From Targets to Treatments: Bridging Autoimmune Research to Advance Understanding of Alopecia Areata

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Alopecia areata is a common autoimmune skin disease resulting in the loss of hair on the scalp and elsewhere on the body that affects over 146 million people worldwide at some point in their lives. Founded in 1981, the National Alopecia Areata Foundation (NAAF) is a nonprofit organization that supports research to find a cure or acceptable treatment for alopecia areata, supports those with the disease, and educates the public about alopecia areata. NAAF conducts research summits every 2 years that are central to achieving the goals of a major strategic initiative, the Alopecia Areata Treatment Development Program, which are: to accelerate progress toward a safe, effective, affordable treatment or a cure for alopecia areata. These summits have played a key role in transforming the understanding of alopecia areata from largely inflammatory and dermatological perspectives to a focus on the genetic and immunological factors that are now recognized as driving and active determinants of the disease process.

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Significant research progress occurred in 2014, offering new leads that are driving current research efforts related to alopecia areata. The fifth Alopecia Areata Research Summit since 2008, *From Targets to Treatments: Bridging Autoimmune Research to Advance Understanding of Alopecia Areata*, brought together leading experts with new investigative partners to discuss exciting recent discoveries and identify opportunities to further advance alopecia areata research. This meeting, held on 4–5 December in Bethesda, Maryland, represented a pivotal moment for alopecia areata research and treatment development with early stage clinical trials of drugs targeting autoimmune pathways showing promising hair regrowth for the first time ever. Among the 90 participants were representatives from five different branches of the National Institutes of Health (NIH), the US Food and Drug Administration (FDA), the Patient-Centered Outcomes Research Institute (PCORI), and several biopharmaceutical companies with relevant clinical initiatives, as well as experts in the fields of hair and skin disease research, clinical care, basic science, immunology, and autoimmunity from more than 35 academic institutions and research centers around the globe, and representatives of the patient community that any potential treatment would be designed to serve.

The three summit co-chairs, Drs David Norris, Julian Mackay-Wiggan, and Jeffrey Frelinger, worked together to organize a packed program focused on (1) autoimmune and immunological aspects of alopecia areata; (2) recent genetic developments and new therapeutic targets; (3) emerging animal models; (4) new research technologies and directions; and (5) clinical aspects, epidemiology and tools to advance research.

Meeting Summary

Presentations with asterisks (*) are published in these symposium proceedings.

Immunology and autoimmunity

Presentation Highlights

- Dr Raphael Clynes, Group Medical Director at Bristol-Myers Squibb, presented research on the identification and targeting of cytotoxic CD8 T cells in alopecia areata.
- Dr Marta Bertolini*, from the University of Münster in Germany, discussed an ongoing project to characterize the clonotypes of autoagressive CD8 T cells that invade the hair follicles in alopecia areata. She discussed the identification of CD8 T cells *in situ* in the hair follicle using laser capture microdissection technology.
- Dr John Harris, from the University of Massachusetts, shared the commonalities between alopecia areata and vitiligo, including interferon-gamma (IFNγ), CXCL9, CXCL10, and CXCL11 chemokine gene expression. He noted that similarities in pathogenesis of vitiligo and alopecia areata could lead to potential treatments.
- Dr Dan Kaplan, from the University of Minnesota, examined the diversity and phenotype of dendritic cell subsets found in the skin and their potential role(s) in alopecia areata.

Future Research Priorities

- Continue research to advance understanding of mechanisms of disease, including roles of dendritic cells, antigen-presenting cells, macrophages, and early innate immune response,

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which could lead to discoveries for early intervention and prevention.
- Identify TCRs and the antigens/epitopes they recognize that are driving the disease. These could be used as predictive markers or as targets for antigen-specific therapeutics.
- Begin collecting samples (white blood cells, peripheral blood mononuclear cells, and swabs) useful for functional and genetic assays for the Alopecia Areata Registry, Biobank, and Clinical Trials Network (the Registry). Work with the Immune Tolerance Network to develop standardized protocols for freezing and banking samples.
- Investigate the potential use of antibodies, cytokines, or other molecules to serve as predictive biomarkers for alopecia areata and correlate T-cell responses with therapeutic responses.
- Study regulatory T cells for potential use as predictors of hair regrowth or response to therapy.
- Investigate the role of chemokines regulating autoreactive homing in alopecia areata.
- Further study the relationship between other autoimmune diseases and alopecia areata.
- Further study the mechanistic relationship between thyroid dysfunction and thyroiditis and alopecia areata.
- Investigate the role of inflammasomes in alopecia areata.

