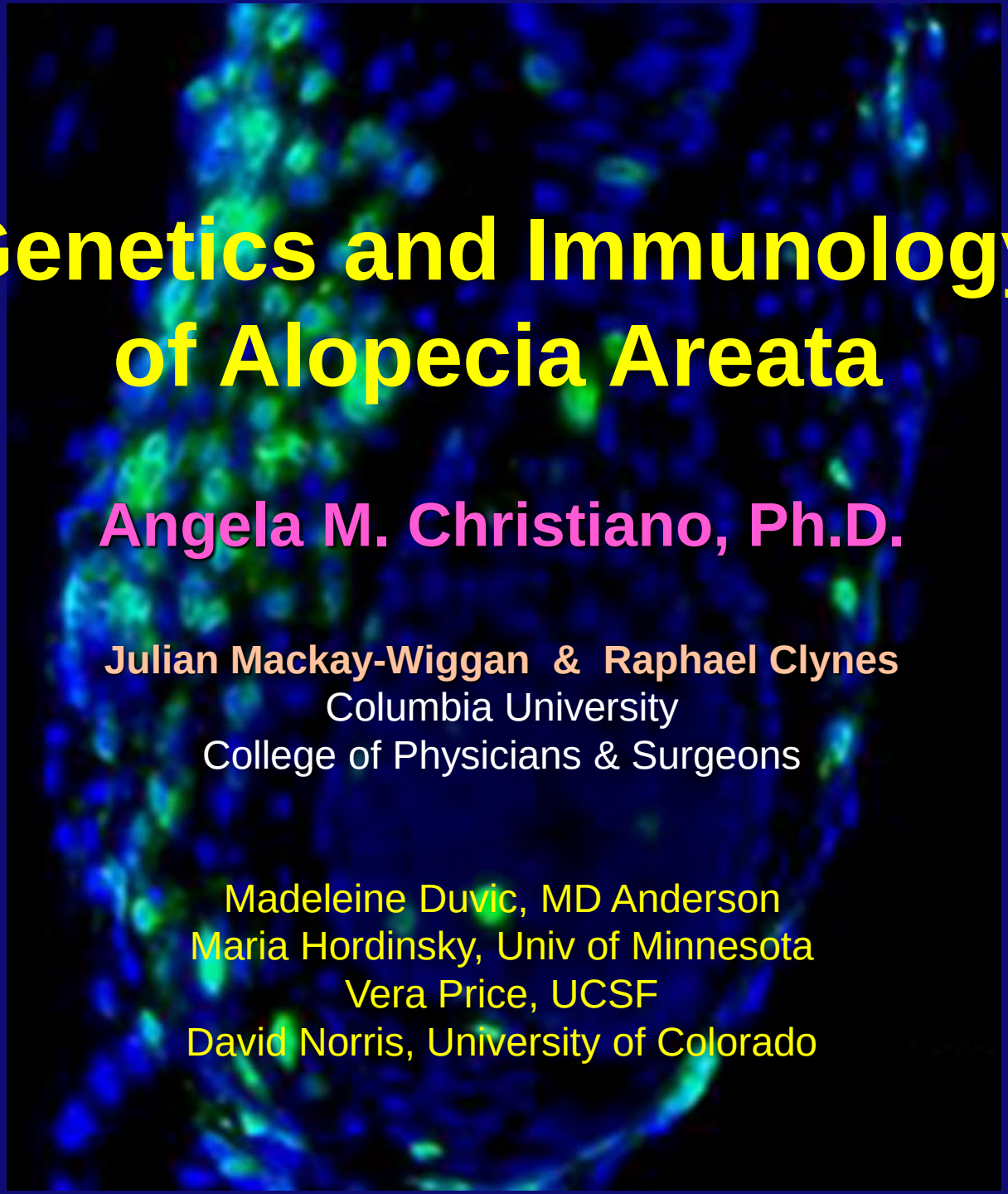
# Aclaris Therapeutics R&D and Investor Day
# October 4, 2017
# Genetics and Immunology of Alopecia Areata

ACLARIS THERAPEUTICS



# Genetics and Immunology of Alopecia Areata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David Norris, University of Colorado

# Disclosures

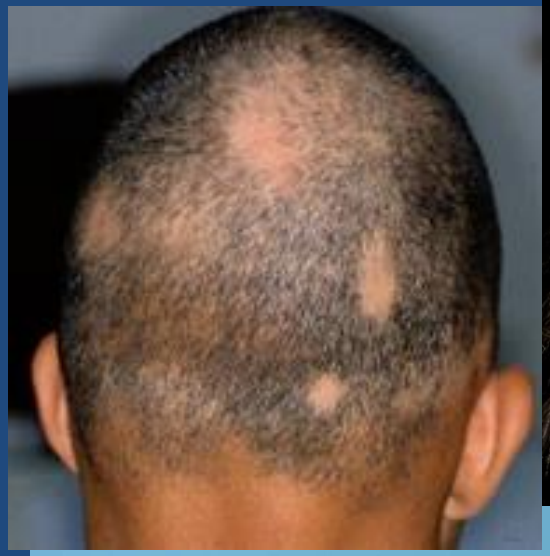
- Board Member: Dystrophic EB Research Association of America
- Scientific Advisory Council Chair:  National Alopecia Areata Foundation
- Board Member:  North American Hair Research Society
- Board Member: Douglass College of Rutgers University

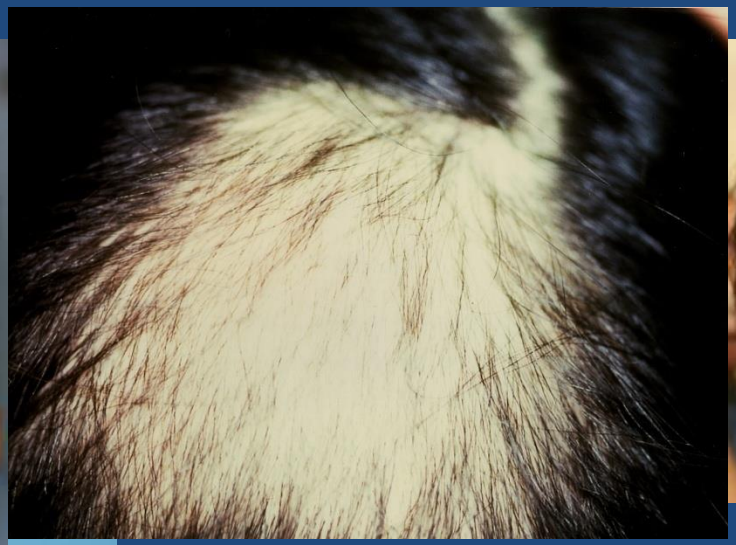
- Research Grant Recipient: Bristol-Myers Squibb
- Research Grant Recipient: Pfizer

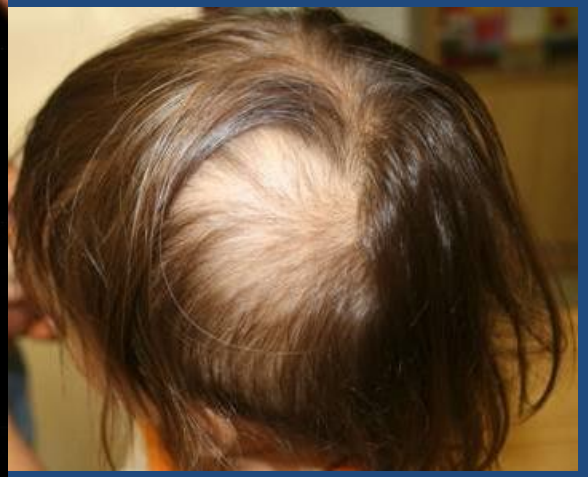
- Scientific Advisor, Shareholder, Consultant:  Aclaris Therapeutics
- Consultant: Dermira

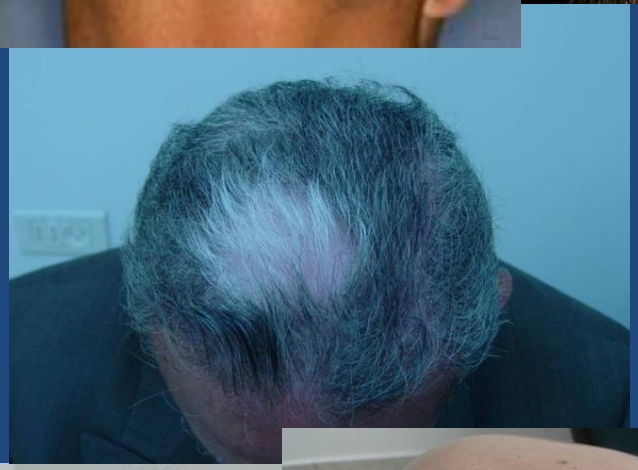
- Co-Founder: Rapunzel Bioscience

- President 2016-2017:  Society for Investigative Dermatology

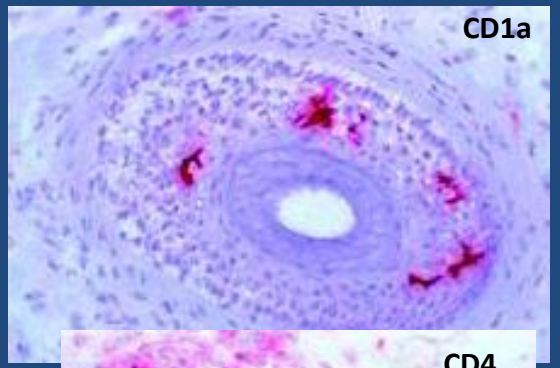


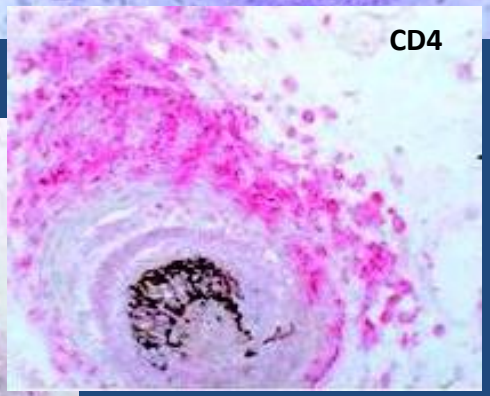


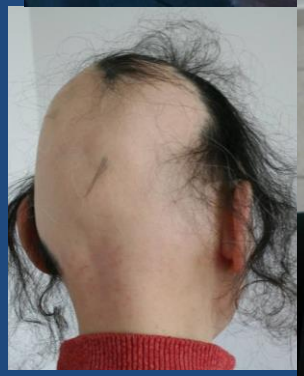


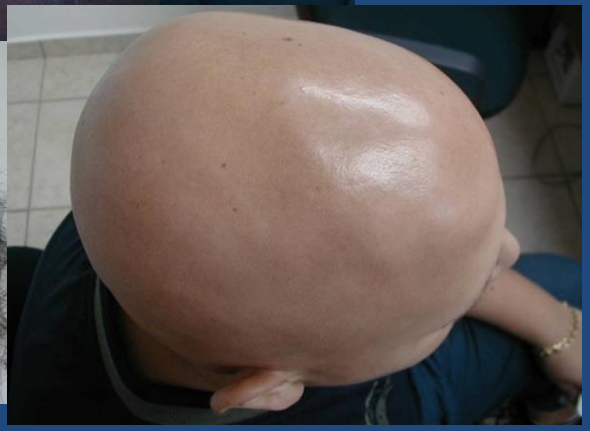


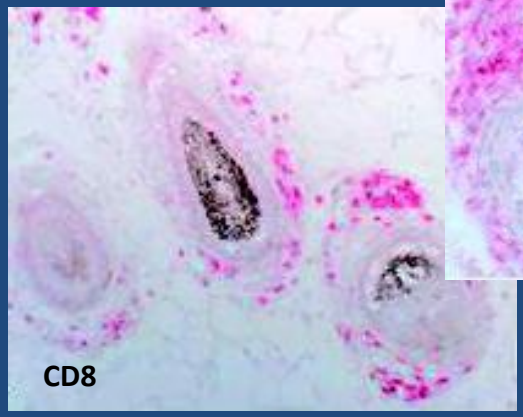
hair follicle immune system








V. Herbst *et al.*, *Eur J Dermatol* **16**, 537 (Sep-Oct, 2006).

# Challenge of Alopecia Areata Pathogenesis: Collapse of Immune privilege of the Hair Follicle

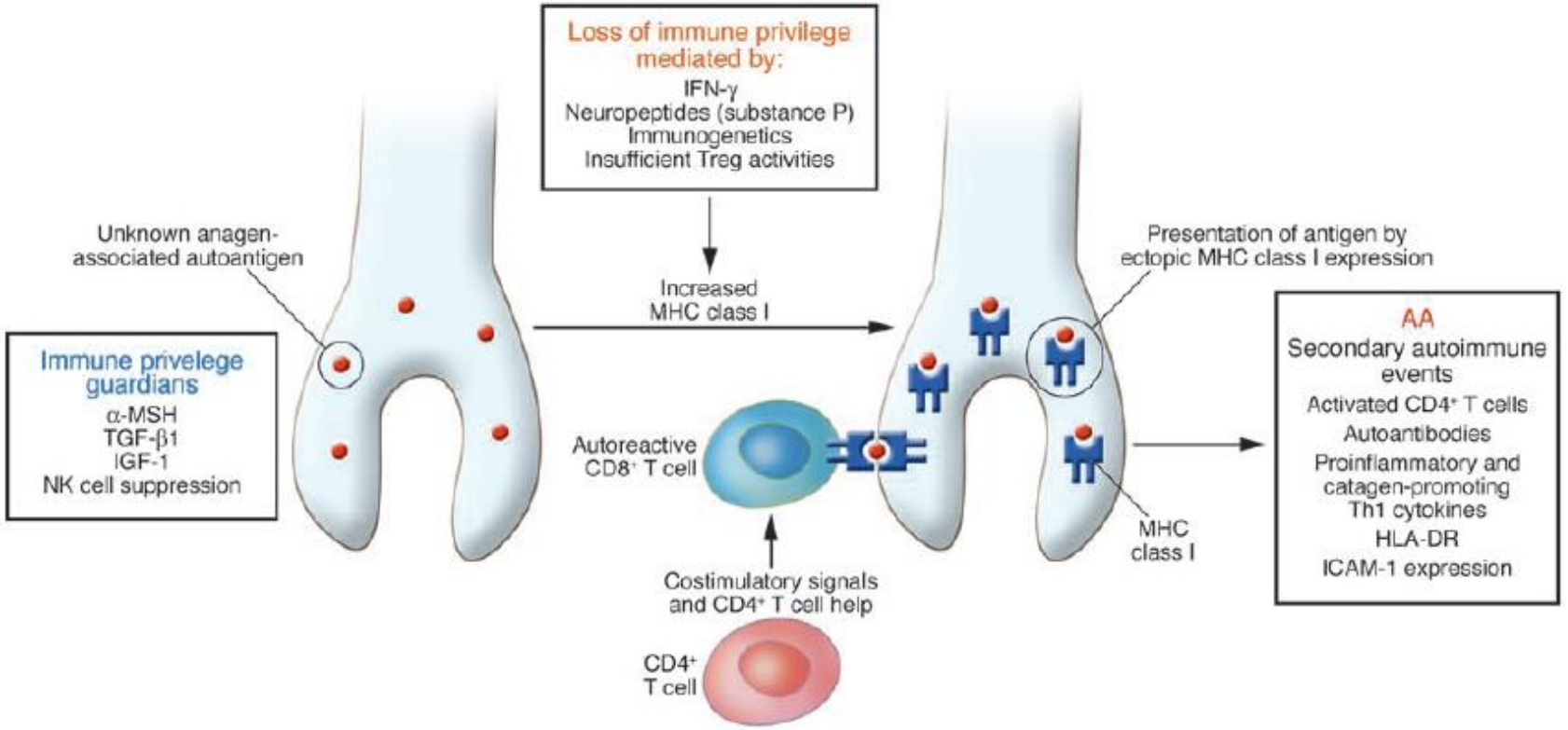
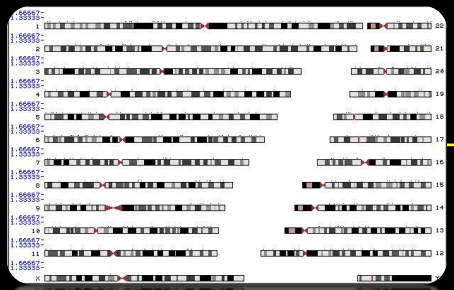



**Figure 3**
Proposed pathogenesis of AA. Cytokines and cellular factors responsible for maintaining immune privilege are listed in the left box. Those factors believed to mediate loss of immune privilege and initiation of disease are listed in the middle box. Loss of immune privilege is associated with expression of MHC class I molecules, which are capable of presenting hair follicle autoantigens to T lymphocytes. Secondary autoimmune amplification circuits that may help establish or amplify the pathology are listed in the right box.

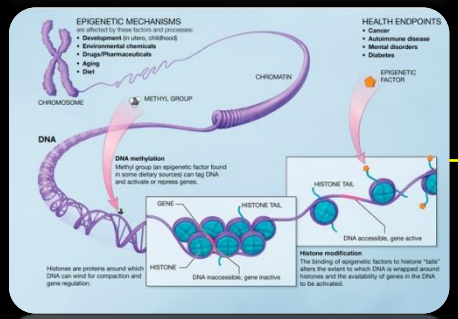
From Gilhar, Paus & Kalish, JCI, 2007

# Functional Genomics: How to Apply to AA?

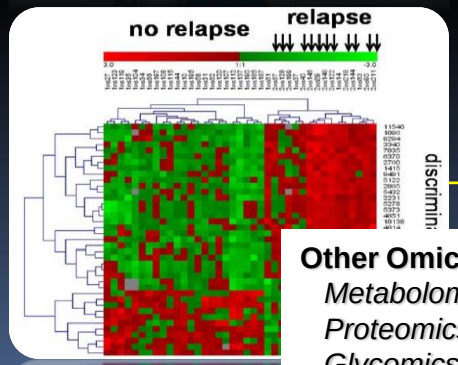
Genomics



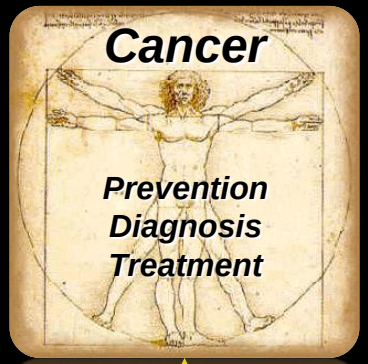
Epigenomics



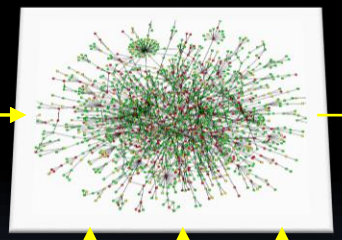

Transcriptomics

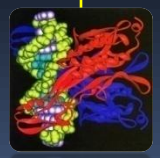



**Other Omics**:
*Metabolomics*
*Proteomics*
*Glycomics*
…



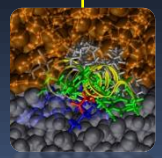

Cell Regulatory Logic





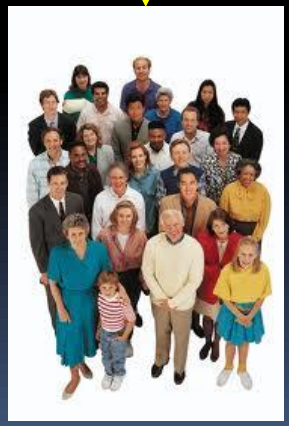
Protein
DNA

Protein
Protein



Protein
Membrane

Drugs & Biomarkers



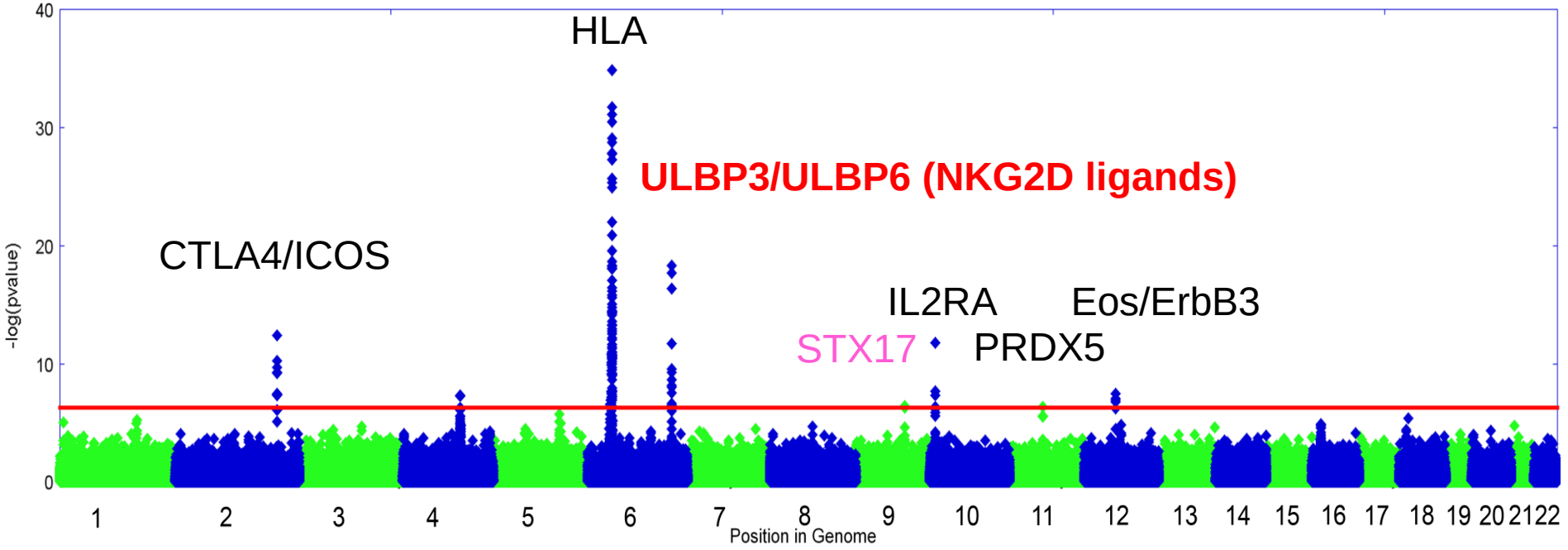
Clinical Trials

Slide courtesy of Dr. Andrea Califano

# Outline

- **Genetics of Alopecia Areata**
- **Immunology of Alopecia Areata**
- **Translational Research and Clinical Studies**

# Genomewide Association Studies (GWAS) in Alopecia Areata




Petukhova, et al, Nature 2010

# Genome-wide Association Study in AA

Vol 466|1 July 2010|doi:10.1038/nature09114 nature

| Region | Gene | Function | Strongest association (*P* value) | Maximum odds ratio | Involved in other autoimmune disease |
|---|---|---|---|---|---|
| 2q33.2 | *CTLA4* | Co-stimulatory family | $3.55 \times 10^{-13}$ | 1.44 | T1D, RA, CeD, MS, SLE, GD |
| | *ICOS* | Co-stimulatory family | $4.33 \times 10^{-8}$ | 1.32 | |
| 4q27 | *IL-21/IL-2* | T-, B- and NK-cell proliferation | $4.27 \times 10^{-8}$ | 1.34 | T1D, RA, CeD, PS |
| 6q25.1 | *ULBP6* | NKG2D activating ligand | $4.49 \times 10^{-19}$ | 1.65 | None |
| | *ULBP3* | NKG2D activating ligand | $4.43 \times 10^{-17}$ | 1.52 | None |
| 9q31.1 | *STX17* | Premature hair greying | $3.60 \times 10^{-7}$ | 1.33 | None |
| 10p15.1 | *IL-2RA* | T-cell proliferation | $1.74 \times 10^{-12}$ | 1.41 | T1D, MS, GD, GV |
| 11q13 | *PRDX5* | Antioxidant enzyme | $4.14 \times 10^{-7}$ | 1.33 | MS |
| 12q13 | *Eos (IKZF4)* | $T_{reg}$ transcription factor | $3.21 \times 10^{-8}$ | 1.34 | T1D, SLE |
| | *ERBB3* | Epidermal growth factor receptor | $1.27 \times 10^{-7}$ | 1.34 | T1D, SLE |
| 6p21.32 | *MICA* | NKG2D activating ligand | $1.19 \times 10^{-7}$ | 1.44 | T1D, RA, CeD, UC, PS, SLE |
| (HLA) | *NOTCH4* | Haematopoietic differentiation | $1.03 \times 10^{-8}$ | 1.61 | T1D, RA, MS |
| | *C6orf10* | Unknown | $1.45 \times 10^{-16}$ | 2.36 | T1D, RA, PS, GV |
| | *BTNL2* | Co-stimulatory family | $2.11 \times 10^{-26}$ | 2.70 | T1D, RA, UC, CD, SLE, MS, GV |
| | *HLA-DRA* | Antigen presentation | $2.93 \times 10^{-31}$ | 2.62 | T1D, RA, CeD, MS, GV |
| | *HLA-DQA1* | Antigen presentation | $3.60 \times 10^{-17}$ | 2.15 | T1D, RA, CeD, MS, SLE, PS, CD, UC, GD |
| | *HLA-DQA2* | Antigen presentation | $1.38 \times 10^{-35}$ | 5.43 | T1D, RA |
| | *HLA-DQB2* | Antigen presentation | $1.73 \times 10^{-13}$ | 1.60 | RA |

Surprises

## Pathways

Costimulatory Pathway
NKG2D axis
Cytokine signaling

## Aligned Diseases

Type 1 diabetes
Rheumatoid arthritis
Celiac disease

- **How have genetic studies advanced our understanding of disease pathogenesis?**

- **How have genetic studies led to translational research and new clinical approaches?**

# Common pathways with other Autoimmune diseases: NK ligands in the target organs

- In RA, synoviocytes aberrantly express MIC ligands, leading to autoreactive T cell stimulation.

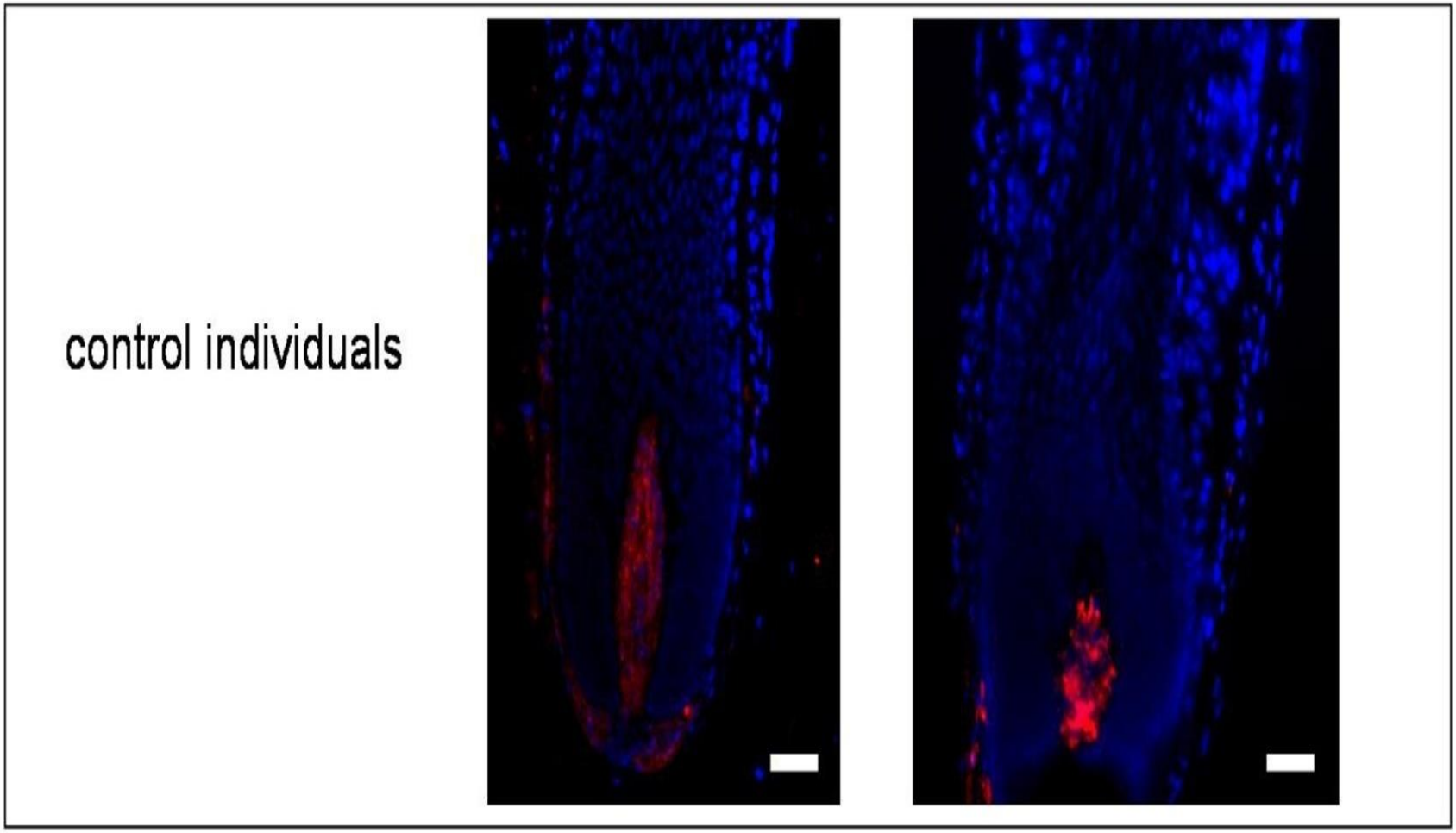
- In Celiac disease, MIC ligands are overexpressed in the gut epithelium during active disease.

- In Type 1 diabetes, Islet cells in prediabetic NOD mice express RAE-1 ligand.

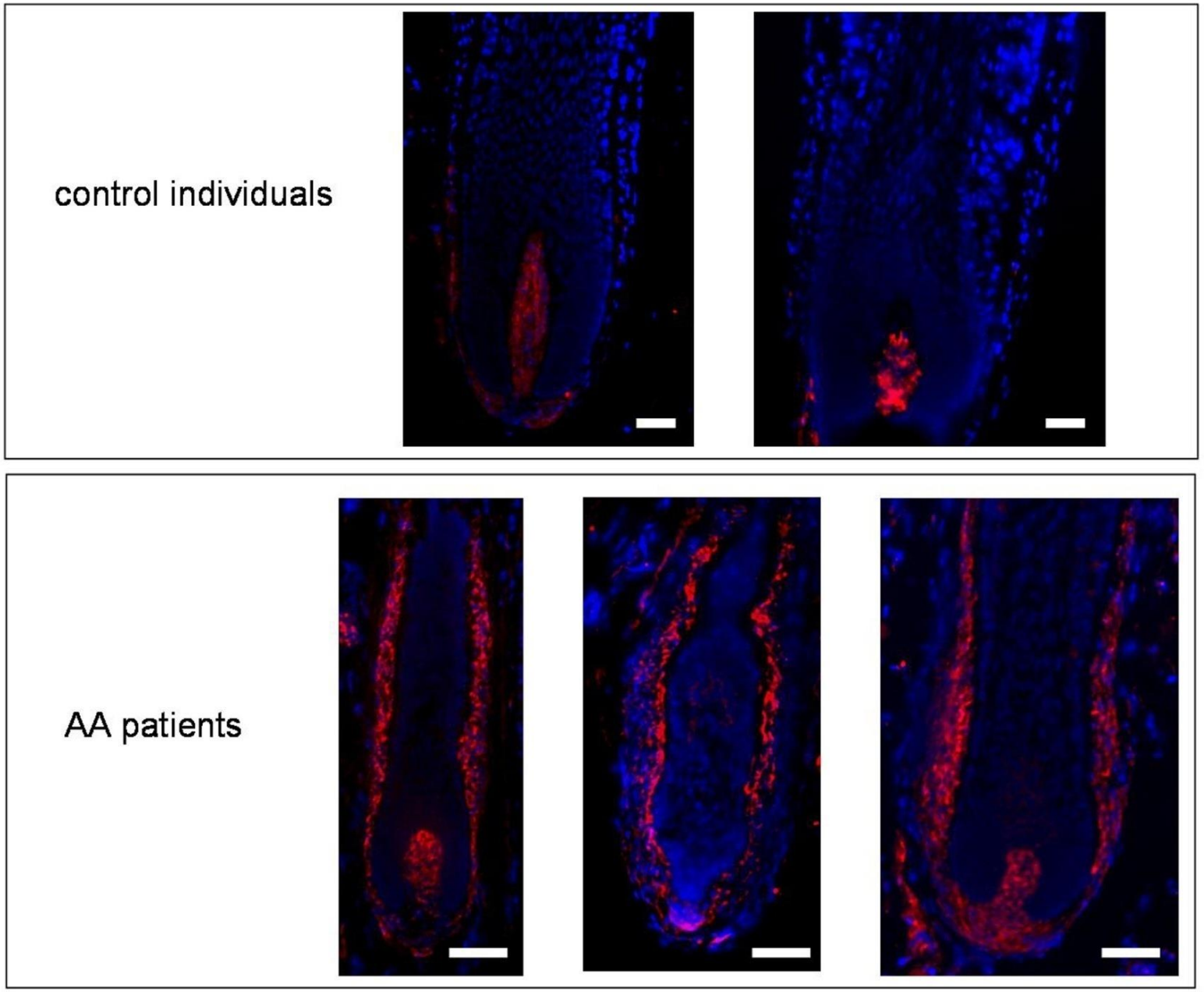
**Hypothesis: In AA, do we find overexpression of ULBP3 in the hair follicle?**

The aberrant expression of NK activating ligands in genetically predisposed individuals may induce or exacerbate disease.

