Proceedings of the Ninth World Congress for Hair Research (2015)

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OVERVIEW

There is growing research interest in the hair follicle as a model system because of the ease of access for study and the ability to use a wide variety of technologies, including sophisticated imaging techniques, to watch hair follicle growth in real time. The follicle is one of the most proliferative organs/cells, along with the bone marrow and gastrointestinal tract. Therefore, with greater understanding of hair cycling, cells outside the follicle that influence the cycle, auxiliary cells, inflammation, and other factors, major cross-transfer of that knowledge to other organ systems and diseases, such as autoimmune disease, asthma, and allergies, will be possible.[a] The Ninth World Congress for Hair Research was hosted by the North American Hair Research Society, with participation from the Australasian Hair and Wool Research Society, the European Society for Hair Research, The Hair Research Society of India, the Japanese Hair Research Society, and the Korean Society of Hair Research, on November 18–21, 2015, in Miami, Florida, USA, at the InterContinental Hotel Miami. The major theme of the Congress was *Reflect, Rejuvenate, and Regenerate.*

Since the inaugural meeting in Brussels in 1995, the scope, size, and quality of the Congress has increased as new member societies have joined. The ultimate goal of the World Congress of Hair Research was and is to offer a comprehensive hair research meeting with international colleagues to present new research, share experiences, and discuss new directions for the advancement of knowledge in hair growth, hair and scalp disease, and clinical care.

The Congress was a major success, with more than 700 attendees from 53 countries. Attendees included hair scientists, researchers, dermatologists, hair transplant surgeons, trichologists, and industry. Of the 700 attendees, 53% were regular attendees, 23% were students, and 24% were from industry. Of the attendees, 37% came from North American and 21% from Europe, 20% from South America, 17% from Asia, 3% from Central America and the Caribbean, and 1% each from Africa, Oceania, and the Middle East.

WEDNESDAY, NOVEMBER 18, 2015

Pre-Congress Course: Basics Course on Diagnosis and Treatment of Hair Disorders

Session Directors: Lynne J. Goldberg, Paradi Mirmirani

This course was designed to present an overview of different hair disorders, including how they are recognized, evaluated, and managed. The speakers were the Co-Directors, Drs. Goldberg and Mirmirani, joined by Dr. Amy McMichael from Wake Forest Baptist Medical Center in Winston-Salem, North Carolina, USA, and Dr. Leonard Sperling from the Uniformed Services University of the Health Sciences in Bethesda, Maryland, USA. Unfortunately, Dr. Andrew Messenger from the University of Sheffield in England, UK, who was originally slated to speak, could not attend.

Dr. Goldberg started off the session by discussing non-scarring alopecia. She reviewed the important elements of the patient visit, including the history, physical examination, counseling of the patient, and stressed the need for a discussion of realistic expectations and for offering emotional support. She then used patient vignettes to highlight standard treatment approaches and options, as well as upcoming or novel therapies for patterned alopecia, telogen effluvium, alopecia areata (AA), and traction alopecia.

Primary scarring alopecia was then covered by Dr. Mirmirani, who explained that this is a group of disorders characterized by an inflammatory infiltrate targeting the pilosebaceous unit that cause permanent hair loss. She presented an algorithmic approach to the diagnosis and treatment of primary scarring alopecia, stressing the need to set expectations and define treatment endpoints. The entities covered included lichen planopilaris, frontal fibrosing alopecia (FFA), folliculitis decalvans, dissecting cellulitis, and erosive pustular dermatitis. The choice of treatment is based on the subtype of alopecia, the degree of clinical and histological inflammation, the progression of hair loss, and consideration of medication adverse effects.

After a short break, Dr. McMichael was the next to speak. She began by citing data indicating that alopecia is among the most common causes for dermatology visits by African American patients. Frequent hair concerns in African Americans include hair breakage, central and frontal hair loss,

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*Collaborators from the Ninth World Congress of Hair Research are listed in the Appendix

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Abbreviations: AA, alopecia areata; AGA, androgenetic alopecia; CIA, chemotherapy-induced alopecia; DHT, dihydrotestosterone; DP, dermal papilla; DZ, dizygotic; FFA, frontal fibrosing alopecia; FPHL, female pattern hair loss; FUE, follicular unit extraction; HFSC, hair follicle stem cell; IP, immune privilege; LED, light-emitting diode; LLLT, low-level light therapy; miRNA, microRNA; MSC, mesenchymal stem cell; McSC, melanocyte stem cell; MZ, monozygotic; N^1-MeSpd, N^1-methyl spermidine; OFPMA, octafluoropentyl methacrylate; PG, prostaglandin; ROS, reactive oxygen species; Shh, sonic hedgehog; siRNA, small interfering RNA; STAT, signal transducer and activator of transcription; TNF, tumor necrosis factor

[a] With permission from The North American Hair Research Society, www.NAHRS.org, organizer of the Ninth World Congress for Hair Research, www.hair2015.org.

Figure 1. Congress imagery. (**a**) Vascular three-dimensional network of the bulb region of an actively growing anagen human scalp hair follicle. Sample: A 4-mm punch biopsy sample was acquired from a healthy white subject, fixed, and vertically sectioned into 200-μm—thick sections. Biomarkers: Sections were stained with the plant lectin, Ulex europeaous conjugated to FITC (yellow). This lectin binds to L-fucose moieties, a carbohydrate abundant in human blood vessels. Acquisition: This image was captured using laser scanning confocal microscopy at original magnification of ×200 and is a projection of 56 2-μm optical sections. Awards: Science and Engineering Visualization Challenge sponsored by AAAS and NSF, Finalist in Photography 2006. Marna Ericson and Maria Hordinsky, Dermatology, University of Minnesota. (**b**) Milene Crispin reporting on "Two-Center Open-Label Trial of Oral Tofacitinib in Patients with Severe, Recalcitrant Alopecia Areata." (**c**) Takashi Tsuji responding to question on "Hair Regeneration as a Future Organ Replacement Regenerative Therapy." (**d**) Wilma Bergfeld and Leonard Sperling in discussion. (**e**) "Coffee with the Experts" discussion table on Trichoscopy led by Fernanda Torres. (**f**) Tudorita Tumbar lecturing on "Molecular Control of Hair Follicle Stem and Progenitor Cells."

scalp pruritus, and seborrheic dermatitis. Tips for treating these issues, including stopping use of chemicals and heat, using moisturizing products, and different anti-pruritus and anti-inflammatory regimens, were provided, as were therapeutic ladders for treating central centrifugal cicatricial alopecia, FFA, and traction alopecia.

Dr. Sperling then gave a talk on the histopathology of alopecia, reviewing terminology, normal anatomy, and techniques for tissue sectioning. He started off with the findings in nonscarring alopecia including AA, patterned alopecia, telogen effluvium, and trichotillomania. He then covered scarring alopecia, giving tips on how to distinguish lichen planopilaris and FFA from lupus erythematosus, as well as showing the histopathologic findings in central centrifugal cicatricial alopecia and traction alopecia.

Dr. Goldberg concluded the session with a discussion on the pathology report for alopecia, stressing the need for the clinician to pay attention not only to the diagnosis but to the microscopic and gross descriptions as well. Providing adequate clinical information for the dermatopathologist and, when necessary, discussing pathology test findings by phone, are very useful for clinicopathologic correlation and accurate diagnosis.

Pre-Congress Course: Epidemiology and Clinical Trial Design

Session Director: Julian Mackay-Wiggan

The Course began with an introduction by Dr. Mackay-Wiggan. The goals of the course were outlined, and each speaker was introduced in turn.

The first speaker was James A. Solomon, MD, PhD, Associate Professor, University Central Florida, College of Medicine, Orlando, Florida, USA; Assistant Clinical Professor, University of Illinois, College of Medicine, Urbana, Illinois, USA; and Director, Ameriderm Research, Ormond Beach, Florida, USA. Dr. Solomon spoke on "National Alopecia Areata Foundation (NAAF) Uniform Protocol Development Project: A Plug and Play Method to Facilitate the Clinical Trial Process." The rationale for the development of the AA uniform protocol, including lack of pharmaceutical industry research into treatments for AA, was outlined. The National Alopecia Areata Foundation initiated a plan to facilitate and drive clinical research aimed at the development of safe and efficacious treatments for AA. The AA uniform protocols were developed as a result of this effort. The design of the uniform protocol is to create a plug-and-play template to allow comparison of data across studies using the uniform protocol through consistency of inclusion and exclusion criteria and safety and outcome measures. Standardized statistical methodology is also provided. The universal protocol and informed consent have been approved in concept by Liberty Institutional Review Board and are available for presentation to pharmaceutical companies.

The next talk was by Tito R. Mendoza, PhD, MS, MEd, Associate Professor, Department of Symptom Research, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA. Dr. Mendoza discussed "Quality of Life Measures in Alopecia Areata and the Development of the Alopecia Areata Symptom Impact Scale (AASIS)." He began by discussing the importance of assessing quality of life to

understand the patient's experience and to evaluate the effectiveness of health care. Patient-reported outcomes were defined, and existing quality of life measures were described. Symptoms were distinguished from health-related quality of life. Dr. Mendoza advised that symptoms are patients' perceptions of what is closest to the disease and treatment process. In contrast, health-related quality of life is an *inclusive* concept that includes many domains *outside* of those that are most likely to be affected by disease and treatment.

The steps in development of the Alopecia Areata Symptom Impact Scale, including use of AA registry data, clinician input, and statistical and psychometric analysis followed by the administration of the newly developed Alopecia Areata Symptom Impact Scale measure to participants, were outlined. Considerations of measurement theory, reliability, validity, and correlation with quality of life were outlined. Dr. Mendoza concluded with future steps in the ongoing development and use of the Alopecia Areata Symptom Impact Scale.

Erika L. Hagstrom, MD, Internal Medicine Preliminary Resident, Loyola University, Chicago, Illinois, USA, discussed the global burden of disease findings for hair loss. Dr. Hagstrom started the discussion with comments regarding the clinical characterization of AA and the substantial psychological morbidity and negative influence on quality of life. She observed that data regarding the epidemiology and global burden of disease due to AA are scarce. The Global Burden of Disease Study has described the disability burden of skin disease worldwide. Dr. Hagstrom discussed the use of this database to examine the disease burden caused by AA in 2010 and 2013. The development of the Global Burden of Disease Study was reviewed, highlighting the breadth of involvement (1,000 collaborators, 188 countries, disability data for 291−301 diseases and injuries). *Disease burden* was defined as impact of a health problem, current health status versus ideal health situation, and the use of disability-adjusted life years as a measure of disease burden. Methodology was published by the Global Burden of Disease Study: http://www.healthdata.org/announcement/new-book-illuminates-global-burden-disease-methods. Worldwide disability-adjusted life years for AA were discussed. Limitations of the Global Burden of Disease Study and next steps were presented.

The next presentation was by Madeleine Duvic, MD, Professor and Deputy Chair Dermatology, UT MD Anderson Cancer Center, Houston, Texas, USA. Dr. Duvic discussed the development of the AA registry, including lessons learned from the AA registry and special considerations for registry-type clinical research. Development and funding of the registry were discussed. The structure of the registry was reviewed. There are five major sites led by MD Anderson, with additional ability for local physicians/sites to collect and contribute samples. The methodology of data collection was outlined beginning with a short online questionnaire; this was followed by selection of a subset of patients to complete a detailed questionnaire regarding history and clinical data, concomitant diseases, family history, medications, and quality of life related to AA in addition to evaluation by a physician with confirmation and characterization of the presence of AA and collection of genetic data (serum or cheek swabs). The status of enrollment to date was discussed. Over 10,000 subjects have been enrolled, with over 3,000 patients examined and genetic samples collected. Dr. Duvic described important developments in the understanding and treatment of AA stemming from the data collected by the registry and concluded with future developments and potential ongoing findings from AA registry data.

Dr. Wilma Bergfeld, MD, Professor, Department of Dermatology, Department of Dermatopathology, Cleveland Clinic, Cleveland, Ohio, USA, kindly presented on Dr. Mesinkovska's behalf—Natasha Atanaskova Mesinkovska, MD PhD, Staff, Department of Dermatology, Department of Dermatopathology, Cleveland Clinic, Cleveland, Ohio, USA. Dr. Bergfeld presented on "Use of Electronic Medical Record for Clinical Trials or Epidemiological Studies—The Cleveland Clinic Experience." In the age of electronic medical records, collection and analysis of data should be easier. Simple techniques for data collection and analysis in patients with alopecia were presented. Dr. Bergfeld discussed the possibility of building registries for longitudinal data analysis, outlined opportunities for working with research fellows and students more efficiently, and described ways in which use of electronic medical records increases opportunities for students, residents, and fellows to become more involved in alopecia research. Dr. Bergfeld concluded with a discussion of the usefulness of data registries in clinical studies.

Finally, Dr. Mackay-Wiggan discussed "Optimal Design versus Reality—Real Life Considerations in Clinical Trial Design," illustrated by recent clinical trials in AA. Dr. Mackay-Wiggan discussed the factors affecting optimal clinical design including adequate sample size and power to detect the desired treatment effect, a well-selected subject group, and optimal outcome and safety measures. Using illustrations from the ongoing ruxolitinib and tofacitinib trials in AA, she discussed the importance of recognizing limitations in study design and outlined methods for mitigating the drawbacks while still obtaining accurate and significant data. The importance of acknowledging limitations of the study upon publication of study results was emphasized.

Pre-Congress Course: Basic Science Course for Hair Researchers

Session Directors: Angela M. Christiano, Valerie Horsley and Sarah E. Millar

The objective of this course was to provide clinicians, trainees, and researchers in other areas of investigative dermatology with an up-to-date background on key aspects of hair follicle biology, including the roles played by the Wnt/β-catenin signaling pathway in hair follicle development and cyclic regeneration; regulation of hair follicle melanocyte proliferation and differentiation; functions of mesenchymal lineages in the skin; the location and functions of stem cell niches in the hair follicle; and the role of the dermal papilla (DP) in controlling hair follicle formation, growth, and cycling.

In the first presentation, Dr. Sarah Millar of the University of Pennsylvania reviewed work from her laboratory showing that activation of the Wnt/β-catenin pathway provides a key signal that initiates hair follicle development in embryonic

skin. Using genetic loss- and gain-of-function approaches, the Millar laboratory and other research groups showed that Wnt/β-catenin signaling is required for the formation of embryonic hair follicle precursor structures (placodes) and that forced activation of this pathway promotes formation of enlarged placodes that differentiate prematurely. Wnt/β-catenin signaling is initially activated broadly in embryonic skin and subsequently becomes localized to sites of placode formation. Tight regulation of this pathway is essential for hair follicles to form in a normal pattern. Dr. Millar discussed published studies from other groups and unpublished work from her own laboratory showing that this pattern is regulated in part by secreted Wnt inhibitors. Wnt/β-catenin signaling is also a central regulator of adult stem cells in many tissues, including the hair follicle. Dr. Millar showed that Wnt/β-catenin signaling is essential for onset of the anagen growth phase of the hair cycle and controls the proliferation, but not the maintenance, of adult hair follicle epithelial stem cells. She further discussed possible dysregulation of this pathway as a contributing factor in androgenetic alopecia (AGA).

Dr. Mayumi Ito, from New York University's Department of Dermatology, provided an overview of recent studies of melanocyte stem cells (McSCs). In both mice and humans, hair follicle melanocytes form two distinct populations: undifferentiated McSCs, which localize to a niche in the bulge and sub-bulge region of the follicle, and terminally differentiated mature melanocytes in the hair bulb, which undergo a melanogenic program to transfer pigment to the growing hair. Dr. Ito described how gain- and loss-of-function studies using genetically modified mouse models identified several signaling pathways that play critical roles in controlling melanocyte survival, proliferation, and differentiation. In particular, maintenance of McSCs requires the Notch and transforming growth factor-β signaling pathways, whereas Wnt and SCF signaling regulate the proliferation and differentiation of McSCs. Under homeostatic conditions, mouse hair follicle McSCs remain within the follicle. However, Dr. Ito showed that upon injury or UVB irradiation, McSCs can migrate from the hair follicle to the epidermis and generate functional epidermal melanocytes. This migration requires Mc1R signaling. During aging, mechanisms that regulate McSCs are compromised. Recent studies have shown that McSC numbers decrease during aging in mice and humans, and this underlies hair greying. Dr. Ito concluded her presentation by discussing how our understanding of McSC regulatory mechanisms may ultimately help us develop novel treatments for hypopigmentation diseases such as vitiligo and for diseases in which melanocyte proliferation becomes excessive or uncontrolled, as in melanoma.

Dr. Valerie Horsley of Yale University discussed how adipocytes in the skin function to regulate hair follicle biology. Many cell types within the skin have been shown to interact with the hair follicle, including neurons, the arrector pili muscle, melanocytes, and adipocytes. Adipocytes are lipid-filled cells that store energy and can act as endocrine cells, secreting factors that regulate several tissues. Dermal adipose tissue is a cluster of adipocytes that resides below the skin's dermis and expands during hair follicle growth. Work from Dr. Horsley's laboratory has implicated adipocyte regeneration in the initiation of hair follicle growth, and work from others has implicated mature adipocytes in repression of hair growth. Several groups have shown that dermal adipose tissue expands during cold treatment and skin infection models in mice. Dr. Horsley discussed how hair follicles may influence adipogenesis directly. Together, the picture of adipocytes in the skin suggests that hair-adipose tissue communication is bidirectional and may play a role in alopecia or other skin disorders.