Genetic developments and therapeutic targets

Presentation Highlights
- Dr Angela Christiano, from Columbia University, provided an update on Genome-wide Association Studies (GWASs) in alopecia areata, including resolution of HLA signal, identification of new candidate GWAS loci, and regulation of autophagy, JAK (Janus kinase)-STAT signaling, and regulatory T cells.
- Dr Ali Jabbari, from Columbia University, shared preliminary results from a comprehensive gene expression profile of skin and blood from patients with alopecia areata and the results correlate with disease severity. Data suggest that alopecia universalis and alopecia totalis are extreme manifestations of patchy alopecia areata at the gene expression level suggesting a single pathogenic mechanism.
- Dr Rick Kittles, from the University of Arizona, presented the use of genetic ancestry in disease studies, including genetic factors driving differences in disease susceptibility across populations and the use of Ancestry-Informative Markers in the design of genetic studies.
- Dr Thomas Waldmann, Chief of the Lymphoid Malignancies Branch at the National Cancer Institute, discussed JAK1-selective inhibitors and their role as a potential therapeutic agent for alopecia areata.
- Dr Massimo Gadina, Director of the Office of Science and Technology at National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS), reviewed studies of JAK inhibitors in autoimmune and autoinflammatory diseases, and related drugs now in development.
- Dr Aziz Ghahary, from the University of British Columbia, presented a new cell therapy using indoleamine 2,3 dioxygenase (IDO)-expressing fibroblasts to suppress the immune cells attacking hair follicles, resulting in regrowth of the hairs.
- Dr Robert Gensure*, from Albert Einstein College of Medicine, shared results of a trial of skin-targeted parathyroid hormone agonists and antagonists in C3H/HeJ-engrafted mice, including effects on hair growth, immune response, and induction of the hair cycle.
- Dr Bill Levis*, from New York and Rockefeller Universities, discussed a new delivery of diphencyprone (DPCP) coupled with interfering RNA targets in alopecia areata skin, enhancing DPCP efficacy and response rates.
- Dr Scott Kachlany*, from Rutgers School of Dental Medicine and Actinobac Biomed, Inc., presented an experimental biologic that specifically targets active lymphocyte function associated antigen-1 on hyper-reactive immune cells. Leukothera (Lekotoxin) is able to rapidly deplete activated immune white blood cells that are involved in autoimmune and inflammatory conditions such as alopecia areata, resulting in relief of disease symptoms.

Future Research Priorities
- Focus on expanding the Registry to 10,000+ DNA samples for deep sequence analysis to identify new candidate genes and variants and determine the downstream impact.
- Study the epigenetics of alopecia areata and investigate the role of race and ethnicity.
- Undertake additional biomarker studies and determine a genotype risk score.
- Study gene expression of patchy alopecia areata and investigate parallels between regional beta cell destruction in Type 1 diabetes.
- Investigate the role of epigenetics, environment, and triggers in discordant twins.
- Study the role of CD4 T cells in alopecia areata.
- Collaborate with international databases/repositories for the use of samples from diverse populations.
- Apply to NIH for funding of large-scale genome sequencing studies.

Animal models

Presentation Highlights
- Dr Michael Brehm*, from the University of Massachusetts, provided an overview of humanized mouse models that are currently available to study human immunobiology, discussing the advantages and limitations of each model and the possibility of future models for personalized medicine using individual samples.
- Dr Lishan Su*, from the University of North Carolina, presented an update of humanized mouse models used in the study of human immunology, the recent progress in studying persistent human virus infections, and the goal of generating disease-specific and patient-specific disease models.
- Dr John Sundberg*, from The Jackson Laboratory, shared the International Knockout Mouse Project to inactivate all known protein-coding genes and analyze skin phenotypes in individual gene knockouts. Systematically screening mice generated at The Jackson Laboratory and the Wellcome Trust Sanger Institute for skin diseases will provide an enormous resource for dermatological research, including models for alopecia areata.