# Danger Signals: ULBP3 Expression in the Hair Follicle

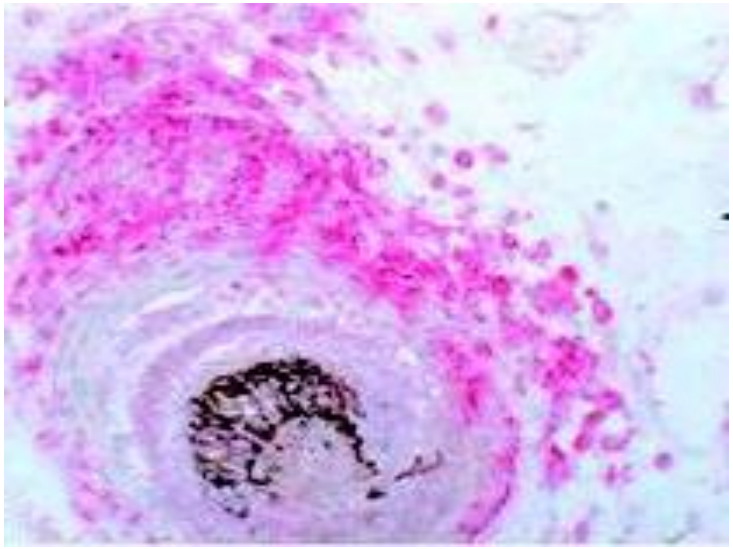


# Danger Signals: ULBP3 Expression in the Hair Follicle

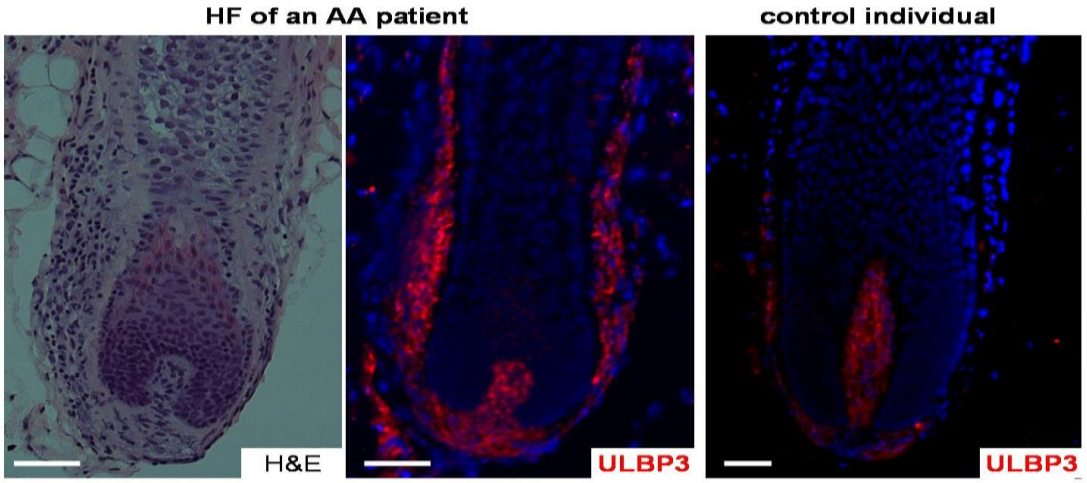


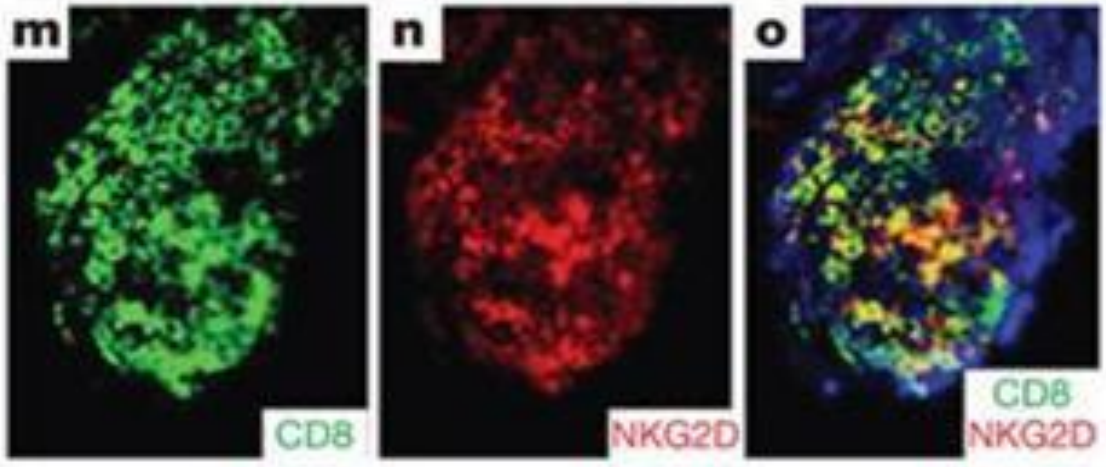
# AA is caused by ‘swarm of bees’
# Killer CD8 T cells attracted by NKG2DL

**AA**
Pre-2010
Swarm of Bees



**AA**
2010: Bees are identified



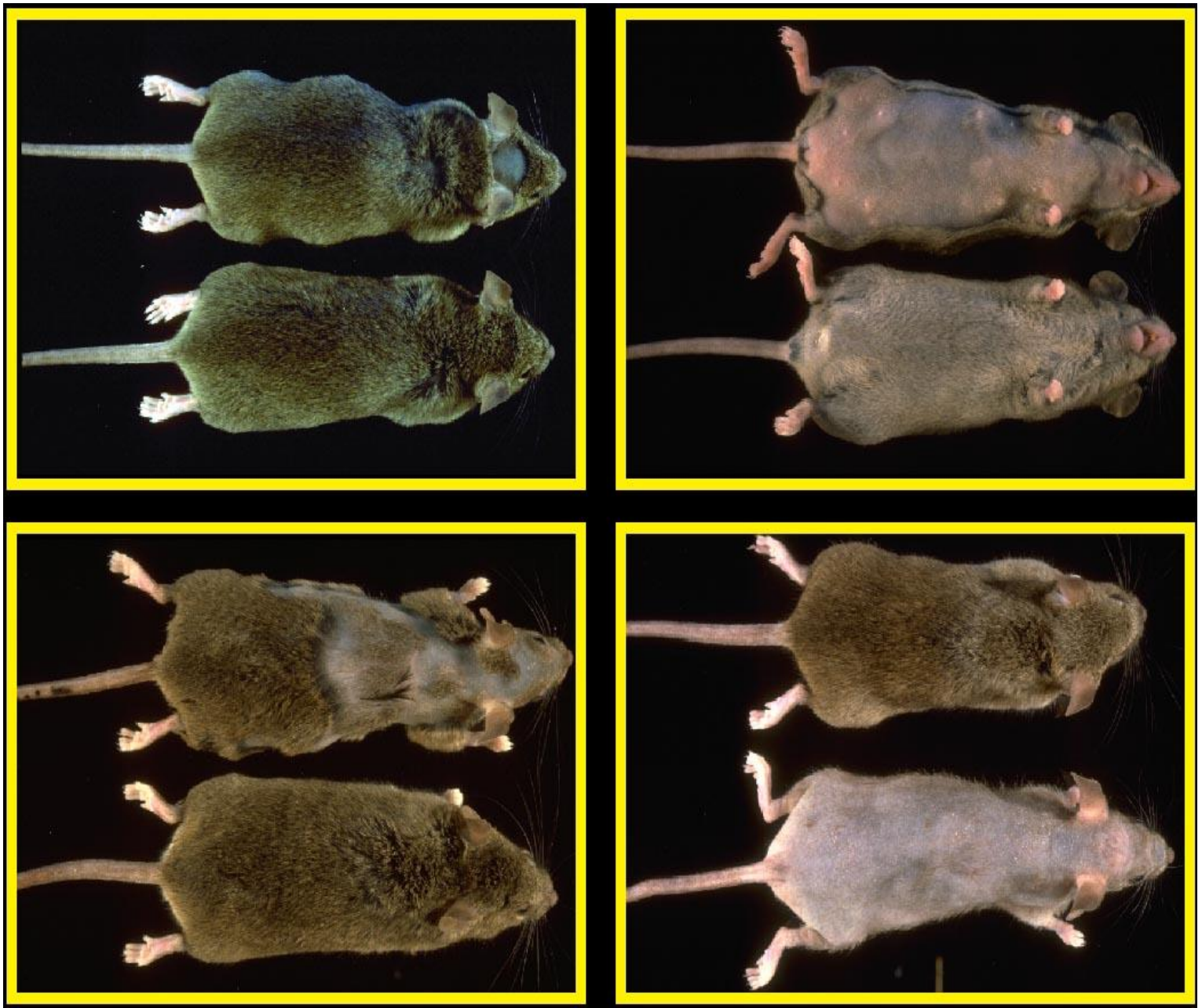




*Collaboration with Raphael Clynes

# Outline

- **Genetics of Alopecia Areata**
- **Immunology of Alopecia Areata**
- **Translational Research and Clinical Studies**

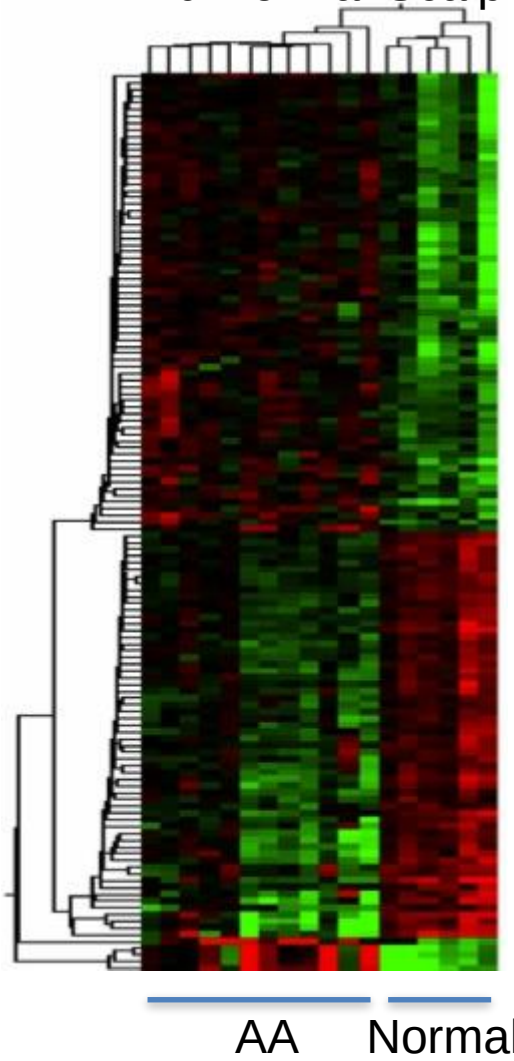
# Alopecia Areata in Mice



# Shared Pathogenesis in Human and C3H/HeJ Mouse

## Comparative Transcriptomics Reveals Shared IFN Response Signature

Human Alopecia Areata And Normal Scalp



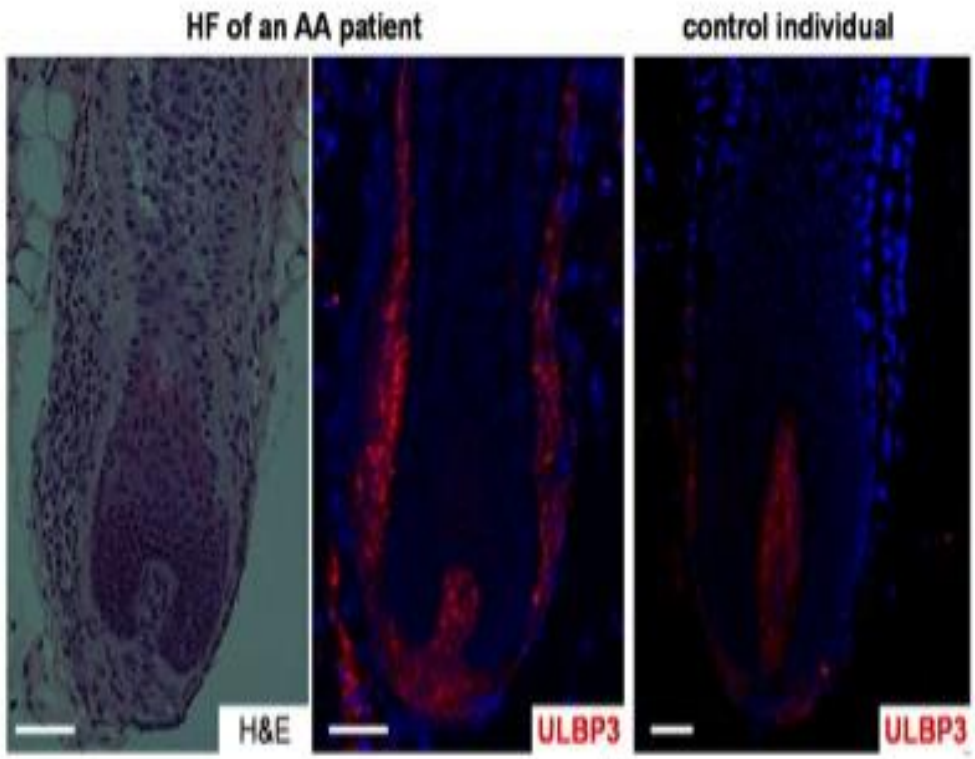

Gene Expression in Human and Mouse AA

| Human | | Mouse | | |
|---|---|---|---|---|
| Gene | Fold Upregulated | Gene | Fold Upregulated | |
| CXCL10 | 6.749738 | Cxcl10 | 37.489223 | ★ |
| CXCL9 | 6.3546677 | Cxcl9 | 75.22101 | |
| CD8A | 2.8763134 | Cd8a | 14.440988 | ★ |
| STAT1 | 2.647132 | Stat1 | 14.508814 | ★ |
| IFI44 | 2.504809 | Ifi44 | 20.594664 | |
| CCL2 | 2.4538028 | Ccl2 | 6.1081963 | |
| GZMA | 2.4454105 | Gzma | 56.385307 | ★ |
| RSAD2 | 2.1267016 | Rsad2 | 2.905758 | |
| PTPRC | 2.0699668 | Ptprc | 2.068105 | ★ |
| TLR3 | 2.0492992 | Tlr3 | 2.5370347 | ★ |
| CD274 | 2.0426362 | Cd274 | 8.396741 | |

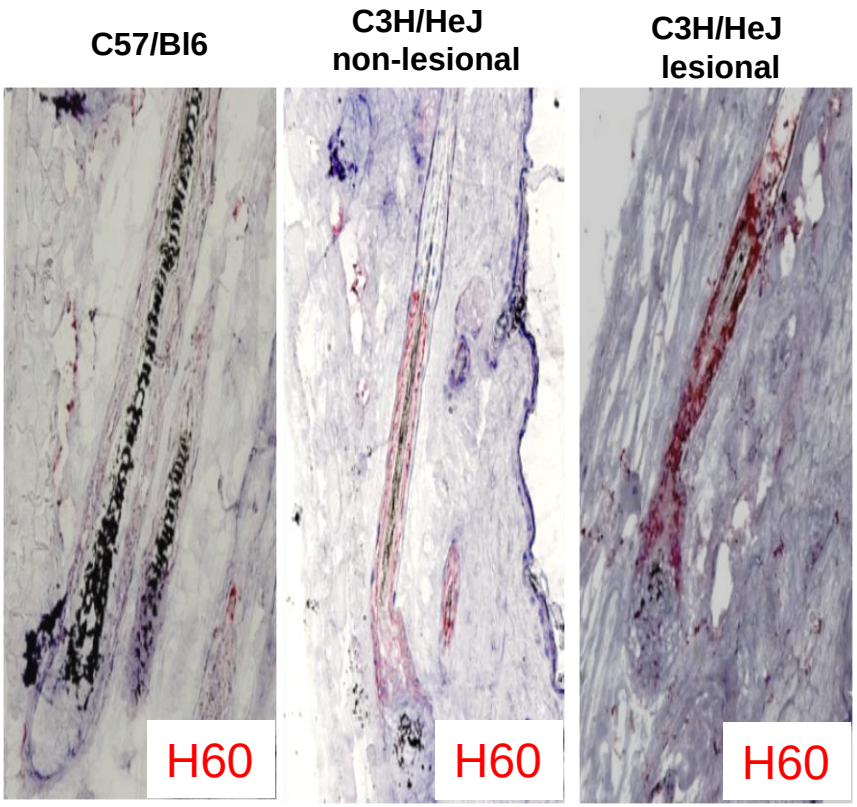
# Shared Pathogenesis in Human and Mouse

## NKG2D “Stress” Ligands are Upregulated in the Hair Follicle

**Human Alopecia**



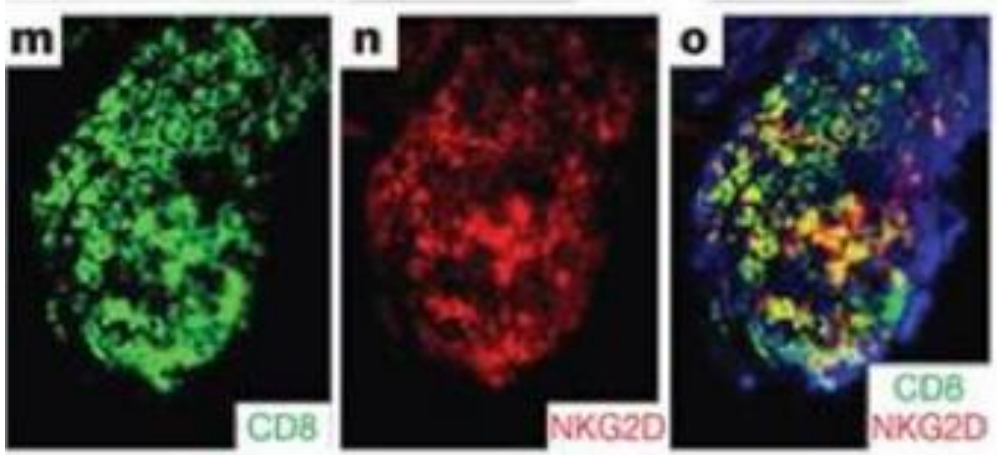

**Mouse Alopecia**




Pethukova, Nature 2010

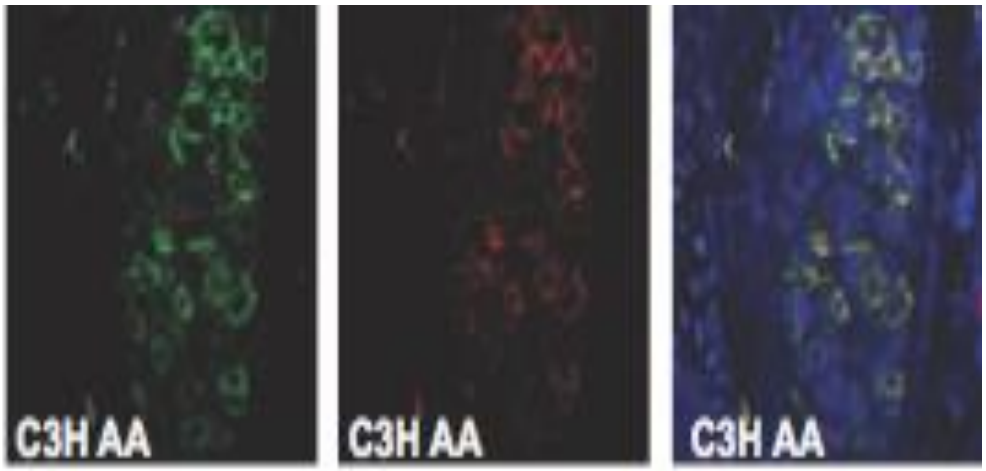
# $CD8^{+}NKG2D^{+}$ Cytotoxic T Cells infiltrate the AA Hair follicle

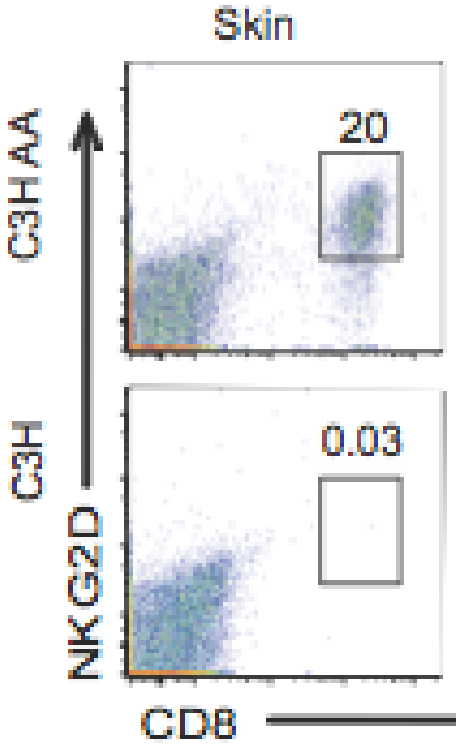
## Human Alopecia



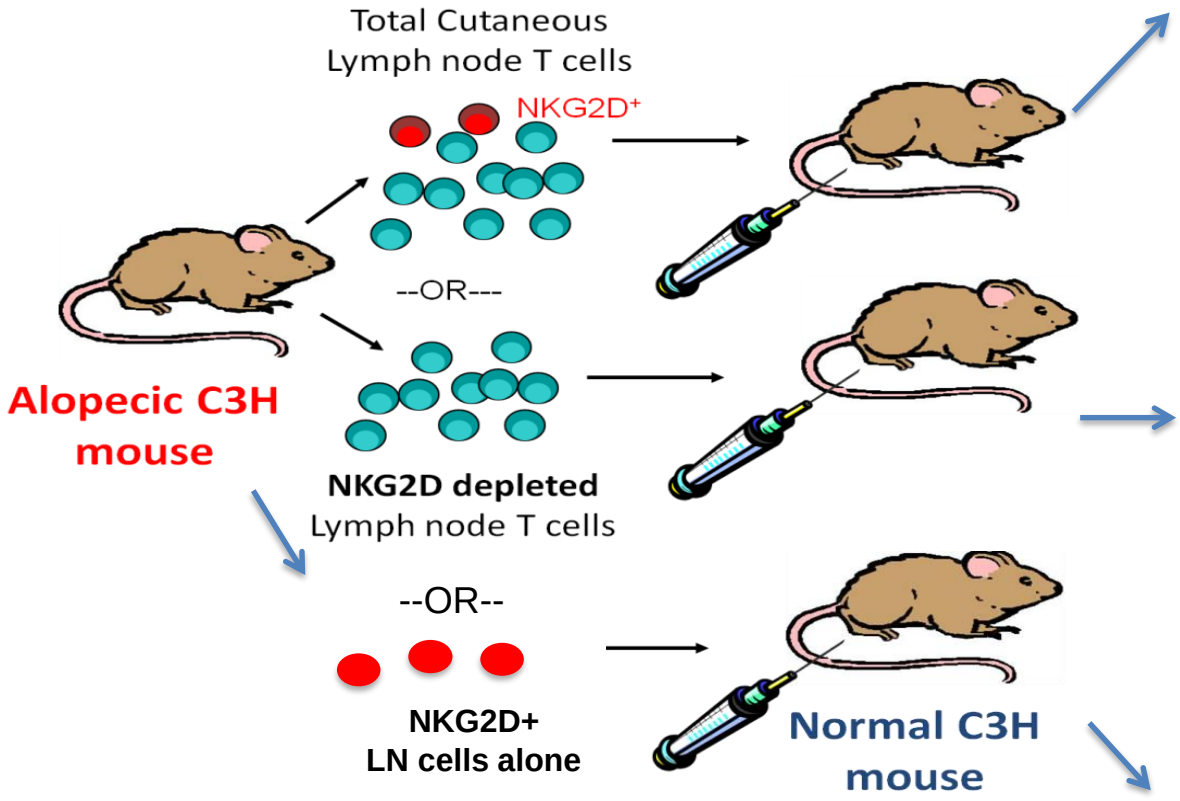

Pethukova, Nature 2010

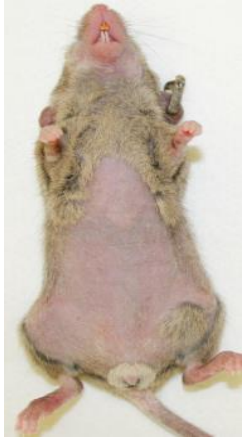
## Mouse Alopecia



# CD8+ NKG2D+ T cells are Necessary and Sufficient for T Cell Transfer of AA



Xing, et al, Nature Medicine 2014

# Shared Characteristics between Human AA and the C3H/HeJ Mouse Model

## Several lines of evidence support the relevance of the model

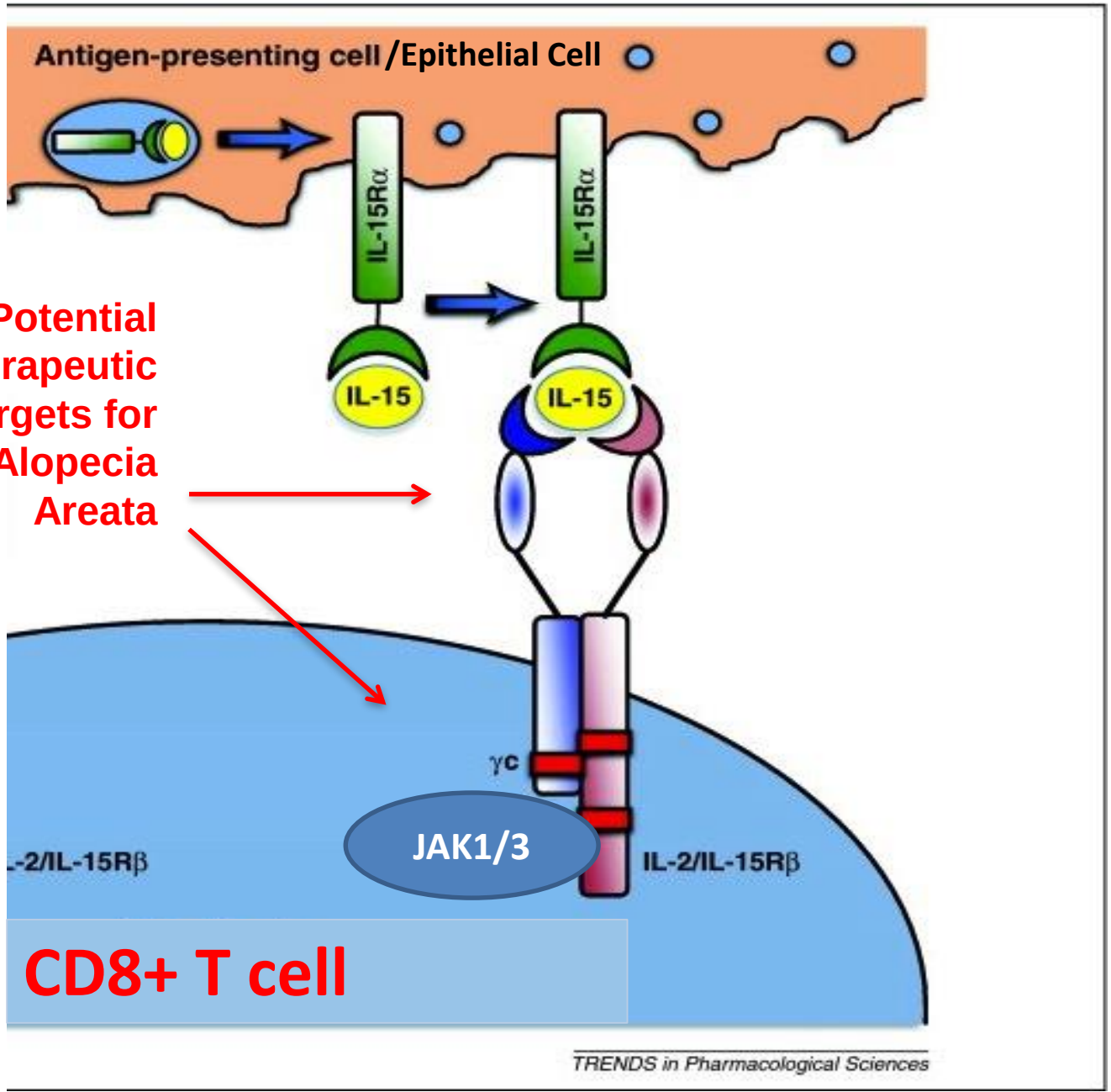
1. **End–organ drivers** → NKG2DL (ULBP3, MICA), and IL-15 in HF
2. **Immune effectors** → CD8 T cell effectors
3. **Biomarkers** → IFN-transcriptional signature
4. **TCR Repertoire** → evidence for antigenic drive

Using the mouse model, we have validated relevant **cytokine pathways in AA (**such as IFNg, IL15, IL2), using **blocking antibodies** to *prevent* disease onset.

To *reverse* established disease, rather than using a biologic..