Dr. Peggy Myung from Yale's Department of Dermatology discussed how hair follicle regeneration is regulated. Specifically, she presented work illustrating how Wnt/β-catenin signaling, a key molecular pathway required for hair follicle regeneration, is propagated throughout a population of undifferentiated cells to promote synchronous and coordinated growth. Imaging of hair follicles in live mice showed a subset of cells that fuel activation of growth behaviors throughout surrounding epithelial cells and is associated with up-regulation of diffusible Wnt ligands. She also discussed the importance of the underlying mesenchyme in regulating normal hair follicle epithelial growth and in skin tumor growth.

The final presentation by Dr. Michael Rendl of Icahn School of Medicine at Mount Sinai discussed how hair follicle stem cells (HFSCs) are regulated by signals from the DP niche. He presented classic experiments that illustrated a role for embryonic dermal cells in the formation of hair follicles in embryonic skin. He also discussed data from his and other laboratories that show an essential role of the DP in hair follicle growth and regeneration. Although the nature of these signals and how they are regulated remain elusive, his laboratory has used transcriptomic approaches to identify several potential mechanisms by which DP niche cells communicate with the hair follicle during embryonic development. The comprehensive analysis of gene expression performed by his laboratory is available at hair-gel.net.

Opening Ceremony

Session Directors: Wilma F. Bergfeld, Angela M. Christiano and Maria K. Hordinsky

The Ninth World Congress of Hair Research Opening Session began with a greeting and welcome from the Co-Directors, Wilma Bergfeld, MD, and Angela Christiano, PhD. They welcomed attendees who represented hair scientists and researchers, dermatologists, hair transplant surgeons, trichologists, and industry members. During the opening session, crystal awards were given to representatives from the hair research societies.

Maria Hordinsky, MD, the Associate Director of the Congress, welcomed and thanked the industry sponsors as well as the National Institute of Arthritis and Musculoskeletal and Skin Disease and the National Center for Advanced Translational Sciences for educational grants. She also thanked the University of Miami, Department of Dermatology and Cutaneous Surgery; the local host and Continuing Medical Education providers. Dr. Robert Kirsner, MD, PhD, Interim Chair, Department of Dermatology and Cutaneous Surgery, University of Miami, welcome the attendees to the Congress and the city of Miami. Short remarks of welcome

Figure 2. Congress imagery. (**a**) Stockade of nerves surround the bulge region of human scalp hair follicle from a male patient with androgenetic alopecia. Sample: A 4-mm punch biopsy sample was acquired from a 34-year-old white man, fixed, and vertically sectioned into 200-μm–thick sections. Biomarkers: The sample was stained with antibodies to visualize nerves (PGP9.5, green) and with the plant lectin, Ulex europeaous-FITC (red), which binds to follicular keratinocytes. Acquisition: This image was captured using laser scanning confocal microscopy at original magnification ×200 and is a projection of 50 2-μm optical sections. Marna Ericson and Maria Hordinsky, Dermatology, University of Minnesota. (**b**) Valerie Randall speaking on "Regulation of Human Hair Growth: Androgens and Prostanoids." (**c**) George Cotsarelis moderating the "Stem Cells and Stem Cell Niches" session. (**d**) Nonhlanhla Kumalo and Valerie Callender leading "Coffee with Experts" discussion table on Central Centrifugal Cicatricial Alopecia. (**e**) Pantelis Rompolas describing "Mechanisms of Hair Follicle Stem Cell Fate by Live Imaging." (**f**) Congress Co-Chairs Wilma Bergfeld, Maria Hordinsky, and Angela Christiano with moderator Ken Washenik. (**g**) Gillian Westgate moderating session on "New Topics Selected from Abstracts: Part I."

were given by the top corporate sponsors and included Women's Rogaine; P&G; and Samumed, Inc.

The Congress welcomed its two keynote speakers: Michael P. Philpott, BSc, DPhil, and R. Rox Anderson, MD. Dr. Philpott was the recipient of the John Ebling Lecture, sponsored by the European Hair Research Society, a prestigious lectureship named for John Ebling, a renowned zoologist who devoted his career to understanding hair growth sebaceous gland activity and the role of the endocrine system.

Dr. Philpott was introduced by Abraham Zlotogorski, MD, Head, Department of Dermatology, Hadassah-Hebrew University Medical Center, Jerusalem, Israel, and President, European Hair Research Society.

The first keynote speaker, Michael Philpott, SCs, PPhil, Professor of Cutaneous Biology, Centre for Cutaneous Research, Blizzard Institute, Barts and The London School of Medicine and Dentistry, London, UK, spoke on "Hairs to Hedgehogs: from In Vitro Modeling of the Human Hair Follicle to Basal Cell Carcinoma." Since methods for the isolation and culture of human hair follicles were first published over 25 years ago, their importance as a model system to study hair biology has been shown in numerous publications. In particular, in vitro hair follicle culture has played a significant role in helping elucidate the role of signaling molecules in regulating hair growth and hair fiber formation and has been especially useful in understanding metabolic aspects of hair growth. Moreover, with the advent of quantitative PCR, the ability to measure changes in gene expression in single cultured follicles has meant that it is now possible to monitor changes in gene expression in cultured follicles in great detail.

Although full human hair follicle cycling in vitro has not yet been achieved, cultured human hair follicles do undergo anagen to catagen transition in vitro both during normal routine culture and in response to growth regulatory factors. This has been successfully used to investigate factors that influence normal follicle regression and, in particular delay, catagen onset, but it has also been used with great success to identify factors that drive the anagen to catagen transition. He concluded that cultured follicles are highly responsive to in vitro manipulation by a wide range of growth regulatory factors, and recent publications showing that small interfering RNA (siRNA) technology can also be applied to cultured hair follicles opens up a new and exciting avenue of hair research. However, obtaining sufficient numbers of hair follicles has become difficult as plastic surgery has become less invasive. There an urgent requirement for the next generation of in vitro models using cell lines and tissue engineering.

The second keynote speaker, R. Rox Anderson, MD, is an expert in laser-tissue interactions, skin responses, and dermatology. He is the Director, Wellman Center for Photo Medicine; Professor, Harvard Medical School, Massachusetts General Hospital; and Professor, Massachusetts Institute of Technology, Boston, Massachusetts, USA. Dr. Anderson spoke on "Low-Level Light Therapy for Hair Loss: Clinical Use, Mechanisms and Important Questions."

Dr. Anderson began by defining *photobiomodulation* as low-level light therapy (LLLT), which is widely used to

stimulate hair growth in men and women with AGA, has been effective in small clinical trials for AA, but remains untested for many forms of hair loss. LLLT requires no medications and can be performed in minutes at home. In multiple clinical trials, efficacy for AGA is similar to that of topical minoxidil. The miniaturization of hair follicles is arrested, and some miniaturized hairs are also converted back into terminal hairs.

In skin-wounded mice, LLLT increases the number of regenerated neofollicles. Red and/or near-infrared light capable of penetrating at least 1 mm into human skin is used, at wavelengths corresponding to the absorption bands of mitochondrial cytochrome C oxidase complex. The mechanism(s) involved are clearly photochemical, but neither the action spectrum nor the fluence (dose)-response have been established for hair stimulation.

He noted that in general, LLLT exhibits hormesis, with high light fluences actively inhibiting the desired response. Hematopoietic stem cells are potently stimulated to migrate after LLLT of bone marrow, but the effect on follicular stem cells, if any, is unknown. Cytochrome C oxidase complex is the site of oxygen use and includes heme and copper center chromophores liked to electron transport.

LLLT rapidly restores mitochondrial membrane potential and adenosine triphosphate level in cells under ischemic, nutrient, or oxidative stress, probably by photodissociation of NO from an inhibitory heme group and/or direct facilitation of electron transfer to the oxidative site. A host of downstream signaling pathways (e.g., via NF-κB) then occur.

In summary, Dr. Anderson noted that LLLT may play a role in initiating hair growth, but it is unlikely that our present devices and treatment regimens for LLLT are optimal. It is also unknown how LLLT should best be used in combination with other treatments.

THURSDAY, NOVEMBER 19, 2015

Hair Transplantation

Session Directors: Paul T. Rose, Nilofer P. Farjo and Chang-Hun Huh

This session featured lectures by several prominent hair restoration surgeons and scientists involved in hair transplant research. The session opened with a discussion by Dr. Huh on the use of a robotic device for hair transplantation. Dr. Huh reviewed the increasing use of the follicular unit extraction (FUE) approach to hair restoration and interest in this technique due to the fact that it avoids a linear scar, is usually less painful, and has a shorter recovery time. To learn how to perform manual FUE can be difficult. The use of the robotic device eliminates the prolonged learning curve. By using the robot, FUE grafts can be harvested efficiently with low transection rates, and the operative time can be reduced compared with manual FUE. He also pointed out that the robotic machine tends to harvest mostly two- and three-hair groupings.

Next, Dr. Nilofer Farjo from Manchester, England, UK, presented a lecture covering indications for strip harvesting versus follicular unit harvesting for hair transplantation. She discussed the advantages and disadvantages of these two methods. She cited the fact that strip harvesting often allows the surgeon to obtain greater numbers of grafts in a shorter time and that the surgery can be well hidden by the overlying hair. With the FUE process, significant portions of the scalp need to be shaved to allow for harvesting. The FUE process can take considerably longer than a typical strip harvest procedure.

Dr. Farjo stressed that donor hair availability is the main limitation to hair restoration surgery. The surgeon should take into account a patient's age, hair type, hair color, potential for hair loss, and desire regarding possible hair style. At times, a combination of techniques may best suit the patient.

Dr. Gorana Kuka, a plastic surgeon from Colic Hospital, Belgrade, Serbia, provided an interesting lecture on her surgical approach on fat grafting before hair implantation into scars. She pointed out that hair restoration into scars can be difficult because of either a lack of subcutaneous tissue or altered tissue and the lack of vascularity to such tissue. To improve the condition of the scar tissue, Dr. Kuka has used adipose tissue implanted into the area to be treated approximately 3 months before the hair transplantation. Using this approach, she found that the hair regrowth was higher and that the tissue texture and vascular supply of the scars was significantly improved.

Dr. Bessam Farjo, Manchester, England, UK, presented a lecture about eyebrow hair restoration. He emphasized the aesthetic importance of the eyebrow, the anatomical positioning and structure of eyebrows, and the current fashion trend of broader eyebrows. He detailed the surgical approach to eyebrow restoration and advised that it is critical to place hairs at a very acute angle and follow the natural pattern of hair direction and curl. Dr. Farjo pointed out that the first eyebrow transplants were probably done in Japan in the 1930s.

A lecture given by Dr. Meena Singh, from Shawnee, Kansas, USA, provided further insight into hair replacement into scar tissue. Dr. Singh discussed hair transplantation into end stage cicatricial alopecia patients. She noted that in such cases, the surgeon should have an accurate diagnosis of the problem, know the extent of the disease, and have assurance that the disease process is inactive. Dr. Singh suggested that if surgery is to be undertaken, the patient must have reasonable expectations, and it is advised that a test area be treated before committing to a large area of implantation. Dr. Singh recommends waiting 9–12 months before performing a larger session. When performing the surgery, she advises low densities of implantation and smaller incision sites. She counsels patients that several sessions of surgery may be needed and that even then the condition can flare.

Bradley Wolf, MD, Cincinnati, Ohio, USA, presented genomic research findings done in cooperation with P&G. The study compared transcriptomic expression analysis of follicular unit grafts obtained by strip harvesting, FUE, and plucking hairs. Samples were obtained from 35 premenopausal women. Transcript probes were used to measure genes of 132 hair-relevant keratin genes and keratin-associated proteins. Gene expression heat map and stem cell markers showed that FUE and strip-harvested grafts had an almost identical signatures, whereas plucks were demonstrably different. This study concluded that FUE and strip-harvested grafts are genetically very similar, both having

the components necessary to regenerate new hair follicles after transplantation.

An interesting lecture related to the possible future use of allogenic grafts was given by Dr. Jin Yong Kim, PhD, student and research fellow in Department of Dermatology, Seoul National University College of Medicine, Seoul, Republic of Korea. Dr. Kim reported on research performed using a monkey model. The study looked at allogenic grafts placed after MD-3 pretreatment versus short-term immunosuppression versus a control group. After photo-epilation with a diode laser of an area of hair on the back of a monkey, allograft monkey eyebrow hair was transplanted into the epilated area. The grafts that were maintained under MD-3 therapy (anti-ICAM 1 antibody) had enhanced survival compared with the control or the short-term immunosuppression group. Histological study showed that anti-ICAM antibody increased allograft survival by preferentially impairing alloreactive T-cell infiltration. Dr. Kim concluded that MD-3 pretreatment may be a potential therapy for preventing allograft rejection and that the nonhuman primate model may be very effective for further hair transplant research.

The final presentation was given by Dr. Alan Bauman, Boca Raton, Florida, USA. Dr. Bauman lectured on the approach to eyelash transplantation and the complications and management of adverse reactions. Dr. Bauman based his lecture on over 350 eyelash surgery cases. He noted that his technique is derived from that of Dr. Marcelo Gandelman in Brazil, who uses a needle to thread the hairs into the lid skin margin. Dr. Bauman noted that he uses a "pairing" and "tripling" method to place hairs more quickly. He pointed out the need to place the hairs in the proper orientation to avoid aberrantly growing hairs that could damage the cornea. He also pointed out the need for careful post-operative care.

Case Presentations
Session Director: Antonella Tosti

During this session, attendees were able to visit and discuss diagnosis and management of 10 patients who agreed to participate in the session.

Dr. Norma Vazquez from Monterey, Mexico, presented two patients. The first patient was a 60-year-old woman with a history of psoriasis and psoriatic arthritis treated with tumor necrosis factor-α (TNF-α) inhibitors including etanercept, adalimumab, and golimumab. The patient developed lichen planopilaris in 2010 during treatment with adalimumab. Her condition was very resistant to treatment. Discussion was focused on lichen planopilaris and TNF-α inhibitors, with review of four similar cases reported in the literature. The second patient was 61-year-old woman with FFA since 2013. She responded very well to finasteride treatment with disease stabilization. Discussion was focused on 5α-reductase inhibitors in the treatment, with review of recent literature suggesting that these are possibly the most effective treatment for this condition.

Dr. Yanna Kelly from São Paulo, Brazil, presented two patients. The first patient was a 35-year-old woman with dissecting cellulitis of the scalp associated with very severe keloids. Discussion was focused on treatment; review of the literature indicates a role for TNF-α inhibitors to minimize the affected area before surgical excision and split-thickness skin grafting. The second patient was a 50-year-old man who developed lichen planopilaris a few months after hair transplantation. Discussion was focused on whether hair transplantation can trigger the disease or, instead, whether surgeons do not identify the condition and therefore perform hair transplantation on patients with early disease.

Dr. Nouf Mohammed Aleid from Prince Sultan Military Medical City, Riyadh, Saudi Arabia, presented two patients. The first patient was a 57-year-old woman affected by FFA and personal history of breast cancer. She was treated with pioglitazone, with good results. Discussion was focused on treatment options in patients with a history of breast cancer, which is a contraindication for 5α-reductase inhibitors. The second patient was a 55-year-old woman with AA, vitiligo, and Hashimoto thyroiditis and nail abnormalities, indicating a diagnosis of type III polyglandular autoimmune syndrome. Discussion was focused on autoimmune polyglandular syndromes, a group of autoimmune disorders characterized by endocrine tissue destruction causing malfunction of multiple glands.

Dr. Margaret Sanchez from the Department of Dermatology of the University of Miami presented two patients. The first patient was a 79-year-old woman with severe scalp itching due to dermatomyositis. Discussion was focused on management of alopecia and scalp involvement in dermatomyositis. The second patient was a 53-year-old woman with nonscarring alopecia in association with systemic lupus erythematosus. Discussion was focused on types of alopecia in patients with systemic lupus erythematosus, which include patchy nonscarring alopecia, diffuse telogen effluvium, and scarring alopecia due to discoid lupus erythematosus.

Dr. Mina Zarei from the Department of Dermatology of the University of Miami presented two patients. The first patient was a 32-year-old man affected by pemphigus vulgaris with severe scalp involvement with erosions, crusts, and alopecia. Scalp lesions completely disappeared with treatment, and the patient had complete hair regrowth. Discussion was focused on hair involvement in pemphigus. The second patient was a 59-year-old woman with FFA and discoid lupus erythematosus of the scalp. Discussion was focused on this rare association.

Immunobiology, Alopecia Areata
Session Directors: Maria K. Hordinsky, Amos Gilhar and Ralf Paus

This session featured three invited speakers and three oral abstract presentations.

Dr. Angela Christiano (Columbia University, New York, New York, USA) showed how genetic studies (e.g., genome-wide association studies) led to the identification of novel NKG2D ligands such as ULBP3/6, which are targeted by the $CD8^+NKG2D^+$-positive killer cells in the inflammatory infiltrate within and around hair follicles of AA lesions, in both humans and the mouse model of AA. Dr. Christiano presented how this interaction leads to the production of IFN-γ and IL-15, potent effectors of the cytotoxic $CD8^+$ cells that

signal through the JAK-signal transducer and activator of transcription (STAT) pathway. Because JAK molecules are important for the activation and proliferation of these killer cells in AA, Dr. Christiano's laboratory tested JAK inhibitors that were originally approved for rheumatoid arthritis and myelofibrosis on the mouse model of AA and found that the JAK inhibitors (ruxolitinib and tofacitinib) can both prevent and reverse alopecia in the C3H mouse model of AA. The reversal of AA with JAK inhibitors can be observed as early as 4 weeks and full regrowth by 7 weeks in the treated area. The skin with hair regrowth showed decreased inflammatory signature and diminished CD8/NKG2D-positive T cells that resemble healthy nondiseased skin.

