh in mice recapitulating many aspects of cyclophosphamide damage.[39] A pilot human study also demonstrated a correlation between reduced Shh transcripts in plucked HFs from patients undergoing chemotherapy treatment and the development of alopecia.[46]

Importantly, follow-up work in both mice and humans could suggest that the loss of Shh signalling may result from chemotherapy-induced general or metabolic stress, which could be governed via mammalian target of rapamycin (mTOR) or AMP-activated protein kinase (AMPK) signalling or reactive oxygen species (ROS) induction/extracellular-signal-regulated kinase (ERK)/p38/c-Jun N-terminal kinase (JNK) activation.[35,46] As such, loss of Shh activity, alongside activation of P53-dependent apoptosis, may provide an indication of the likely severity of CIA.[35]

It can be seen that P53 activation, loss of Shh, and subsequent stimulation of apoptotic pathways represent useful biomarkers, which are likely to correlate with the extent of CIA. Furthermore, they could serve as useful indicators for the efficacy of preventive treatments, ie, through the analysis of P53 phosphorylation status and Shh gene expression in plucked HFs.

### *Proliferation/cell cycle alterations*

In addition to massive apoptosis, loss of matrix keratinocyte proliferation is a hallmark of chemotherapy-induced insult in the HF.[19] Such high levels of proliferation in the matrix keratinocytes is vital for maintaining anagen and, yet critically, also makes the HF highly sensitive to chemotherapy. Loss of Ki67, an M-phase marker of the cell cycle, is a prominent feature of exposure to chemotherapeutic agents in murine and human models.[19,47,48] However, this change is often more subtle and temporally delayed in comparison with the rapid induction of apoptosis described above.[37] As such, measurement of proliferation, specifically using Ki67, may not provide as reliable an early indication of damage when compared against induction of these apoptotic markers. As proliferation primarily occurs in matrix keratinocytes, a region of the follicle not consistently obtained by hair plucking, accurate analysis of Ki67 immunostaining may not be possible without the use of invasive biopsies.

In contrast, cell cycle inhibitors CDKN1A (P21) and CDKN1C (P57) are expressed primarily within the HF precortex,[49] which can be efficiently removed by plucking. Both markers are upregulated following doxorubicin exposure in the human HF,[37] indicating inhibition of the cell cycle. P21 is also a p53 target gene, although its inhibition of the cell cycle promotes differentiation, thereby preventing apoptosis (Figure 3). It is likely, therefore, that the balance between p53-mediated apoptosis and p21-mediated cell cycle inhibition could determine the damage response of individual follicles. This would directly impact on whether HFs enter dystrophic anagen or dystrophic catagen.

Further analysis of these cell cycle inhibitors in plucked human HFs could allow their use as additional biomarkers of the HF response to chemotherapy and the capacity for preventive therapies to blunt the damage leading to dystrophic anagen or dystrophic catagen.

## Biomarker Use in the Development of Anti-CIA Strategies

The likelihood of experiencing hair loss following specific chemotherapy regimens has been previously described.[7] That said, given that different publications often report different rates of hair loss, advice to patients will frequently depend on their clinicians' personal experience with the cytotoxic agent being administered. In the development of novel treatments for preventing CIA, trials in cancer patients have often not looked beyond a macroscopic assessment of the extent of hair drop and the requirement for patients to need a head covering, such as a wig, which does not capture the full extent of the pathobiology.[50]

Recent trials involving scalp cooling are a case in point.[8,51,52] These trials have been tremendously successful, yet to move this technology forward and drive improvements in efficacy, a greater understanding of the biological mechanism(s) through which they prevent CIA is needed. Indeed, studies are yet to examine, in detail, how molecular pathways governing apoptosis and cell cycle changes are impacted by this cooling (either in vivo or ex vivo/in vitro). Furthermore, although trichoscopy is increasingly used in the assessment of hair disorders, it has not yet been used to examine hair changes post cooling. A detailed study in this respect would be invaluable in determining the extent to which cooling prevents or reduces the varying external signs of dystrophic anagen or dystrophic catagen.

Such an approach could and should be correlated with the analysis of both plucked hairs (for transcriptomics and proteomics) and hair shaft drug concentrations. Together, this would allow a more complete picture to emerge of how signalling events occurring within HFs impact on hair loss and changes in hair shaft appearance.

## Conclusions

Correlations between these numerous biomarkers of HF damage (trichoscopic analysis, gene expression signatures for apoptotic/cell cycle markers, intrafollicular drug concentrations) are likely to provide insightful read-outs linked to the efficacy of anti-CIA therapies. Furthermore, enhancing our understanding of the link between molecular pathways associated with damage/protection and the outward signs of damage (trichoscopic features, hair drop) would inform future efforts to develop new or improved treatments to prevent or minimise CIA. Use of biomarkers as suggested in this article should therefore aid in the design of future studies in which new strategies for preventing chemotherapy-induced hair loss are investigated.

## Author Contributions

ISH conceived and designed the content and structure of the article; ISH and ES drafted, revised, and edited the article.

## ORCID iDs

Iain S Haslam https://orcid.org/0000-0002-1008-2447
Eleanor Smart https://orcid.org/0000-0003-4571-4384

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