**How do we target our CD8+ killers?**

# JAK kinases: Therapeutic targets in Alopecia Areata



# IL-15 and IFNg are Critical Cytokines for CD8+NKG2D+ Killer T Cells

## Hypothesis: Can we target their Effector JAK Kinases?

Highly expressed in diseased human and murine AA HF

Drives CD8 killer activation

Ab blockade prevents disease

CD8 Killer T cells

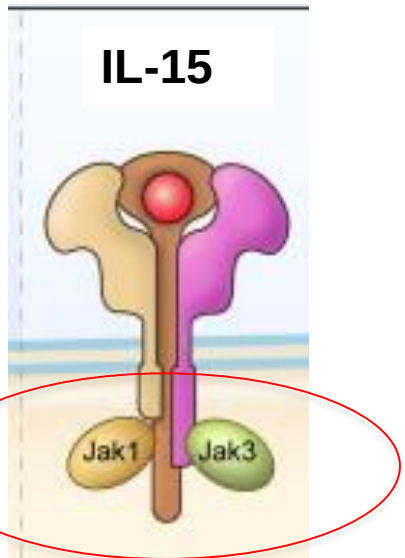
IL-15

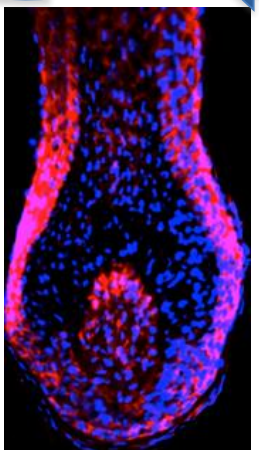
IFN-g

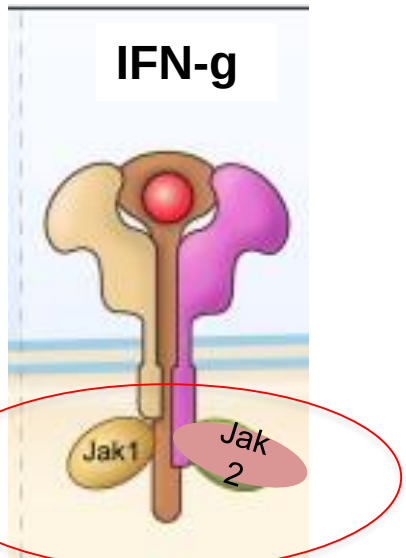
Produced by killer T cells

Dominates human and murine AA signature and drives inflammation

Ab blockade prevents disease



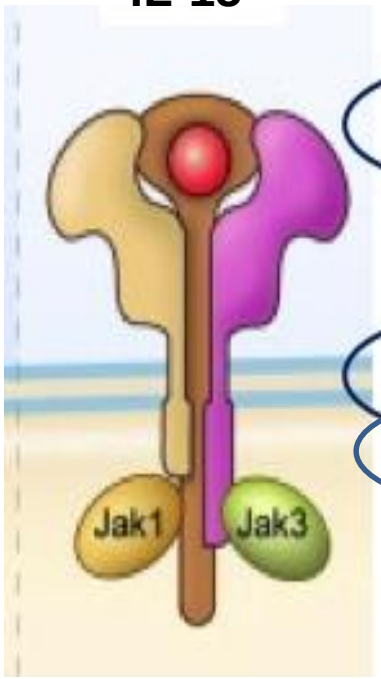





# JAK Inhibitors in Clinical Development

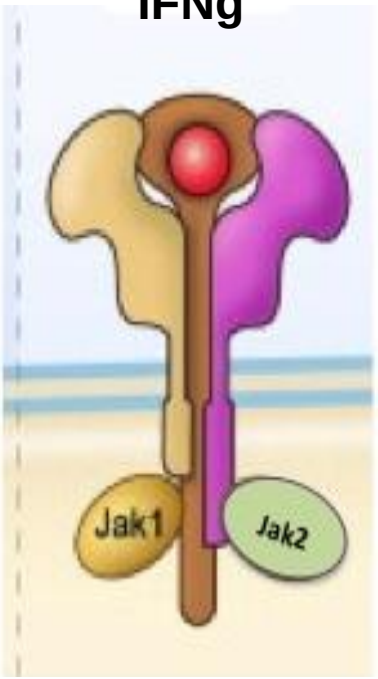
O'Shea, Immunity 2012

**IL-15**




| DRUG | TARGET | Route | STATUS |
|---|---|---|---|
| Tofacitinib | JAK 1,2,3 | oral | FDA approved RA |
| | | topical | Psoriasis Phase II |
| | | oral | IBD Phase II |
| VX-509 | JAK3 | oral | RA Phase II |
| R348 | JAK3 | oral | RA Phase I |
| Ruxolitinib | JAK1,2 | oral | FDA approved MF, PV |
| | | topical | Psoriasis Phase II |
| Baricitinib | JAK1,2 | oral | RA Phase II |
| GLPG0634 | JAK1 | oral | RA Phase II |

**IFNg**



# Pre-clinical C3H-HeJ mouse model of alopecia areata

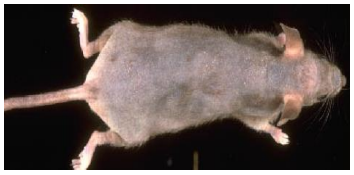
C3H/HeJ *Spontaneous* alopecia areata → 15% incidence in 6-12 months

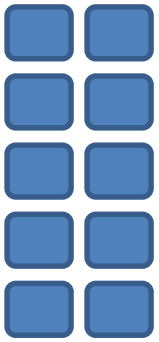
C3H/HeJ *Skin Graft* Model→ **100% incidence in 6-12 weeks**
**McElwee, JID 1998**

**Prevention Model**→ Give drug at the time of grafting

**Treatment Model** → Give drug after onset of disease (6 weeks or later)

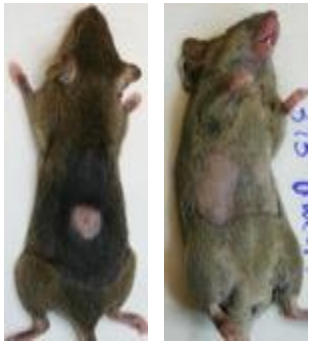
AA skin donor



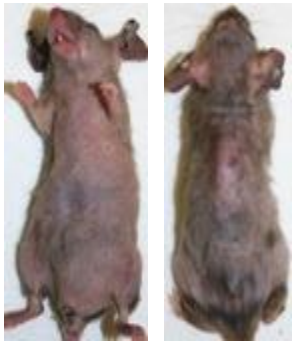


1cm$^2$
skin grafts

AA graft recipients



6 weeks
Patchy AA



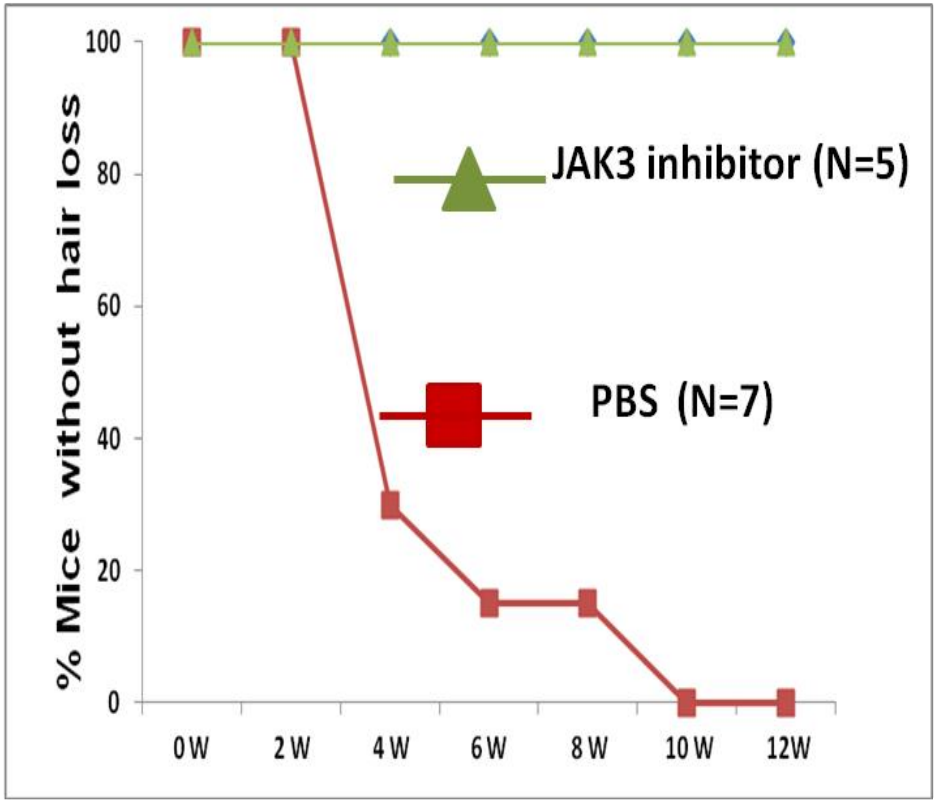
16 weeks
AT/AU

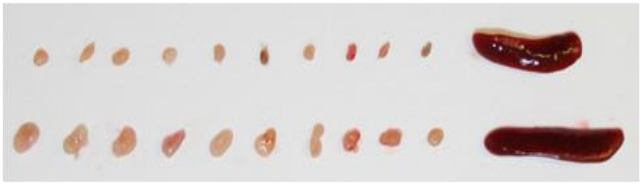
# Pre-clinical studies with systemic delivery

## JAK1/2 Inhibitor (ruxolitinib)

## JAK3 inhibitor (tofacitinib)

# JAK 3 Inhibitor (Tofacitinib) Prevents Alopecia Areata

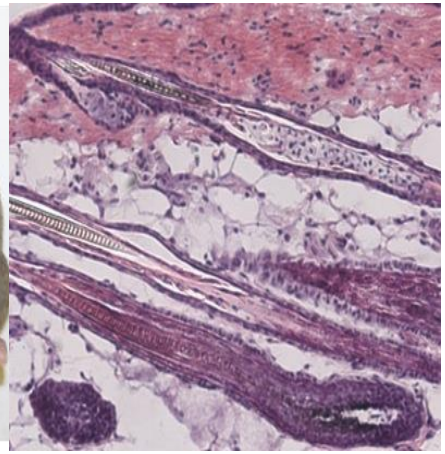


JAK3 Inhibitor

PBS

JAK 3 Inhibitor



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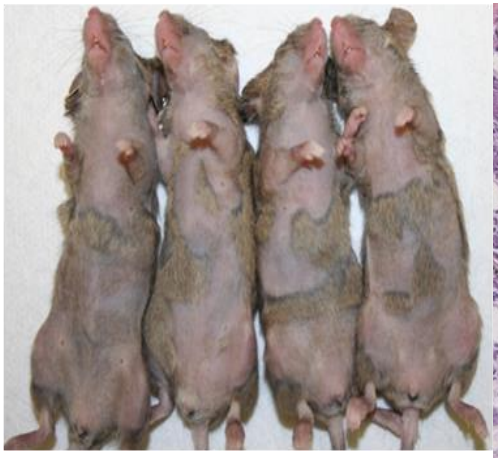
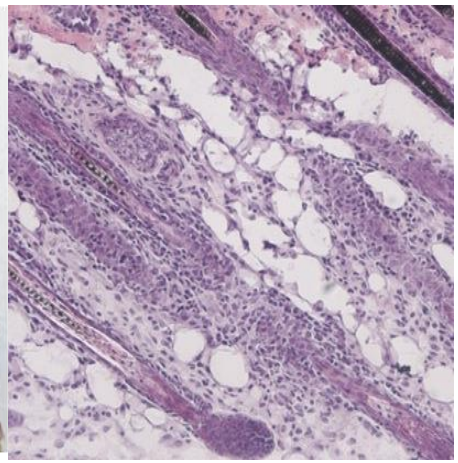
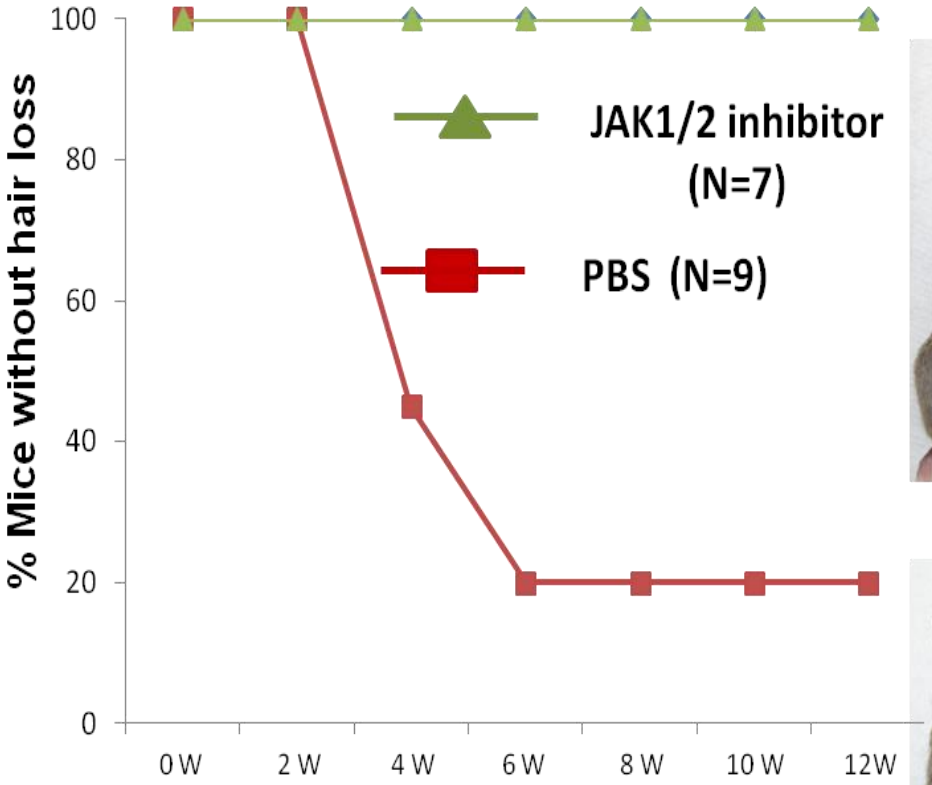


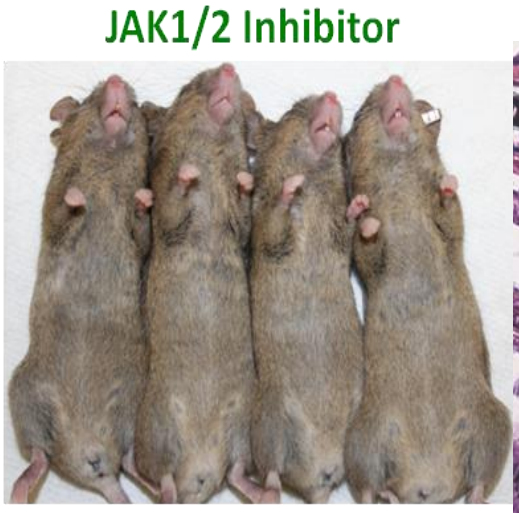


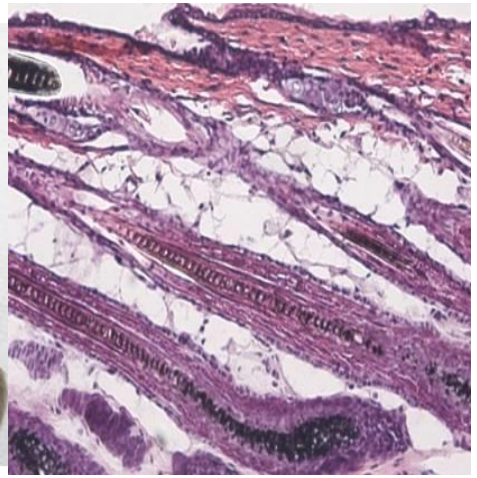
Xing, et al, Nature Medicine 2014

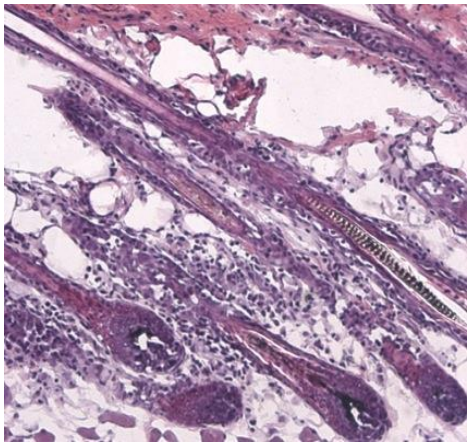
# JAK 1/2 Inhibitor (Ruxolitinib) Prevents Alopecia Areata