Dr. Christiano also presented promising results from a pilot clinical trial with 12 patients with moderate to severe AA, conducted by Dr. Julian Mackay-Wiggan at Columbia University. Oral ruxolitinib showed high efficacy in 9 out of 12 patients, with significant hair regrowth by week 20. The patients showed only minor adverse effects, with changes in gene expression profile that showed higher keratin signature and lower IFN and inflammatory signature (summarized in the Alopecia Areata Disease Activity Index score). The use of JAK inhibitors showed high efficacy in inhibiting crucial killer cells involved in the pathogenesis of AA and successfully reversed disease phenotype in a relatively short amount of time. This evidence-based targeting of immune cells and repurposing of existing US Food and Drug Administration-approved drugs provided rationale to expand clinical trials to include other JAK inhibitors and larger patient cohorts.

Dr. Amos Gilhar (Skin Research Laboratory, Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel) pointed out that various cell types are taking part in the pathogenesis of AA. AA is now widely accepted as a CD8/NKG2D T-cell–dependent, antigen- and organ-specific autoimmune disease that selectively attacks growing hair follicles. However, $NKG2D^+$ cells represent a rather mixed collective, which produce large amounts of IFN-γ. These include $CD8^+$ T lymphocytes, $6B11^+$ natural killer T cells, $CD56^+/CD46^+/CD3^-$ natural killer cells, subpopulations of gamma/delta TCR^+ lymphocytes, and type 1 innate lymphocytes.

Recent evidence suggests that some of the subpopulations promote autoimmunity, whereas others may suppress it. Indeed, autoimmune diseases are characterized by an imbalance between disease-protective and disease-promoting $NKG2D^+$ cell populations. So far, various protective $NKG2D^+$ cells have been identified, such as regulatory natural killer cells, FOXP3 regulatory T cells, FOXP3 natural killer T cells, FOXP3 γδ T cells, and a subgroup of $CD8^+$ cells with positive expression of FOXP3; and a recent study showed a novel subgroup of regulatory, presumably autoimmunity-protective, natural killer T cells producing IL-10 (named NKT10 cells).

Dr. Gilhar's group used the humanized AA mouse model to show the regulatory and therapeutic effects of invariant natural killer (i.e., iNKT10) cells. Administering synthetic glycolipid molecule, α-galactosylceramide, to autologous, IL-2–activated immune cells expressing high levels of NKG2D before their injection into the hair-bearing human scalp skin xenotransplants prevented the development of AA-like phenotype. Furthermore, injections of α- galactosylceramide to xenotransplant-grafted mice once a week after the administration of the autologous immune cells prevented alopecia in the treated human scalp skin grafts. On the other hand, the protective effect of α-galactosylceramide was inhibited by adding invariant natural killer cell-neutralizing antibodies or depletion of invariant natural killer cells from the culture of IL-2–activated immune cells/α-galactosylceramide before the injections. The therapeutic effect was evidenced by hair regrowth in experimentally induced AA lesions in the scalp skin grafts after treatment with either α-galactosylceramide or IL-10. These recent findings may raise a new potential therapy for AA.

Dr. Ralf Paus (University of Manchester, UK, and University of Münster, Münster, Germany) critically examined how close we have come to understand the pathobiology of AA. Summarizing some essential features, he characterized AA as an inflammatory disorder of hair follicle cycling, where essentially only growing, melanogenically active (anagen) hair follicles are attacked by a peribulbar inflammatory cell infiltrate dominated by NKG2D+/$CD8^+$ T cells ("no infiltrate, no AA"). This infiltrate induces hair follicle dystrophy, hair shaft breakage, and premature anagen termination and clinically manifests as a characteristic hair loss phenotype. He argued that AA represents a prototypic territorial disease, whose core pathobiology arises from *within* a circumscript skin territory and can therefore be fully shown only by intracutaneous research. He discussed blood-based analyses (e.g., genome-wide association studies) as being invaluable for dissecting individual risk AA factors and for identifying novel therapeutic targets in a hypothesis-free manner.

Dr. Paus proposed that, contrary to conventional wisdom, AA may be best viewed not as a single disease entity but rather as a stereotypic response pattern of damaged anagen hair follicles to several different proinflammatory stressors that all elicit a characteristic damage response: the AA hair phenotype. This pattern develops only if the hair follicle's physiological immune privilege (IP) collapses ("no IP collapse, no AA"), for example, as a result of excessive IFN-γ–induced and/or NKG2D-mediated signaling. Therapeutically, therefore, restoring hair follicle IP (e.g., by administering α-melanocyte stimulating hormone analogs, FK506, and other "IP guardians") and inhibiting IFN-γ/JAK signaling should work in most AA patients. However, Dr. Paus pointed out that curative AA therapy may be achievable only for selected pathobiology pathways that induce the clinical AA phenotype, namely those that depend on major histocompatibility complex class I-presented hair follicle (auto-)antigens recognized by pre-existing autoreactive $CD8^+$ T cells. This perspective of AA pathobiology suggests a paradigm shift in how we think about this stereotypic hair loss pattern and mandates a personalized medicine approach to future AA management.

Dr. Gwang Seong Choi and his colleagues from Inha University School of Medicine, Incheon, Korea, presented their research, "Prevention and Treatment of Alopecia Areata with Mesenchymal Stem Cells in the C3H/HeJ Mouse Model." Dr. Choi described the emerging evidence of the potent immunosuppressive activity of mesenchymal stem cells (MSCs) by modulating immune responses, which

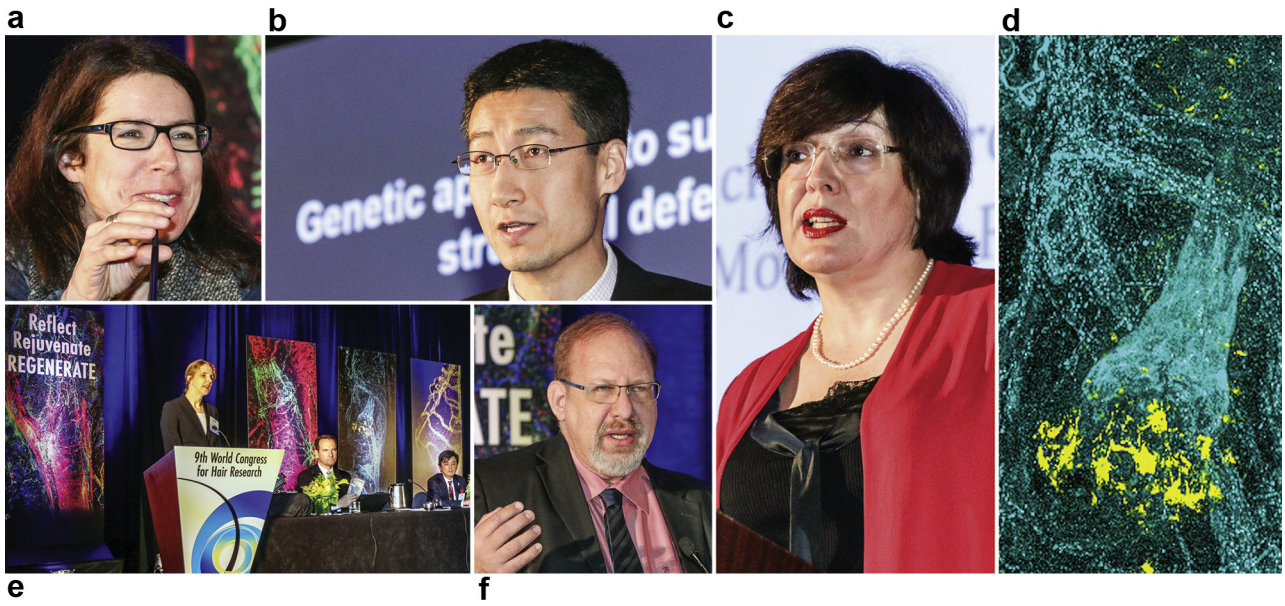

Figure 3. Congress imagery. (**a**) Regina Betz sharing the "Latest Findings in the Field of Monogenic Hair Disorders." (**b**) Jiang Chen speaking on "The Development of a Genetic Approach to Suppress an Inheritable Structure Defect of the Hair." (**c**) Ulrike Blume-Peytavi describing the "Translational Approach to Androgenetic Alopecia—Clinical and Molecular Read-outs." (**d**) Melanocytes and nerves of a human anagen scalp hair follicle. Sample: A 4-mm punch biopsy sample was acquired from a 44-year-old white man, fixed, and vertically sectioned into 200-μm sections. Biomarkers: Sections were multistained with antibodies to visualize nerves (PGP9.5, blue) and melanocytes (MEL1, yellow) of the hair bulb and the epidermis. Acquisition: This image was captured using laser scanning confocal microscopy at original magnification of ×200, is a projection of 38 1-μm optical sections, and is a montage of three fields of view. Marna Ericson and Maria Hordinsky, Dermatology, University of Minnesota. (**e**) Annika Vogt, Co-Director in "Emerging Technologies and Therapies" session with Co-Directors Ken Washenik and Takashi Tsuji. (**f**) Abraham Zlotogorski presenting on Mapping of Hair Disorders—Not Everything Is Gold.

enables MSCs to be developed as a promising therapeutic modality for immune-related or inflammatory diseases. In their study, they investigated the effects of MSCs on AA development and treatment in C3H/HeJ mice. They identified potentially important cytokines and chemokines in the treatment of AA by MSCs. Mice with skin graft induction of AA were injected with phosphate buffered saline, bone marrows from wild-type C3H/HeJ mice, and MSCs from wild-type C3H/HeJ mice. Serum of C3H/HeJ mice was collected at 0, 7, 35, and 49 days after treatment and assessed for alterations in hematopoietic cytokine secretion using Luminex assays (Luminex, Austin, TX). In the result, mice with AA development had increased secretion of IP-10 and monokine induced by IFN-γ in serum. MSC injection resulted in a significant decrease in AA development compared with that with phosphate buffered saline and bone marrow injection. This result also correlated with significant decreases in IP-10 and monokine induced by IFN-γ after MSC injection. In conclusion, their results showed that MSCs provided effective prevention of onset of AA in the C3H/HeJ model and warrant further studies to determine whether MSCs might be developed as a cell therapy for AA.

Dr. Taisuke Ito of Hamamatsu University School of Medicine, Japan, presented his team's work on "Chemokine Receptor CCR5 is the Novel Target for the Treatment of Alopecia Areata." He described how AA is an organ-specific autoimmune disease with cell-mediated autoimmune reactions. T lymphocytes densely surround hair bulbs in the lesion of acute- phase AA, referred to as "swarm of bees." The pathological mechanisms of "swarm of bees" can be induced by the up-regulation of type 1 helper chemokine expression from hair follicles that result in the infiltration of $CXCR3^+$ and $CCR5^+$ type 1 helper or T-cytotoxic cells into AA lesions. Here, C3H/HeJ mice with AA were treated with a CCR5 inhibitor, maraviroc, which is used to treat HIV by negative allosteric modulation of the CCR5 receptor. AA was induced by intracutaneous injection of activated lymph node cells derived from C3H/HeJ mice. Then, maraviroc is orally administered. Four out of five maraviroc-treated C3H/HeJ mice with AA showed improvement of hair loss lesions after 2 weeks. Immunohistological assessments showed a decreased number of $CD4^+CCR5^+$ and $CD8^+CCR5^+$ T cells in the lesions after maraviroc treatment. Furthermore, fluorescence-activated cell sorting analysis also supports the reduced frequency of $CD4^+CCR5^+$ T cells in skin-infiltrating cells. In addition, EZTaxiscan (ECI Frontier, Kanagawa, Japan) showed significant inhibition of the chemotactic activity of $CD4^+$ lymph node cells toward RANTES by maraviroc compared with phosphate buffered saline. In conclusion, it was stated that the inhibition of chemokine receptors/chemokines can be a novel target for the treatment of AA.

Ms. Gina M. DelCanto and her colleagues from the University of Miami, Miami, Florida, USA, presented on "Treatment with Simvastatin Decreases pStat1 Levels and Reverses AA in the C3H/HeH Mouse Model." Ms. DelCanto described that AA is an autoimmune disorder characterized by T-cell infiltrate of the hair follicle. She noted that at present there is no cure for AA, but JAK-STAT pathway inhibitors have recently shown considerable efficacy as treatment. The authors have shown previously that simvastatin, a lipid-lowering drug that has been suggested to modulate the JAK-STAT pathway in multiple cellular models, functions in

combination with ezetimibe to reverse hair loss in a statistically significant number of AA patients. In this study, the effects of topical simvastatin treatment on hair regrowth in the C3H/HeH mouse model were examined. Mice with spontaneous AA received either a topical simvastatin or vehicle treatment daily for 12 weeks. Skin samples were taken from a selection of mice after 8 weeks of treatment. Samples were fixed and stained to visualize phosphorylated STAT1 and inflammatory cell infiltrate. Of the mice treated with simvastatin, 8 of 11 responded with hair regrowth, five completely and three only partially. No new hair growth was observed in untreated mice. Staining of skin sections showed a relative decrease in both inflammatory cell infiltrate and levels of activated STAT1 in the skin of mice treated with simvastatin. These findings serve to highlight simvastatin as a possible treatment option for AA whose effect on AA may be mediated, at least in part, by modulation of the JAK-STAT signaling pathway.

Satellite Symposium: New Insights and Cosmetic Approaches for Healthy Hair and Scalp
Session Director: John Gray

The New Insights and Cosmetic Approaches for a Healthy Hair and Scalp was a satellite symposium sponsored by P&G. The session featured invited talks by Drs. James Schwartz, Jennifer Marsh, and Vicky Jolliffe. The talks focused on recent advances in cosmetic hair and scalp care research and technologies and the new benefits that these approaches can provide. Understanding the effects of oxidative stress on the properties of the pre- and postemergent hair has led to new technology and treatment possibilities. These advances, along with practical considerations, were discussed by each presenter.

In a talk entitled "Growing Better Hair: Impacting Pre-Emergent Hair via Scalp Condition," Dr. James Schwartz provided insight into the latest research, which shows that poor scalp health is accompanied by or caused by oxidative stress, as is evident from detection of oxidatively damaged scalp lipids and elevated natural antioxidant enzymes. In a comparison of dandruff and nondandruff subjects, Dr. Schwartz showed that dandruff/seborrheic dermatitis subjects have substantially elevated levels of oxidative stress on the scalp and hair as measured by oxidative stress biomarkers including the biomarker hydroxyoctadecadienoic acid (i.e., HODE). His research showed that oxidative stress in the scalp can affect the quality of the growing hair as it matures within the hair follicle. More specifically, the research has shown that secondary oxidative damage can be manifested in cuticular damage before hair emerges from the scalp. This may make the hair more susceptible to subsequent post-emergent damage. Dr. Schwartz noted that effective treatment with a potentiated zinc pyrithione product (Head and Shoulders; P&G, Cincinnati, OH) has shown the ability to reduce and normalize oxidative stress in the scalp and resultant effects on the hair for improved hair quality. This is the first treatment study showing a causative relationship between scalp and hair.

Dr. Jennifer Marsh followed Dr. Schwartz with a talk entitled "Healthy Hair Care: Advances in Conditioning Technology" focusing on new research into the sources and effects of oxidation on the hair fiber. In addition to hair coloring, UV light exposure, and exposure to oxidative stress from the scalp, Dr. Marsh described the role that exogenous copper ions play in catalyzing oxidative damage, which has been shown to attack the fiber's protein structure. Consequently, the amino acid histidine has been developed as an antioxidant ingredient in cosmetic conditioners to protect the fiber's protein structure. Data were presented to show histidine's antioxidant mechanisms. Histidine works by penetrating into the fiber, where it reduces copper levels, thereby reducing oxidative stress. As a result, hair fiber properties, such as shine, manageability, and perception of overall hair health, were shown to improve over time.

In the final talk, Dr. Vicky Jolliffe provided practical considerations in a presentation entitled "Clinical Importance of Effective Cosmetic Hair Care Product." Dr. Jolliffe focused on the clinical signs of oxidative hair damage across hair types and the important role cosmetic products play in a complete treatment regimen. To prevent oxidative stress to both the hair and scalp, Dr. Jolliffe provided practical tips for the best use of cosmetic hair and scalp care products and guidance for recommending products from the range of forms currently available.

Morphogenesis, Neogenesis, and Tissue Engineering
Session Directors: Sarah E. Millar, Vladimir Botchkarev and Marja L. Mikkola

The session on Morphogenesis, Neogenesis, and Tissue Engineering provided a forum for presenting recent developments in research on hair follicle development, regenerative growth, wound-induced neogenesis, and tissue engineering and discussing how these discoveries may eventually be applied to treating hair disorders. This session featured three invited speakers and an oral abstract presentation. Dr. Sarah Millar began the session with a discussion of the phenotypes associated with mutation of the human *WNT10A* gene. These defects include adolescent onset of ectodermal defects including thinning hair, smooth tongue, palmoplantar keratoderma, and sweating abnormalities, suggesting roles for WNT10A in natural regenerative processes. Dr. Millar's research group generated a mouse model for WNT10A deficiency that closely mimicked the human disease and enabled them to study the mechanisms underlying the observed defects. Dr. Millar presented evidence that lack of WNT10A results in decreased signaling through the Wnt/β-catenin pathway and reduced proliferation of epithelial progenitor cells in a wide variety of ectodermal organs. Dr. Millar further used genetic lineage tracing approaches in mice to show that Wnt/β-catenin signaling marks self-renewing stem cells in these tissues.