Future Research Priorities

- Expand research to identify better humanized animal models to minimize graft-versus-host disease as current models are often difficult to reproduce.
- Develop models that use induced pluripotent stem cells from patients to perform drug screening.
- Facilitate contracts between academics and industry and the coordination of resources.
- Develop standardized protocols for more reproducibility and begin pilot studies in basic and preclinical trials.

New technologies and directions

Presentation Highlights

- Dr Vladimir Botchkarev*, from the University of Bradford in the United Kingdom, shared how epigenetic mechanisms have an important role in the control of the skin and hair follicle. Knowledge about how epigenetic mechanisms are involved in the pathogenesis of alopecia areata could lead to understanding how to activate good genes and silence bad genes in skin cells.
- Dr Lita Proctor* (authored by Dr Jeffrey Frelinger), Director of the Human Microbiome Project at the National Human Genome Research Institute, discussed the human microbiome and its role in autoimmune diseases. The microbiome is an integral, normal, and necessary part of human physiology, and new research suggests a loss in microbiome diversity is associated with an increase in autoimmune diseases.
- Dr Annemieke de Jong, from Columbia University, explored the use of Next-Generation TCR sequencing in alopecia areata mice and humans to gain insight in the dynamics of the T-cell repertoire during alopecia areata pathogenesis.
- Dr Heather Hickman, Staff Scientist at the National Institute of Allergy and Infectious Diseases (NIAID) Laboratory of Viral Diseases, described recent work using intravital microscopy to track virus-infected cells and immune effectors in real-time *in vivo* during skin infection with vaccinia virus, including intravital imaging of T-cell dynamics during skin infection.
- Dr Alessandro Sette*, from the La Jolla Institute for Allergy and Immunology, presented the Immune Epitope Database (IEDB), a searchable warehouse of published antibody and T-cell epitope data, as well as new tools for antigen discovery and epitope prediction.
- Dr Stephen Miller, from Northwestern University, detailed the efficacy and mechanisms of using the intravenous infusion antigen-encapsulated biodegradable poly(lactide-co-glycolide) nanoparticles containing antigens to induce immune tolerance including ongoing efforts to advance clinical translation of this novel therapy.

Future Research Priorities

- Perform a literature review and industry scan to identify the status of epitope and microbiome research in alopecia areata and related diseases.
- Create a Microbiome of the scalp of control and alopecia areata patients' database using Next Generation Sequencing of 16 s RNA.
- Analyze the regional and geographical pattern of alopecia areata to study the epidemiological, ecological, and environmental factors related to microbiome diversity.
- Investigate differentially expressed proteins in hair follicles and nerves using proteomic approaches.
- Utilize the IEDB analysis resource to generate predicted epitopes and sets of specific predicted epitopes from proteins identified in the above action item for testing in alopecia areata.
- Leverage available technology in infectious diseases and allergies to generate targeted experimental data using alopecia areata blood samples to screen the epitope sets described in the above action item.

Clinical aspects, epidemiology, and tools

Presentation Highlights

- Dr Maria Hordinsky*, from the University of Minnesota, provided an overview of current treatment practices in alopecia areata and examined the rationale for choosing one treatment over another, including weighing the risk/benefit ratio of current and evolving choices.
- Dr Wilma Bergfeld*, from the Cleveland Clinic, presented a retrospective study of 50 Cleveland Clinic patients with alopecia areata that were treated with DPCP that revealed three statistically significant predictors of poor treatment outcome: extent of hair loss; history of thyroid disease; and extent of body hair involvement. Dr Bergfeld also discussed Intralesional Kenalog (ILK) as the first-line therapy in the treatment of alopecia areata and presented data suggesting that, in addition to the immunosuppressive effect of intralesional steroids, the act of penetrating the skin may also be a component of the therapeutic benefit of ILK injections.
- Dr Julian Mackay-Wiggan, from Columbia University, reported on the exciting preliminary results from ongoing pilot trials at Columbia University Medical Center to test the efficacy of Jakafi (ruxolitinib, a Jak1/2 inhibitor) and Orencia (abatacept, the fusion protein CTLA4-Ig) to treat alopecia areata. Interim results show promise and the incidence of adverse events are minimal. The design for the upcoming the Xeljanz (tofacitinib, a Jak 3 inhibitor) study was also discussed. Early findings from the ruxolitinib study were recently published (Xing L *et al. Nature Medicine* 20 September 2014: 1043–9).
- Dr Vera H. Price*, from the University of California, San Francisco, shared the accomplishments and current enrollment status of the Alopecia Areata Registry, Biobank, and Clinical Trials Network (Registry) including epidemiological data and tissue samples of patients with alopecia areata.
- Dr Melissa Piliang*, from the Cleveland Clinic, discussed eosinophilic esophagitis as a potential trigger of alopecia areata and the association of atopy and alopecia areata.
- Dr Natasha Mesinkovska*, from the Cleveland Clinic, presented a retrospective cross-sectional study that evaluated the prevalence of comorbid conditions among patients with alopecia areata over a 10-year period and found no significant differences