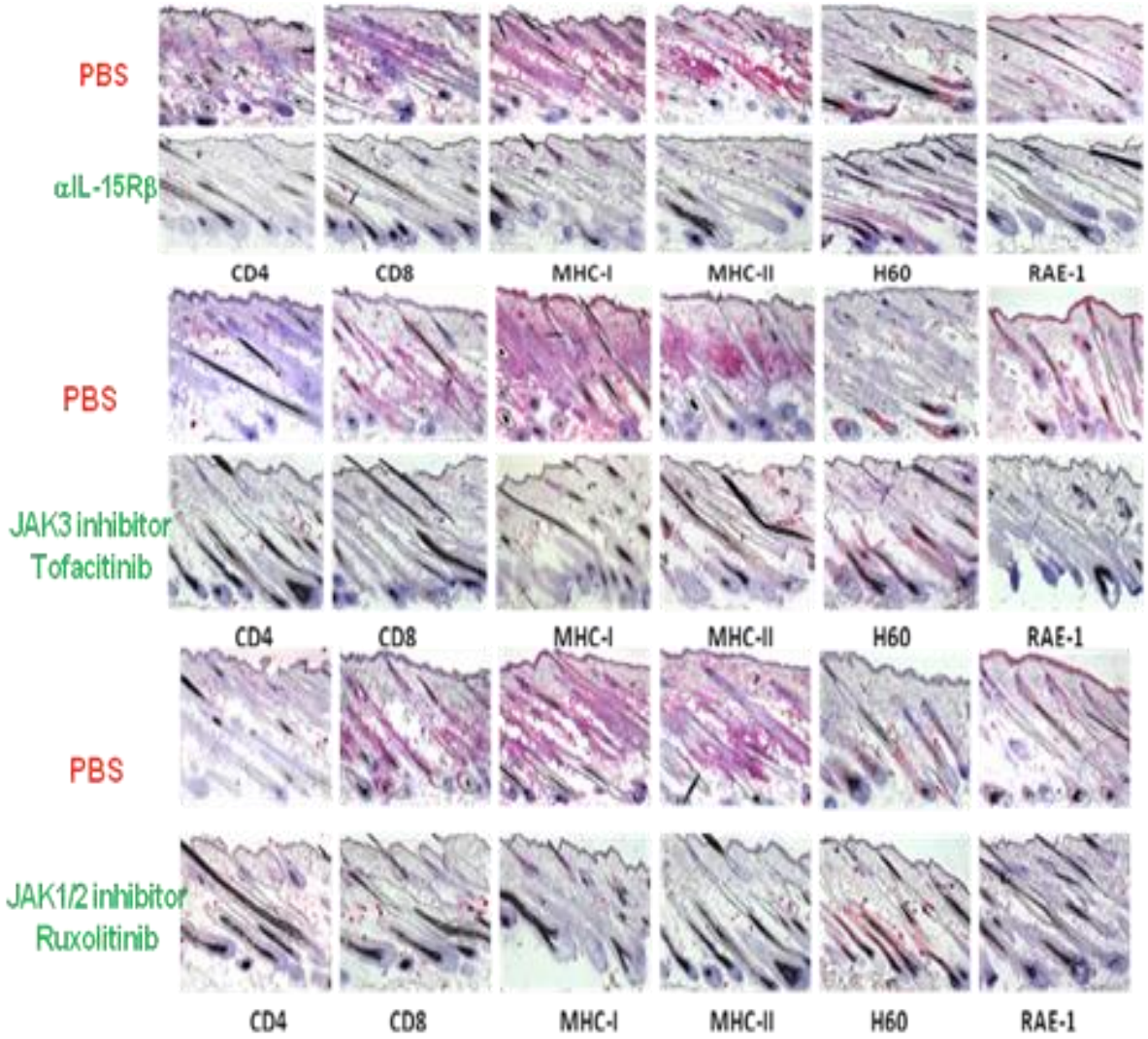


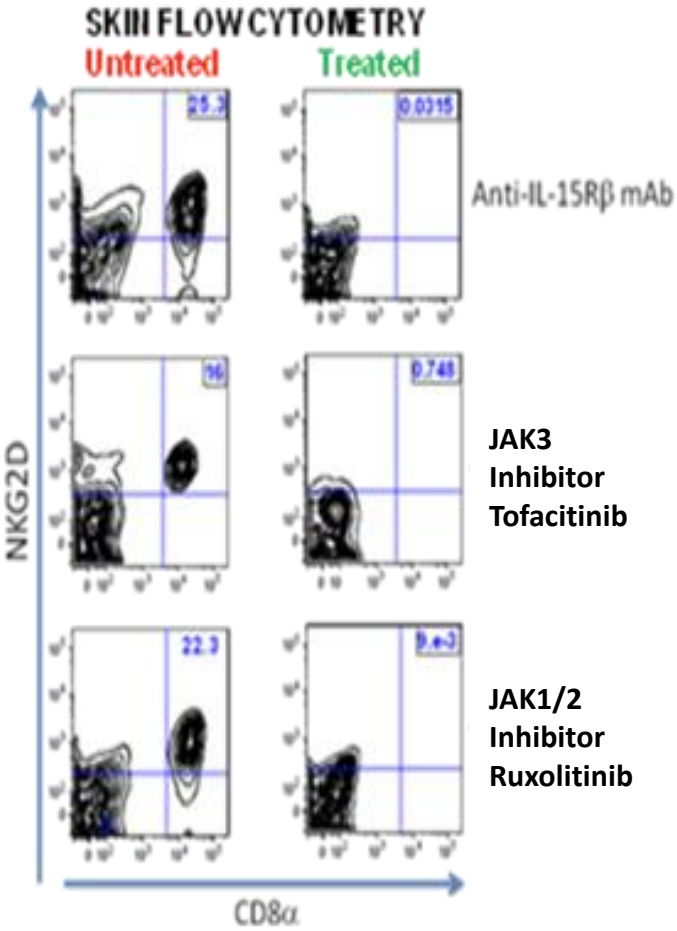



Xing, et al, Nature Medicine 2014

# Normalization of inflammatory infiltrates in AA prevention model using systemic delivery




Xing, et al, Nature Medicine 2014

# Pre-clinical studies with topical delivery

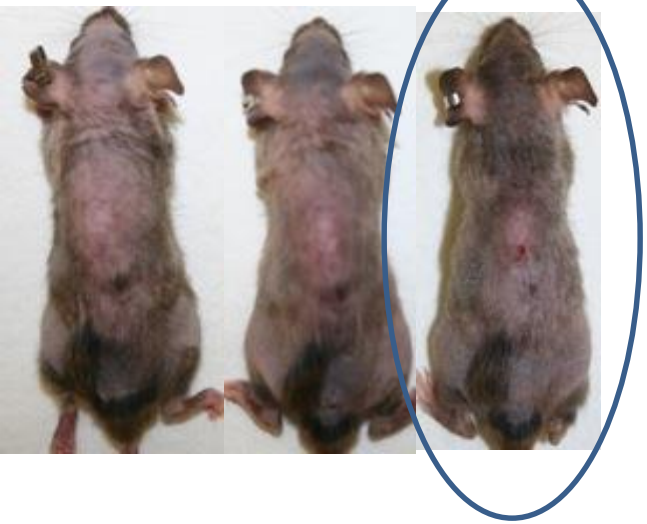
**JAK1/2 Inhibitor (ruxolitinib)**

**JAK3 inhibitor (tofacitinib)**

# Topical treatment results in reversal of long-standing AA (2-3 months duration)

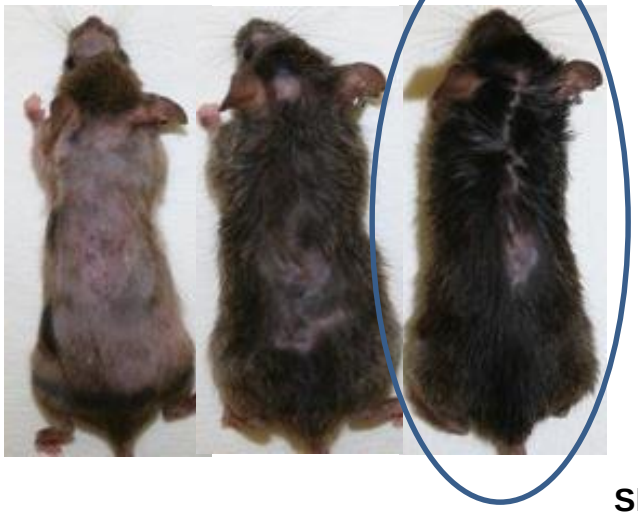
## Control Mice

Before Treatment | 4 weeks | 7 weeks



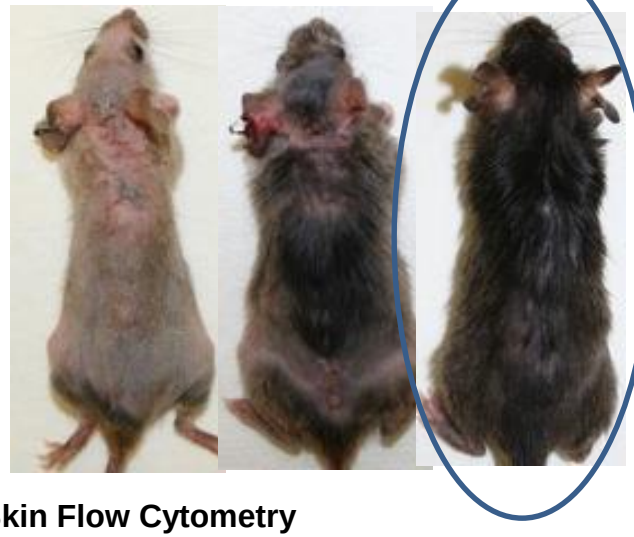
## JAK3 inhibitor Treated

Before Treatment | 4 weeks | 7 weeks



## JAK1/2 Inhibitor Treated

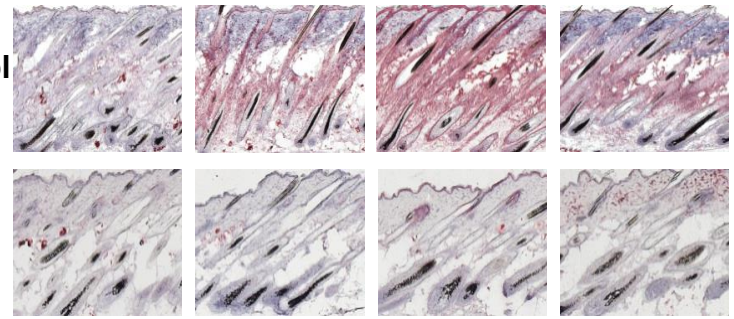
Before Treatment | 4 weeks | 7 weeks



CD4 | CD8 | MHC-I | MHC-II

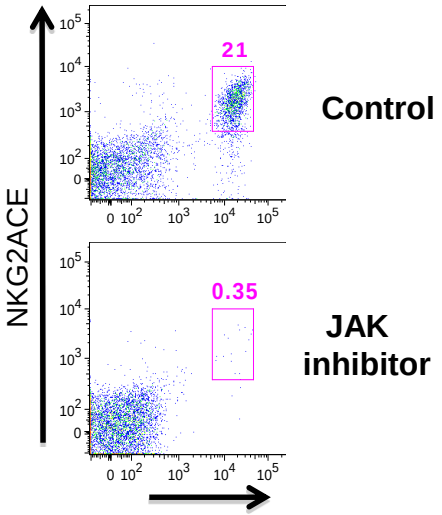
Control

JAK inhibitor

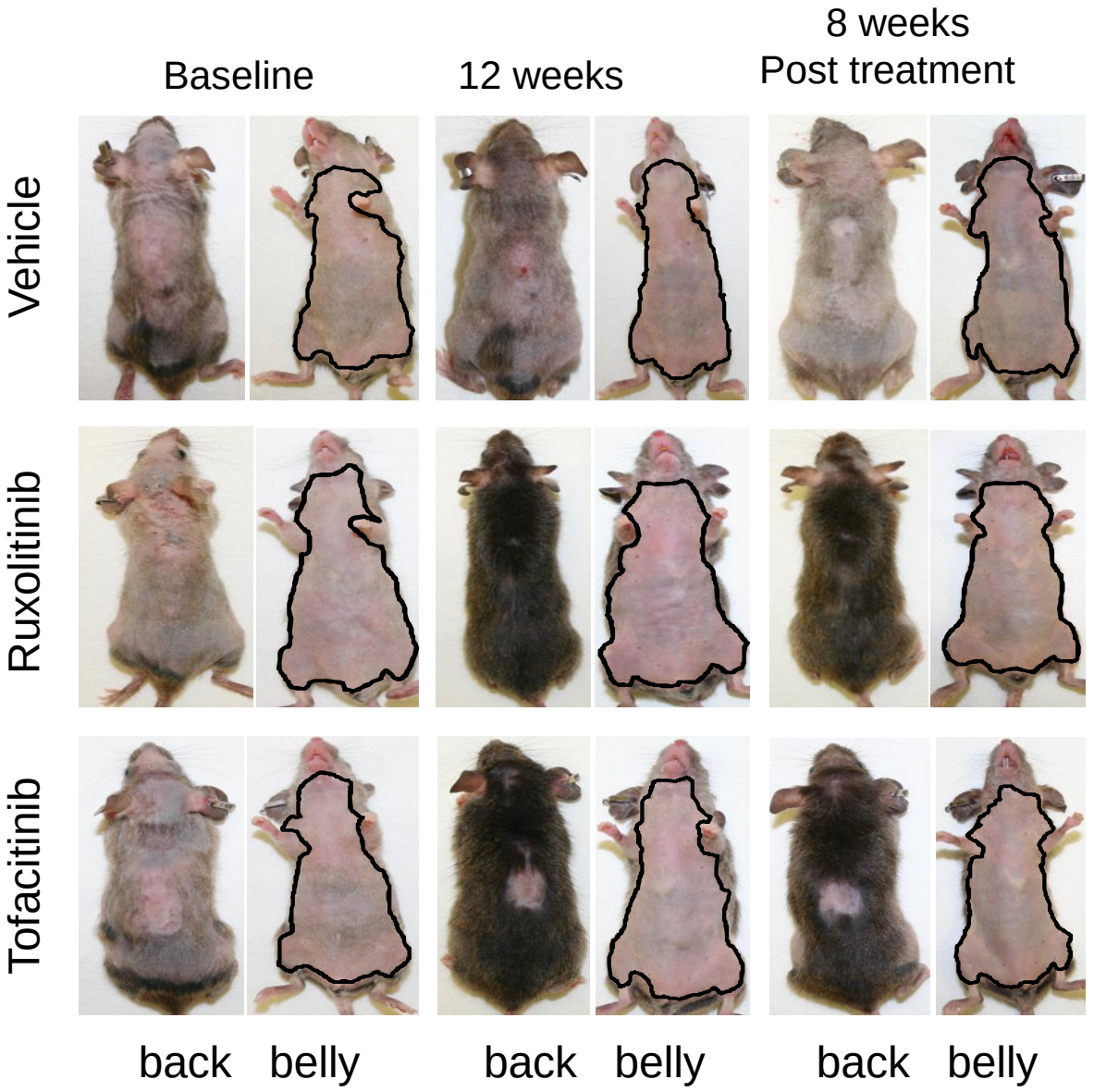


Skin Immunohistochemistry

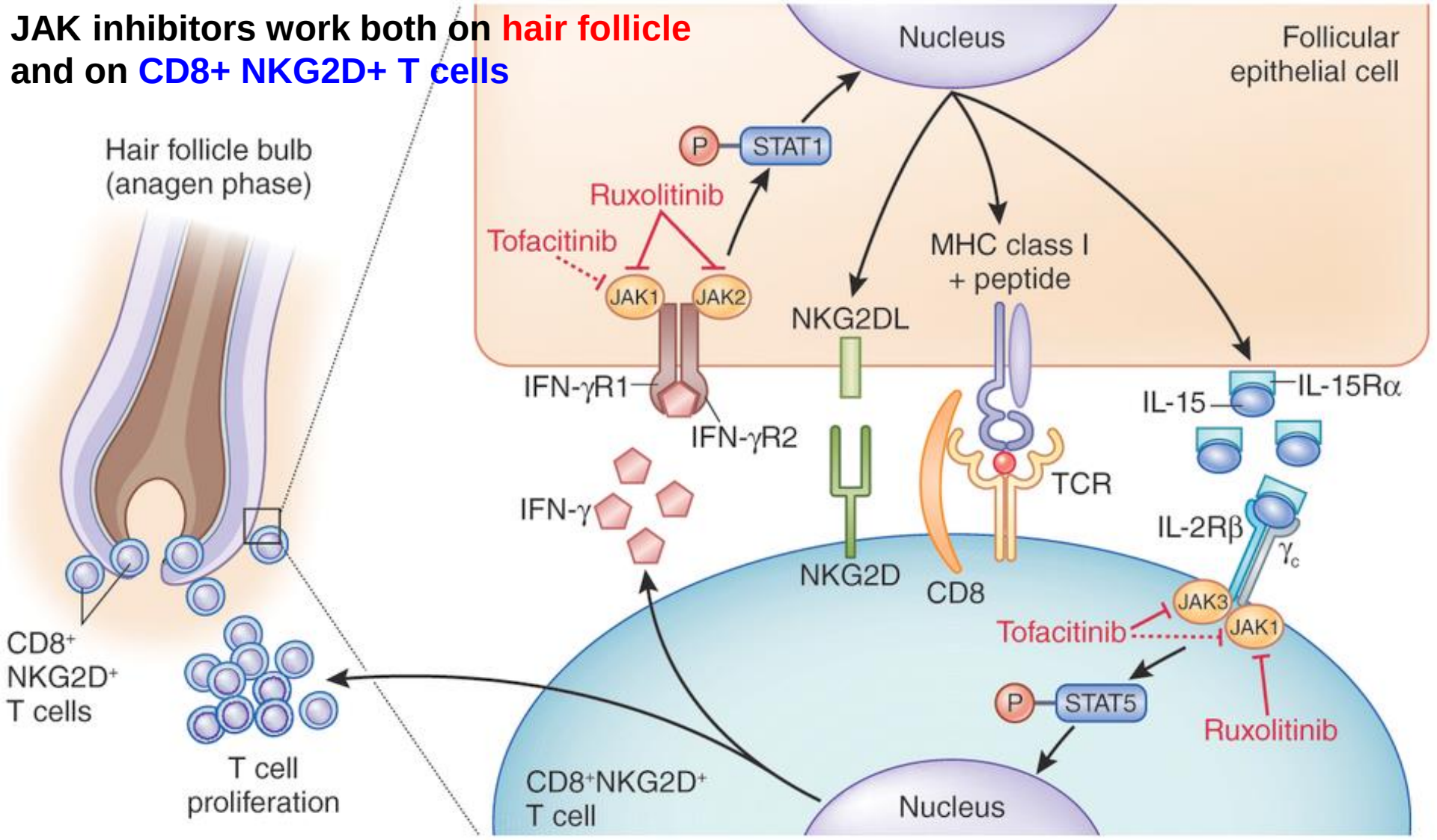
Skin Flow Cytometry



# Topical treatment with JAKi is durable and *does not* show evidence of systemic absorption



# JAK inhibitors work both on hair follicle and on CD8+ NKG2D+ T cells

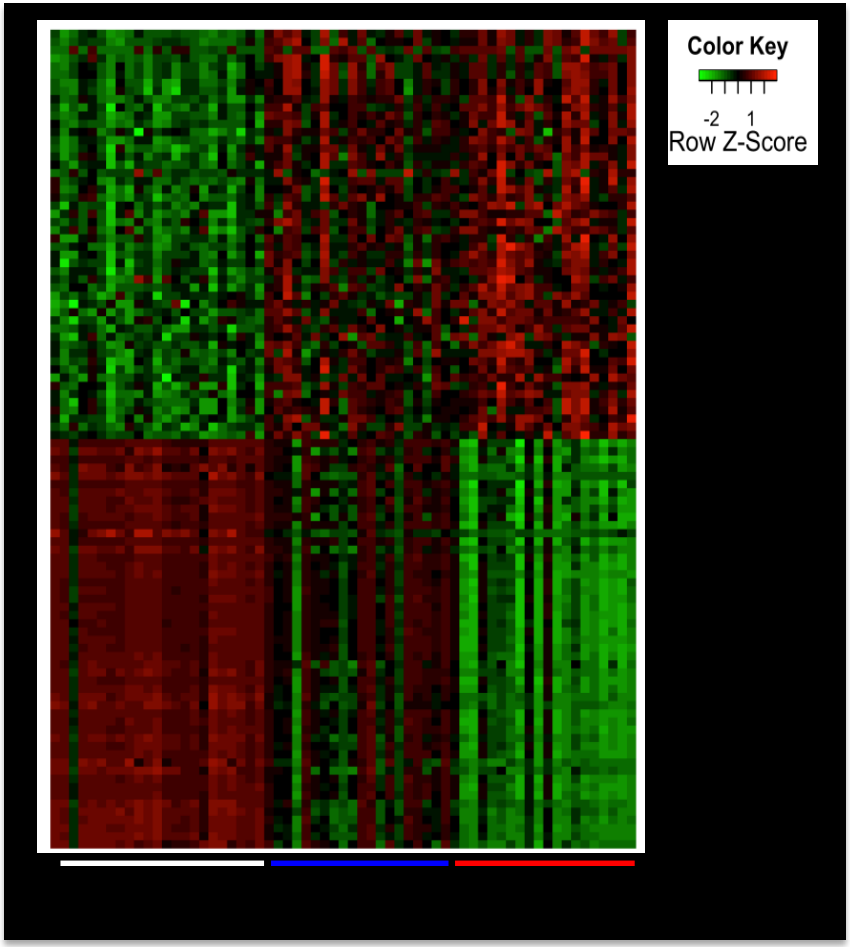


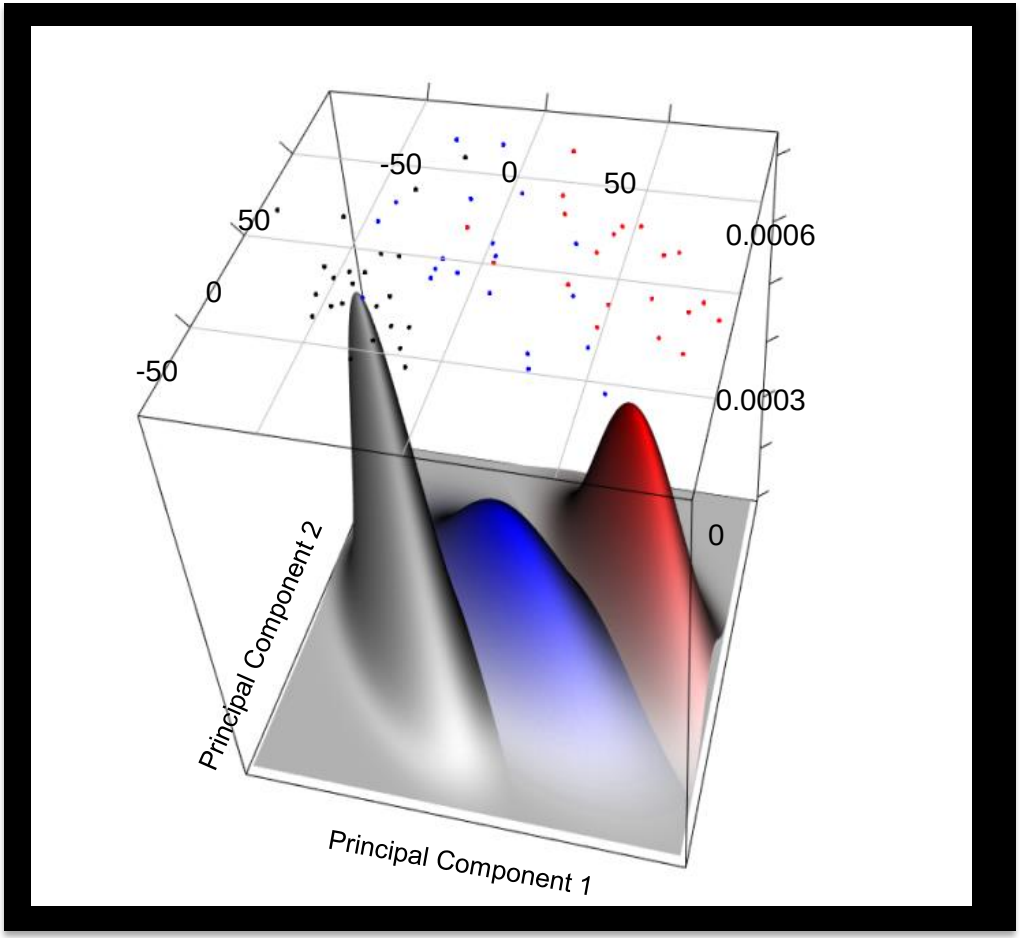

Xing *et al*.[2] show that CD8⁺ NKG2D⁺T cells infiltrate the dermis and localize to the hair follicle bulb, where they form immune synapses with follicular epithelial cells through major histocompatibility complex (MHC) class I–peptide complexes and NKG2DL. Activated CD8⁺ T cells release IFN-γ, which binds the IFN-γR on the surface of the follicular epithelial cell, which in turn signals via JAK1 and JAK2 to promote production of IL-15, a mediator of CD8⁺ T cell induction, and its chaperone IL-15Rα. This binds the IL-15R complex (IL-2Rβ and $\gamma_c$) on the CD8⁺ T cell surface, causing signaling via JAK1 and JAK3 to enhance the production of IFN-γ and amplify the feedback loop. Ruxolitinib and tofacitinib are small-molecule JAK inhibitors that interfere with this feedback loop; ruxolitinib inhibits JAK1 and JAK2, and tofacitinib inhibits JAK3 more strongly than JAK1. These inhibitors are able to alleviate the AA symptoms. STAT1, signal transducer and activator of transcription-1; TCR, T cell receptor; STAT5, signal transducer and activator of transcription-5. From Divito and Kupper**, Nature Medicine** 20, 989–990 (2014).

# Outline

- Genetics of Alopecia Areata
- Immunology of Alopecia Areata
- Translational Research and Clinical Studies

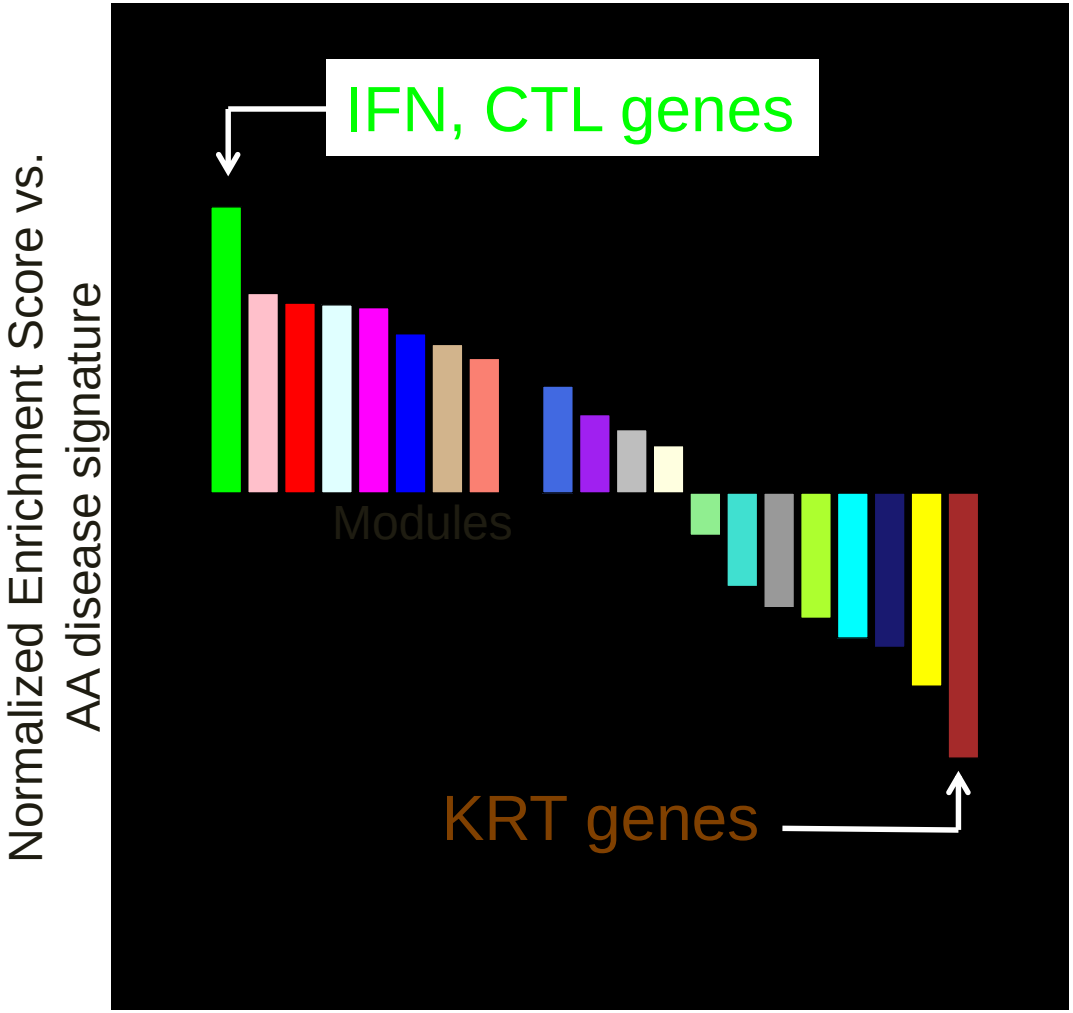
# Alopecia Areata Disease Signatures



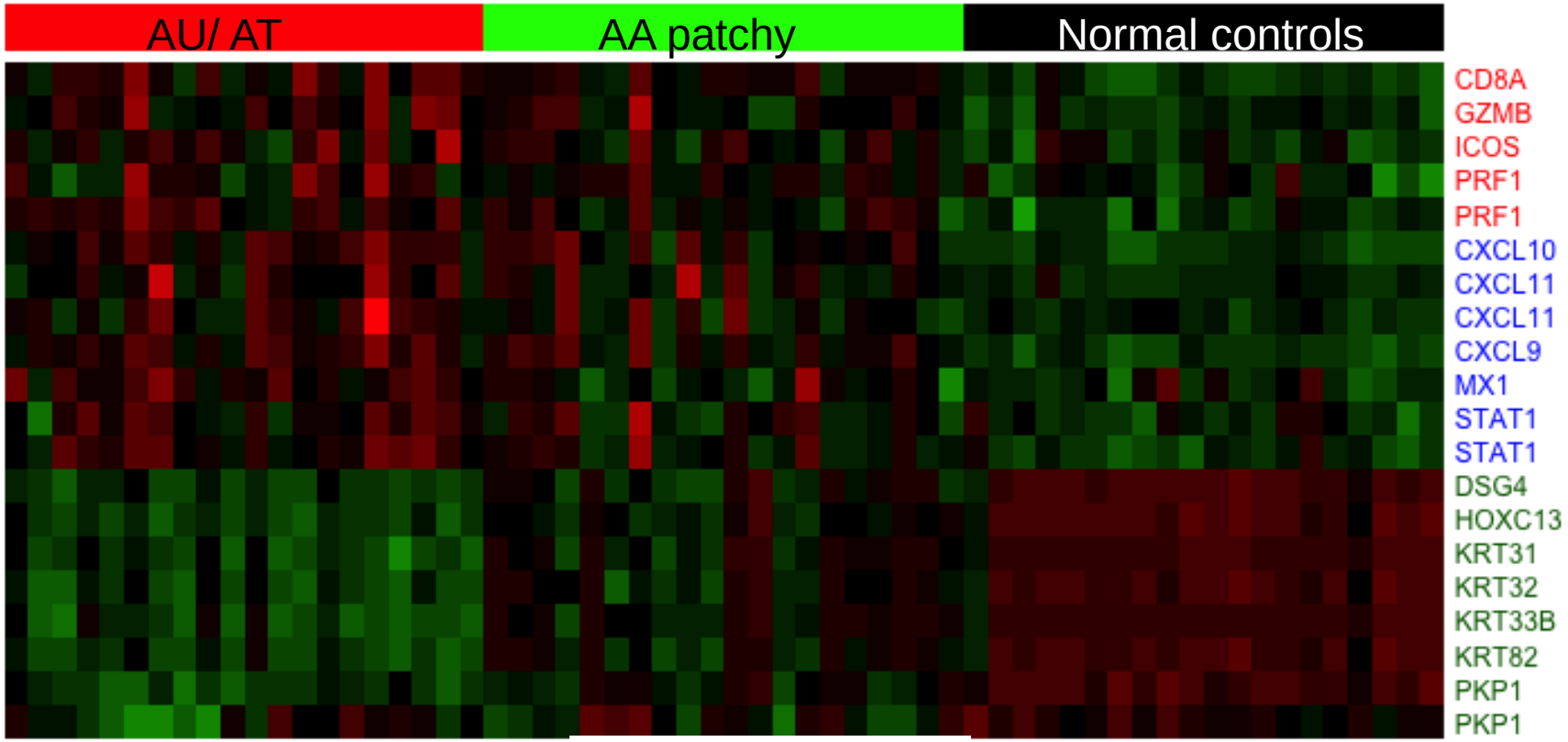

Jabbari, et al. EBioMedicine. 7:240-7, 2016

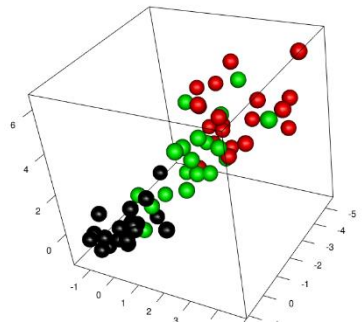
# Gene Expression Modules in Human Alopecia Areata




Jabbari, et al. EBioMedicine. 7:240-7, 2016

# ALADIN analysis of AA patient biopsies



**CTL signature**
**IFN signature**
**KER signature**

Jabbari, et al. EBioMedicine. 7:240-7, 2016

# Alopecia Areata Disease Activity Index (ALADIN)

- Alopecia Areata Disease Activity Index (ALADIN),

a multi-dimensional quantitative composite score that may be used as an AA "molecular distance to health" (Pankla et al 2009).

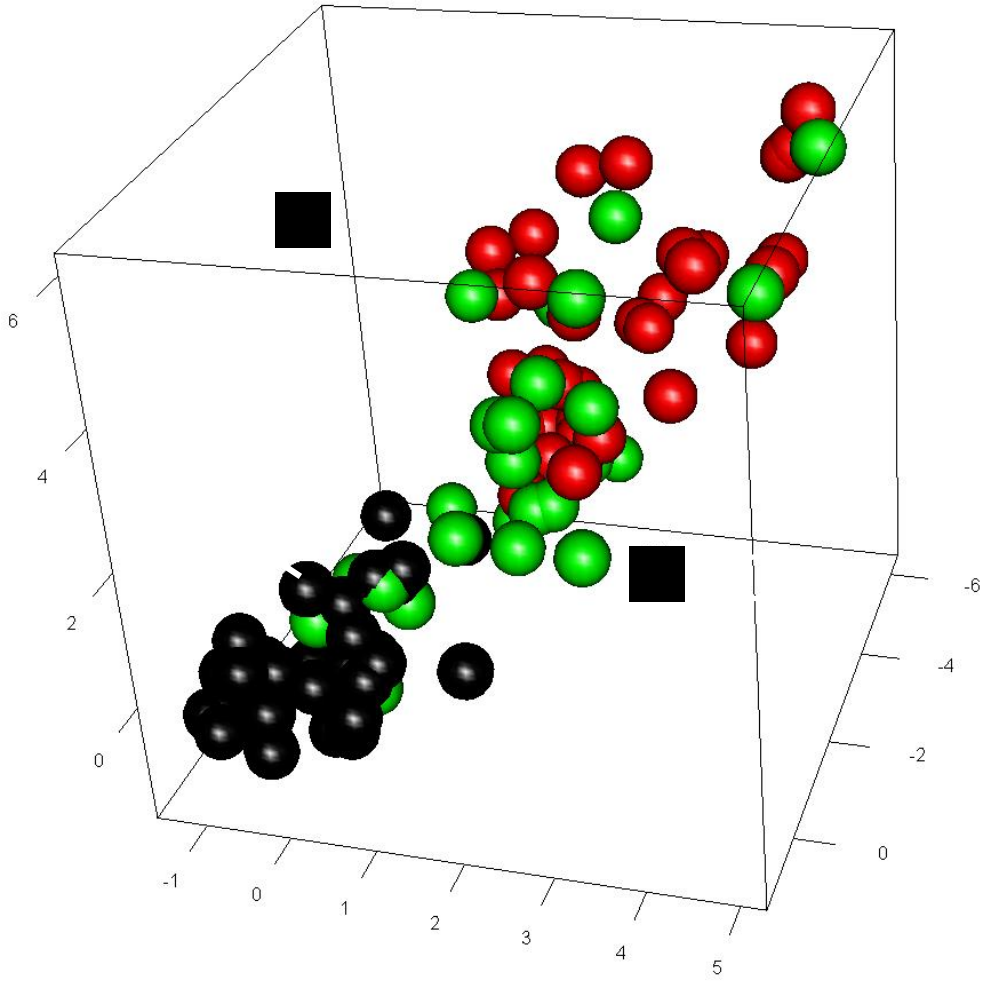
- ALADIN is comprised of three signature scores:

  a CTL score, an IFN score and a KER score.

- ALADIN is being assessed and refined in our biomarker and clinical studies to evaluate its utility in human AA.

Jabbari, et al. EBioMedicine. 7:240-7, 2016

# Alopecia Areata Disease Activity Index (ALADIN)




AU/AT

AAP

Normal

Jabbari, et al. EBioMedicine. 7:240-7, 2016

# Molecular signatures define alopecia areata subtypes and transcriptional biomarkers

**Conventional thinking in AA..**

- Efficacy of topical immunotherapies in AU/AT may be lower in longstanding disease
- AU/AT biopsies often appear devoid of hair follicles, as well as immune infiltrates, “burned out” disease
- AU/AT is more recalcitrant to therapy than AAP

Contrary to dogma, gene expression profiling reveals a ***more exaggerated*** immune profile in AU/AT compared with AAP, suggesting that even long-standing disease may be treatable using rational therapies

Jabbari et al, ebiomedicine 7:240-247, 2016

# FDA-Approved JAK Inhibitors

XELJANZ®
(tofacitinib citrate)
5 mg tablets

JAK3 (pan-JAK) inhibitor

Rheumatoid arthritis

Jakafi®
ruxolitinib (tablets)
JAK targeted to make a difference

JAK1/2 Inhibitor

Myelofibrosis


**JAK inhibitors are potent immunosuppressive agents with associated risks:**
**Are they safe and effective in alopecia areata?**

# Ruxolitinib – study design

PI: Julian Mackay-Wiggan, MD, MPH

- Open-label, single arm pilot study
- 12 patients (initially 10), duration of disease from 2-34 years
- 10 Moderate to severe patch type AA, 2 AT/AU (s/p modification)
- Treatment with ruxolitinib 20mg BID for 3 to 6 months.

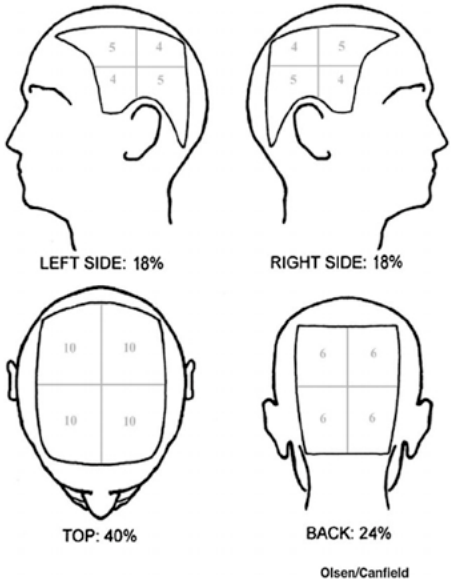
- Efficacy measured by hair re-growth as determined by physical exam, SALT score and photography, and by patient and physician evaluation scores.
- Patients followed for 3 months after treatment to evaluate durability of response.
- Biopsies and peripheral blood obtained at baseline and at 12 weeks for immune monitoring and molecular studies. Additional biopsies and blood draws (1-3 anticipated) obtained as clinically indicated
- QOL measures at baseline and at various additional time points

# Efficacy of Ruxolitinib in AA

- 9 of 12 patients (75%) achieved the primary outcome of at least 50% regrowth measured by SALT score
- Response is seen as early as 1 month (subtle patchy regrowth)
- Regrowth progresses steadily with continued patchy regrowth usually by 3 months
- 3 non-responders
- 3 patients with mod/severe relapse, 6 patients with mild

Mackay-Wiggan, et al. JCI Insight. 2016 15:e89790.

# Severity of Alopecia Tool - SALT Score




SPECIAL ARTICLE

## Alopecia areata investigational assessment guidelines—Part II

Elise A. Olsen, MD,[a] Maria K. Hordinsky, MD,[b] Vera H. Price, MD,[c] Janet L. Roberts, MD,[d] Jerry Shapiro, MD,[e] Doug Canfield,[f] Madeleine Duvic, MD,[g] Lloyd E. King Jr, MD, PhD,[h] Amy J. McMichael, MD,[i] Valerie A. Randall, PhD,[j] Maria L. Turner, MD,[k] Leonard Sperling, MD,[l] David A. Whiting, MD,[m] and David Norris[n]

*Durham, North Carolina; Minneapolis, Minnesota; San Francisco, California; Portland, Oregon; Vancouver, British Columbia, Canada; Fairfield, New Jersey; Houston, Texas; Nashville, Tennessee; Winston-Salem, North Carolina; West Yorkshire, United Kingdom; Bethesda, Maryland; Dallas, Texas; and Denver, Colorado*

Alopecia areata is an immunologically mediated disease characterized by extreme variability not only in the time of initial onset of hair loss but in the duration, extent and pattern of hair loss during any given episode of active loss. These variables, as well as the unpredictable nature of spontaneous regrowth and lack of a uniform response to various therapies, has made clinical trials in alopecia areata difficult to plan and implement. In fact, there are currently no drugs FDA-approved specifically for the indication of alopecia areata.

To help facilitate well-controlled clinical trials for alopecia areata, this National Alopecia Areata Foundation (NAAF) sponsored subgroup of investigators/clinicians experienced in clinical trials and/or in the clinical care of patients with alopecia areata has outlined some general principles and potential endpoints for clinical studies in alopecia areata. These guidelines build on the Alopecia Areata Investigational Assessment Guidelines published in 1999[1] which established baseline clinical staging and background information important to gather on any alopecia areata patient involved in clinical research.

### Recommended criteria for assessing a therapeutic response

A. General: The following information should be collected at baseline (in addition to that baseline information outlined in A-D (Part V) in the Alopecia Areata Investigational Assessment Guidelines[1] (see Table I). Stratification is suggested for some characteristics that may have prognostic implications (ex: duration hair loss, percentage hair loss, pattern of hair loss). The stratification into subgroups is meant to prevent inappropriate clustering of patients in clinical therapeutic trials but should not substitute for the collection, and later analysis, of the unqualified data.

1. Duration of current episode of scalp hair loss (beginning with when last had normal complement of scalp hair excluding hair loss from etiologies other than alopecia areata (such as androgenetic alopecia/pattern hair loss). May be stratified by subgroups of duration of current episode including:
   a. < 3 months
   b. 3-12 months
   c. 12-24 months
   d. >2-5 years
   e. >5 years
2. Percent scalp hair loss. This takes into account the percent of the scalp surface with no hair. The hair loss in patients with diffuse alopecia areata without discrete patches of alopecia cannot be captured by this method. Fig 1 is recommended as a visual aid. The diagrams of Fig 1 and the percentage scalp surface area

From the Duke University Medical Center, Durham[a]; University of Minnesota, Minneapolis[b]; University of California at San Francisco[c]; Oregon Health & Science University, Portland[d]; Skin Care Center, Vancouver[e]; Canfield Scientific, Fairfield[f]; University of Texas M. D. Anderson Cancer Center, Houston[g]; Vanderbilt University, Nashville[h]; Wake Forest University Medical Center, Winston-Salem[i]; University of Bradford, West Yorkshire[j]; National Institute of Health, Bethesda[k]; Uniformed Services University of Health Sciences, Bethesda[l]; Baylor Hair Research and Treatment Center, Dallas[m]; and the University of Colorado Health Sciences Center, Denver.[n]

Funding sources: None.
Conflicts of interest: None identified.

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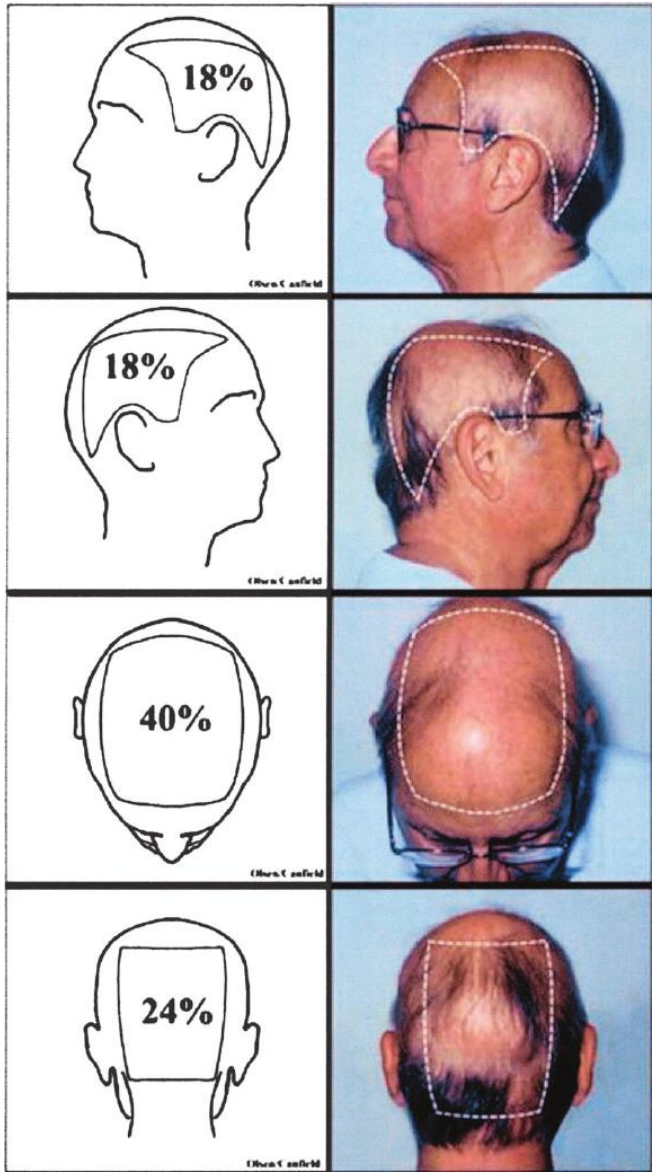
J Am Acad Dermatol 2004;51:440-7
0190-9622/$30.00

doi:10.1016/j.jaad.2003.09.032



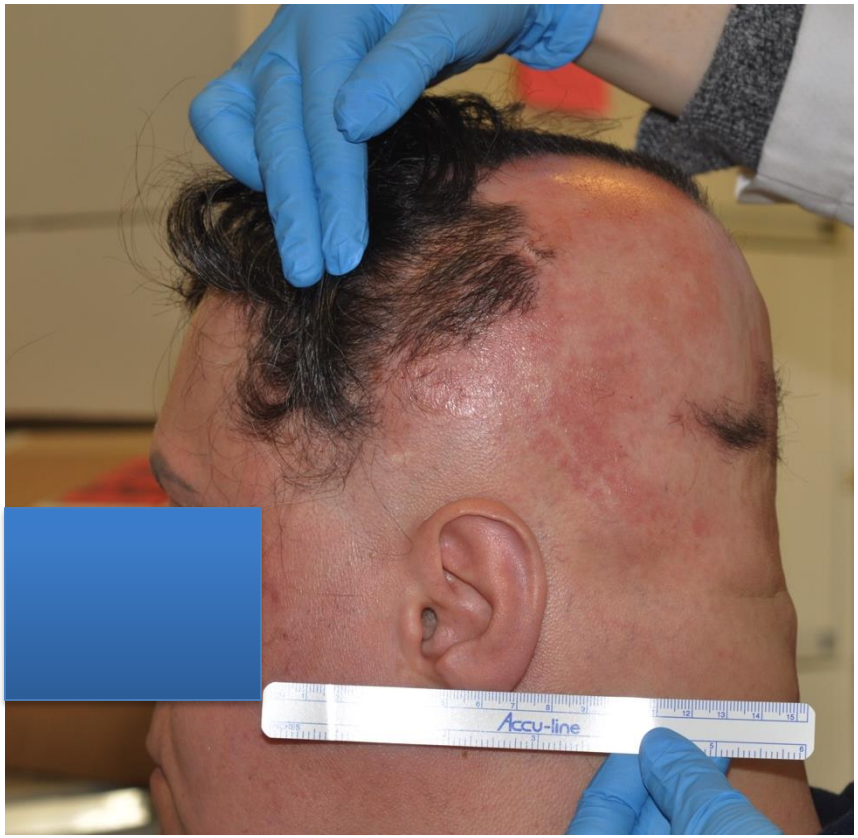
| SALT score | No. (%) |
|---|---|
| 0-24 | 3 (10.34) |
| 25-49 | 8 (27.59) |
| 50-74 | 1 (3.45) |
| 75-99 | 1 (3.45) |
| 100 | 16 (55.17) |