Dr. Cheng-Ming Chuong from the University of Southern California provided a wide-ranging discussion of different modes of skin appendage regeneration and reviewed the distinct molecular mechanisms that underlie these events. Dr. Chuong first discussed cyclic regeneration of hair or feather follicles under physiological conditions and showed how hair and feather phenotypes can change with age and/or under the influence of hormones. AGA is an example of such a

phenotype and results from failure of stem cell activation in the hair follicle. A second type of regeneration, intrafollicular regeneration, occurs within follicles after plucking of the hair or feather filament or damage by chemicals or irradiation. In a third type of regenerative event, population regenerative behavior, hair follicles in a population can influence the regeneration of other follicles. Examples of this include the regenerative hair wave, in which hair follicles interact with intradermal adipose tissue, and quorum-sensing behavior that involves interactions of hair follicles with the immune system. In a fourth type of regenerative mechanism, changes in the cellular fate can occur after wounding, as seen in the conversion of appendage progenitors into epidermis or nail. Finally, wound-induced follicle neogenesis involves reprogramming of endogenous cells, allowing them to participate in the formation of new hair primordia and follicles.

Dr. Bruce Morgan from Harvard Medical School presented data showing that the size and shape of the hair shaft depends on the number and activity of hair progenitor cells, which is in turn dependent on the number and activity of the DP cells that compose their niche. Using elegant genetic studies in the mouse, Dr. Morgan showed that a reduction in hair follicle DP cell number can cause follicular decline and telogen arrest, similar to those observed in human hair thinning and loss. Dr. Morgan also presented evidence that DP cell number is actively regulated in the context of the regenerative phase of the hair cycle. His research group is now using genetically modified mice to manipulate gene expression in the DP to identify the molecular mechanisms by which DP and progenitor cell numbers are set to ensure production of appropriately sized hairs.

In the final talk of this session, Dr. Sung-Jan Lin from National Taiwan University presented exciting new studies aimed at promoting de novo formation of hair follicles. Hair follicle neogenesis depends on the initiation and perpetuation of cross-talk between keratinocytes and dermal cells. When skin is injured, it is usually repaired with fibrosis, except in embryos, which exhibit scarless healing with formation of new hair follicles. Dr. Lin has explored whether similar neogenesis of hair follicles can be reinitiated in postnatal life. His group discovered that protein extracted from embryonic skin at a specific developmental stage was able to induce hair follicle neogenesis, both in a full-thickness wound and in a modified patch assay in mice, without the help of inductive mesenchymal cells. Hair follicle organogenesis in this system was mediated mainly through the effects of the protein extract on fibroblasts. Dr. Lin presented evidence that when adult fibroblasts, but not keratinocytes, were cultured with the protein extract, they acquired the ability to induce new hair follicles. Dr. Lin used phosphoproteomic analysis to begin to delineate the underlying molecular mechanisms. These experiments showed that application of the protein extract resulted in activation of insulin/insulin growth factor signaling in adult fibroblasts and that this was required for their hair follicle inductivity. Through proteomics analysis with mass spectrometry, Dr. Lin identified three extracellular proteins enriched in embryonic skin that together were required and sufficient to induce hair follicle neogenesis in vivo. Dr. Lin's findings indicate that hair follicle regeneration can be initiated by creating a pro-regeneration environment with defined extracellular factors that are normally enriched in embryonic skin. In the future, such environmental signals can be incorporated with other approaches to enhance hair follicle regeneration.

Cicatricial Alopecia
Session Director: Elise A. Olsen

Dr. Olsen opened the session with the first invited lecture on "Frontal Fibrosing Alopecia: preliminary results of a multicenter study." This represented a prospective multicenter cooperative study of female patients with FFA with coinvestigators E. Olsen, W. Bergfeld, V. Callender, G. Cotsarelis, M. Hordinsky, A. McMichael, P. Mirmirami, J. Roberts, J. Shapiro, L. Sperling, and A. Tosti. Patients who met criteria for FFA including a scalp biopsy result consistent with lichen planopilaris/FFA; frontal hairline recession in a band-like or irregular "moth-eaten" pattern; plus at least one of (i) eyebrow loss, (ii) perifollicular or interfollicular erythema in the area of hair loss, or (iii) perifollicular papules in the area of hair loss were eligible to participate. Patients who consented had measurements of degree of hair loss performed by the investigators using new standardized assessment tools and completed a detailed online Research Electronic Data Capture (REDCap) questionnaire assessing potential etiological factors.

This presentation was on the first 222 patients with FFA to have completed questionnaires. Of participants, 88% were white, 8% were African American, and 82% were postmenopausal. Most had college education or beyond (91%). A total of 92% had eyebrow loss, including 41% before the hairline recession began, and 63% had hair loss elsewhere, including 30% eyelash, 52% axillary, 45% pubic, 70% arm, and 81% leg hair loss. The most common medical conditions were atopy (30%), thyroid disease (26%), hyperlipidemia (24%), hypertension (22%), and depression (16%). Hair care products, used by over 60%, included hair dye, conditioners, and hair spray. Topical preparations applied to the face included sunscreens (82%), wrinkle creams (55%), and liquid foundation (74%). Exposure to pet pesticides was high, at 30%. Topical and intralesional steroids were the most common treatments, followed by hydroxychloroquine, topical minoxidil, tetracycline class of antibiotics, and finasteride. Regrowth was reported most frequently by patients with topical minoxidil (22.5%), finasteride (9.3%), and intralesional steroids (7.1%). The group continues to enroll patients, with interest particularly in environmental exposure and more critical assessment and response to treatment using the new clinical tools.

The second invited lecture was presented by Nonhlanhla P. Khumalo, MBChB, PhD, Professor and Head of Division of Dermatology at the University of Cape Town, South Africa, on "Low-Hanging Fruit: Identifying Preventable Causes of Scarring Alopecia." The focus of her lecture was how externally applied agents, particularly relaxers, may effect Afro-textured hair. It was noted that relaxers all have a pH greater than 10.5, which is a level likely to directly damage skin. NaOH (lye) relaxers have the highest pH of greater than 12.5, whereas LiOH relaxers have a lower pH of 12.0—12.5. NaOH relaxers also have a larger amount of active ingredient

(2.5%) versus LiOH relaxers (1.5%). If a neutralizer is not included with the relaxer, hair breakage may occur. Additives such as those in the Rio Hair Naturalizer System (World Rio Corporation) may have been the cause of the profound hair loss seen in women so treated (~60% with more than 40% loss, 22% with total loss). Relaxed hair causes changes in the amino acid content of hair, including a loss of cysteine, arginine, and citrulline and an increase in glutamine in both proximal and distal treated hair. Of note, of several studies published on central centrifugal cicatricial alopecia, relaxers alone were not noted as risk factors, and consideration that this is likely multifactorial (traction, chemicals, heat, moisturizers, and genes) was hypothesized.

The third presentation was by Nikki Tang, MD, Mt. Sinai St. Luke's-Roosevelt Hospital, New York, New York, USA. The presentation was titled "Comparison of Four Regimens for Treatment of Central Centrifugal Cicatricial Alopecia" and discussed 13 patients with biopsy-proven central centrifugal cicatricial alopecia who were randomized to four treatment arms: (i) oral doxycycline 100 mg twice daily, (ii) clobetasol lotion in 2-week cycles, (iii) triamcinolone 5-mg/ml intralesional injections every 4 weeks, or (iv) rifampin 300 mg and cephalexin 500 mg twice daily for 2 weeks, repeated up to 24 weeks. The treatment period of 6 months was followed by topical minoxidil 5%. The mean improvement in the four treatments was (i) 32.8 cm^2, 19.1%; (ii) 48.3 cm^2, 34.5%; (iii) 70.3 cm^2, 32.5%; and (iv) −12.3 cm^2, −8.9%. Despite the differences among groups, none approached statistical significance.

The fourth presentation was given by Varvara Kanti, MD, Charité—Universitätsmedizin Berlin, Department of Dermatology and Allergy, Clinical Research Center for Hair and Skin Science, Berlin, Germany. Dr. Kanti discussed "Frontal Fibrosing Alopecia: Epidemiologic Data from a Patient Registry." This was a retrospective data collection of patients with FFA by patient chart review from France (n = 135) and Germany (n = 85). In addition, patients with FFA in the German national registry since 2013 were included (n = 156), for a total of 376 patients. Most patients were women (96.5%), with the majority postmenopausal. However, there were patients as young as 15 years old, women with regular periods, and men (3.5%). The diagnosis was delayed 24 years in at least one patient, which would put the onset before the original FFA publication by Kossard (Kossard S. Postmenopausal frontal fibrosing alopecia. Scarring alopecia in a pattern distribution. Arch Dermatol 1994;130:770—4.).

Using the Lichen planopilaris activity index score developed at University of California—San Francisco (Chiang C, Sah D, Cho BK, Ochoa BE, Price VH. Hydroxychloroquine and lichen planopilaris: efficacy and introduction of Lichen Planopilaris Activity Index scoring system. J Am Acad Dermatol 2010;62:387—92.), the score was relatively low overall (2.3 ± 1.8). Perifollicular erythema was the most common finding (84%). Eyebrow loss was present in approximately 80% of women, less in men, and complete in almost 25% of women and less than 10% of men. Axillary/pudendal and arm/leg hair loss occurred in over 40% of affected women. In men, beard hair was lost in 89% of patients, including half with complete loss. Thyroid disorders occurred in 33%, lipid metabolic disorders in 22%, and hypertension in about 20% of patients.

The most frequent therapies used included topical and intralesional steroids (most common), tetracyclines, hydroxychloroquine, finasteride, mycophenolate mofetil, and methotrexate. The fifth presentation by Curtis Thompson, MD, Departments of Biomedical Engineering, Pathology and Dermatology, Oregon Health & Science University, Portland, Oregon, USA, was on "Absence of Catagen/Telogen Phase and Loss of Cytokeratin 15^+ Expression in Hair Follicles in Lichen Planopilaris (LPP)." The HoVert method was proposed for tissue processing that would give both vertical and horizontal sectioning in one punch biopsy sample. A method for distinguishing acute from chronic telogen effluvium was proposed, because at the time of greatest hair loss, 100% of hairs are in anagen in acute telogen effluvium, whereas in chronic telogen effluvium, the catagen/telogen ratio is greater than 20%.

The value of immunohistochemistry in cicatricial alopecia was noted in that in early lichen planopilaris, the infiltrate is all T cells but that in advanced disease, this becomes a mixture of T and B cells. This is distinct from lupus erythematosus, in which the B-cell infiltrate far outweighs the T cells. In lichen planopilaris as well as lupus erythematosus, central centrifugal cicatricial alopecia, and FFA, there is also a loss of cytokeratin 15^+ cells at the bulge area of the follicle. Dr. Thompson hypothesized that this represents a loss of cytokeratin 15^+ stem cells after the follicle enters catagen, leading to irreparable damage to the follicle in these cicatricial alopecias even when immunosuppressive therapy has been used as treatment.

Auxiliary Cells
Session Director: Valerie Horsley

This session featured invited talks by Drs. Valerie Horsley, Mirna Perez-Moreno, and Mayumi Ito and an oral presentation from the abstracts by Dr. Francisco Jimenez-Acosta. Dr. Perez-Moreno of Spanish National Cancer Research Centre, Madrid, Spain, provided insights into how macrophages contribute to activation of hair follicle growth. Using the murine hair cycle as a model system, her laboratory has examined whether immune cell interactions are able to regulate hair follicle cycling, specifically the physiological entry of telogen hair follicles into anagen. By inhibiting skin-resident macrophages that are part of the skin stem cell niche, and given the fact that their numbers fluctuate before the onset of the activation of hair follicle progenitor cells, the macrophage-specific pharmacological inhibition of Wnt processing is sufficient to delay hair follicle growth. Overall, her data implicate macrophage-derived Wnts in the activation of hair follicle progenitor cells.

Dr. Mayumi Ito, of New York University, New York, New York, USA, discussed how McSCs communicate with epithelial stem cells during pigmented hair regeneration. Her laboratory identified Wnt signaling as a key pathway that couples the behavior of the two stem cell populations. Studies with genetic mouse models showed that Wnt activation in epithelial stem cells not only drives hair follicle formation but also regulates McSC proliferation during hair

regeneration via Edn secretion. Furthermore, their recent data showed that endothelins function as ligands that activate endothelin receptor B and that this receptor is necessary for hair pigmentation. These results provide insight into the understanding of how pigmented hair follicles can be regenerated through the collaboration of heterotypic stem cell populations. Next, Dr. Horsley discussed recent progress in her laboratory analyzing adipocytes in the skin during hair growth, aging, and wound healing. She described molecular mechanisms that control the maintenance of the adipocyte lineage in the skin and how adipocytes contribute to skin regeneration after injury.

The final talk by Dr. Jimenez-Acosta, of Las Palmas de Gran Canaria, Canary Islands, investigated morphological evidence that eccrine glands are associated with hair follicle units, especially when the whole eccrine gland trajectory is examined, and showed that the distal eccrine duct and the secretory coils come very close to the hair follicle and sometimes surrounds the hair follicle. By observing the whole eccrine gland trajectory, evidence was presented that the distal eccrine duct and the secretory coils are associated to the hair follicle units. The authors' propose a new term for this association, "hair field unit."

Great Cases from South America
Session Directors: Ricardo Romiti, María E. Cappetta and Isabella Doche

A stimulating spectrum of presentations from Brazil, Argentina, and Chile based on hair research and clinical cases was presented during the "Great Cases from South America" session. As the first speaker, Dr. Pirmez from Rio de Janeiro, Brazil, showed a new clinical presentation of FFA in which an unusual retention of the hairline produces a misleading pseudo-"fringe sign." These findings have recently been published by this author in the *British Journal of Dermatology*.

Thereafter, Dr. Mariana Martin from Argentina presented a challenging case of breast cancer metastasis closely mimicking cicatricial alopecia, further including this disorder in the differential diagnosis of cicatricial alopecias. Dr. Alessandra Anzai from São Paulo, Brazil, illustrated achromic vitiligo-like lesions as a cutaneous hallmark of the interface dermatitis associated with FFA and stressed that this manifestation should always be considered in the differential diagnosis of both vitiligo and subacute lupus erythematosus.

At the second half of this session, Dr. Isabella Doche from São Paulo, Brazil, in collaboration with the University of Minnesota, presented histologic findings of normal-appearing scalp in both lichen planopilaris and FFA and showed that these sites may equally be affected by the inflammatory process. This finding will probably demand a change in the therapeutic protocols of affected patients in the near future. Dr. Felipe Mardones from Chile presented epidemiologic data, clinical profile, and treatment regimens and outcomes of fibrosing alopecia in a pattern distribution in a Chilean Study Group, highlighting specific issues in this population. Further on, Dr. Aline Donati from Brazil presented specific alert signs for quickly identifying areata-like lesions by trichoscopy. Dr. Cecilia N. Tuculte from Argentina gave a talk about "Alopecia Areata in Solid Organ Transplant Patients." The final presentation of the day was conducted by Dr. Ricardo Romiti from the University of São Paulo, São Paulo, Brazil. During this interactive session, the audience actively participated, discussing challenging topics such as historical aspects of Graham-Little syndrome, treatment of scalp psoriasis, and differential diagnosis of pressure alopecia.

FRIDAY, NOVEMBER 20, 2015

Satellite Symposium: Quality of Life Considerations and Treatment Options in Female Pattern Hair Loss
Session Directors: Richard G. Fried, Amy McMichael and Ulrike Blume-Peytavi

The Quality of Life Considerations and Treatment Opportunities in Female Pattern Hair Loss was a company-sponsored satellite symposium by Women's Rogaine (Johnson & Johnson Consumer Inc., New Brunswick, NJ). The symposium featured invited talks by Drs. Richard Fried, Amy McMichael, and Ulrike Blume-Peytavi focusing on the use of a novel once-a-day 5% minoxidil foam for female pattern hair loss (FPHL). Dr. Fried commenced the session with a fascinating introduction about hair and its impact on beauty, youth, health, and wellness. He introduced the quality-of-life impact on hair loss sufferers in emotional, economic, social, marital, and sexual aspects.

Dr. Blume-Peytavi, a world renowned hair loss expert and lead researcher on multiple peer-reviewed articles on the subject of male pattern hair loss and FPHL, spoke next. She provided an overview of a large-scale phase III, both placebo- and benchmark-controlled, double-blind, randomized clinical design and evidence for the use of 5% minoxidil foam in FPHL patients. In the clinical trials, women were split into three groups: foam placebo; 2% minoxidil (Rogaine/Regaine) solution; and a novel, once-a-day 5% minoxidil (Rogaine/Regaine) foam. The 24-week multicenter clinical study showed the once-a-day 5% minoxidil (Rogaine/Regaine) foam had a significant change from baseline in the target area hair count compared with placebo. Her work found the foam to be safer, milder, and effective, with hair growth outcomes similar to that of the traditional 2% minoxidil solution applied twice daily. Patients saw an increase of 48% in mean diameter of new nonvellus hairs at 24 weeks. Additionally, the foam provided easier use and decreased dosing per day, providing an additional option for women with FPHL and improving compliance among patients.

Next, Dr. Amy McMichael, Chairman of Dermatology Department of Wake Forest Medical School, discussed the unique challenges facing female hair loss in African and Hispanic populations. Dr. McMichael, a leading expert in FPHL and its diagnostics and treatment, detailed her experiences with treating patients with traction alopecia, cicatricial alopecia, and FPHL in her clinic. Since Women's Rogaine 5% foam was approved in the United States, Dr. McMichael has seen strong success with this novel product form for women with hair loss, as well as adjacent therapies. She emphasized the need for compliance when using the treatment to achieve more satisfactory response.