between alopecia areata and controls in sun-induced skin cancers.
- Dr William Russell, Vanderbilt University, discussed the similarities between Type 1 diabetes and alopecia areata, including the role of beta cells and prevention studies as well as enrolling alopecia areata patients in a Type 1 Diabetes TrialNet prevention screening program.
- Lindsay Boyers, medical student at Georgetown University, shared results of the Global Burden of Disease Study: the burden of disease caused by alopecia areata is equivalent to 18.6 years of healthy life lost despite not including mental health burdens. Data from this study are publicly available on the Global Burden of Disease website: www.healthdata.org/gbd.
- Dr James Solomon*, Director of Ameriderm Research, discussed the development of a Core Uniform Protocol for alopecia areata. This will allow pharmaceutical and device companies to develop standardized clinical trials, and to maintain consistent parameters to compare and contrast studies, including inclusion/exclusion; outcome assessment measures; and safety parameters.

Future Research Priorities
- Modify the Core Uniform Protocol to expand age ranges and duration of hair loss criteria for inclusion.
- Collaborate with pharmaceutical companies, both large and small, to supply drugs to be tested and facilitate larger trials with more patients.
- Estimate burden of disease and annual cost to payers to entice insurance companies for coverage as well as pharmaceutical interest.
- Publicize information about disease burden and collaborate with the American Association of Dermatologists to disseminate data quickly.
- Advocate for insurance coverage of systemic or topical JAK inhibitors and partner with PCORI for comparative effectiveness research.
- Survey patients and medical professionals to capture clinical data about off-label use of JAK inhibitors and other potential therapies.
- Encourage and support medical professionals in obtaining Institutional Review Board approval to prospectively capture efficacy and safety data for alopecia areata patients treated off label with JAK inhibitors and other potential therapies.
- Study connections between alopecia areata and other systemic autoimmune diseases such as hypothyroidism.
- Investigate response to therapy among different ethnic groups.
- Collaborate with investigators performing clinical studies and develop a unified database to capture information.
- Improve communication between patients and caregivers, including sensitivity training about emotional impacts to facilitate information sharing.
- Improve privacy and emotional sensitivities of survey tools to validate potentially underestimated prevalence and incidence statistics.