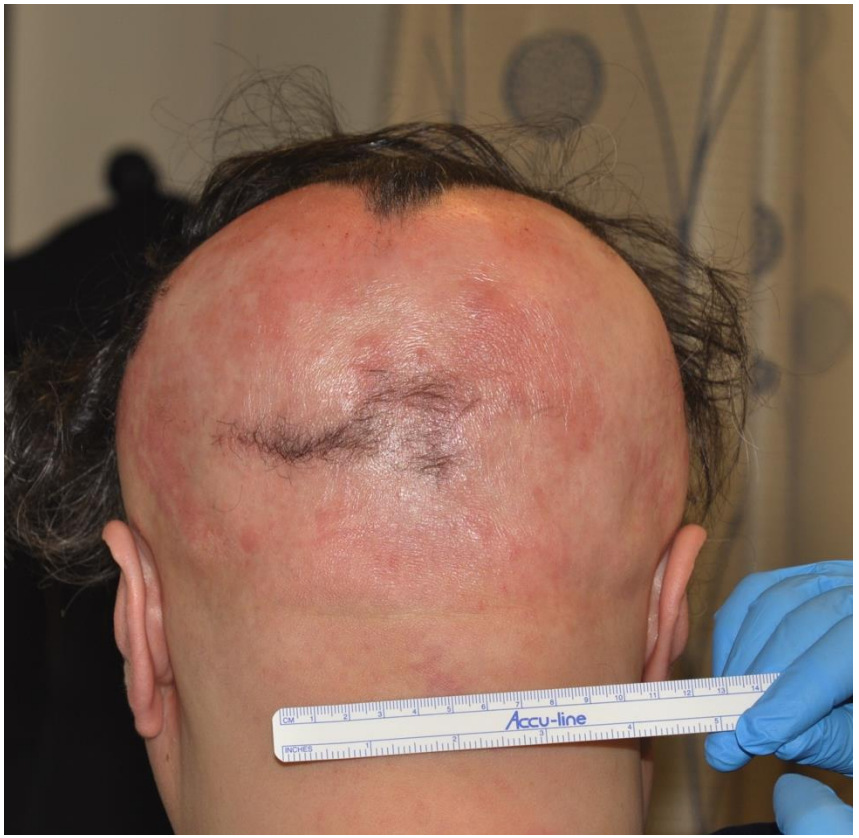
SALT: Severity of alopecia tool, SALT score ranges from 0 to 100, with 0 indicating no alopecia areata and 100 indicating severe disease



# Ruxolitinib - Subject 3

## Baseline

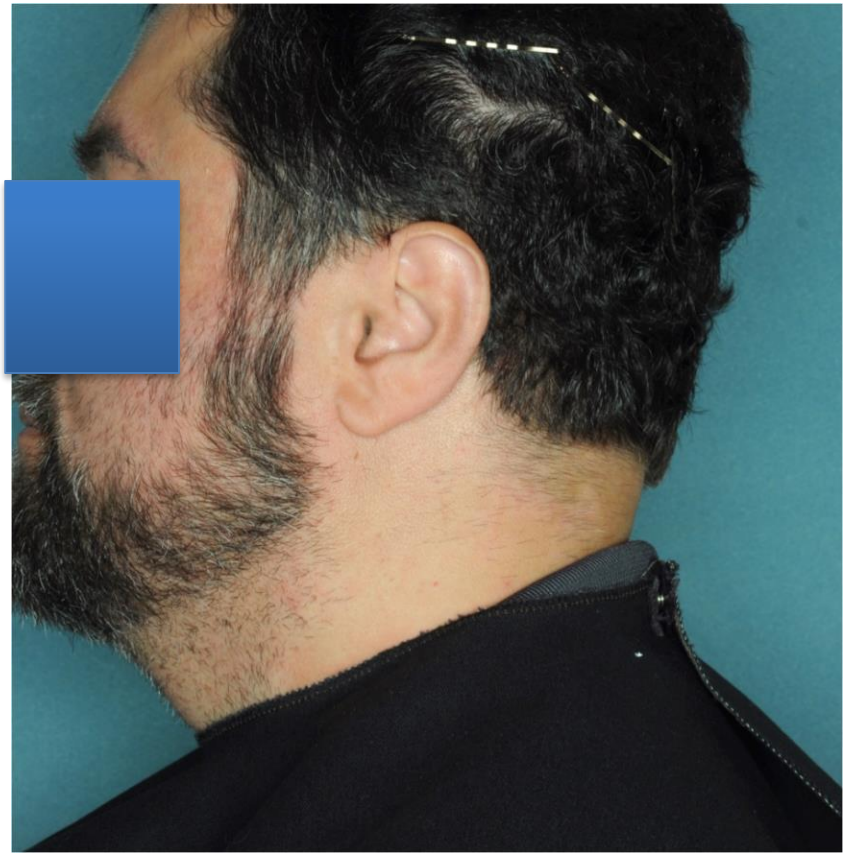


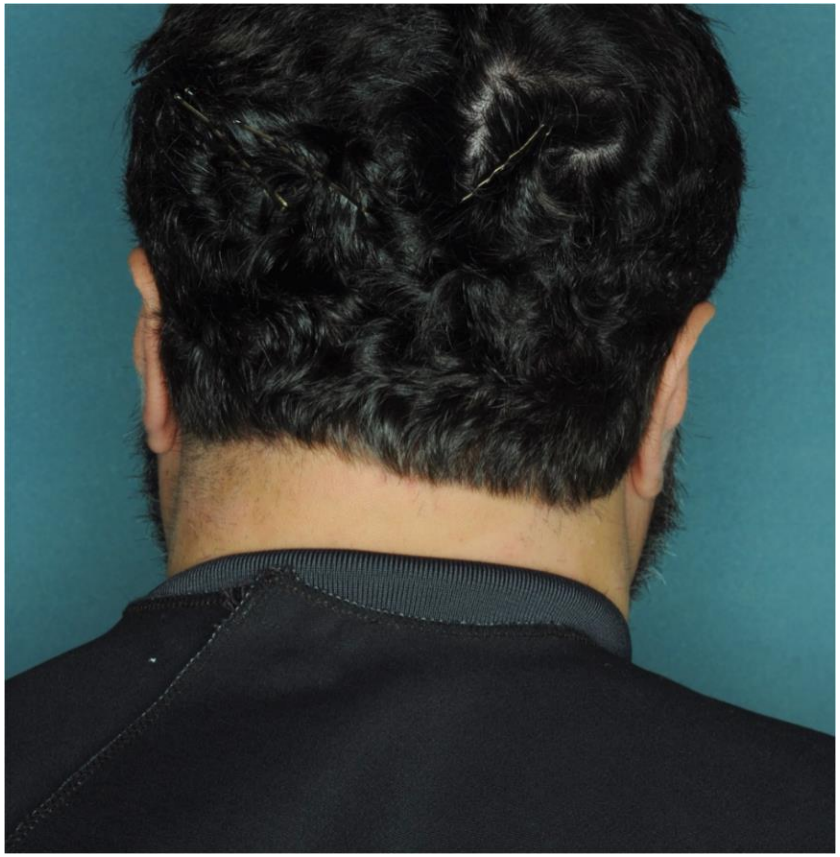




Baseline SALT 64%. Duration of hair loss 12 years

# Ruxolitinib - Subject 3

**Week 24**

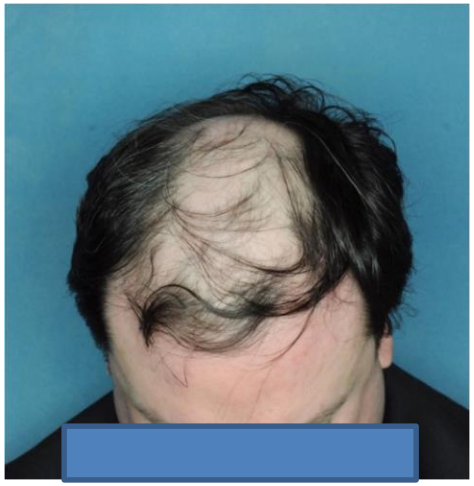




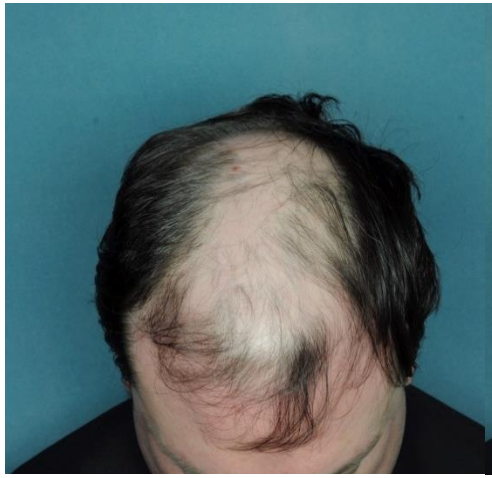
Mackay-Wiggan, et al. JCI Insight. 2016 15:e89790.

# Ruxolitinib - Subject 4

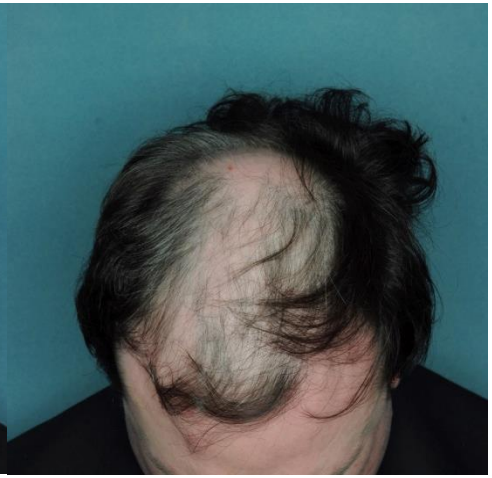
Baseline



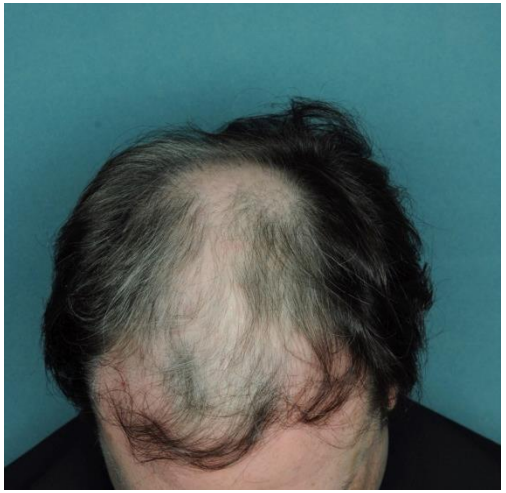
Week 4



Week 8



Week 12



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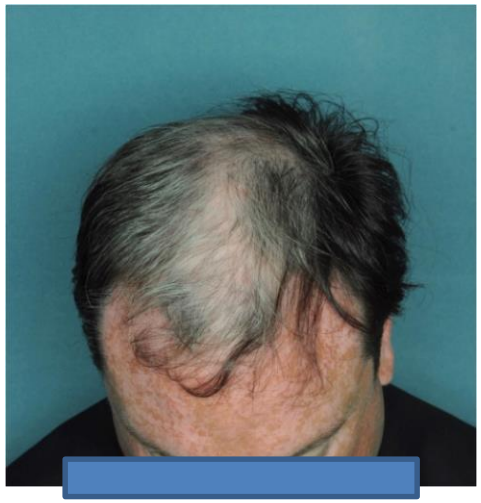
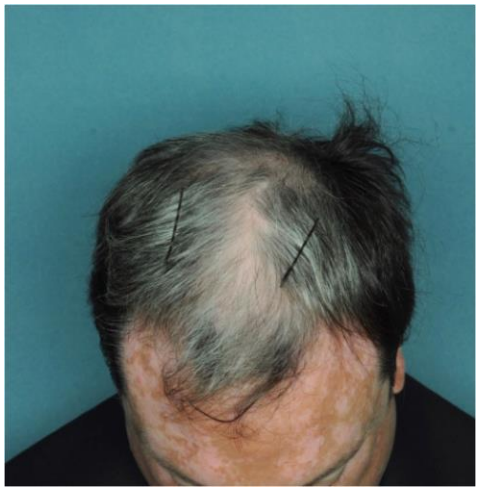
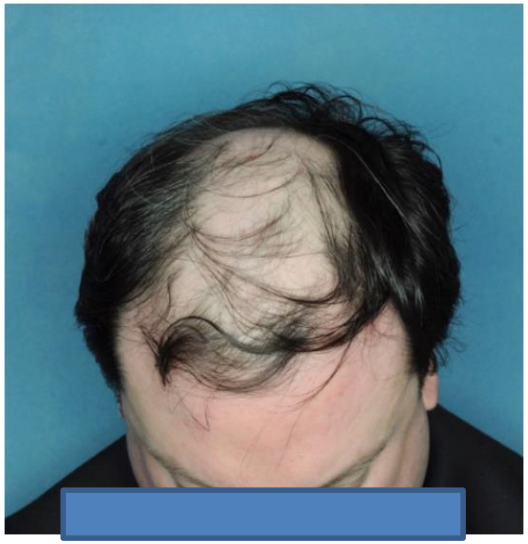


Week 20

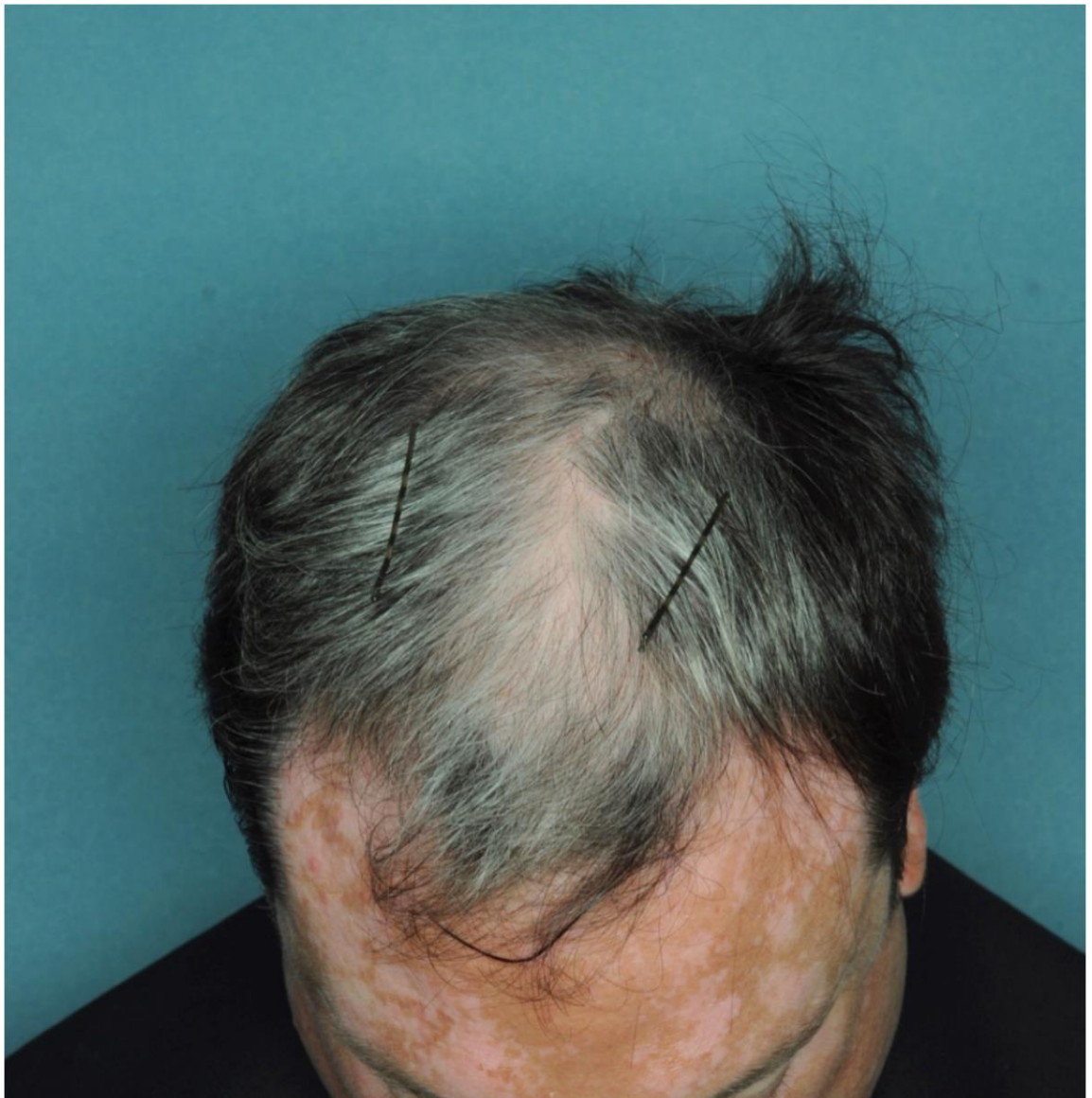


# Ruxolitinib - Subject 4

**Baseline**



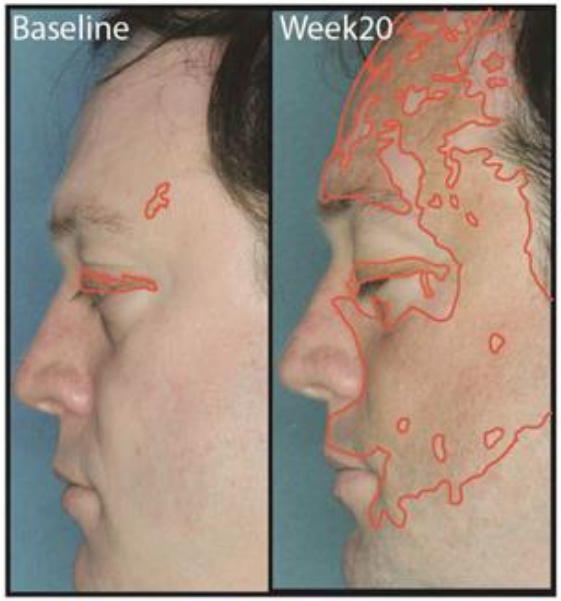
**Week 20**

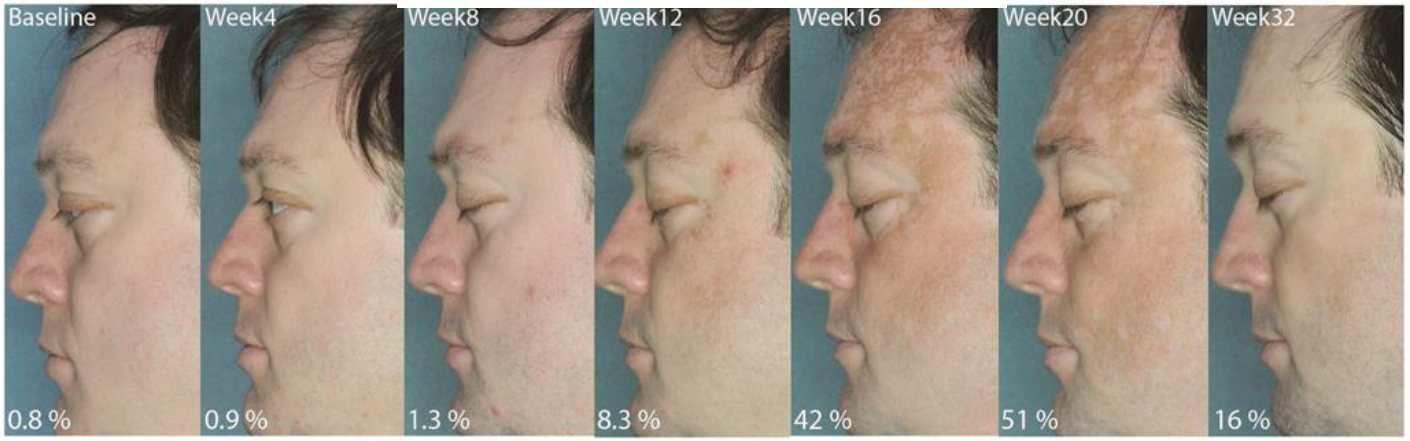


Duration of AA 12 years.

Mackay-Wiggan, et al.
JCI Insight. 2016 15:e89790.

# Rapid skin repigmentation on oral ruxolitinib in a patient with coexistent vitiligo and AA

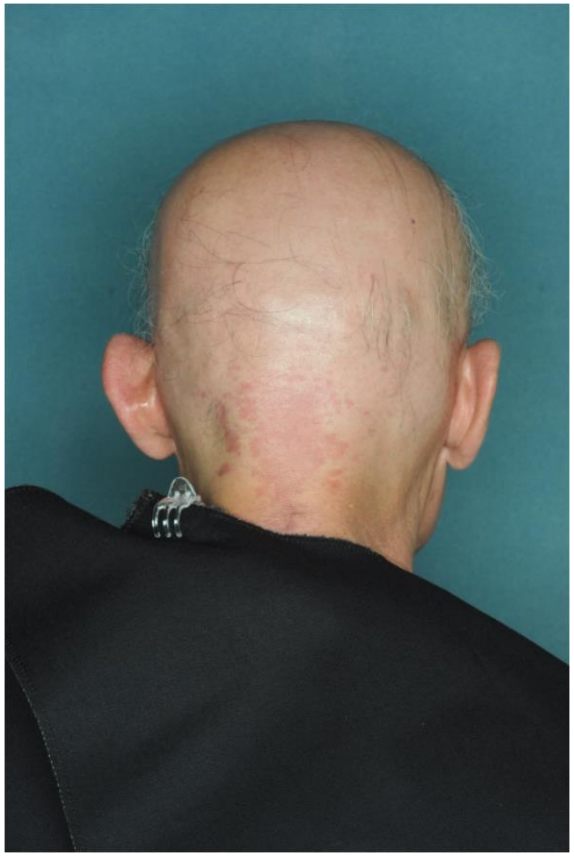


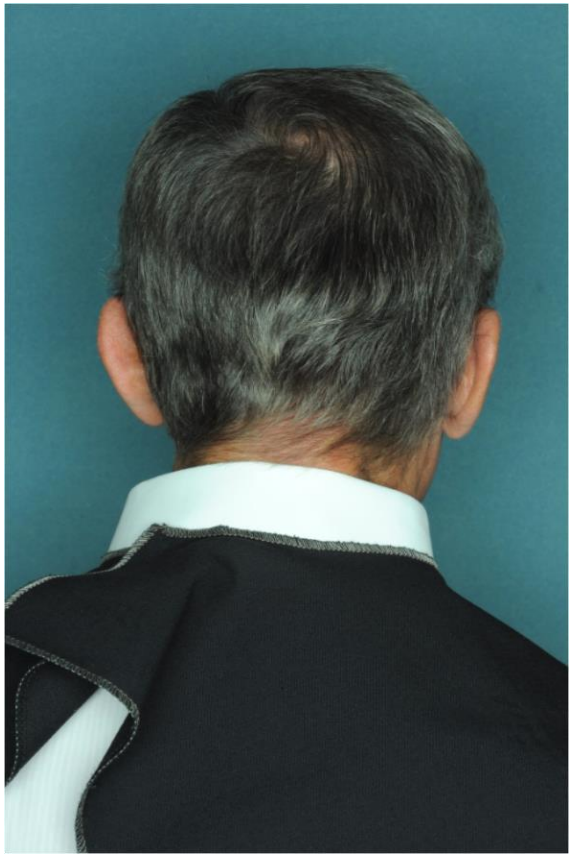

Harris et al, JAAD 2016

# Ruxolitinib - Subject 9

**Baseline**



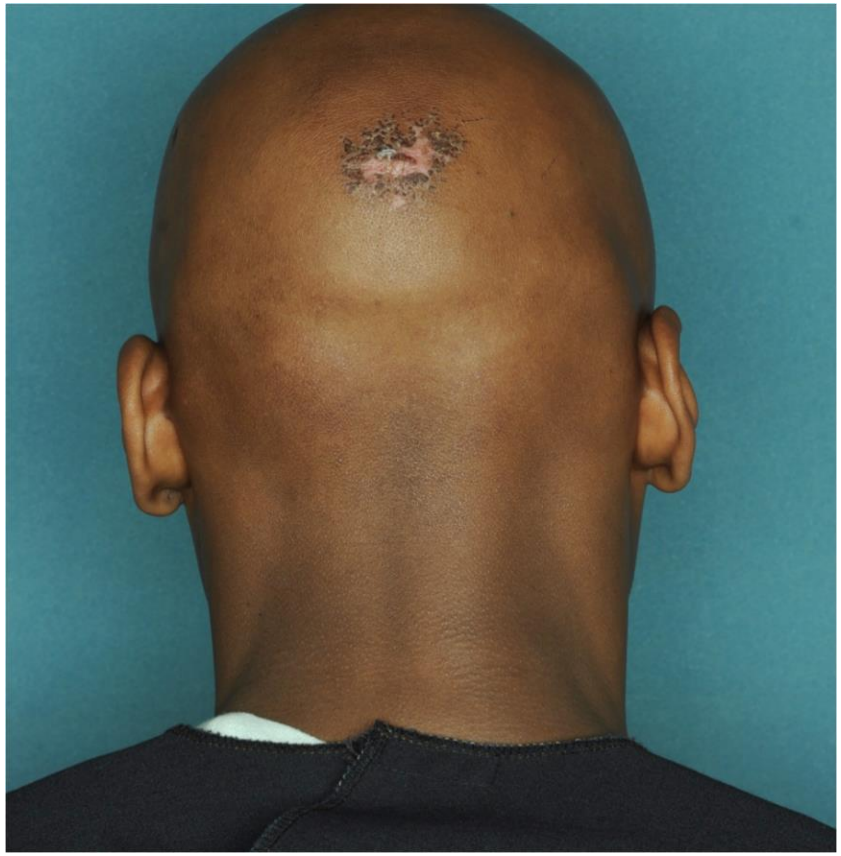
**Week 24**



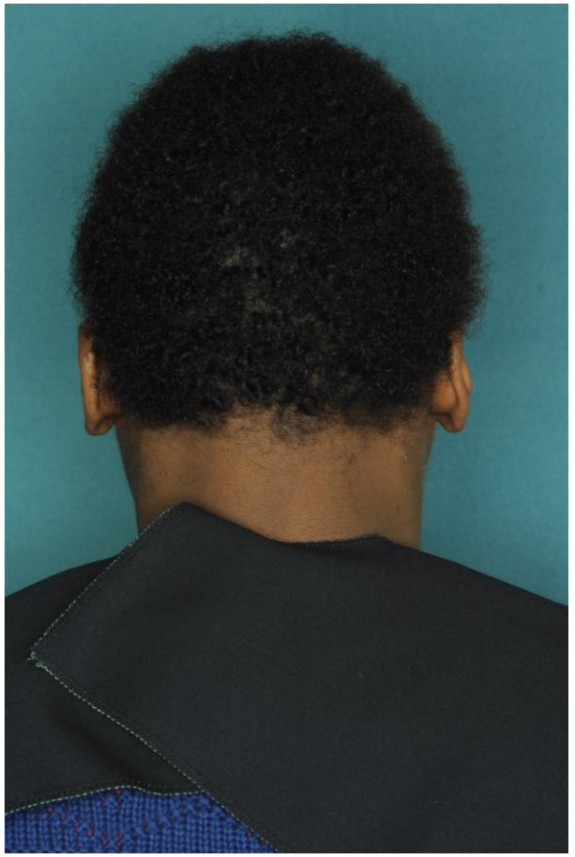
Baseline SALT 95%, duration 4 years

# Ruxolitinib - Subject 12

**Baseline**



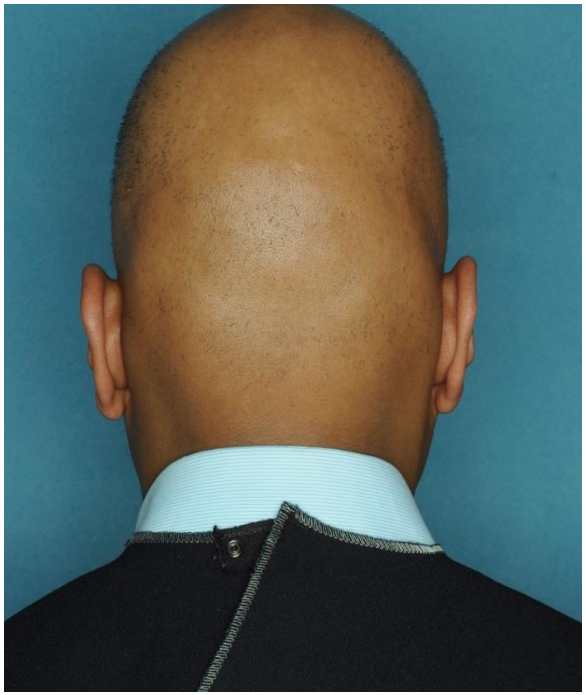
**Week 24**



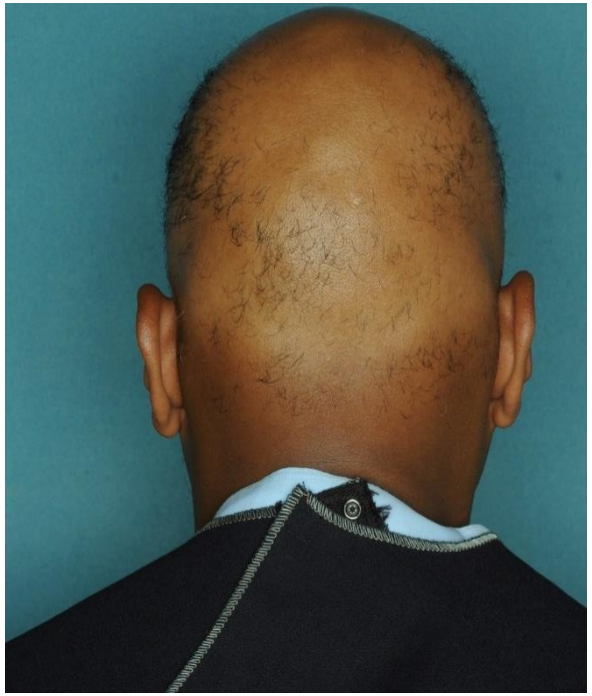
Baseline SALT 100%. Duration of hair loss 9 yrs

# Ruxolitinib - Subject 5

**Baseline**



**Week 12**



Baseline SALT 81%. Duration of hair loss 4 yrs

Mackay-Wiggan, et al. JCI Insight. 2016 15:e89790.