In closing, Dr. Fried returned to the stage to summarize the session and returned to the topic of quality of life. He began

by showing a series of pictures of women with and without hair. Ranging from models to women suffering from FPHL, he weaved a story for the audience, highlighting the incredible importance of hair to women, showing how it can be used to express personality, emotion, and wellness. Highlighting this point, he showed survey data showing the importance of overall wellness with quality and satisfaction with one's own hair. The audience of dermatologists and hair researchers were asked to think about hair in a slightly different way and were shown the important work they can do in helping with compliance and treatment for women with FPHL.

The session concluded with an engaged question and answer session. Many dermatologists in the audience were curious and excited to learn more about Women's Rogaine 5% foam. Questions were answered by the panelists regarding superior efficacy compared with nonbranded options, differences in foam product versus solution product, and when the product would be released in their home country. This early morning session was well attended, with a large number of attendees standing in the back of the theatre.

Hormones, Hair Growth, and Pattern Hair Loss
Session Directors: Wilma F. Bergfeld, Rodney Sinclair and Ulrike Blume-Peytavi

The session on "Hormones, Hair Growth, and Patterned Hair Loss" was opened by the Chair, Wilma Bergfeld, MD, who introduced her co-chairs, Rodney Sinclair, MBBS, MD, and Ulrike Blume-Peytavi, MD. The session focused on clinical and molecular findings seen in FPHL, male pattern AGA, and regulation of human hair growth, which are orchestrated by androgen hormones and prostanoids. Other important subjects included the role of inflammation and immunity in the pathogenesis of FPHL and clinical methodology validation techniques to judge responses to therapies.

The first session lecture was by Dr. Blume-Peytavi, who spoke on "Translational Approach to Androgenetic Alopecia—Clinical and Molecular Read Outs." She noted that disease activity in male pattern AGA predominantly affects frontal and vertex areas but that lower occipital hair appears unaffected. Hair follicle density and hair shaft diameter as measures of treatment success rely on hair outgrowth rate with best clinical outcomes assessed by patients' self-assessment or phototrichogram, usually obtained at week 16 after the start of treatment. The identification of early response parameters would tremendously facilitate testing of novel therapeutic formulations and regimen.

Telogen shedding 4–6 weeks after topical treatment initiation underscores the concept of interference with hair cycle regulation during the early phase of treatment. Focusing on the early treatment phase (weeks 0–8) could provide substantial information on hair and scalp changes during this sensitive time period in which shedding is most likely to occur but also in which regrowth is activated and initiated.

In their first approach, the investigators studied six healthy male volunteers with AGA, Norwood-Hamilton stage IIIv–IV, to link the clinical manifestation with molecular markers at the RNA and protein levels in noninvasively obtained material, namely plucked hair follicles and skin surface and follicular cast material, collected from vertex and occiput.

Clinical findings (e.g., hair density decrease, increased miniaturized hair, and anagen-telogen shift), were reflected in the RNA microarray analyses. Genes responsible for hair follicle cycling and hair keratin synthesis were down-regulated in AGA. The most significant markers up-regulated in AGA were involved in ubiquitin pathways; in methylation, which was confirmed by methylation assay; and in developmental pathway genes like *SOX13,* a repressor of the Wnt pathway. Besides imbalances of ion channels, an imbalance of protease expression was identified, which was confirmed by protein profiler analyses of scalp material. These findings indicated a high presence of CTSB, CTSL, KLK10, and KLK11 in vertex skin and CTSD in the protein samples from the occiput.

Dr. Blume-Peytavi summarized this translational study as encouragement to include molecular studies in clinical trials. Her hypothesis is that molecular signatures could open perspectives for novel therapeutic targets in AGA.

The second speaker was Valerie A. Randell, PhD, FIBMS, FRSB, from the University of Bradford, Bradford, UK. She is Professor of Biomedical Sciences at the University and leads a well-established and recognized team investigating the biology of hair growth, particularly mechanisms of androgen action and drugs, which stimulate human hair growth. Dr. Randall spoke on "Regulation of Human Hair Growth: Androgens and Prostanoids." She discussed that the main role of most human hair is social and sexual communication; axillary and pubic hair development signals adulthood, and beard signals masculinity. Therefore, hair loss or excessive hair growth (hirsutism) causes psychological distress and decreased quality of life.

Unfortunately, hair disorders are poorly defined and treated because the mechanisms regulating hair follicles are not fully understood. Androgens are the main regulator of human hair growth, stimulating pubertal hair changes but also promoting gradual, patterned scalp hair loss (AGA). All androgen responses require androgen receptors within follicle cells, particularly the DP. She noted whether the follicle is stimulated (many areas), inhibited (scalp), or unaffected (eyelashes) varies depending on the body site; this corresponds to differences in gene expression within individual follicles.

Recently, prostanoids, prostaglandins (PGs), and closely related prostamides attracted attention because prostanoid glaucoma drugs promoted eyelash growth side effects. Dr. Randall and her research associates found that one, bimatoprost, a prostamide $F_{2\alpha}$ analog, also stimulated mouse pelage growth in vivo and scalp follicle growth in organ culture. They identified PGs within isolated scalp follicles using lipidomics and showed that bimatoprost up-regulated prostamide-synthesizing enzymes in cultured scalp follicles and DP cells, while simultaneously down-regulating PG pathways and altering prostanoid receptor levels. This indicated that prostanoid paracrine mediators have natural signaling roles in human follicles. Professor Randall summarized her presentation with the statement that further investigations of androgens and prostanoids should facilitate novel therapeutic approaches.

The third speaker, Neil Sadick, MD, of Sadick Dermatology and Director of Sadick Research Group, New York, New York, USA, spoke on "The Role of Inflammation and Immunity in the Pathogenesis of Female-Pattern Hair Loss." Dr. Sadick began his lecture by summarizing the status of FPHL and noted that it affects a significant percentage of women, with increased prevalence with aging. Although follicular pathology and pathophysiology of male AGA are widely considered resolved, FPHL is still under investigation, particularly as no androgen excess is found in more than 50% of the affected women.

The objective of his study was to determine the role of immunity and inflammation in FPHL, because immunoglobulin deposition within the epidermal basement membrane zone was a finding in AGA. A second objective was to evaluate a modulated therapy according to inflammatory and immunoreactant profiles.

He concluded that there is a lymphocytic microfolliculitis targeting the bulge epithelium, along with deposits of epithelial basement membrane zone. He observed that immunoreactants are frequently found in FPHL, and this finding suggested an immunologically driven trigger. Patients showing a positive immunoreactant profile responded best to combined modalities of therapy that included anti-inflammatory agents and hair growth promoters compared with those who had a negative clinical response.

The next speaker, Ying Zheng, PhD, Senior Research Investigator, Department of Dermatology, University of Pennsylvania, Philadelphia, Pennsylvania, USA, spoke on the results of the study "CRTH2/ PTGDR2 Antagonists Reverse the Hair Growth Inhibition Caused by Elevated PGD2 Level." Dr. Zheng began his lecture with a summary of PG activity and the hair follicle. He noted that prostaglandin D2 (PGD2) and its synthesizing enzyme, PGD2 synthase, are present at higher levels in balding versus nonbalding scalp in men with AGA. The research collaborators had previously observed in a mouse model that PGD2 inhibits hair growth via CRTH2/ PTGDR2, which led them to hypothesize that PTGDR2 is the key receptor mediating the hair growth inhibitory activity of PGD2 in human follicles.

In his study, the researchers tested several pharmacological PTGDR2 antagonists for their anti-PGD2 activity on human hair growth. They found that PTGDR2 antagonists reversed the growth inhibition mediated by PGD2 in a dose-dependent manner by reducing PGD2-triggered apoptosis and maintaining proliferation of keratinocytes. Topical administration of a PTGDR2 antagonist to mice extended the anagen follicular growth cycle, resulting in longer hair. He noted that they also found that hair follicles from two of five of the alopecia patients exhibited little susceptibility to PGD2's effect in the culture assay. By sequencing the entire *PTGDR2* gene, including the flanking regions, he and his colleagues identified single nucleotide polymorphisms in the human *PTGDR2* gene that are associated with sensitivity of hair growth to PGD2.

In summary, these findings further underscore the key role of PTGDR2 in regulating hair growth and suggest that pharmacological intervention of PTGDR2 may be an effective approach in preventing and/or treating alopecia in patients sensitive to PGD2. Furthermore, the single nucleotide polymorphisms identified here may serve as markers for identifying patients responsive to treatment with PGD2-targeted drugs.

The last speaker, D. Hugh Rushton, PhD, DSc, is the founding member of European Hair Research Society School of Pharmacy and Biomedical Sciences, University of Portsmouth, Portsmouth, UK. Dr. Rushton is a well-recognized international hair researcher who presented on an interesting subject that may change the basic understanding of hair regrowth, "Significant Growth Following Effective Medical Treatments in Men and Women with Patterned Hair Loss Does not Involve the Conversion of Vellus Hair to Terminal Hair. Where Does the Observed Hair Growth Originate?" Dr. Rushton began his lecture with a short background of what has been the understanding of hair growth. He noted that hair regrowth after effective medical treatments for female and male pattern hair loss has historically has been attributed to the reversal of miniaturized (vellus) hair to terminal hair. This explanation stemmed from publications reporting reductions in the percentage of vellus hair in those patients who had significant increases in total hair density (hair per cm^2) and remained unchallenged except by one small study in 2006 by Van Neste.[b]

Dr. Rushton reported his retrospective analysis of the raw data from peer-reviewed publications, using the same quantitative, validated hair evaluation method (unit area trichogram, or UAT), which used a standardized presampling protocol. In his study, the raw data were reanalyzed to determine the absolute vellus hair count at baseline and after 12 months in treated or 24 months in untreated men and women with pattern hair loss.

The investigators found that, for male and female subjects exhibiting pattern hair loss who were treated for 12 months with various medical therapies (minoxidil, finasteride, and anti-androgens), significant increases in total hair density per cm^2 occurred, but they did not experience a significant change in the vellus hair population per cm^2. Dr. Rushton concluded that there is no evidence supporting the hypothesis that the reversal of vellus to terminal hair is responsible in the observed increase in total hair density initiated by effective medical treatment of male pattern hair loss and FPHL.

Stem Cells and Stem Cell Niches

Session Directors: George Cotsarelis, Claire A. Higgins and Manabu Ohyama

The stem cell and stem cell niches session consisted of talks displaying the latest in technology and cutting edge research. First, Dr. Tudorita Tumbar from Cornell University discussed the role of Runx1 in HFSC biology. In the hair follicle, Runx1 epithelial deletion in morphogenesis impairs normal adult hair follicle cycling and arrests adult HFSCs in quiescence. She showed that these effects are overcome later in adulthood. Deleting Runx1 after morphogenesis "resulted in cyclin-dependent kinase inhibitor Cdkn1a (p21) upregulation. Interfering with Runx1 function in cultured HFSCs impaired their proliferation and normal G(0)/G1 and G(1)/S

[b] Van Neste D. Natural scalp hair regression in preclinical stages of male androgenetic alopecia and its reversal by finasteride. Skin Pharmacol Physiol 2006;19: 168–76.

cell cycle progression. The proliferation defect could be rescued by Runx1 readdition or by p21 deletion. Thus, Runx1 has a direct role in promoting anagen onset and HFSC proliferation."[c]

Dr. Pantelis Rompolas from the University of Pennsylvania described the power of using in vivo multiphoton microscopy to visualize and modulate stem cell behavior in the intact skin of live mice. This remarkable technology produces fascinating time lapse videos of hair follicles as they enter anagen and produce a new lower follicle. Individual stem cells in the bulge can be labeled and followed as they divide and generate progeny. Specific cells can be ablated, and the follicle's response can be tracked in vivo. These studies established that the precise position of individual cells and therefore the cellular organization of the hair follicle determined their fate and specific contribution to hair growth. Dr. Rompolas proposes a model for the compartmentalization of the hair follicle, which describes a hierarchical process for HFSCs toward terminal differentiation.

Dr. Michael Rendl from Icahn School of Medicine at Mount Sinai described his results of a comprehensive survey of global gene expression patterns acquired from developing skin. Using fluorescence-activated cell sorting, Dr. Rendl's laboratory isolated hair follicle placode, dermal condensate, interfollicular epidermis, melanocytes, fibroblasts, and Schwann cells from developing skin, then performed RNA sequencing to define genes enriched in each cell population and thus define cell-specific gene signatures. In a gesture perhaps unprecedented in the HFSC field, Dr. Rendl established an interactive website to allow anyone to interrogate this rich database. Investigators can put in their favorite gene or genes to see where they are expressed within the developing skin and hair follicle (see Hair-Gel.net). This transformative approach to science allows for the greatest impact and widespread dissemination of information.

Dr. Sung-Jan Lin, MD, PhD, from National Taiwan University gave a presentation from an abstract selected for oral presentation. He presented data from experiments in which mice were irradiated with different doses of ionizing radiation. He described different types of responses by the hair follicle based on the dose of ionizing radiation. Lower doses resulted in transient arrest of anagen secondary to bulb cell injury with recovery of hair growth within a day. Higher doses caused dystrophic catagen, a disorganized form of hair follicle regression resulting in reformation of the lower hair follicle from HFSCs in the bulge. Forced Wnt signaling shortened the time required for the hair follicle to repair and reenter anagen.

Satellite Symposium: New Clinical Evidence and the Role of Neutraceuticals for the Treatment of Thinning Hair
Session Director: Mark Holland

This satellite symposium was company-sponsored by Lifes2good (Galway, Ireland), the makers of Viviscal, a scientifically formulated nutritional supplement for thinning scalp hair. The lunchtime event was opened by Mr. Mark Holland, CEO for North America Lifes2good, who provided background to the sponsor's interest in hair research and associated nutritional product development. Thereafter, the session was moderated by Prof. Desmond J. Tobin, Professor of Cell Biology, Centre for Skin Sciences (Director), Faculty of Life Sciences, University of Bradford, Bradford, West Yorkshire, UK, and included presentations on (i) the impact of nutrition on human scalp hair growth (Dr. Desmond Tobin), (ii) clinical trial portfolio of the hair thinning/loss nutritional supplement Viviscal (Dr. Glynis Ablon), and (iii) a presentation of recent laboratory data on the influence of digested Viviscal on the behavior and function of human scalp hair follicle DP fibroblasts in vitro (Dr. Ablon for Dr. Helena McMahon).

Dr. Tobin opened with a discussion on the capacity of each hair follicle to produce hair fibers of different types and cosmetic value during different life stages under the influence of internal (e.g., hormonal) and external (e.g., diet) stimuli. He remarked on the dearth of knowledge on the impact of Western (highly processed) diets, lifestyles, environmental exposure, and other factors on hair thinning/loss. In this context, he defined what we understand by *hair quality* in terms of contribution to the fiber's biomolecular components from dietary amino acids, lipids, microelements, and others. Dr. Tobin discussed the prototypical types of hair follicle aging and how these can affect hair growth, as well as the increasing interest in nondrug options for hair growth, before moving onto a more detailed exploration of how nutrition (including marine sourced) can influence hair growth quality. Specific examples included the impact of certain vitamins (e.g., biotin, vitamin C, vitamin E, etc.), metals (e.g., zinc), amino acids, and others on keratin and collagen production.

Next, Dr. Glynis Ablon, Assistant Clinical Professor, UCLA and Ablon Skin Institute, Los Angeles, California, USA, summarized approximately 25 years of clinical research in which Viviscal was assessed as a nutritional supplement for optimal hair growth. Viviscal emerged from research into Inuit marine diets at the University of Helsinki. Dr. Ablon focused on the most recent trials, including a multicenter, randomized, placebo-controlled, 6-month trial (conducted by Thomas J. Stephens and Associates in Dallas, TX and Colorado Springs, CO) that involved 72 women with self-perceived hair thinning.[d] Key outcomes of that study included a statistically significant reduction in hair shedding and an increase in vellus fiber diameter (7.4%) after 6 months of supplementation. Dr. Ablon also reported on trials conducted at her institute in which she also observed significantly increased terminal counts, and she provided some encouraging preliminary data from the first trial in young males with AGA.

Dr. Ablon was followed by a presentation prepared by Dr. Helena McMahan, Senior Research Fellow, Shannon ABC, Institute of Technology Tralee, County Kerry, Ireland, which discussed new data from her laboratory at Shannon

[c] Copyright © American Society for Microbiology, Molecular and Cellular Biology, vol. 30, no. 10, May 2010, pages 2518–2536, doi:10.1128/MCB.01308-09.

[d] Rizer RL, Stephens TJ, Herndon JH, Sperber BR, Murphy J, Albon GR. A marine protein-based dietary supplement for subclinical hair thinning/loss: results of a multisite, double-blind, placebo-controlled clinical trial. Int J Trichology 2015;7:156–66.

Applied Biotechnology Centre in Ireland. Using Viviscal in a digested format, Dr. McMahon reported that the supplement significantly increased human hair follicle DP cell proliferation (+37%) and increased the expression and activity (+87%) of alkaline phosphatase compared with unstimulated controls. These combined clinical and laboratory data suggest that components in this supplement (i.e., AminoMar complex of shark cartilage and oyster extract, horsetail stem extract millet seed extract with vitamin C, niacin, biotin, iron and zinc (Viviscal; Lifes2good, Galway, Ireland), may support hair growth and that this may occur via support of the hair follicle DP.

The symposium concluded with a vibrant question and answer session.