Funding and partnership opportunities

Presentation Highlights
- Dr Stephen Katz, Director of the NIAMS, kicked off the meeting and discussed relevant NIH funding opportunities, including the Accelerating Medicines Partnership, as well as a new initiative to share data about well-scored grants that fall below the NIAMS pay-line with patient advocacy groups to provide important bridge funding.
- Dr Ricardo Cibotti, Director of the Skin Immunobiology and Immune Mediated Diseases of Skin Program at NIAMS, provided a comprehensive review of current funding opportunities to support clinical trials, including R21, R34, and U01 grants. He informed participants about common pitfalls to avoid, including insufficient study premise documentation, inexperienced investigators, limited evidence for efficacy, poor study design, sample size issues, and failing to address relationships between the principal investigator, sponsor, and other parties.
- Dr Daniel Rotrosen, Director of the Division of Allergy, Immunology, and Transplantation at the NIAID, discussed funding opportunities and sponsored research tools, reagents, and resources to facilitate basic and clinical research on autoimmune disorders, including the Immune Tolerance Network for clinical trials and ImmuneXpresso, a new tool that filters published data to create enrichment maps of network interactions between cells and cytokines and gene expression for specific diseases.
- Dr Kara Odom Walker, Deputy Chief Science Officer at the PCORI, introduced PCORI to participants, including comparative effectiveness research (comparing two or more options for prevention, diagnosis, and treatment), and patient-centered outcomes research (including patient input throughout the development process). She presented funding opportunities in five areas: prevention, diagnosis, and treatment; improving healthcare; communication; health disparities; and accelerating methodological research.
- Dr Anton Simeonov, Acting Deputy Scientific Director at the National Center for Advancing Translational Sciences, presented high-throughput screening opportunities, including the comprehensive public access screening collection of small molecule agents and a platform capable of testing thousands of compounds in pairwise matrix blocks for the rapid and systematic identification of synergistic, additive, and antagonistic drug combinations. He noted that it can take several years to establish good cell-based assays for screening and is open to discussing projects with researchers.
- Dr Theresa Mullin, Director of the Office of Strategic Programs at the US FDA Center for Drug Evaluation and Research, discussed new FDA initiatives to help advance drug development. She presented an overview of three key initiatives: structured benefit-risk assessment, patient-focused drug development, and breakthrough therapy designation.
- Amanda Wagner*, Senior Director of Product Planning and Program Management at Concert Pharmaceuticals, shared the business case for pursuing alopecia areata from the biotech industry perspective. Important topics

covered included how companies and investors think about drug development and commercialization for alopecia areata; insurance reimbursement considerations for novel therapies; and strategies to obtain approval for repurposing of existing drugs.

In addition to the scientific presentations, two patient advocates shared their stories. Maria Beckett* shared her experiences as a young girl with hair loss and growing up with the stigma that the disease brought. She shared how this disease affects more than just the hair and how the community must remember that while they are looking for a cure they are also seeking to help improve a patient's self-esteem and confidence. A father of a child with alopecia areata also shared his perspective as a parent who struggles to weigh the pros and cons of toxic drugs to potentially treat his young daughter. He stressed how difficult it is not only to watch your child suffer but also to make the medical decisions when there is no certainty the drugs will help to alleviate the burden. He encouraged the researchers to look outside the box for medical advances.

Concluding Remarks

This 2-day summit fostered innovation and collaboration across multiple disciplines with a series of key research presentations followed by substantive question-and-answer sessions and discussions. Inclusion of women, minorities, and people with disabilities is a NAAF priority and the meeting drew a diverse and balanced group of knowledgeable attendees. Several early-career investigators brought fresh ideas and new talent, and individuals with alopecia areata and family members provided an important bridge between those studying the disease and those personally affected by it. The fruitful discussions had several common underlying themes, including increased collaboration between existing researchers with common interests, increasing biopharma interest and participation, expanding the Registry to include more diversity and more samples, amplifying the NAAF message, and prioritizing research to learn more about how alopecia areata begins and how to stop its progression. Participants left feeling excited about recent research progress and new possibilities.

Many of the research accomplishments explained above have been part of the TDP, with NAAF either providing direct funding or acting as a concierge, leveraging all of our available research resources and clinical partnerships. Our strategic goal is to produce a safe, effective, affordable treatment beneficial to the millions of people with alopecia areata. This summit was another strategic step on the structured and focused path to that goal. Many of the Research Priorities proposed and discussed are projects that have been in progress. Planning for the next Alopecia Areata Research Summit is in progress for the Fall of 2016. NAAF has and will continue to provide the support and leadership toward accomplishing these Research Priorities to enhance the understanding of alopecia areata. We look forward to future discoveries.

Please contact us if you can help in any way or are interested in applying for funding to study any of the Research Priorities mentioned above.

CONFLICT OF INTEREST

JAF owns Amgen stock, received grant support from the NIH, and received royalties from the sale of monoclonal antibodies, paid through the University of Southern California. AE and DK received grant support from NIAMS, National Center for Advancing Translational Sciences, Rxi Pharmaceuticals, and Summer Labs. JM-W received grant support from Locks of Love and AA Initiative—Gates Foundation. DAN states no conflict of interest.

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