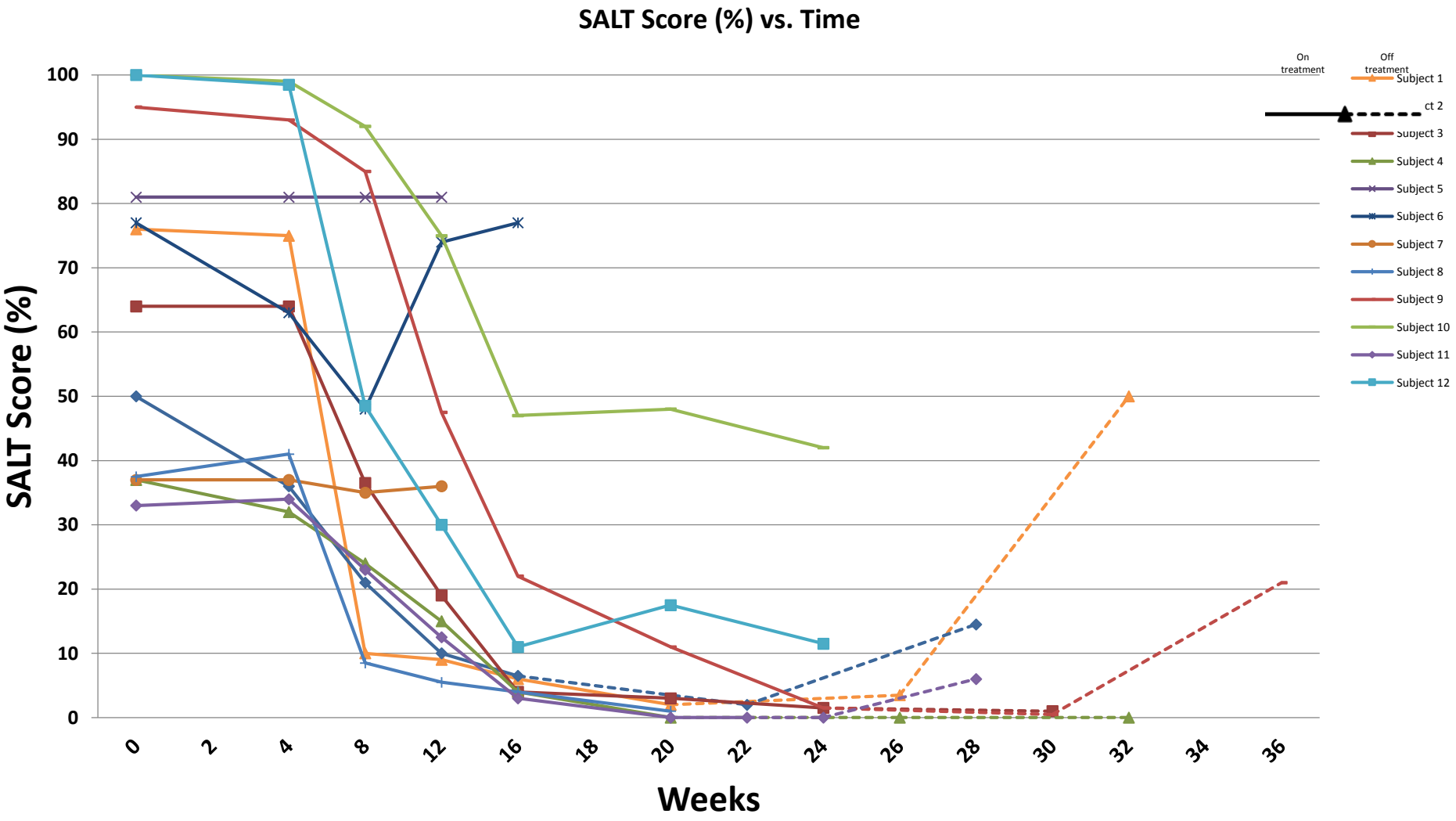
# Ruxolitinib – Adverse Events and Relapse

AEs - Less frequent than anticipated

- Lowered white counts initially but remaining WNL therefore no dose adjustment required
- Lowered hgb – 1 patient required dose modification
- 1 patient with 2 episodes furuncles/abscesses and reported possible biopsy site infection
- Mild URIs, 1 episode pneumonia
- No observed lowered platelets

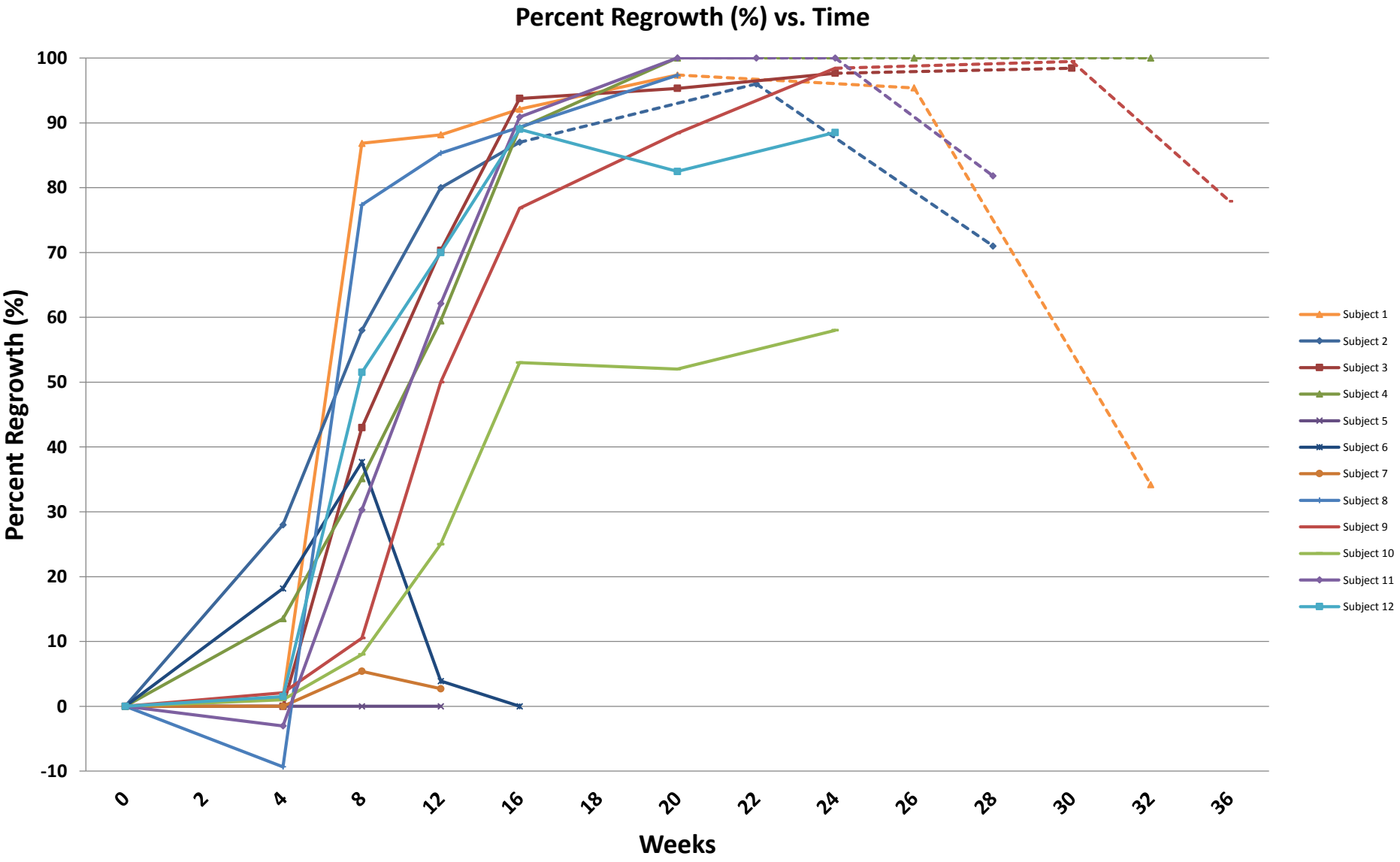
Relapse - observed in most patients by 2-3 months, ranging from mild (not noticeable) to moderate/severe

# Ruxolitinib SALT scores




Mackay-Wiggan, et al. JCI Insight. 2016 15:e89790.

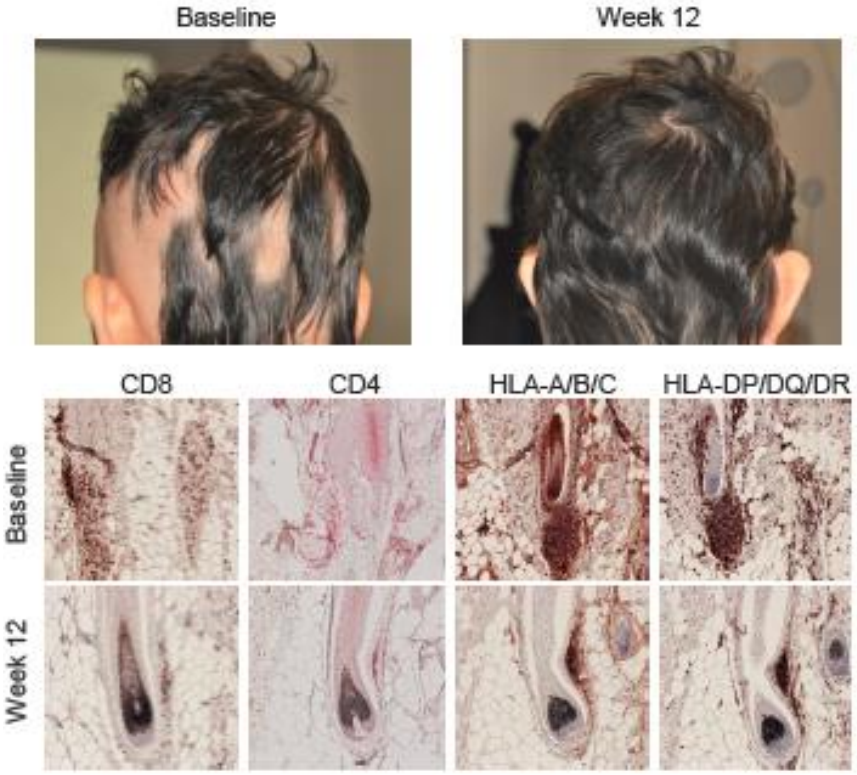
# Ruxolitinib % regrowth

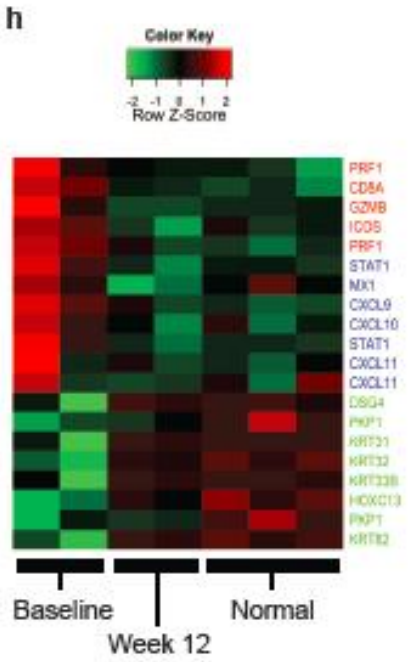


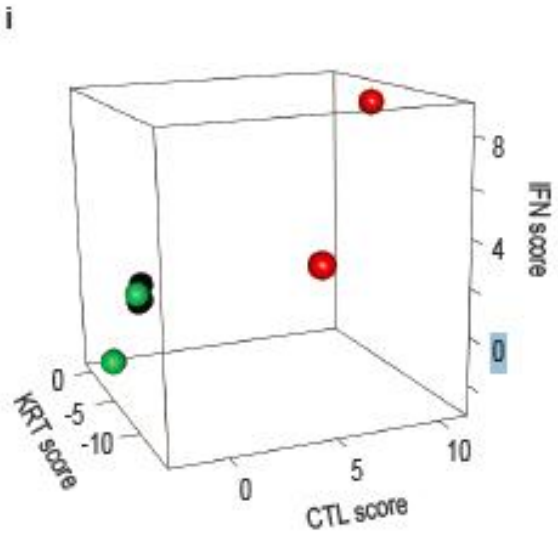

Mackay-Wiggan, et al. JCI Insight. 2016 15:e89790.

# Oral Ruxolitinib Clinically Reverses Moderate/Severe Alopecia Areata

On-Target Reversal of Inflammatory Biomarkers
Early Data from Single Arm Open Label Study of 12 patients

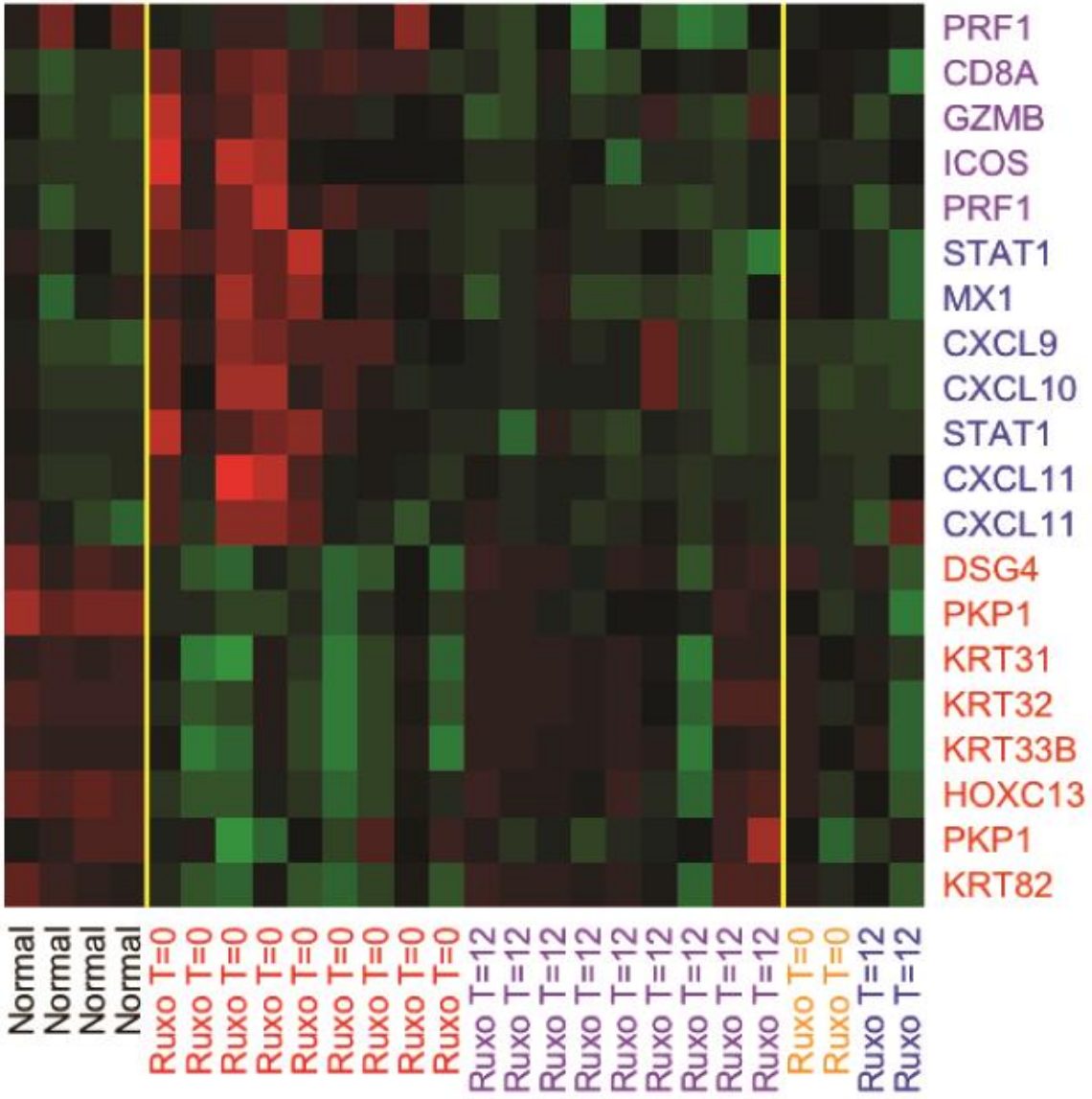


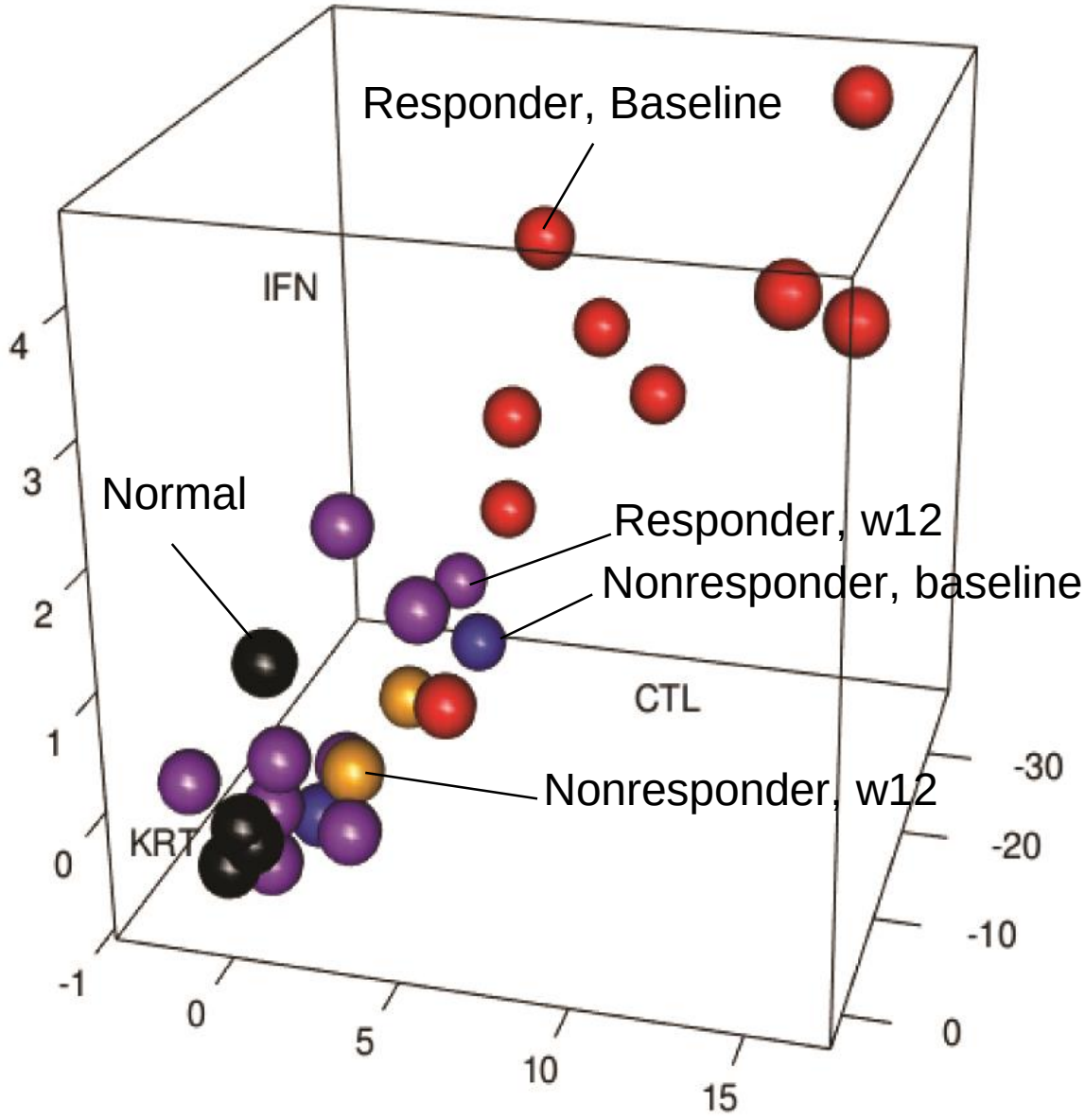

Xing, et al. Nature Medicine 2014

# Gene expression profiling before and after Ruxolitinib treatment




Mackay-Wiggan, et al. JCI Insight. 2016 15:e89790.

# ALADIN score before and after ruxolitinib treatment




Mackay-Wiggan, et al. JCI Insight. 2016 15:e89790.

# Tofacitinib Study Design

PI: Julian Mackay-Wiggan, MD, MPH

- Open-label, single arm pilot study
- 12 patients –6 moderate to severe patchy AA and 6 AT/AU, duration of disease from 3-34 years
- Treated for 6 to 12 months with tofacitinib 5mg BID, up to 10mg BID.

- Efficacy measured by hair regrowth as determined by physical exam, SALT score and photography, and by patient and physician evaluation scores.
- Patients will be followed for another 6 months after end of treatment to evaluate the durability of response.
- Biopsies and peripheral blood will be obtained at baseline, weeks 4 and 24, for immune monitoring and molecular studies. Additional biopsies and blood draws (1-3 anticipated) may be obtained as clinically indicated
- QOL measures at baseline and at various additional time points

# Tofacitinib – Subject 1


Baseline SALT 100%, 5 mth 5mg BID, 2mth 10/5 mg BID, 3 mth 10 BID ongoing. Last SALT 39%

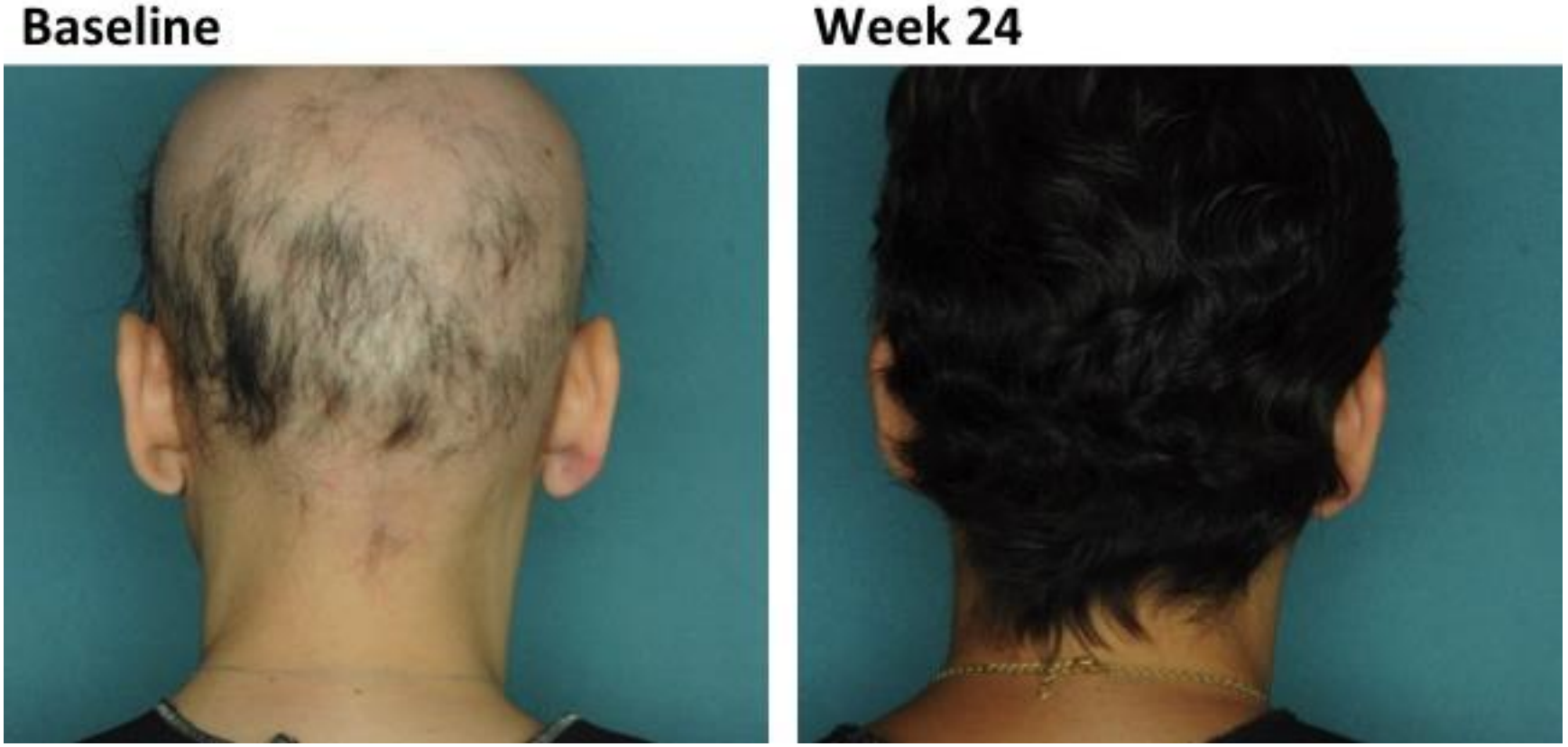
Jabbari et al., submitted

# Tofacitinib – Subject 2


BL SALT 46, 5 months at 5mg BID, 2 months at 10/5mg , 2 months 10mg ongoing. Last SALT 12.

Jabbari et al., submitted

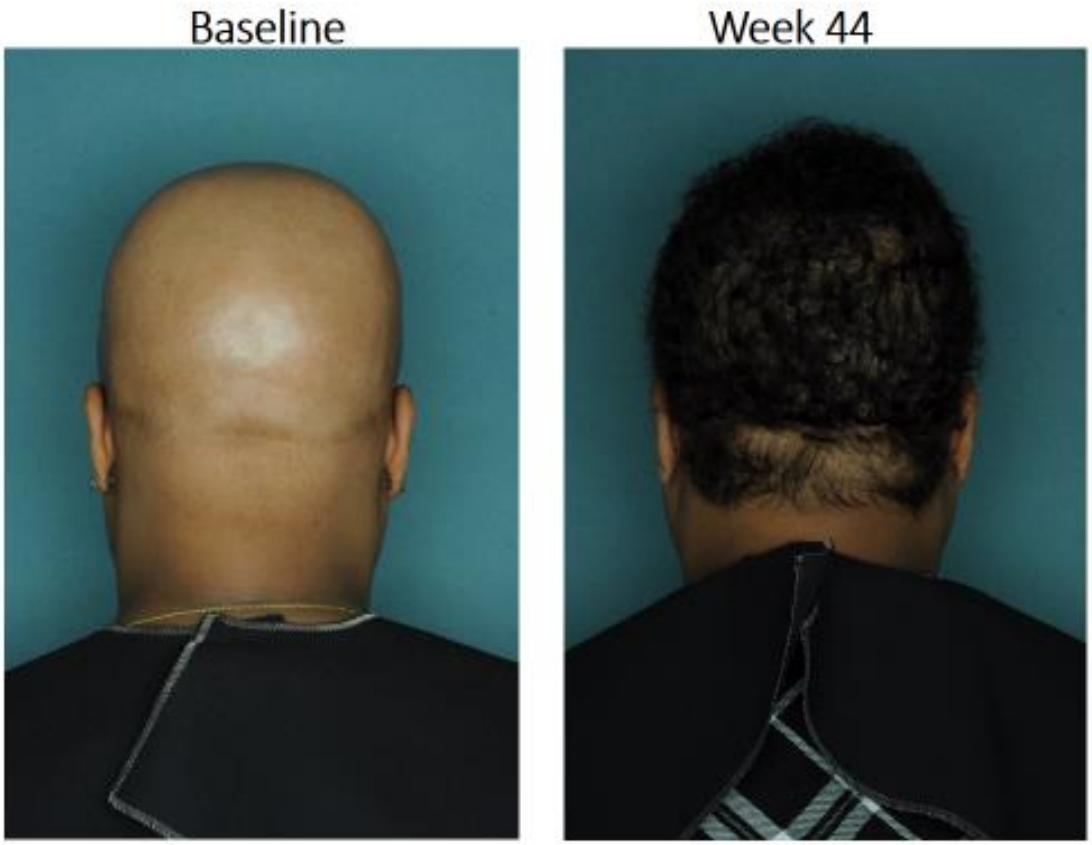
# Tofacitinib – Subject 2




BL SALT 84, 6 months 5mg BID, 0 months 10/5mg, 0 months 10mg, End of treatment SALT 0.

Jabbari et al., submitted

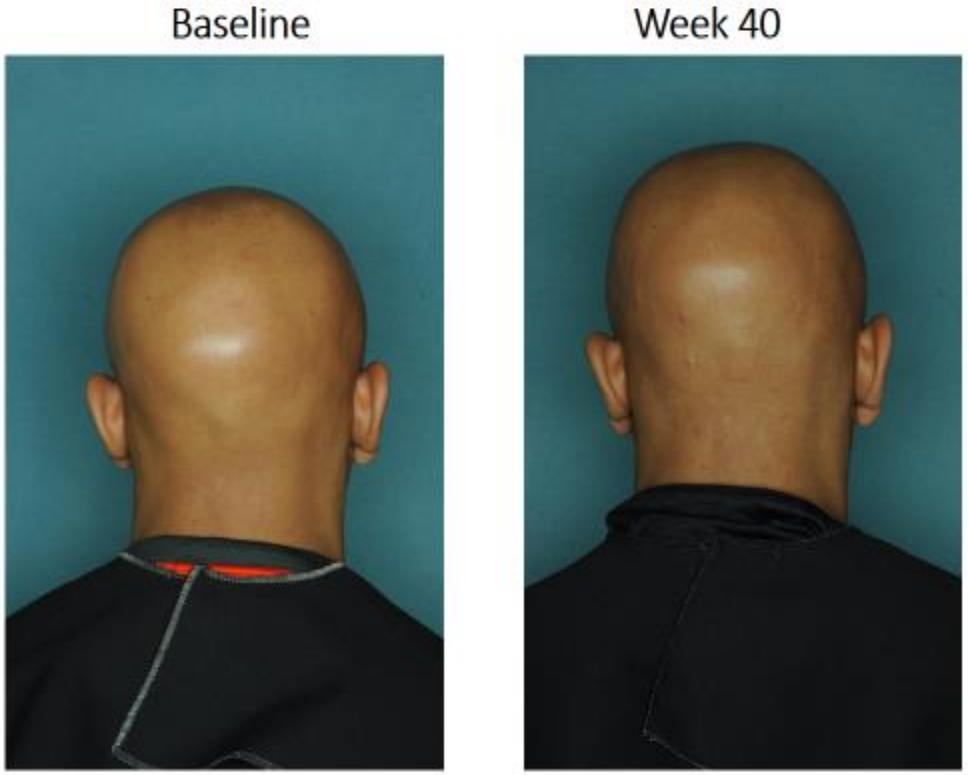
# Tofacitinib – Subject 11




Baseline SALT 100%, 1 month – 5 mg BID, 2 months - 10 mg/5 mg, 9 months – 10 mg BID. Last SALT 13%

Jabbari et al., submitted

# Tofacitinib – Subject 5 (non-responder)

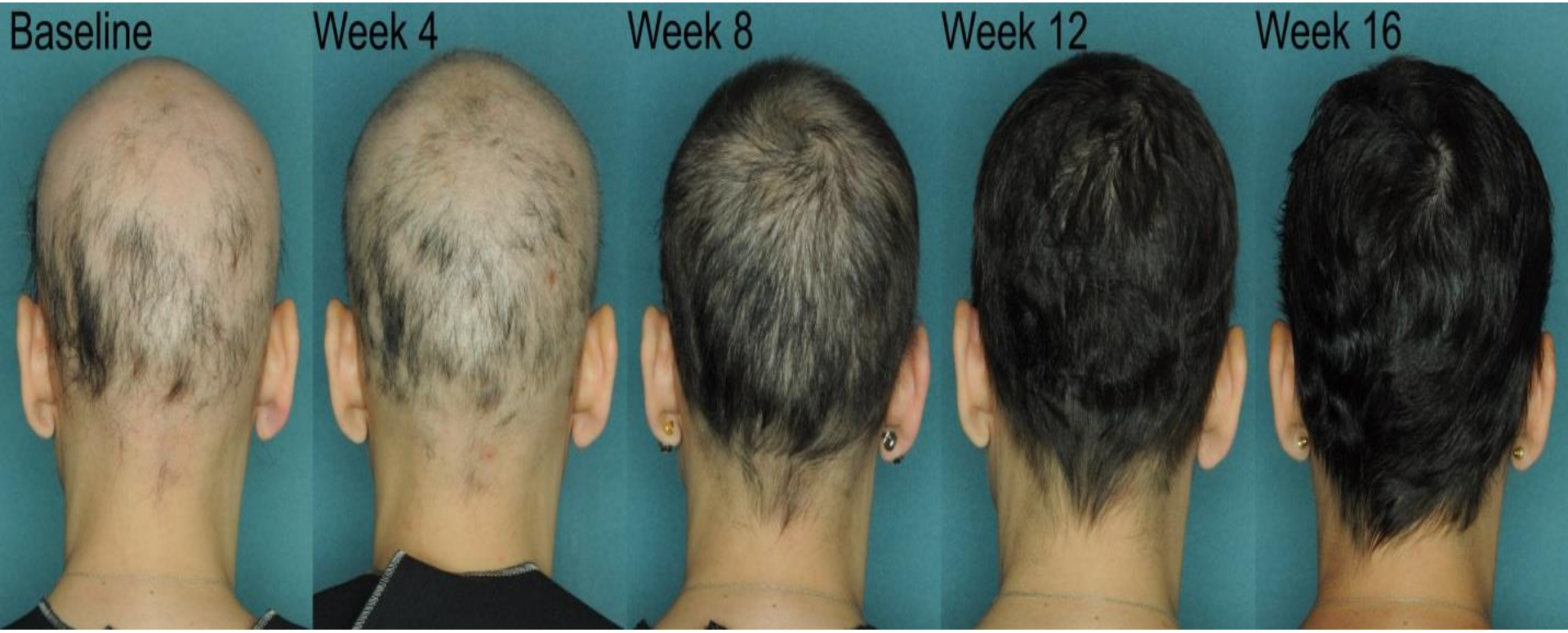



Baseline SALT 100%, 3.5 months - 5 mg BID, 2 months 10 mg /5 mg, 2.5 months 10 mg BID. Last SALT 99%