Emerging Technologies and Therapies
Session Directors: Ken Washenik, Takashi Tsuji and Annika Vogt

The Emerging Technologies session featured invited talks by Drs. Takashi Tsuji and Annika Vogt and three oral abstract presentations. Dr. Tsuji discussed strategies and recent progress in his laboratory for the establishment of hair follicle regenerative therapies as a future organ replacement regenerative therapy. He described the "Organ Germ Method" to reconstitute a bioengineered organ germ comprising organ-inductive potential epithelial and MSCs in vitro. He reviewed that in a mouse model his laboratory successfully developed a bioengineered tooth, hair follicle, and secretory organs including salivary gland and lachrymal glands that have correct physiological functions in vivo. He noted that the bioengineered hair follicle develops correct structure and forms proper connections surrounding host tissues including the epidermis, arrector pilli muscle, and nerve fibers. The bioengineered hair follicle could restore hair cycle, hair color, and piloerection through the rearrangement of stem cells and their niches.

Next, Dr. Vogt provided insights into noninvasive strategies that could improve our understanding of hair growth disorders and could become a valuable addition to conventional clinical read-outs in hair research. She reviewed the differences between affected and nonaffected scalp in males with male pattern AGA and how to link the clinical and molecular biological findings in patients during the course of disease and under therapy. She discussed a newly developed procedure for noninvasive collection of protein material from scalp surface and hair follicle openings using of cyanoacrylate skin surface stripping on shaved scalp skin areas of 1.8 cm^2.

Dr. Hong Jim Joo of St. Paul's Hospital in Seoul, Korea presented the work of his group, which conducted a study to better understand the underlying mechanisms of why light-emitting diodes (LEDs) have been shown to promote hair growth in clinical trials. Specifically, the aim of his study was to determine the effect of LED irradiation on IFN-γ–treated human DP cells. He studied four ranges of wavelengths of LED light, from 415 nm to 830 nm. He noted that the proliferation of human DP cells was significantly increased by LED irradiation at 660 nm with 1, 5, and 10 J/cm^2 compared with nonirradiated cells. LED irradiation at 660 nm significantly counteracted the inhibitory effect of IFN-γ on human DP cell proliferation. His group suggested that LED inhibits IFN-γ–induced catagen-like changes on human DP cells.

Dr. Andy Goren of Applied Biology, Inc., in Irvine, California, USA, reviewed how topical minoxidil is activated via sulfotransferase enzyme SULT1A1 in the scalp and that the enzyme expression is variable among individuals. He noted that the efficacy of 5% minoxidil foam remains low; that is, 30–40% of patients regrow hair. His group created a test to determine which patients may respond to minoxidil. He reviewed the results of his 24-week multicenter prospective study to test the clinical response of females with AGA to minoxidil based on the sulfotransferase enzyme activity in the hair follicle. He concluded that the SULT1A1 enzymatic test predicts with high confidence patients who are likely not to benefit from 5% topical minoxidil therapy.

The final talk of this session was given by Dr. Beren Atac of TissUse GmbH, Berlin, Spreenhagen, Germany, on "The Microfollicle: In Vitro Modeling the Hair Follicle for High-Throughput Screening." He described the use of high-throughput microfollicle cultures that enable tracking hair organoids during their development and studying hair follicle biology. He noted that as an in vitro model of the hair follicle, the high-throughput microfollicle cultures enable studying acute and chronic effects of substances on hair follicles.

Structure, Biology, and Hair Curl, Color, and Luster
Session Directors: Thomas L. Dawson, Jr., Jolon Dyer and Amy McMichael

The Structure, Biology, and Hair Curl, Color, and Luster session included two invited presentations and three selected from submitted abstracts. These presentations taken together highlight current progress in linking follicle biology to the tensile/physical properties of hair. They show progress in understanding the development of the hair fiber and also show that to fully understand and appreciate hair shaft development, we need to leverage broad expertise in "live" follicle biology and "dead" hair shaft and the most current technologies across the breadth of the core sciences: chemistry, biology, and physics.

In the first invited presentation, Dr. Jolon Dyer discussed cross-linking in wool fibers, novel methods to map protein-protein cross-links using proteomics, and how protein modification affects hair fiber structure. The technology involves mass spectrometry of hair proteins and identification of oxidative protein modification via an approach termed *redox proteomics.* Redox proteomics allows qualitative and quantitative measurement of oxidative changes in individual hair samples with high precision and specificity. Dr. Dyer showed changes in hair structure with several common cosmetic hair treatments, including bleaching and relaxing. By controlling the strength and rate of reduction of remaining disulfides, the team was able to deduce the location of specific disulfide bonds in the complex hair shaft protein structure and define the location of particular proteins, peptides, and details of hair shaft protein structure. The work highlighted new technology for measuring hair structure and new methods and insight into how common hair treatments affect hair structure.

In the second invited presentation, Dr. John J. Lemasters presented vital imaging of hair follicle metabolism, including viability, mitochondrial activity (mitochondrial membrane potential, $\Delta\Psi$), generation of reactive oxygen species (ROS), and location of acidic secretory vesicles. The lecture highlighted the advantages of new ex vivo imaging technologies, such as multiphoton and light sheet fluorescent microscopy, and how these techniques will lead to new insights into the metabolic requirements for follicular differentiation and formation of the hair shaft. Dr. Lemasters showed the progression of cellular metabolic activity as cells progress and differentiate upward through the follicle germinative matrix in dissected bovine follicles and then showed similar changes in plucked human hairs. The DP was shown to have viable cells with active mitochondria but with relatively low mitochondrial membrane potential and minimal ROS generation. Progressing upward through the follicle bulb cells, germinative epidermal follicular cells showed strong mitochondrial membrane potential and ROS production, culminating in a sharp transition of high mitochondrial membrane potential and ROS generation just before mitochondrial depolarization, loss of viability, and degranulation to form an extracellular hair matrix. The strongest ROS generation occurred in a ring of highly polarized epidermal cells whose depolarization, degranulation, and loss of viability led to formation of the cuticle of the hair shaft, a structure he termed a "ring of fire."

The initial selected talk was given by Dr. Lucien Bildstein regarding how the hair shaft matures during progression through the follicle. Dr. Bildstein leveraged atomic force microscopy coupled with light microscopic histology, infrared spectromicroscopy, and transmission electron microscopy. He reported how microscopic changes in the follicular keratin network correlated with the mechanical properties of the hair shaft. The follicle matrix/hair shaft began to harden within 1 mm of the follicle base, correlated with coalescing of the fiber keratin macrofibrils in terms of their thickening, network orientation, and reduction in mesh size. Further, the development of structure hardness was reduced by the reducing agent dithiothreitol, concurring with the first presentation regarding the importance of disulfide cross-linking in development of hair shaft properties, in this case hardness. Although ample previous knowledge was available on the biological and structural events occurring in the hair follicle, this is the first study of their consequence: the mechanical stiffening of the nascent hair fiber.

Dr. Babu Varghese of Philips Research in The Netherlands presented new ways to measure hair fiber cortex and medulla optical properties with confocal microscopy. He reviewed the physics of light scattering, reflectance, and birefringence and then explained how confocal microscopy could be used to define optical properties of hair. He also presented novel methods using radially polarized illumination to image hair cortex independent of signal from the hair on the orientation of incident polarization. Dr. Varghese reported on both isolated hairs and ex vivo measurement of follicle depth and hair angle and its variation at different locations on facial skin. The new measures added precision over prior measures. Appreciation of the distinct optical properties and physical distribution of facial hair should provide new opportunities to improve laser-enabled modification of hair, including epilation, growth enhancement, and diagnosis.

Finally, Dr. Maria Bovcon from Argentina presented structural changes in hair shaft properties during childhood. Structural information was presented on hair strand morphology via scanning electron microscopy, highlighting variation in the normal sequence of hair development during first 8 years of childhood, either in shape or pattern. The lanugo hair in the first year was characterized by a lack of special distribution of cuticles, whereas the imbricated structures of cuticle cells became very clearly visible and well defined by the age of 4 years Hair cross-sectional area rapidly rose until the age of 4 years and thereafter more gradually. Children's hair is on average finer, rounder, less frequently medullated, and lighter in color than adult hair, considered normal signs of hair immaturity.

New Topics Selected from Abstracts: Part I

Session Directors: Victoria H. Barbosa, Woo-Young Sim and Gillian E. Westgate

This session consisted of nine oral presentations submitted from the abstracts. The first presentation was given by Omer Ibraham, MD, of Cleveland Clinic, Cleveland, Ohio, USA. He spoke on "Treatment of Moderate to Severe Alopecia Areata (AA) with Janus Kinase Inhibitor, Tofacitinib: The Cleveland Clinic Experience." Novel therapies are constantly under study. Eight AA patients treated with the oral JAK inhibitor tofacitinib were reviewed. Seven of the eight patients were female. Disease duration ranged from 3 to 34 years. AA severity ranged from 50–60% scalp involvement to alopecia universalis. One patient ended her treatment after 1 week of therapy because of development of a rash on her trunk and severe peripheral edema in her hands and feet. Two patients prematurely ended treatment after 3 months because of loss of insurance. The remaining five patients currently continue to receive treatment, with cumulative duration of therapy ranging from 1 to 4 months.

Early preliminary results show up to 50% regrowth in one patient after 4 months of treatment and 0–25% regrowth in the remaining patients after 1 week to 2 months of total treatment. Adverse effects included mild increase in creatinine from 0.91 to 1.5 mg/dl (1 patient), rash (1 patient), peripheral edema (1 patient), and upper respiratory infection (2 patients). These patients were presented and suggest a role for JAK inhibition in the treatment of AA.

The second presentation, "Two-Center Open-Label Trial of Oral Tofacitinib in Patients with Severe, Recalcitrant Alopecia Areata," was given by Milene Crispin, MD, of Stanford University School of Medicine, Stanford, California, USA. AA is a common autoimmune disease, with a lifetime risk around 2%. Recent therapeutic insights derive from the discovery that blockade of common signaling pathways downstream of cytokine receptors inhibit established AA. Although treatment of a patient with the JAK3 inhibitor tofacitinib or three patients with the JAK1/2 inhibitor ruxolitinib induced inflammatory remission and hair regrowth, confirmation of efficacy and safety in larger-scale studies is required. Dr. Crispin presented interim results of a two-center, open-label trial of the oral JAK3 inhibitor tofacitinib. The study enrolled

70 patients to undergo treatment with oral tofacitinib at 5 mg twice daily for 3 months. The participants had AA including patch stage, with greater than 50% scalp involvement in 16 (22.8%), totalis in 5 (7.1%), and universalis in 49 (70%). Median age was 37 years, and median length of current episode was 9.6 years. Overall, 45% of patients completed the trial, with significant hair growth over 3 months in 75% of these patients. Responders included those with pretreatment biopsy samples that included inflammatory infiltrates and those with no detectable infiltrates. Nonresponders were more likely to have had alopecia universalis for 20 years or longer. Tofacitinib was well tolerated without significant clinical or laboratory adverse events. The interim results suggest that tofacitinib is a safe and efficacious therapy for the treatment of severe AA.

The third presentation was given by Xingqi Zhang, MD, Sun Yat-sen University, Guangzhou, China, on the topic of "Sequential Cyclic Change of Hair Roots of Dystrophic Anagen Followed by Catagen and Telogen in the Mechanism of Alopecia Areata Incognita Revealed by Dermoscopy." Hair roots were collected by pull test or combing from 23 patients with AA incognita, throughout their hair loss duration, and examined by dermoscopy. Scalpel dermoscopy and histopathology were also carried out. Sequential cyclic changes of hair roots were found; that is, dystrophic anagen effluvium followed by catagen and telogen effluvium, with prominent depigmentation, was also found in hair roots and proximal hair shafts in the later course. The morphology of the hair roots was well correlated to dermoscopy of the hair shafts on the scalp, that is, dystrophic anagen with black dots, catagen/telogen hair roots with broken hairs, and discolored hair shafts.

Histology features of AA incognita at early stages of hair loss with dystrophic anagen effluvium showed prominent acute inflammation and early stages of hair follicle regression; anagen follicles could be seen in the close vicinity of catagen follicles. At the later stage with telogen/exogen hair effluvium, less inflammatory infiltration and increased hair follicle regression were found. The conclusion was that the sequential cyclic staging of shed hairs in patients with AA incognita indicates the insult may be hair cycle-specific and a "one-hit" event, leading to dystrophic anagen release for some follicles, disturbance of pigment production, and subsequent catagen or telogen release for the other follicles, according to the hair cycle stages they are in. Anti-inflammatory management should be instigated early in the disease duration cycle.

The fourth presentation, "P-3074, A New HPCH Topical Formulation for the Treatment of Androgenetic Alopecia in Male Subjects," was given by Francesco Scarci, MD, from Bellinzona, Switzerland. AGA is an androgen-dependent disorder that leads to progressive miniaturization of hair follicles. It depends on an increased rate of conversion of testosterone into dihydrotestosterone (DHT) in scalp, through the action of the 5α-reductase enzyme. Oral finasteride is an effective treatment for AGA, potentially associated with sexual adverse effects. A new topical formulation of finasteride (P-3074), vehicled in hydroxypropyl chitosan (i.e., HPCH) technology, that is able to control the release of finasteride in hair and scalp, minimizing the systemic exposure, has been developed. A pharmacokinetic phase I study, tested P-3074 twice daily versus oral finasteride 1 mg once daily, showing a finasteride systemic exposure 15 times lower in the topical formulation.

A pharmacodynamic study compared P-3074 twice daily and once daily versus oral finasteride 1 mg once daily in DHT inhibition in scalp (vertex) and in serum. The results showed comparable serum/scalp DHT inhibitions across formulations, suggesting that the achievement of comparable levels of DHT inhibition versus the oral form could be attained by a lower dose of P-3074. A following dose-response study evaluated whether P-3074 lower doses could achieve consistent inhibitory effects on scalp DHT, minimizing the systemic effect. At doses up to 200 μl, topically applied P-3074 was shown to significantly and consistently decrease DHT in scalp (comparable to oral finasteride) and only marginally in serum, potentially minimizing the untoward adverse effects linked to a systemic DHT reduction.

The fifth presentation was given by Eric Spengler, MAS, of Living Proof, Cambridge, Massachusetts, USA, on "A Novel Ingredient of Improved Hair Surface." The integrity of hair decreases with weathering. This includes repeated grooming, shampooing, UV exposure, and chemical treatments. Weathering causes gradual damage and ultimate removal of the protective cuticle, exposing the weaker cortex—leading to breakage and loss. The most controllable way to minimize cuticle damage is to protect it and decrease the frequency of shampooing.

Octafluoropentyl methacrylate (OFPMA) is a novel material that possesses lipophobic, hydrophobic, and very low surface energy properties. OFPMA imparts surface protective properties to hair, reducing weathering and extending the interval between shampooings.

A series of experiments were performed to assess OFPMA's interaction with the surface of human hair and its consumer benefits. Time-of-flight secondary ion mass spectrometric and atomic force microscopy were used to assess deposition of OFPMA. OFPMA preferentially deposited at the edge of cuticles, significantly reducing friction. Dynamic vapor sorption experiments on hair treated with OFPMA showed a significant decrease in hysteresis isotherms, indicating a decrease in moisture vapor flux. A cornstarch particulate experiment showed that OFPMA resists accumulation of particulates, helping keep hair cleaner longer. Finally, a consumer use study showed that shampooing frequency decreased and impressions of hair quality improved when an OFPMA product was used. Results show novel and beneficial effects from OFPMA that are not available via current treatments.

The sixth presentation was on the topic of "A Novel Treatment Principle in Anti-hirsutism Management: An Osteopontin-Derived Peptide Potently Inhibits Human Hair Growth in Vitro and in Vivo," given by Marta Bertolini, MD, PhD, of University of Münster. Undesired hair growth (hirsutism, hypertrichosis) can cause major psychological distress. Because only few, and then often unsatisfactory, therapeutic options are currently available, new treatment strategies need to be developed. Because the multifunctional, immunomodulatory glycoprotein osteopontin reportedly is expressed by rat hair follicles only during catagen, the researchers hypothesized that osteopontin-derived fragments may inhibit human hair growth. This hypothesis was tested

using a newly generated, short modified osteopontin-derived peptide (FOL-005). In microdissected organ-cultured human scalp hair follicles, FOL-005 highly reproducibly induced premature hair follicle regression (catagen). This was confirmed in organ-cultured full-thickness human scalp skin from 6−9 subjects, where FOL-005 (15 nmol/L, 150 nmol/L) significantly promoted catagen development, along with increased hair matrix keratinocyte apoptosis.

When human male scalp skin was transplanted onto SCID/beige mice (three 3-mm^2 grafts per mouse) and FOL-005 was injected intracutaneously, this significantly decreased the number of hairs growing per graft compared with vehicle-treated control transplants. Moreover, FOL-005 administration potently counteracted the hair growth-promoting effects of minoxidil, one of the strongest hypertrichosis-inducing agents.

There was no morphological evidence of FOL-005-induced hair follicle toxicity, and a standard battery of toxicological tests showed no overall FOL-005 toxicity. These data identify this osteopontin-derived peptide as a potent, novel inhibitor of human hair growth in vitro and in vivo, which deserves clinical testing as a new treatment principle for excessive hair growth (hirsutism, hypertrichosis).

The seventh presentation was given by Jane Li, MBBS, of The University of Melbourne at The Peter Doherty Institute for Infection and Immunity and Department of Medicine (St. Vincent's Hospital), The University of Melbourne & Department of Dermatology, Epworth Hospital, Melbourne, Australia, on the topic "Alopecia Areata Bulbs Show Significant Transcriptional Abnormalities Before, During and After Active Hair Loss." Anagen bulbs are the primary targets of autoimmune attack in AA. This study investigated the transcriptional profile of AA bulbs during different disease stages. Biopsy samples were collected from AA patients and healthy volunteers, with AA biopsy samples obtained from areas of active hair loss, regrown areas, and previously unaffected areas. We used laser capture microdissection to isolate mRNA specifically from anagen bulbs, then performed PCR with primers targeting immune- and hair-related genes, including all known chemokines. Multiple chemokines were significantly upregulated in active AA compared with healthy control subjects.

A strong correlations in the expression of several chemokine receptor pairs suggested that these chemokines were recruiting immune cells bearing the corresponding receptors. Although the transcription pattern in regrown AA was attenuated compared with active AA, it remained significantly abnormal. This finding implies that permanent changes may persist in regrown AA despite clinical remission, potentially predisposing to future relapse.

Unaffected AA bulbs also showed transcriptional abnormalities compared with those of healthy control subjects, including a relative decrease in CST6 expression. Interestingly, CST6 deficiency is known to cause scarring alopecia in mice. Finally, the researchers identified five genes that were significantly overexpressed in all AA categories: *CCL5, CXCL9, CCL19, HLA-C,* and *CD4*. This core signature supports the existence of an underlying abnormality in AA that is present before overt hair loss.

The eighth presentation was given by Andy Goren, MD, Applied Biology, Inc., Irvine, California, USA, on the topic of "α1-AR Agonist Induced Piloerection Protects against the Development of Traction Alopecia." Traction alopecia affects a large number of women undergoing cosmetic hair procedures, such as blow drying, flat ironing, braiding, ponytails, hair extensions, and repeated brushing. Traction alopecia develops from the force applied to hair follicles during mechanical procedures. Each hair follicle in the human scalp contains an arrector pili muscle that, when contracted, erects the hair. The smooth muscle in the arrector pili expresses α1-adrenergic receptors. As such, we hypothesized that contraction of the arrector pili muscle via an α1-adrenergic receptor agonist would increase the threshold of force required to pluck hair during cosmetic procedures.

Female subjects, ages 18−40 years, were recruited to study the effect of topically applied phenylephrine, a selective α1-adrenergic receptors agonist, on epilation force and hair loss during cosmetic procedures. In our blinded study, 80% of subjects showed reduced hair loss on days they used phenylephrine compared with days using a placebo solution.

The average reduction in hair loss was approximately 44%. In addition, the force threshold required for epilation increased by approximately 172% after topical phenylephrine application. To our knowledge, this is the first study showing the utility of α1-adrenergic receptor agonists in the treatment of traction alopecia and excessive hair loss resulting from cosmetic procedures.

The ninth and final presentation was given by Etienne Wang, MBBS, MA, of Columbia University Medical Centre, New York, New York, USA, on the topic of "Pharmacologic Inhibition of JAK-STAT Signaling Promotes Hair Growth." The JAK-STAT pathway has been implicated in regulation of the immune system but has not been examined in the noninflammatory context in the skin. The researchers' recent studies showed that JAK inhibition can both prevent and reverse disease in murine AA. Unexpectedly, topical treatment with JAK inhibitors resulted in more hair regrowth compared with systemic treatment, suggesting a localized effect of JAK-STAT inhibition on initiation of the hair cycle. That topical JAK-STAT inhibitors in normal mice in telogen resulted in anagen onset within 10 days of treatment was confirmed. This effect was not T-cell dependent.

To define the state of JAK-STAT signaling in the hair follicle, the activation of signaling components Stat3 and Stat5 in stem cell compartments such as the bulge, hair germ, and DP was identified. Functional studies suggest that JAK-STAT inhibition promotes HFSC activity and enhances the inductive capacity of DP in patch assays. To establish relevance in human hair follicles, the grafted human scalp skin was treated with JAK-STAT inhibitors and showed that inhibition of JAK-STAT signaling is sufficient to stimulate human hair growth. JAK-STAT inhibition also resulted in the elongation of the hair shaft in human organ culture assays. The findings suggest that blockade of the JAK-STAT pathway represents a new therapeutic target for the promotion of hair growth.

SATURDAY, NOVEMBER 21, 2015

Genetics, Genomics, and Personalized Medicine/Biomarkers

Session Directors: Angela M. Christiano, Regina C. Betz and Abraham Zlotogorski

The Genetics, Genomics, and Personalized Medicine/Biomarkers session was co-chaired by Drs. Angela Christiano, Regina Betz, and Abraham Zlotogorski and included invited talks from renowned speakers covering a wide range of topics regarding genetics and genetic approaches to hair-related disorders. Dr. Christiano opened the session with a warm introduction of the speakers.

Dr. Regina Betz, Heisenberg Professor for Dermatogenetics at the Institute of Human Genetics at the University of Bonn, presented gene findings of diverse monogenic forms of hypotrichosis to illustrate the current technologies available for diagnosing disease in patients who present with rare, inherited hair pathologies. Identification of these genes, namely *CDSN, LPAR6,* and *SNRPE,* has also lead to deeper understanding of the mechanisms of hair growth and regeneration. In addition, Dr. Betz described a rare autosomal recessive disorder, Bartsocas-Papas syndrome, characterized by alopecia, hypotrichosis, hypogonadism, diabetes mellitus, and intellectual disability associated with mutations in the *C2orf37* gene, which might influence transcription and ribosome biogenesis, thus influencing multiple developmental pathways, including that of the hair follicle.

Dr. Abraham Zlotogorski, the President of the European Hair Research Society and Chair of the Department of Dermatology at the Hadassah—Hebrew University Medical Center of Jerusalem, followed with a talk with a similar theme, this time describing the pitfalls and caveats in the use of next-generation sequencing and modern genetic techniques. He illustrated his points with several case studies, for example, the difficulty in finding mechanistic links to genes uncovered in the genome-wide association studies for AGA. He ended his talk on a positive note, describing how the diagnosis of a gain-of-function *STAT3* mutation in a patient with familial autoimmunity and AA lead to reasoned treatment with tocilizumab, an IL-6 receptor inhibitor, with good results.

Dr. Yuval Ramot, a senior dermatologist in the Department of Dermatology and a researcher in the Center for Genetic Diseases of the Skin and Hair in Hadassah Medical Center, presented familial cases of keratoderma and woolly hair that was surprisingly not associated with a desmosomal mutation and thus were not associated with cardiomyopathy. Whole-exome sequencing and direct sequencing identified a homozygous mutation in *KANK2* in affected patients, and functional studies showed that this mutation allowed for unfettered translocation of a steroid receptor co-activator into the nucleus, causing aberrant activation of transcription. He further described the role of KANK2 in controlling the activation of several nuclear receptors such as vitamin D receptor. He has found that vitamin D-induced transactivation was increased in patients' keratinocytes, supporting the loss of function of KANK2. This discovery suggests a novel pathomechanism of keratoderma and woolly hair and leads clinicians and researchers to consider a reclassification of keratodermas by their pathophysiology rather than their clinical presentation.

The next speaker, Dr. Jiang Chen, Associate Professor in the Departments of Pathology and Dermatology of Stony Brook University School of Medicine, spoke about bench-to-bedside genetic approaches for genodermatoses. He described a mutant mouse model of *Krt75* (K75-N159del) mimicking pseudofolliculitis barbae. Using *in vitro* studies to establish effective siRNAs that knock down the mutant protein in cultured keratinocytes, Dr. Chen showed feasibility for rescuing hair follicle regeneration in a chamber assay, which was maintained in the cycling hair follicle. By using a hair shaft blebbing phenotype that is associated with Krt75 mutation as an example, Dr. Chen attempted to correct the disease by using siRNA-producing cells. siRNA specific to mutant Krt75 was developed and expressed as short hairpin RNAs in keratinocyte progenitor cells isolated from mutant Krt75 mice. These modified cells expressing mutant-Krt75 specific short hairpin RNAs successfully reduced the hair shaft blebbing phenotype in vivo. The implications of his findings suggest the topical siRNA might be a therapeutic option for certain keratinopathies.

This stimulating session was closed with a talk on age-related changes in the skin and hair, specifically in relation to microRNAs (miRNAs). Dr. Natalia Botchkareva's laboratory from the Centre for Skin Sciences of the University of Bradford used global profiling of occipital human hair follicles in young and old men and identified a set of miRNAs that are significantly perturbed with age. Dr. Botchkareva's laboratory performed a thorough identification and validation of relevant miRNAs by using miRNA microarray analysis followed by hair follicle transfection with miRNA mimics to assess the functions. She has found a set of miRNAs that is down-regulated in the older 60+-year-old age group, and miR-17 was among one of the miRNAs that was strongly associated with aging. By using mimics of miR-17 to rescue its down-regulation in the older subjects, she has found the miR-17 mimics protected hair follicles from going into catagen induced by oxidative stress. Gene expression studies showed that miR-17 mimics decreased the expression of genes found in the hair follicles of older subjects (*BMP2, Oxr1,* and *Map3k8*) and genes with antiproliferative properties (*E2F1* and *p21*). This study suggested an epigenetic control of hair follicle aging via miR-17.

Of interest, miR-17 was found to be down-regulated with age, and its target genes include quiescence factor *BMP2*, which is consistent with the finding that BMPs appear to be more abundant in aged skin. Thus, low miR-17 levels might serve as a marker for aging skin or suggest premature aging in other individuals with unexplained hair loss or thinning.

Clinical Trials

Session Directors: Jerry Shapiro and Valerie D. Callender

The clinical trials session comprised five talks, with two invited speakers and three oral abstract presenters.

Dr. Rolf Hoffman, Professor, Replicel Life Sciences, Inc., Vancouver, British Columbia, Canada, spoke regarding the unique characteristics of self-renewal abilities within the hair follicle. The hair follicle is known as a reservoir of "multipotent cells. The dermal compartment of the hair follicle comprises DP, a small aggregation of mesenchymal derived cells at the base of the hair follicle bulb, and dermal sheath (DS), which surrounds the bulb and envelops the hair follicle.

A specialized group of DS cells that localize at the base of the bulb and supports the growth of the DP is termed the dermal sheath cup (DSC)."[e] Because of their unique properties, hair follicle-derived cells may be ideal candidates to treat human diseases that are the result of a cell deficit. In the lecture, current developments for pattern baldness, skin aging, and tendon injuries were presented.

Won Soo Lee, MD, PhD, of Yonsei Wonju University, Wonju, Korea, reported that although there had been promising reports on successful use of JAK inhibitors in AA, maintenance of hair growth and relapse rates after cessation of therapy should be monitored carefully. Superficial cryotherapy is a simple, noninvasive, convenient method that has relatively good therapeutic response with fewer adverse effects. It could be recommended as an alternative or first-line therapy for the treatment of a mild form of AA. Low-dose DPCP elicits subclinical or mild sensitization in the alopecia patches that could still sustain sufficient effectiveness and eventually promote hair growth. There are some differences in depth of quality of life between AA and AGA. In both conditions, a younger age group is more emotionally affected. Proper treatment of the alopecia condition could increase quality of life for patients with both diseases.

One of the oral abstract speakers was Dr. Yuzuf Yazici, Chief Medical Officer of Samumed, LLC, and Assistant Professor at New York University School of Medicine Department of Rheumatology. He discussed the molecule SMO4554 and presented data showing it may have great potential in regrowing hair via the Wnt pathway. A phase I clinical trial showed that there were very few adverse effects after 28 days and that there was a statistically significant increase in hair growth and slowed hair loss at a concentration of 0.125% compared with placebo. Another abstract speaker was Dr. Gail Naughton, of Histogen, Inc., who spoke about using hair stimulating complex, a human cell-derived formulation. This involves intradermal injections of hair stimulating complex and showed an increase in hair shaft width and density with hair stimulating complex compared with placebo. Multiple injections showed greater increases in hair shaft width and density even after 48 weeks. Further clinical trials are necessary to show efficacy and safety.

Dr. Rodney Sinclair, Professor of Dermatology at University of Melbourne, Melbourne, Australia, gave a compelling talk on the use of oral minoxidil at 0.25 mg daily and spironolactone 25 mg daily and showed significant hair growth with these oral agents in FPHL in an open study for 12 months. Adverse effects were minimal.

New Topics Selected from Abstracts: Part II
Session Directors: Lloyd E. King and Ryoji Tsuboi

Dr. Yuval Ramot of Hadassah Medical Center, Jerusalem, Israel, presented the studies performed in collaboration with Prof. Ralf Paus' group on how polyamines modulate the hair follicle and its stem cells to prolong anagen. A stable polyamine analog, N^1-methylspermidine (N^1-MeSpd) was tested in organ-cultured human scalp hair follicles and a human keratinocyte cell line to detect changes related to anagen growth. N^1-MeSpd induced expression of the epithelial stem cell-associated keratin-15. It decreased keratinocyte cell lysis and death and the expression of two peripheral clock core genes related to catagen induction. It also reduced intracellular ROS production and TNF-α gene and protein expression after lipopolysaccharide stimulation. These results suggest that the anagen-promoting effects of polyamines mediate interaction or combined effects of antioxidative, anti-inflammatory, and peripheral clock-related mechanisms to regulate human hair growth.

Dr. Ji won Oh of University of California Irvine, California, USA, discussed a biological dynamics system to study mouse hair follicle patterning. Cyclic growth of each individual hair follicle is regulated by signaling interactions with neighboring hair follicles and other cells in biologically complex interactions. These signaling interactions define the microenvironmental networking and long-range signals between neighboring hair follicles and other skin cells to create the macroenvironmental networks. A pattern analysis of hair morphogenesis in the first two hair cycles detected an unrecognized spatiotemporal complexity with rapid growth patterning and unknown biological interactions between anatomically distinct hair follicle populations at the onset of second anagen cycle. A three-dimensional mathematical model based on coupling activator and inhibitor signals captured critical features of hair follicle growth including cyclic hair follicle growth and communication among a population of hair follicles. To analyze dynamic properties of each hair follicle domain, the phase separation mechanism and interactions among different hair follicle domains were identified based on their geometric vicinity. This biological dynamics approach provides new insights into complexities of the mouse hair growth patterns.

Dr. Kevin McElwee of University of British Columbia, Vancouver, British Columbia, Canada, described studies to examine if IP of hair follicle mesenchymal cells could affect survival of transplanted islet allograft survival. IP-related gene and protein expression against nonfollicular fibroblasts using cultured DP, dermal sheath cup cells, and non-bulbar dermal sheath cells. The IP effects of dermal sheath cup cells documented a significant effect on islet cell allografts as measured by mRNA H2d (major histocompatibility complex class II) expression, inhibin A mRNA, and protein. The dermal sheath cup cells expressed higher Fgf2 and BMP6 supportive of islet cell survival. Mouse islets co-transplanted with syngeneic hair follicle dermal sheath cup cells or fibroblasts under the kidney capsule of immune-competent, streptozotocin-induced, diabetic C57BL/6J recipients survived significantly longer than the fibroblast control group, had increased blood vessel formation, and lower inflammatory cell infiltration. Renal lymph nodes of transplanted dermal sheath cup cell mice had higher frequencies of $CD4^+Foxp3^+$ and $CD25^+Foxp3^+$ cells but lower $CD8^+CD69^+$ effector cell percentages than control mice. This study indicates that the IP of cultured hair follicle-derived mesenchymal cells can improve islet allograft survival.

Dr. Eddy Hsi Chun Wang of Columbia University, New York, New York, USA, studied DNA methylation profile of

[e] International League of Dermatological Societies, 2015 World Congress of Dermatology, June 2015, Vancouver, Canada, Poster presentation, "Clinical Potential of Hair-Follicle Derived Mesenchymal Cells in Cell Therapy: Multiple Therapeutic Applications," Kevin McElwee[1], Rainer Marksteiner[2], Birte Magnus[2], Darrell Panich[1], David Hall[1], Hisae Nakamura[1], Rolf Hoffmann[1]. [1]Replicel Life Sciences Inc., Vancouver, BC, Canada. [2]InnovacellBiotechnologieAG, Innsbruck, Austria.

monozygotic (MZ) and dizygotic (DZ) twins discordant for AA. Previous epidemiological and genetic studies show a 55% concordance rate among MZ twins, suggesting that epigenetic factors are involved in AA manifestations. Methylation assays on MZ and DZ twins discordant for AA detected several differences with scarce overlap between sites differentially methylated in MZ compared with DZ. The DNA of AA siblings was mostly hypermethylated in MZ but hypomethylated in DZ. The distributions of hypermethylated sites in MZ AA were also overrepresented in promoter regions of the nearest genes, consistent with a transcriptionally repressive state. Gene ontology analysis showed dysregulation of biological processes such as limb morphogenesis and antigen presentation in MZ (neural/sensory process in DZ). Interrogation of gene expression profiles of an independent AA cohort with the genes were differentially methylated in MZ twins and showed an increase of HLA-DRB1 expression consistent with decreased methylation. HLA-DRB1 dysregulation is associated with autoimmune diseases such as rheumatoid arthritis and multiple sclerosis, affecting antigen presentation. These results indicate that differential DNA methylation status and epigenetic factors may influence expression of susceptibility genes and contribute to AA pathogenesis.

Dr. Iain Haslam of University of Manchester, UK, presented. His studies focused on protecting against chemotherapy-induced alopecia (CIA), a psychologically traumatic adverse effect. CIA is thought to be due to the pan-antimitotic toxicity of chemotherapeutic agents, and previous work has emphasized the critical role of p53-mediated apoptosis in CIA. Recent work on feather follicles showed that a sonic hedgehog (Shh)-dependent mechanism also contributes to chemotherapy-induced tissue damage. The study sought to define the role(s) of the Shh pathway in CIA in mice and humans and began with whole-genome expression profiling. In the best-studied murine CIA model (C57BL/6 mice), Shh signaling is significantly down-regulated 24 hours after treatment. Disruption of Shh signaling by cyclopamine recapitulated key features of CIA, whereas exogenous Shh protein partially prevented it. Using organ-cultured human hair follicles supplemented with chemotherapeutic agents (4-hydroxycyclophosphamide and doxorubicin), Shh pathway genes were down-regulated. Quantitative real-time reverse transcriptase—PCR analysis of plucked hair follicles from chemotherapy-treated patients consistently showed down-regulation of Shh gene expression in response to several chemotherapy regimens. These studies suggest that down-regulation of Shh signaling is a common pathomechanism of chemotherapy-induced adverse effects such as hemocystitis, infertility, memory loss, and gastrointestinal syndrome and may become an important novel target for future treatments.