Jabbari et al., submitted

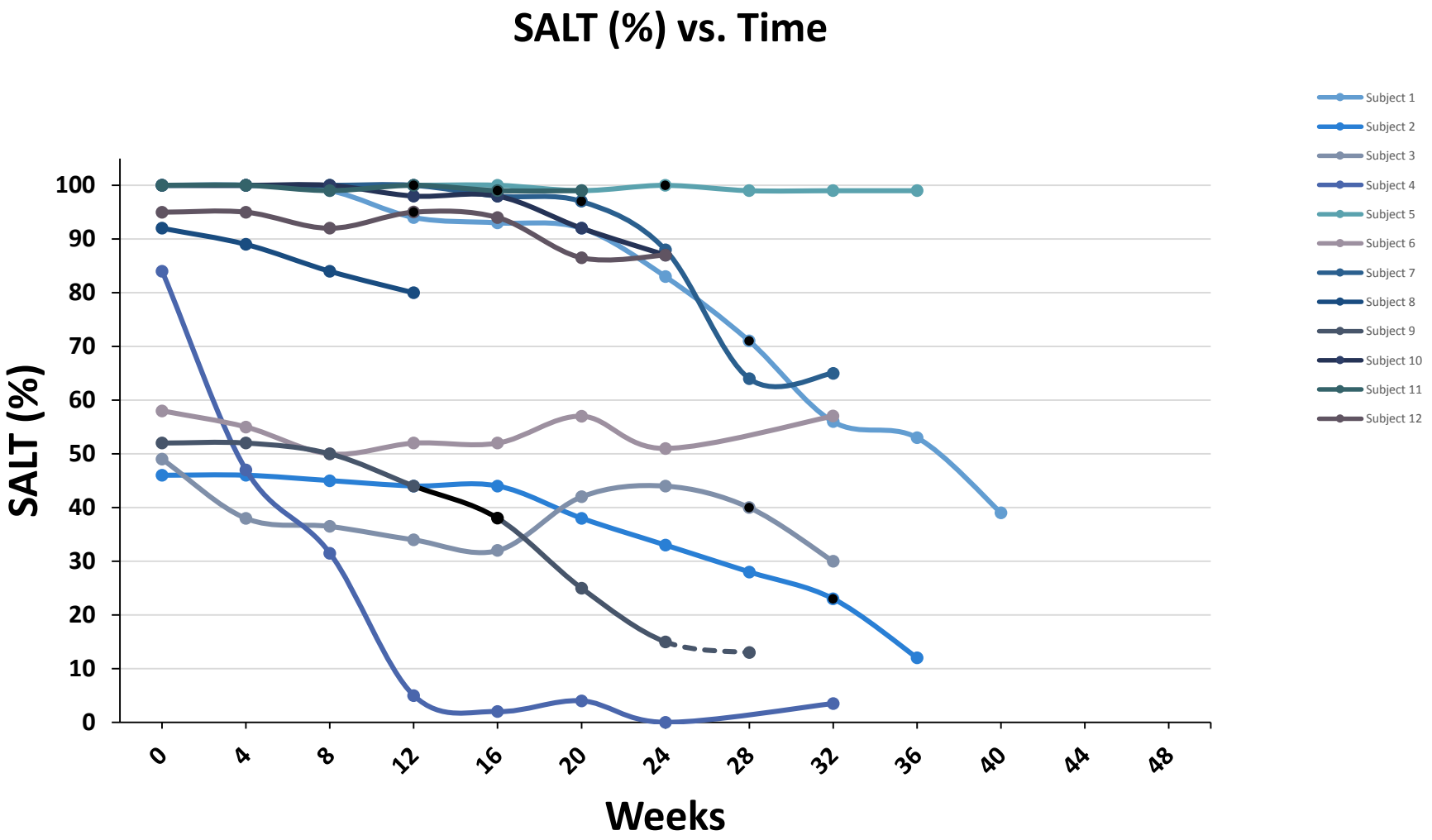
# Tofacitinib

## Timecourse of Treatment Response over 4 months



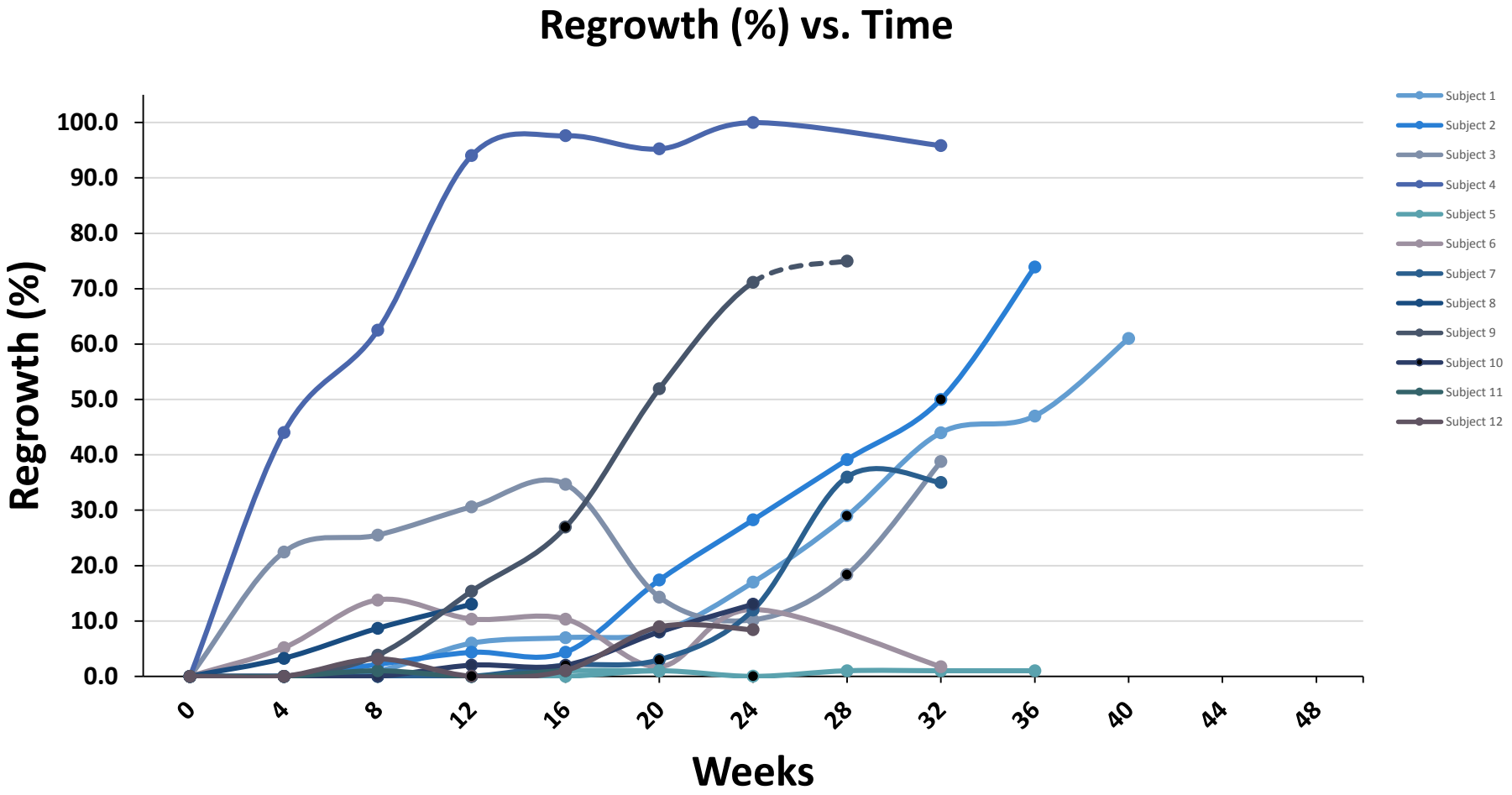

Jabbari et al., submitted

# Tofacitinib SALT Scores




Jabbari et al., submitted

# Tofacitinib Regrowth

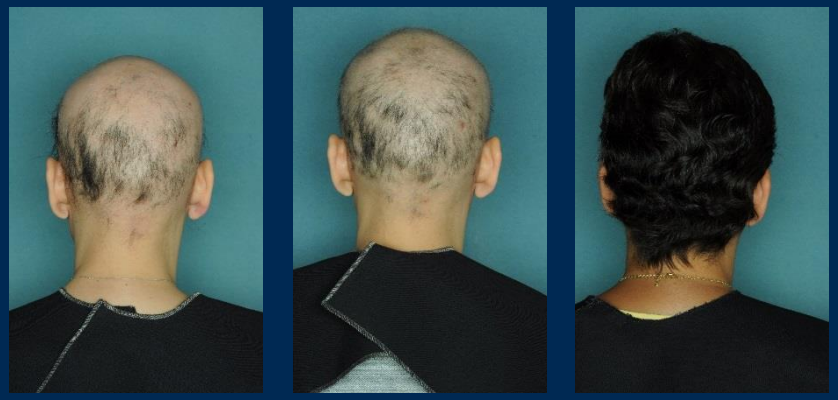


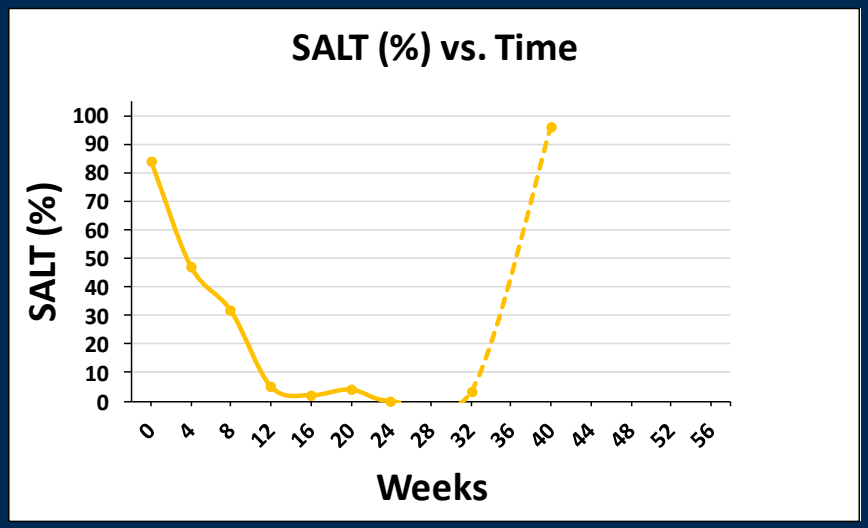

Tofacitinib requires higher dose and longer duration of treatment (15mg or 20mg; >6 months)

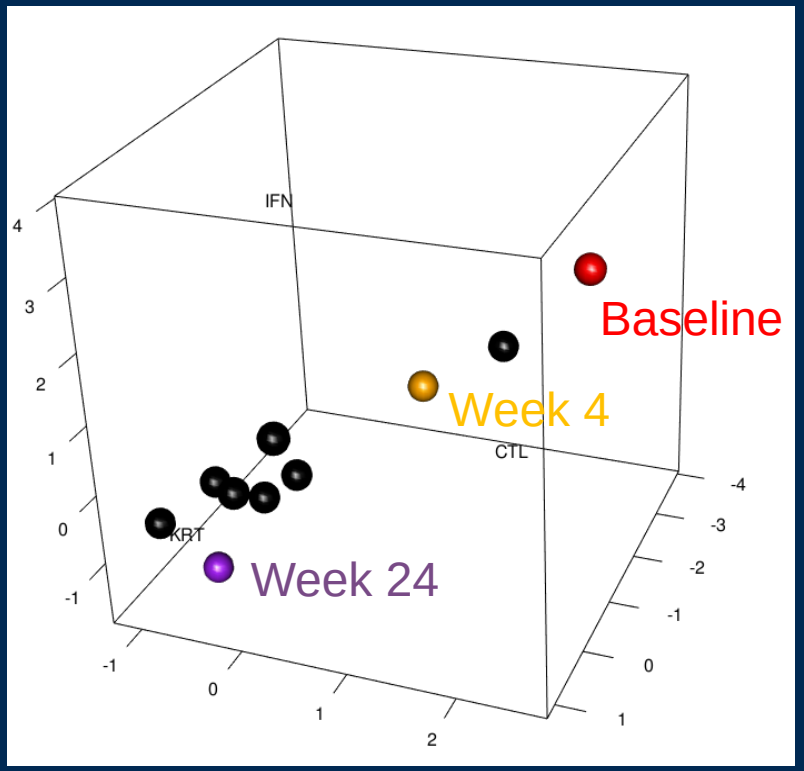
Jabbari et al., submitted

# Tofacitinib– Low Dose Responder

Baseline Week 4 Week 24



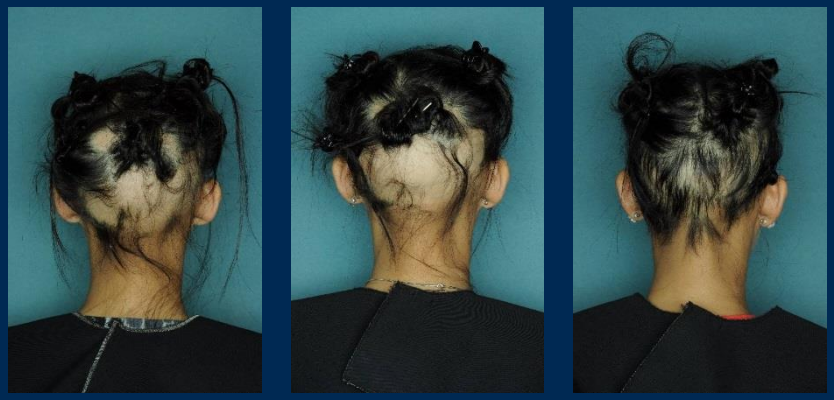


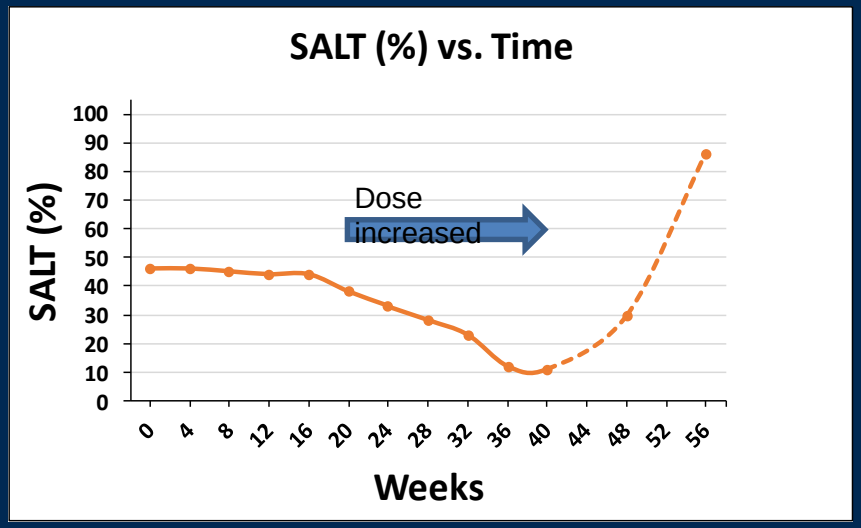



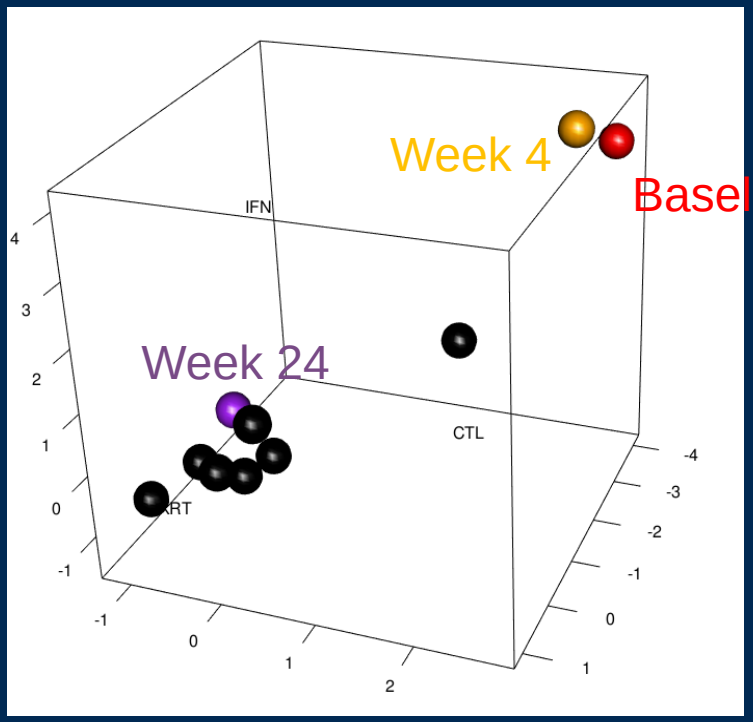

Jabbari et al., submitted

# Tofacitinib– High Dose Responder

Baseline Week 4 Week 24

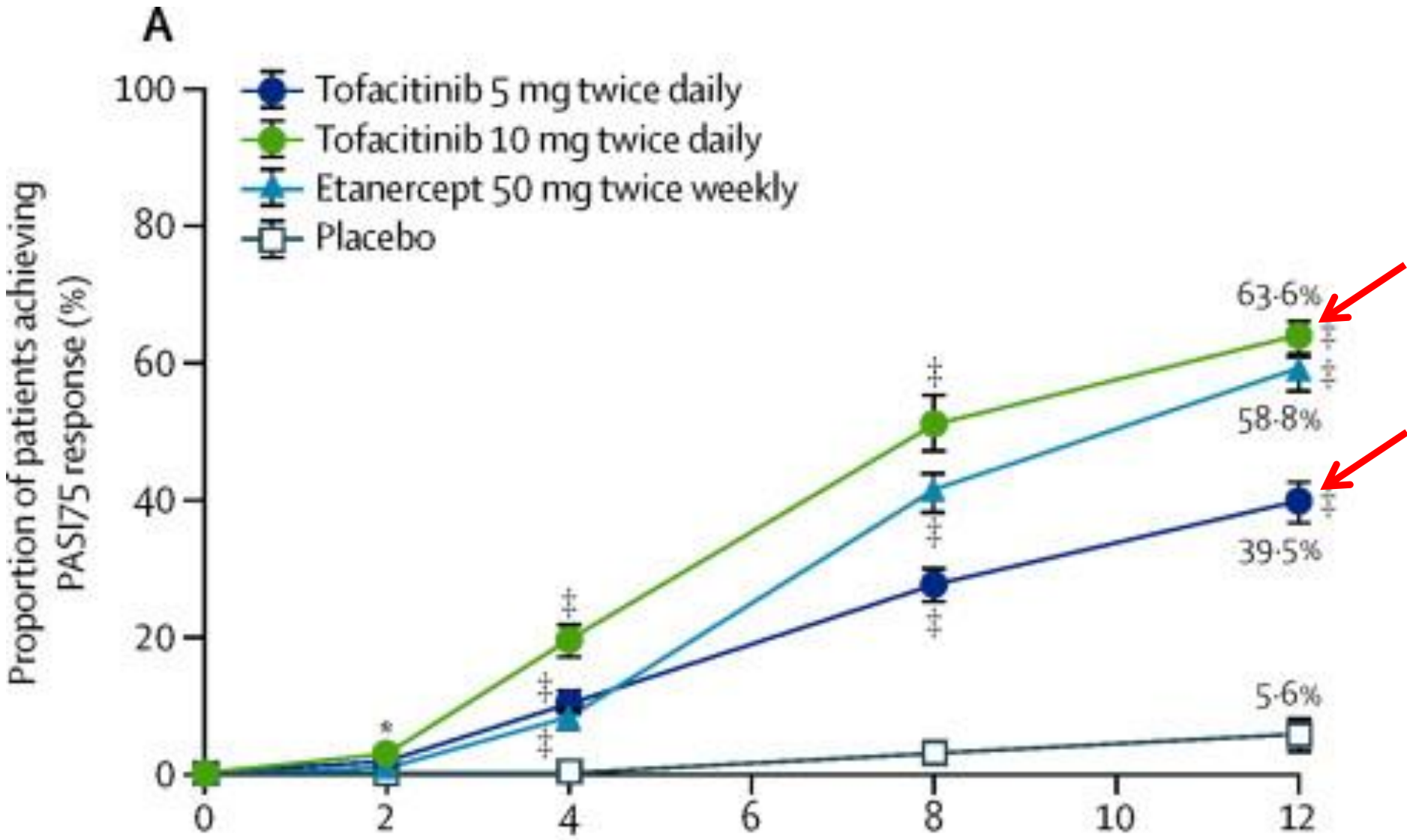





Jabbari et al, submitted

# Tofacitinib in Psoriasis




Bachelez et al., *Lancet*, 2015

# Session III: Success Stories: Lessons from Clinical Studies with JAK inhibitors

November 14-15, 2016
Alopecia Areata Research Summit
Building & Crossing the Translational Bridge
New York City · New York · USA
National Alopecia Areata Foundation
ALOPECIA AREATA Treatment Development Program

**Julian Mackay-Wiggan – Update on Clinical Research in AA**

Ruxolitnib study – 12 patients, 20mg BID. Regrowth as early as 4 weeks. 9 of 12 had 50% regrowth.8 of 9 achieved their endpoint by week 12 (75% response rate). Tofacitinib study – 12 patients 5mg BID up to 10mg BID. Followed for 6 months. 7 of 12 had 50% regrowth. 6 of 7 responders needed dose escalation. (approx 60% response rate). Relapse 4-8 weeks after stopping. Abatacept study – one complete responder out of 15 treated – continued to regrow even after end of treatment. Biomarker analysis using gene expression performed in all studies.

**Wilma Bergfeld – Cleveland Clinic AA Tofacitinib Results**

Open retrospective study. Moderate to severe, recalcitrant patients, some with RA and AA. Thirteen patients, all recalcitrant to other therapies. Average regrowth at 4 months, some as late as 9 months . Some are on drug for 18 months. One AU patient was african american, regrew his eyebrows and lashes but not scalp and not body hair. Three patients had total regrowth. One was duration of 30 yrs. Response rate =approx 54%.

**Justin Ko – Oral Tofa in Severe AA – Stanford/Yale Study**

All patients are on 5mg BID and for 3 months duration only. Enrolled 70 patients – 66 finished study. Long durations 1-43 years; avg 5 years duration. ¾ were AU/AT patients. Biomarker analysis using gene expression studies. Outside the study – treating approx 80 patients. About 2/3 of patients grow clinically acceptable patients at 6 months or longer. Roughly 66% overall response rate.

**Brett King – Tofa in AA in Adults and Adolescents**

Approx 90 adult patients treated and 13 adolescents with tofa alone or tofa with pulse steroid. Overall response rate approx 60% in adults, 75% in teenagers. Patients with disease duration less than 11 years have better responses. Relapses seen while on treatment, and after drug stopped. Topical studies treating one patient with compounded ruxo, regrow brows. Three compounded tofa formulations, no positive results.

**Elise Olsen/Incyte Study - Topical INCB018424 in AA in open-label treatment period**

Study evaluated topical ruxo 1.5% cream in AA patients. Part A: open label 24 week study in 12 subjects. 18-70 yrs of age. Current episode of either 6 months 50-99% loss, or 12 months 25-50% loss. Exclusion of AT AU or ophaisis. 6 of 12 patients (approx 50% response rate) reached SALT50 response at end of 24 weeks. Promising data to encourage further development of topical JAKs.

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## Take home lessons from combined studies

•Relapse seen in all studies after treatment; sometimes worse than baseline, starting 4-8 weeks after stopping drug.

•Flares observed while on treatment in several studies, sometimes in different pattern (ophiasis) than original AA (12% in Yale study, 4 pts Stanford, 1 pt Columbia).

•African american individuals in non-responder groups (though also in responder groups).

•Ruxo treated patients regrowth visible earlier than tofa treated patients. Tofa required dose escalation in all studies, longer treatment periods.

•Each study had responses in patients with long durations of disease; however, two studies suggested that less than 10 years duration had better responses; one study suggested shorter duration of current episode correlated with better responses.

•Regional differences in regrowth: Eyebrows, lashes and body and facial hair don't correspond to scalp hair. Patients respond differently on different body sites.

•One study (Yale) added pulse prednisone 300mg Q4 weeks for 3 doses, in addition to elevated dose of tofa to improve responses. Comment by Dr. Shapiro that he has combined oral tofa with ILK and seen faster responses to tofa.

•Two studies (Stanford/Yale and Columbia) conducted gene expression biomarker analysis.

• No study reported improvement in AGA hair loss in patients taking JAK inhibitors. Perhaps not surprising; this may require topical administration.

•AE: generally mild. Acne (8% in Yale study); trace hematuria (Columbia), eczema herpeticum (Stanford); LFT, lipid abnormality (Cleveland), URIs in all studies (consistent with seasonal patterns).

Despite widely heterogeneous groups of patients, durations of disease, different dosing regimens, and length of treatment, response rates were highly consistent across sites (range 50-75%):

**Columbia:** **Response to oral ruxo = 75%, oral tofa = 65%.**

**Cleveland:** **Response rate =approx 54% oral tofa.**

**Stanford:** **66% overall response rate oral tofa.**

**Yale:** **approx response rate 60% in adults, 75% in adolescents for oral tofa.**

**Duke/Incyte:** **approx response rate 50% for topical ruxo.**

| | Type of study | Treatment | Number of patients | Length of treatment | Response rate |
|---|---|---|---|---|---|
| Crispin et al, JCI Sept 2016 | Open label, single-arm | Tofacitinib 5mg BD | n = 66 | 3 months | 32% at least 50% regrowth |
| Mackay-Wiggan et al, JCI Sept 2016 | Open label, single arm | Ruxolitinib 20mg BD | n = 12 | 3-6 months | 75% at least 50% regrowth |
| Liu et al, JAAD 2017 | Retrospective | Tofacitinib 5mg BD | n = 90 | Median 12 months (4-18) | 77% at least 50% regrowth 20% complete response |
| Ibrahim et al, JAMA Dermatology 2017 | Retrospective | Tofacitinib 5mg BD | n = 13 | Mean 4.2 months (1-9) | 53.8% at least 50% regrowth |
| Craiglow et al, JAAD 2017 | Retrospective | Tofacitinib 5mg BD | n = 13 (adolescents) | Median 5 months (2-16) | 76.9% response with median 100% regrowth (20-100%) |
| Craiglow et al, J Invest Dermatol 2014 | Case Report | Tofacitinib 10mg AM, 5mg ON | n = 1 (AU) | 8 months | Complete regrowth |
| Jabbari et al, EBioMedicine 2015 | Case Report | Baricitinib 7mg AM, 4mg ON for CANDLE* | n = 1 | 9 months | Complete regrowth |
| Pieri et al, Am J Heamatol 2015 | Case Report | Ruxolitinib 20mg BD for ET** | n = 1 (AU) | 10 months | Complete regrowth |
| Gupta et al, JEADV 2016 | Case Report | Tofacitinib 5mg BD | n = 2 (AU) | 8 months | Complete regrowth in both patients |
| Harris et al, JAAD 2016 | Case Report | Ruxolitinib 20mg BD | n = 1 | 6 months | 85% regrowth, maintained 12 weeks after cessation |
| Dhayalan et al, JAMA Dermatol 2016 | Case Report | Tofacitinib 5mg BD | n = 3 | 5-6 months | Improvement in nail dystrophy, 2 out of 3 experienced hair regrowth |
| Scheinberg et al, Ann Intern Med 2016 | Case Report | Tofacitinib 5mg BD | n = 2 (AU) | 9 months | Partial regrowth |
| Ferreira et al, Case Rep Dermatol 2016 | Case Report | Tofacitinib 5mg BD | n = 1 (AU) | 10 months | Complete regrowth, and improvement in nail changes |
| Mrowietz et al, Acta Derm Venereol 2017 | Case Report | Tofacitinib 10mg OD for PsA*** | n = 1 (AU) | 9 months | Complete regrowth |
| Erduran et al, Acta Dermatovenerol Alp Pannonica Adriat 2017 | Case Report | Tofacitinib 10mg AM, 5mg ON | n = 1 (AU) | 6 months | Complete regrowth |

* Chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature ** Essential Thrombocytopenia *** PsA = Psoriatic Arthritis

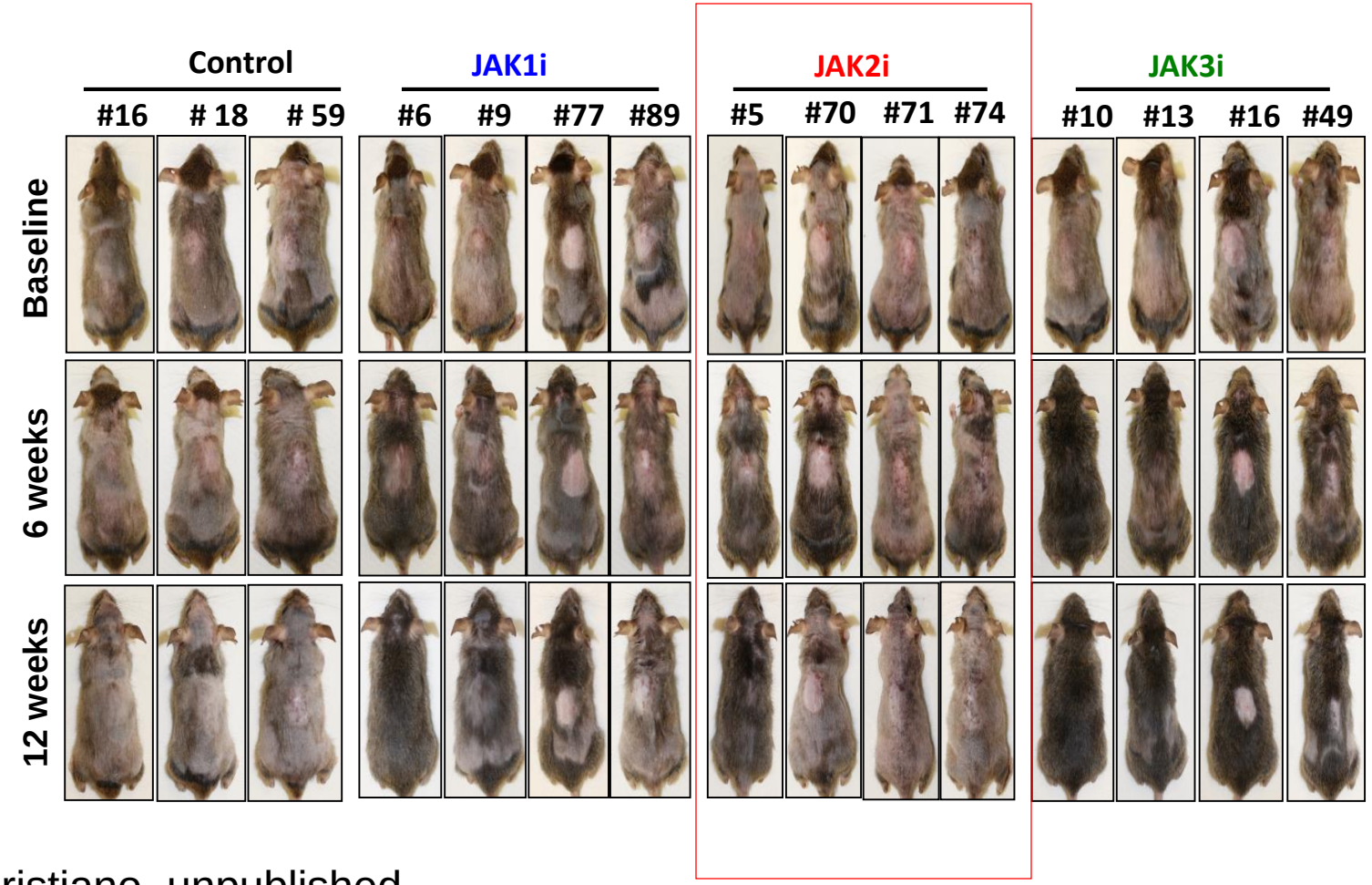
# Efficacy of Selective Next-generation JAK inhibitors in the treatment of AA