Dr. Desmond Tobin of University of Bradford, Bradford, UK, reported on progress to identify immunogenic targets on the anagen hair follicle in AA. Evidence from the literature suggests that some AA patients with celiac disease have poorer outcomes but benefit from treatment for their celiac disease. A recent genome-wide association study that identified AA-associated risk loci in common with celiac disease (which can exhibit skin disease associations), together with the striking epithelial relatedness between skin and gut, prompted him to explore whether AA and celiac disease may share cross-reacting epitopes (i.e., on hair follicle and gluten/gliadin). Dr. Tobin observed that specific antibody raised to a celiac disease-associated immunogenic target epitope expressed in the gluten-associated peptide α-gliadin could react specifically with the inner root sheath of human scalp anagen hair follicle, in a pattern that co-localized with the inner root sheath-associated structural protein trichohyalin. Moreover, antibody to the deamidated gliadin peptide reacted with human anagen hair follicle protein by immunoblotting. Together with their previous identification of trichohyalin (or a constituent cross-reacting/mimic epitope) as a potential immunodominant antigen in AA, these current findings further support the view that the inner root sheath may be a target for the immune response to early anagen hair follicles in AA.

Dr. Paul Bigliardi of A*STAR Institute of Medical Biology, Singapore, indicated that AGA is common in Singapore and that understanding of its pathophysiology is needed. Therefore, his group designed a study with 20 male subjects with AGA and 10 control subjects without AGA. The vertex and occipital region of each subject was precisely photodocumented, then FUEs were taken, and the extracted FUEs were photodocumented and dissected into papillar region, intermediate region with sebaceous glands, and infundibulum/ostium area. The main objectives were to compare morphological differences of follicular units in AGA and normal control subjects in macro- and microphotographs taken from the vertex and occipital regions, perform full RNA sequencing of the papillae of FUEs, do extensive bioinformatics analysis by Cuffdiff (Trapnell Lab, Seattle, WA) of RNA sequencing and correlate results to clinical and morphological parameters. The RNA sequencing results showed very interesting and statistically significant differences and similarities in the vertex regions from AGA patients and healthy volunteers, with clustering of genes in the miniaturized hair from AGA patients and normal hair follicles from healthy volunteers. The gene expression from hair papilla from the occipital region from healthy volunteers and AGA patients was not significantly different. The results document that this translational approach integrating clinicomorphological information can reveal new pathophysiological pathways.

Dr. Carlos Clavel of A*STAR Institute of Medical Biology, Singapore, spoke on "BMP Signaling and Sox2 in the Dermal Papilla Regulates the Hair Follicle Stem Cell Niche." The hair follicle McSC niche is the main melanocyte reservoir in the skin, and a better understanding of the mechanisms regulating pigmentation is critical for designing novel therapy strategies for pigmentation disorders, which affect 1 in 3 people worldwide. How DP regulates the HFSC niches remains unclear. Using novel genetic tools to study DP cells in the HFSC niche, Dr. Clavel recently showed how in the DP compartment the gene *Sox2* is a key regulator of hair growth by controlling the BMP-mediated mesenchymal-epithelial crosstalk between the DP niche cells and the stem cell progeny. Now, the group has identified a pigment switch in the pelage of DP-specific *Sox2* knockdown mice and observed abnormal BMP cell signaling within the melanocyte compartment of the hair follicle. This phenotype suggests that Sox2 is also a master regulator of the melanocyte SC niche. In addition, new preliminary data in human skin biopsy samples indicates the presence of active BMP signaling at the McSC compartment within the hair

follicle. Finally, the group's novel in vitro data show BMP regulation of melanogenesis, melanin transfer, and migration in human melanocytes and keratinocyte co-cultures.

Dr. Evgeniya Schastnaya of Insilico Medicine, Inc., Baltimore, Maryland, USA, presented new candidate drugs for the treatment of AGA that were identified by analyzing the activation of signaling pathways. She used gene expression samples of DP cells from balding and nonbalding regions of men's scalps to analyze pathway dysregulation during the development of AGA. To explore signaling pathway regulation during balding, she used the GeroScope platform, based on the OncoFinder algorithm used in personalized medicine in oncology. She evaluated the activity of over 18,000 compounds for their ability to mimic signaling pathway activation profiles characteristics of nonbalding regions of the scalp to shortlist potential drug candidates, and the candidate molecules were cross-referenced with the recently launched Geroprotectors.org database. She showed that signaling pathway activation analysis is a promising method of drug discovery for hair disorders.

Closing Ceremony

Session Directors: Wilma F. Bergfeld, Angela M. Christiano and Maria K. Hordinsky

The Closing Ceremony was the last session of an exceptional and informative hair research meeting with global representation and enlightenment. The theme of *Reflect, Regenerate, and Restore* was explored most successfully by the international presenters and included both clinical and research advancements. Acknowledgements went to the Session Directors, Co-directors, Invited Speakers, and all those who prepared and presented oral and poster abstracts. Special thanks was given to Dr. Antonella Tosti and her fellows for organizing the Live Patient Viewing Session and to the Abstract Awards Committee (Rodney Sinclair, Australia; Claire Higgins, UK; Valerie Horsley, USA).

The Abstract Awards committee awarded the following awards for three Oral Presentations and three Poster Presentations.

ORAL PRESENTATION AWARDS

Inducing Hair Follicle Organogenesis with Defined Protein Factors

Sung-Jan Lin, MD, PhD, National Taiwan University

Sabrina Mai-Yi Fan[1], Chia-Feng Tsai[2], Chien-Mei Yen[1], Su-Hua Pan[1], Yu-Ju Chen[2] and Sung-Jan Lin[1]

[1]*National Taiwan University, Taipei, Taiwan and* [2]*Academia Sinica, Taipei, Taiwan*

Abstract

Hair follicle neogenesis depends on the initiation and perpetuation of cross-talk between keratinocytes and dermal cells. When skin is injured, it is usually repaired with fibrosis except in embryos, that exhibit scar-less healing with formation of new hair follicles. The researchers asked whether similar neogenesis of hair follicles can be reinitiated in postnatal life.

Their findings were that the protein extract from embryonic skin of specific developmental stage was able to induce hair follicle neogenesis both in a full thickness wound and a modified patch assay in mice without the help of inductive mesenchymal cells. Hair follicle organogenesis here was mediated mainly through the effect on fibroblasts. When adult fibroblasts, but not keratinocytes, were cultured with the protein extract, they were conferred the ability to induce new hair follicles.

In search for the molecular mechanisms involved through phosphoproteomic analysis, it was found that insulin/insulin growth factor signaling was activated and required for the hair follicle inductivity in adult fibroblasts. Through proteomics analysis with mass spectrometry, the identification of 3 extracellular proteins enriched in embryonic skin that together, were required and sufficient to induce hair follicle neogenesis in vivo. Therefore, hair follicle regeneration could be initiated by creating a pro-regeneration environment with defined extracellular factors enriched in the developmental stages. The researchers concluded that the identification of such environmental signals can be incorporated with other approaches to enhance hair follicle regeneration.

Two-Center Open-Label Trial of Oral Tofacitinib in Patients with Severe, Recalcitrant Alopecia Areata

Milene Crispin, MD, Stanford University

Milene Crispin[1], Brittany G. Craiglow[2], Justin Ko[1], Anthony E. Oro[2] and Brett King[2]

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Abstract

Alopecia areata (AA) is a common autoimmune disease, with a lifetime risk around 2%. Recent therapeutic insights derive from the discovery that blockade of common signaling pathways downstream of cytokine receptors inhibit established AA. While treatment of a patient with the JAK3 inhibitor tofacitinib or three patients with the JAK1/2 inhibitor ruxolitinib induced inflammatory remission and hair regrowth, confirmation of efficacy and safety in larger scale studies is required.

Interim results were presented from two-center, open-label trial of the oral JAK3 inhibitor tofacitinib. 70 patients were enrolled to undergo treatment with oral tofacitinib 5 mg twice daily for three months. The participants had AA including patch stage with >50% scalp involvement in 16 (22.8%), totalis in 5 (7.1%), and universalis in 49 (70%). Median age was 37 years and median current episode was 9.6 years.

45% of patients completed the trial, with significant hair growth over three months in 75% of these patients. Responders included those with pre-treatment biopsies that included inflammatory infiltrates as well as those with no detectable infiltrates. Non-responders were more likely to have had alopecia universalis for twenty years or longer. Tofacitinib was well-tolerated without significant clinical or laboratory adverse events. Our interim results suggest tofacitinib is a safe and efficacious therapy for the treatment of severe AA.

Alopecia Areata Bulbs Show Significant Transcriptional Abnormalities Before, During and After Active Hair Loss

Jane Li, MBBS, The University of Melbourne

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Abstract

Anagen bulbs are the primary targets of autoimmune attack in alopecia areata (AA). In this study, we investigated the transcriptional profile of AA bulbs during different disease stages. Biopsies were collected from AA and healthy volunteers, with AA biopsies obtained from areas of active hair loss, regrown areas, and previously unaffected areas. A laser capture microdissection was used to isolate mRNA specifically from anagen bulbs, then a PCR with primers was performed targeting immune- and hair-related genes, including all known chemokines. Multiple chemokines were found to be significantly upregulated in active AA compared to normal controls. Furthermore, strong correlations were observed in the expression of several chemokine-receptor pairs, suggesting that these chemokines were recruiting immune cells bearing the corresponding receptors. Although the transcription pattern in regrown AA was attenuated compared to active AA, it remained significantly abnormal.

This finding implies that permanent changes may persist in regrown AA despite clinical remission, potentially predisposing to future relapse. Unaffected AA bulbs also showed transcriptional abnormalities compared to normal controls, including a relative decrease in CST6 expression. Interestingly, CST6 deficiency is known to cause scarring alopecia in mice. Finally, 5 genes were idenitifed that were significantly overexpressed in all AA categories: CCL5, CXCL9, CCL19, HLA-C and CD4. This "core signature" supports the existence of an underlying abnormality in AA that is present before overt hair loss.

POSTER PRESENTATION AWARDS

The Development of a Genetic Approach to Suppress an Inheritable Structure Defect of the Hair

Jiang Chen, MD, Stony Brook University

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Abstract

Genetic mutations are responsible for a number of inheritable hair disorders characterized by structural defects in the hair. It is unknown whether correcting genetic mutations is sufficient to suppress related hair phonotypes in vivo.

In this study, we examined whether it is feasible to suppress a hair shaft blebbing phenotype associated with the expression of a dominant mutant form of Krt75. First, allele-specific siRNAs that are capable of silencing the mutant, but not wild-type, Krt75 were developed. Subsequently, mutant Krt75-specific shRNA was expressed in epidermal keratinocyte progenitor cells isolated from mutant Krt75 mice.

These genetically modified mutant cells were then used to regenerate new hair follicles in vivo. Hair formed with these genetically modified mutant keratinocyte progenitor cells developed a significantly reduced number of defective hair shafts in comparison to controls. Moreover, phenotypic improvement was associated with suppressed expression of mutant Krt75 in reconstituted skin grafts. Data obtained from this study provided proof-of-concept that inheritable hair structural defects may be suppressed through genetic manipulation.

A Stable Polyamine Analogue, N^1-methylspermidine, Prolongs Anagen and Regulates Human Hair Follicle Stem Cells via Anti-oxidative, Anti-inflammatory, and Peripheral Clock-Related Mechanisms

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Abstract

Polyamines are instrumental for hair follicle growth and function. However, they are readily interconvertible and physiologically unstable because they can be quickly metabolized. Therefore, a metabolically-stable polyamine was used, the spermidine analogue N^1-methylspermidine (N^1-MeSpd), to assess its functional effects on hair follicles, using microdissected, organ-cultured human scalp hair follicles as a clinically relevant assay system. Potential effects were further dissected in a human keratinocyte cell line (NCTC-2544). First, we confirmed that N^1-MeSpd is a stable compound, with a half-life of 90 hours. 0.5 μM N1-MeSpd had a strong anagen-prolonging effect on hair follicles after 6 days in culture, accompanied with increased expression of the epithelial stem cell-associated keratin, K15. Furthermore, N^1-MeSpd decreased lactate dehydrogenase activity in the culture supernatant, a parameter of cell death and cell lysis.

It was shown that N^1-MeSpd decreased the mRNA and protein expression of *PER1* and mRNA expression of *CLOCK*, two peripheral clock core genes that are associated with catagen induction. Gene and protein expression of MTCO1, a subunit of respiratory chain complex IV, were decreased after N^1-MeSpd application, in addition to reduced intracellular reactive oxygen species production in cultured keratinocytes. N^1-MeSpd also reduced TNF-α gene and protein expression after lipopolysaccharide stimulation.

Taken together, these results suggest that the anagen-promoting effects of N^1-MeSpd on hair follicles are mediated by a combined effect of antioxidative, anti-inflammatory and peripheral clock-related mechanisms.

Activin A Is Overexpressed in Three-Dimensional (3D) Cultured Human DP Spheres and Affects Hair Inductive Potency of Neonatal Mouse Dermal Cells

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Abstract

Acquisition of potent human dermal papilla (DP) cells which can induce hair follicle neogenesis is an overarching concern and various approaches have been accordingly attempted to solve the concern. As a way to acquiring hair-inducing DP cells, we have previously applied three-dimensional (3D) culturing method.

It was observed de novo formation of hair follicles when conducting patch hair reconstitution assay using 3D cultured DP spheres with mouse epidermal cells. Stepping further, in this study, we focused on the secretory proteins from DP spheres. Activin A, the most up-regulated protein in DP spheres, has been selected for further study assuming that overexpressed Activin A by sphere formation might bind to its receptors on mouse epidermal cells resulting in successful hair induction using DP spheres.

As the expression of Activin A was observed in neonatal mouse dermal cells and its receptor in mouse epidermal cells, a patch assay was performed using Activin A knock-down mouse dermal cells in combination with mouse epidermal cells. The results of our patch assays showed that the Activin A knockdown mouse dermal cells are severely impaired in hair follicle neogenesis. Consequently, it was demonstrated that Activin A affects hair induction potency of mouse dermal cells.

GRANTS AND AWARDS

Travel Grants for Young Investigators

North American Hair Research Society presented the following:

1. Marta Bertolini, PhD, University of Münster
2. Thomas Chu, MD, Far Eastern Memorial Hospital
3. Milene Crispin, MD, Stanford University
4. Omer Ibrahim, MD, Cleveland Clinic
5. Karzan Khidhir, MD, University of Sulaimani
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8. Liye Suo, MD, Baylor College of Medicine
9. Nikki Tang, MD, Mount Sinai Beth Israel
10. Dorota Zalitacz, MD, Jagiellonian University

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1. Jane Li, MBBS, The University of Melbourne

European Hair Research Society

1. Serena Buscone, PhD, Bradford University
2. Varvara Kanti, MD, Charité — Universitätsmedizin Berlin
3. Anna Lyakhovitsky, MD, Tel Aviv University, The Sackler School of Medicine
4. Helena Topouzi, Imperial College London

The Korean Hair Research Society

1. Hee-Chul Chung, MD, Yonsei University, Wonju College of Medicine
2. Yong Hyun Jang, MD, PhD, Yonsei University, Wonju College of Medicine
3. Kwan Ho Jeong, MS, Catholic University of Korea, St. Paul's Hospital
4. Hong Jin Joo, MD, St. Paul's Hospital
5. Jin Yong Kim, Seoul National University, College of Medicine

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CONCLUDING REMARKS

This 4-day meeting provided comprehensive topic areas in which colleagues had the opportunity to present new research, share experiences, and discuss new directions for the advancement of knowledge in hair growth, hair and scalp disease, and clinical care. Discussions occurred in the lecture halls and collaborations during the social activities including the Welcome Reception held on the first evening after the Opening Ceremony and the Congress Dinner held on Friday evening. A unique "Coffee with the Experts" session occurred on Saturday morning, during which 27 specialty topics were each assigned to a banquet table with one or two experts facilitating small group discussions. The small group format

allowed for a varied learning environment. A robust scientific poster hall included nearly 250 posters, with formal poster sessions occurring on both Thursday and Friday mornings. An exhibits hall included 31 companies displaying their products and services. In addition, five related nonprofit organizations displayed information about their subject matter at nearby table top displays, including Alopecia UK, Cicatricial Alopecia Research Foundation, International Society of Hair Restoration Surgery, National Alopecia Areata Foundation, and World Trichology Society. The North American Hair Research Society is grateful to the many faculty and participants and also to the many corporate supporters who assisted with monetary donations, educational grants, and in-kind support.

CONFLICT OF INTEREST
AMC is a consultant for Aclaris Therapeutics, Inc. MKH serves as Chair, Clinical Research Advisory Council of the National Alopecia Areata Foundation. MKH is also a consultant for Procter & Gamble. The authors state no other conflict of interest.

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ABSTRACTS AND INFORMATION
Abstracts of oral and poster presentations, meeting photos, and highlights video are located at: http://www.hair2015.org/.

APPENDIX

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