# AA molecular signatures reveal JAK1/3 predominance

| Signature | Human | Mouse |
|---|---|---|
| CTL | GZMA(3.65x), CD8A(3.04x), GZMB(2.75x), GZMK(2.68x), PRF1(2.30x), LCP2(2.29x), THEMIS(2.14x), CD2(2.22x) | Gzma(56.37x), Cd8a(9.05x), Gzmb(21.74x), Gzmk(2.61x), Prf1(1.62x), Lcp2(2.32x), Themis(1.84x),Cd2(3.32x) |
| IFN | CXCL10(12.37x), CXCL9(9.53x), MMP12(4.04x), IFI44(3.03x), SPP1(2.69x), IRF8(2.64x), PTPRC(2.63x), CCL2(2.54x), RSAD2(2.49x), CCL5(2.36x), IFIT2(2.28x), C1S(2.28x), LTLR3(2.25x), IFIT3(2.19x), OAS2(2.12x), GBP1(2.11X), XCL1(2.05x), CCR2(2.04x), JAK1 (0.85x), STAT1 (1.86x), IFNG(1.57x), | Cxcl10(37.58x), Cxcl9(42.30x), Mmp12(10.18x), Ifi44(20.58x), Spp1(10.61x), Irf8(4.44x), Ptprc(2.1x), Ccl2(6.12x), Rsad2(3.20x), Ccl5(27.95x), Ifit2(2.39x), C1s(2.49x), Tlr3(2.34x), Ifit3(4.75x), Oas2(3.97x), Gbp1(17.81x), Xcl1(2.88x), Ccr2(4.66x), Cxcl11(53.33x), Stat1 (14.48x), Jak1 (1.40x), Ifng (4.71x), |
| γc | IL15(2.24x), JAK3 (2.10x), IL2RG(2.08x), IL2RB(1.98x), IL15RA(1.60x), IL21R(1.84x), IL2RA(1.12x), IL7(2.20x), IL7RA(1.61x) | Il15(0.80x), Jak3 (1.39x), Il2rg(2.94x), Il2rb(3.14x), Il15ra(1.40x), Il21r(1.69x), Il2ra(1.57x), Il7(1.99x), Il7ra(2.39x) |
| Other | SIA4(2.94x), GPR65(2.60x), GLIPR1(2.29x), IKZF1(2.29x), CD274(PDL1) (2.17x), SAMD9L(2.16x), LCP1(2.15x), SASH3(2.09x), ATP8B4(2.07x) | sia4(1.86x), Gpr65(3.25x), Glipr1(2.33x), Ikzf1(2.34x), Cd274 (PDL1) (8.41x), Samd9l(4.37x), Lcp1(2.25x), Sash3(2.76x), Atp8b4(3.44x) |

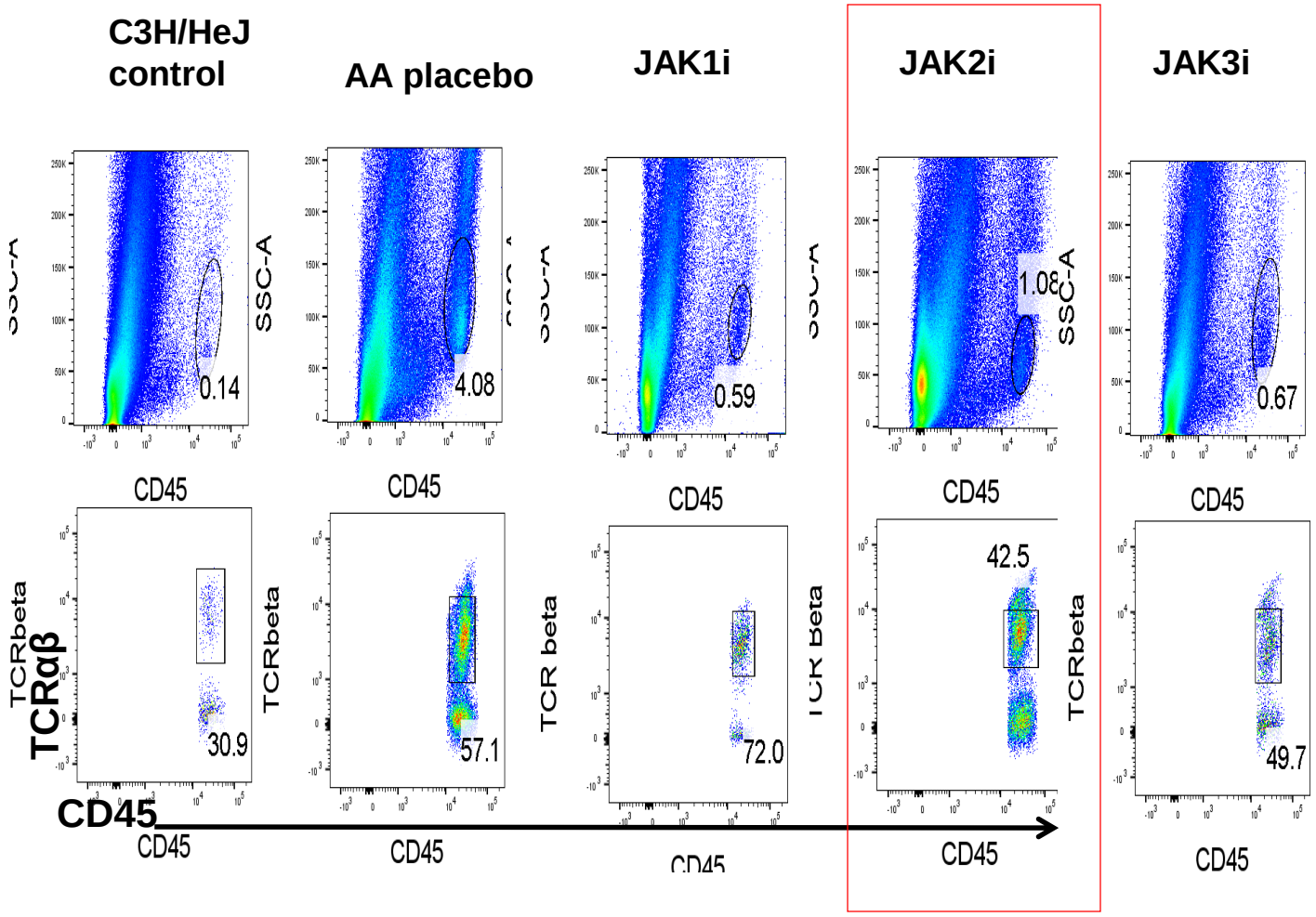
**How critical is JAK2 in AA?**

# Targeting with Topical JAK1i or JAK3i (but not JAK2i) reverses AA




Dai/Christiano, unpublished

# JAK1i and JAK3i (but not JAK2i) inhibit skin infiltrating leukocytes




Dai/Christiano, unpublished

# JAK2 inhibition is *not* required for efficacy in Alopecia Areata

## Investigation of JAK1/3 selective compound, ATI-50001/50002

| Compound | JAK1/JAK3 Enzyme $IC_{50}$ (nM) | IL2 pSTAT5 (JAK1/JAK3) $IC_{50}$ (nM) | INFg pSTAT1 (JAK1/JAK2) $IC_{50}$ (nM) | GMCSF pSTAT5 (JAK2/JAK2) $IC_{50}$ (nM) |
|---|---|---|---|---|
| ATI-50002 | 2/36 | 9 | 38 | >20000 |
| Ruxo | 2/701 | 8 | 9 | 88 |
| Bari | 2/5 | 5 | 11 | 57 |
| Tofa | 3/1 | 12 | 55 | 241 |

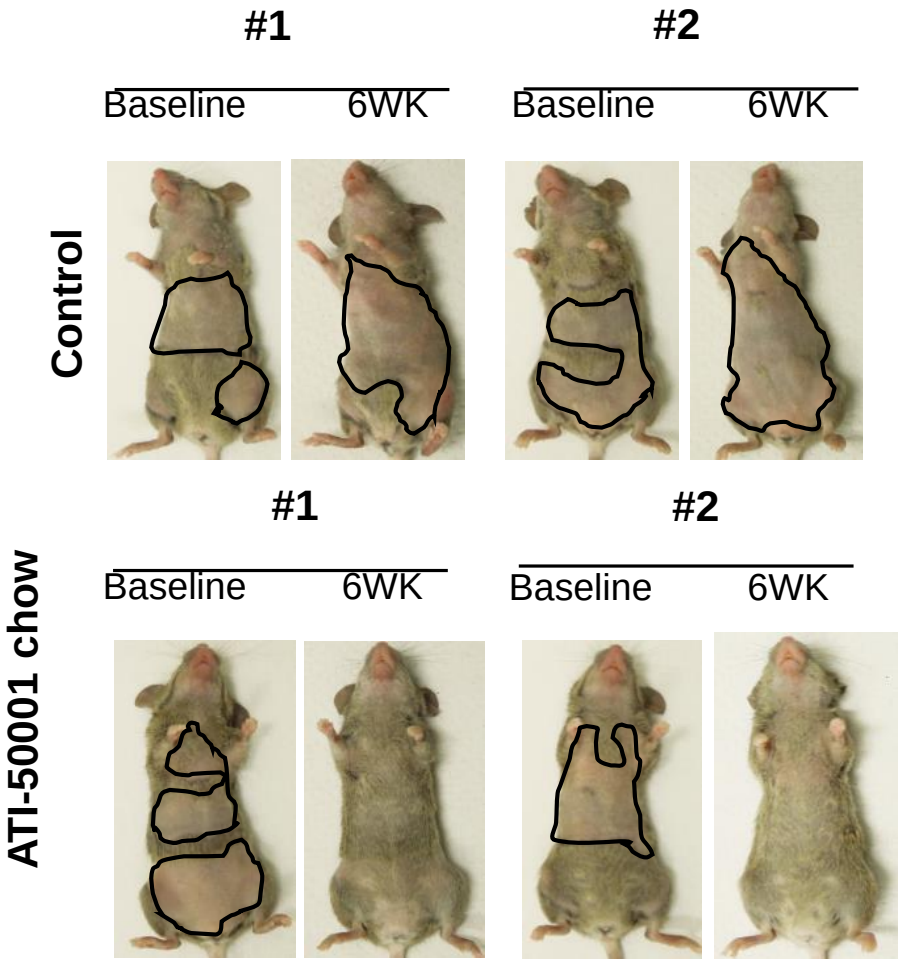
# Systemic ATI-50001 (JAK1/3i) prevents and reverses AA

## Prevention

Control

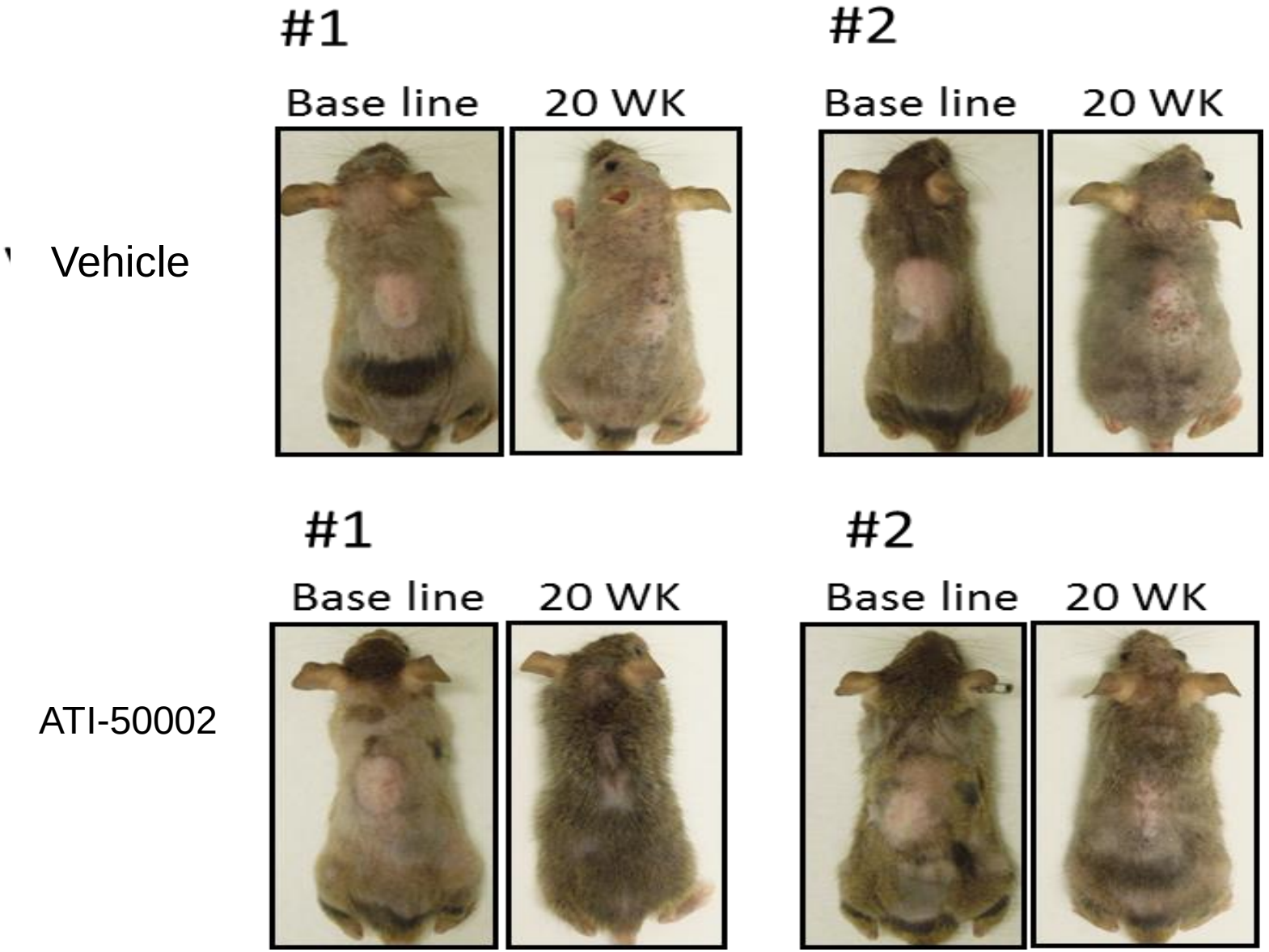
ATI-50001 chow

## Reversal




Dai/Christiano, unpublished

# Topical ATI-50002 (JAK1/3i) reverses AA



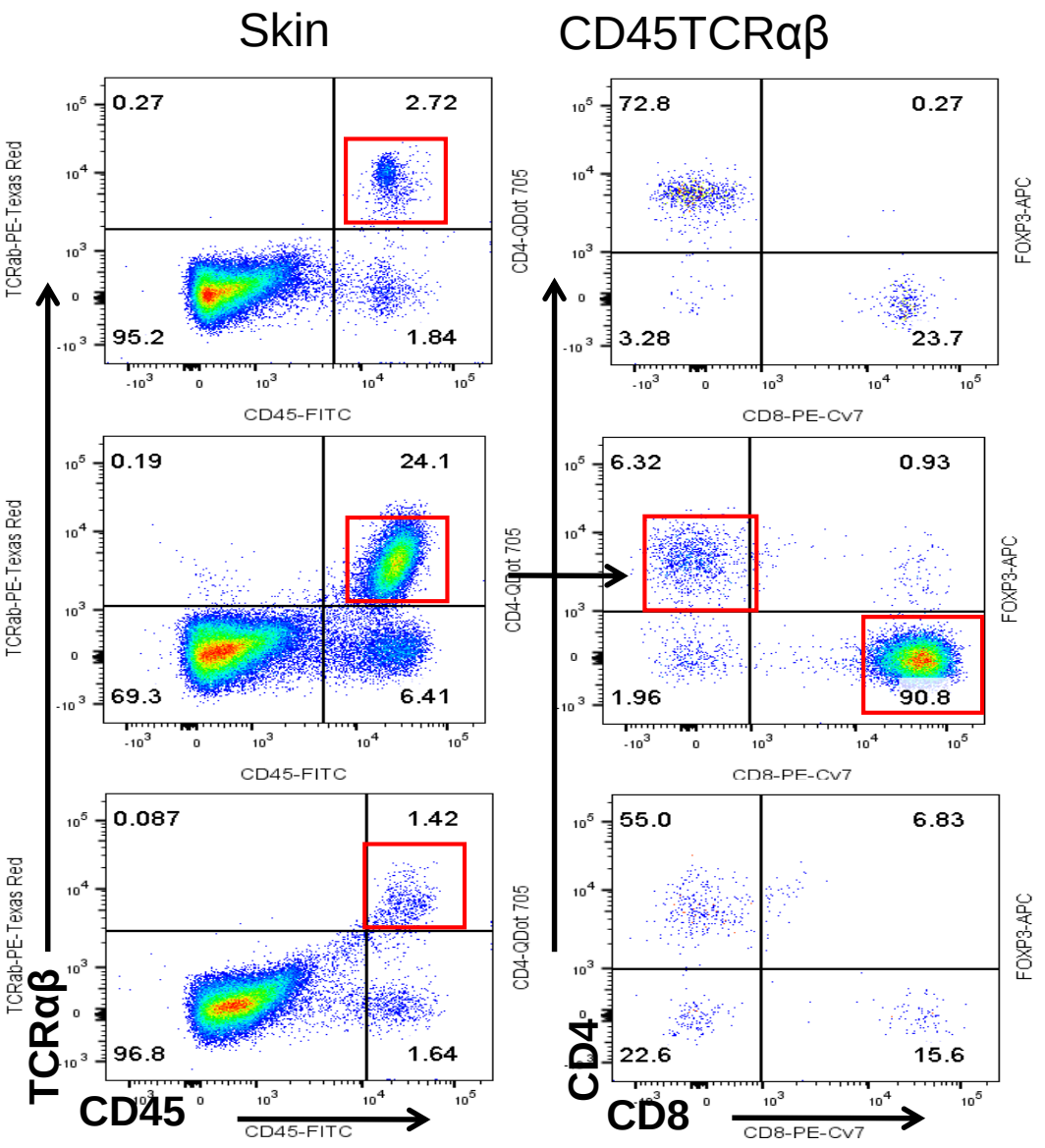

Dai/Christiano, unpublished

# ATI-50001 inhibits AA effector T cells in skin

**C3H/HeJ**
(ungrafted control, without AA)

**C3H/HeJ**
(grafted with AA, placebo group)

**C3H/HeJ**
(grafted with AA; treated with ATI-50001)




Dai/Christiano, unpublished

# JAK inhibitors in dermatology

**psoriasis**
**atopic dermatitis**
**vitiligo**
**Sezary syndrome**
**epidermolysis bullosa/SCC**
**CANDLE syndrome**

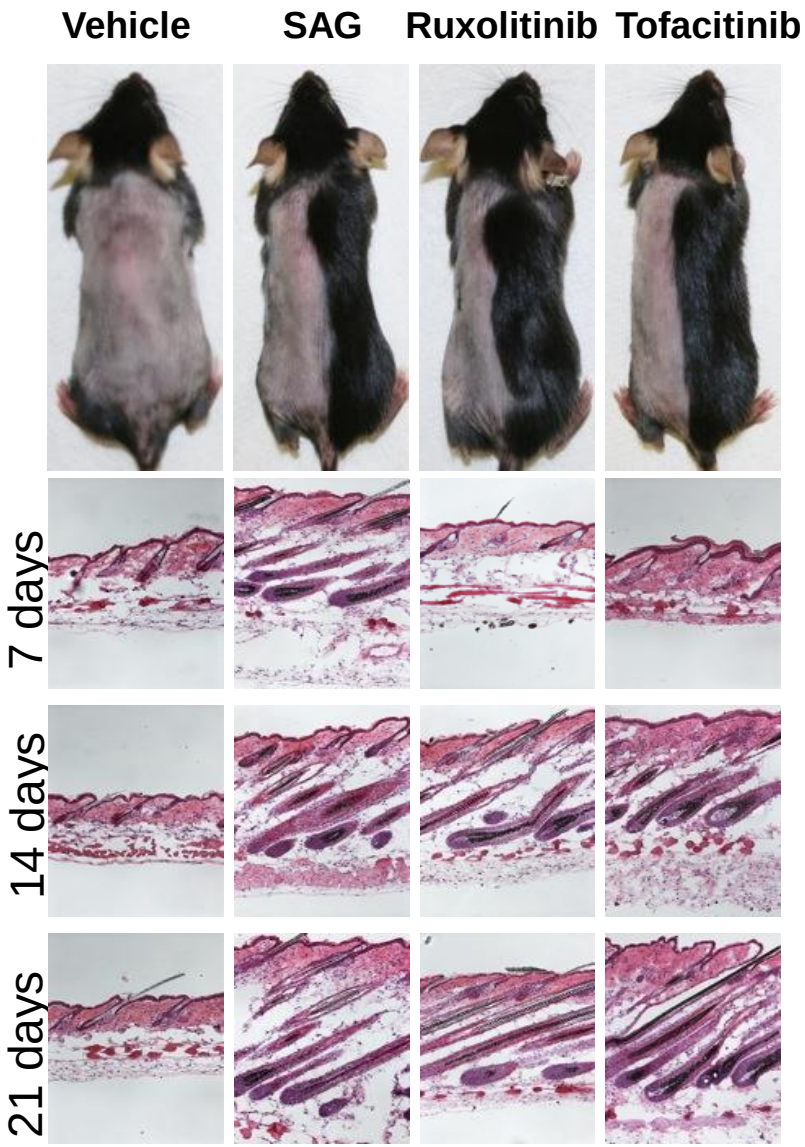
**alopecia areata**

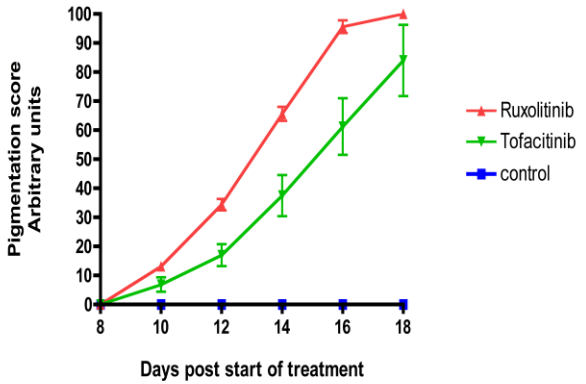
…other types of alopecia?

Scarring alopecias (LPP, FFA)
Androgenetic alopecia

# JAK inhibitors initiate hair growth in *normal mice*

## Relevance for disorders with arrested telogen, like AGA?

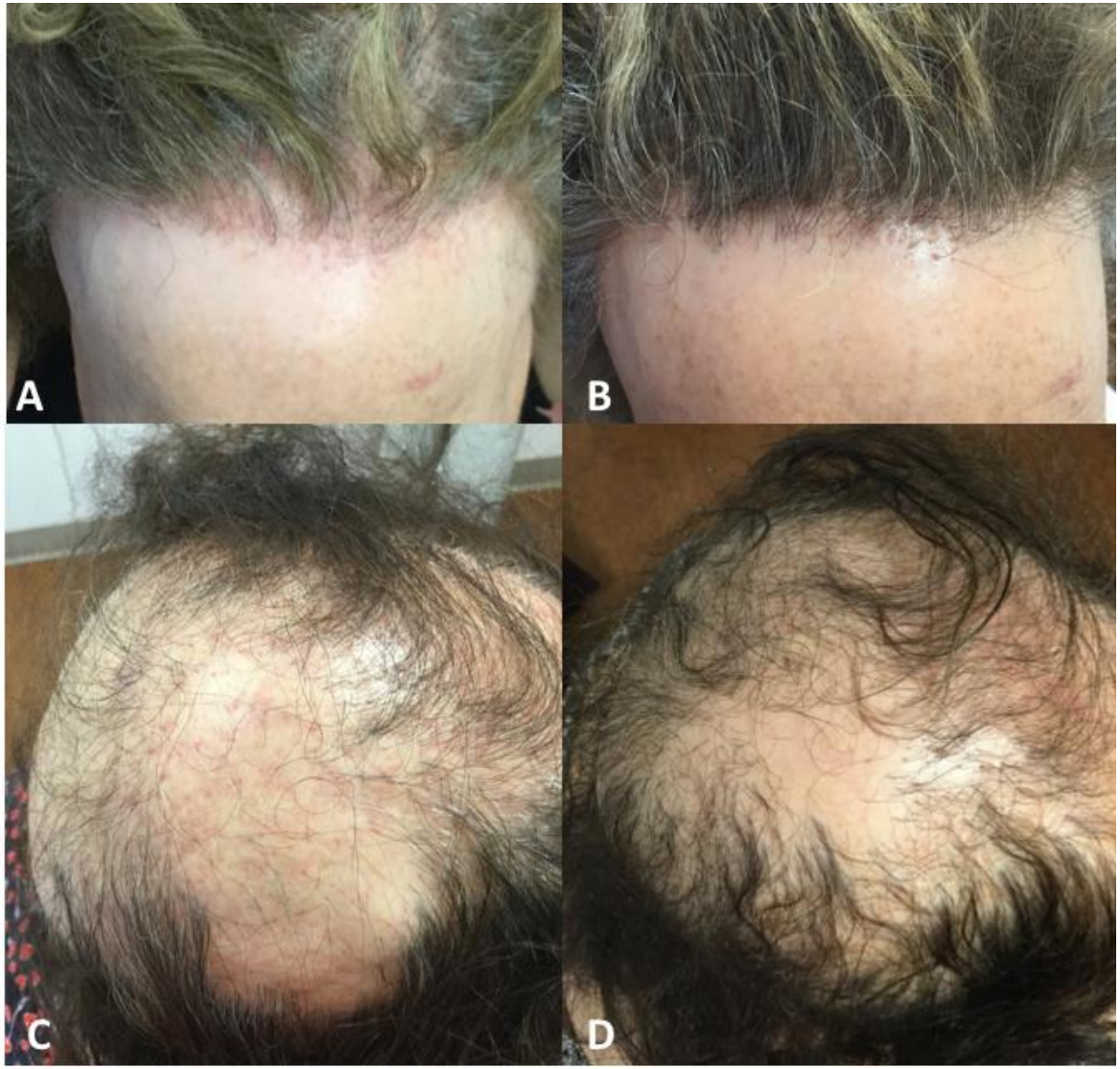



| Category | Differentially expressed in Ruxolitinib and Tofacitinib T0-T5, FC>1.5 or <0.5, P<0.05 | P-value |
|---|---|---|
| Melanogenesis | Tyrp1, Syt4, Mlana, Pmel, Dct, Tyr Sos1, Dbf4, Pax3, PIK3ca, Rps6kb1, Mlph, Stx17 | 0.003 |
| Wnt pathway | Acvr2a, Sfrp4, Sox5, Cdh2, Fzd5, Wif1, Wnt2, Fzd8, Apc, Sox9, iLK | 0.017 |
| Hair development | Shh, Krt25, Dlx2,Prom1, S100a9, Vegfc, Ptgfr, Pdgfrl, Igfbp4, Gli2 | 0.001 |

Harel et al, Science Advances (2015)

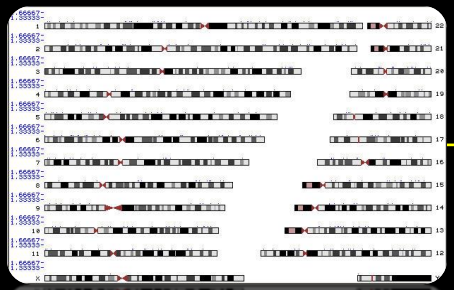
# JAK inhibitors in LPP



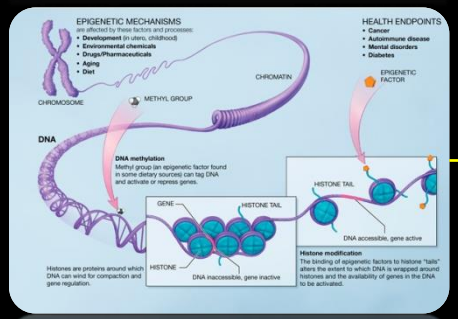

Courtesy of Dr. Lindsey Bordone, Columbia University

# Functional Genomics: New Drugs for AA

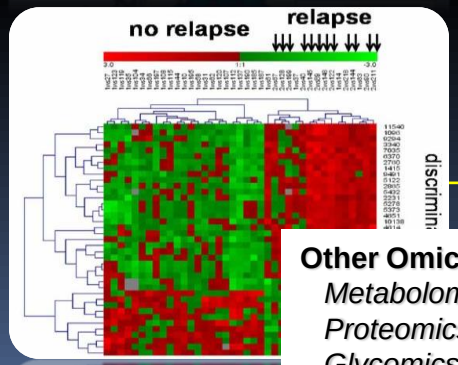
Genomics



Epigenomics




Transcriptomics

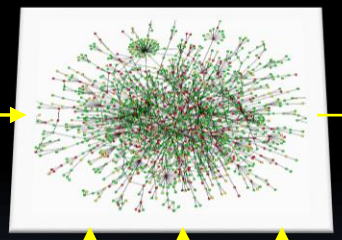


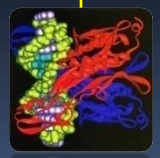

**Other Omics**:
*Metabolomics*
*Proteomics*
*Glycomics*
…

Cancer

*Prevention*
*Diagnosis*
*Treatment*

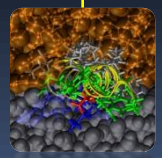
Cell Regulatory Logic





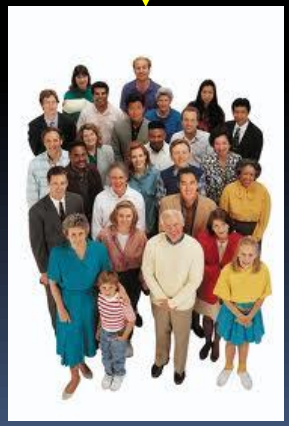
Protein
DNA

Protein
Protein



Protein
Membrane

Drugs & Biomarkers



Clinical Trials

Courtesy of Dr. Andrea Califano

Columbia University Medical Center

# Timeline of Alopecia Areata Translational Research at Columbia University

AACORT

## Translational Milestones

**2001**
Founding of AA Registry, CUMC site
(NCT00069589)
(N01AR062279)

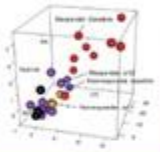
**AA Initiative Foundation**

**2011**
Identification of AA Biomarkers in Skin and Blood
(R21AR061881)

**2011**
RCT of intralesional triamcinolone
(NCT01898806)


**2013**
Open label clinical trial for Abatacept
(NCT02018042)


**2013**
Open label clinical trial for Ruxolitinib
(NCT01950780)



**2014**
Developing an AA Disease Activity Index (ALADIN)
(U01AR067173)

**LOCKS OF LOVE**

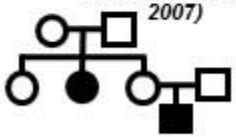
**2015**
Open label clinical trial for Tofacitinib
(NCT02299297)

**AACORT P50**

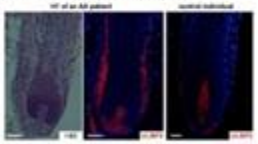
**2016**
Publication of Clinical Trials
(NCT01950780)

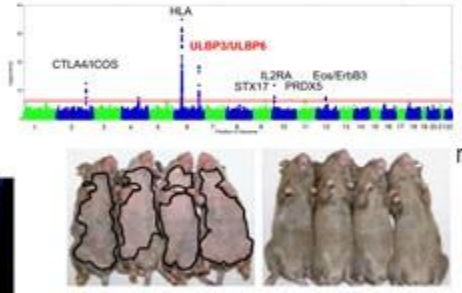
**1996**
**Angela gets AA**

**2007**
First genome-wide linkage study of AA
(R01AR52579)
*(Martinez-Mir et al, Am J Hum Genet 2007)*



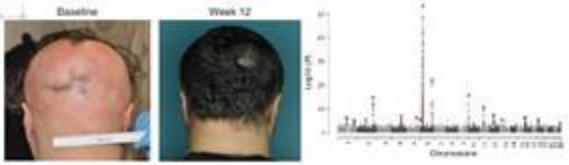
**2010**
First GWAS Study in AA
(R01AR056016)
*(Petukhova et al, Nature 2010)*






**2014**
First demonstration of JAK inhibitors reversing AA in mice and humans
(R01AR056016)
*(Xing et al, Nature Med 2014)*

**2015**
First AA meta-GWAS, higher resolution of HLA locus
(R01AR056016)
*(Betz et al, Nature Comm 2014)*



**2015**
First systems level gene expression study of AA
(R21AR061881)
*(Chen et al, Cell Systems 2015)*

**2015**
First functional genomics study in AA (started July)
(R01AR065963)


**2016**
**Ground Breaking Year for AA**

## Basic and Preclinical Milestones


Translational Studies
Clinical Trials
Key Partnerships

# Acknowledgements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**Marcus Nothen**
**Jane Cerise**
**Esther Drill**
**Andrea Califano**

AACORT
Alopecia Areata Center of Research Translation at Columbia University

Locks of Love

epiCURE
Columbia University
Skin Disease Resource-Based Center

NIAMS National Institute of Arthritis and Musculoskeletal and Skin Diseases
National Institutes of Health, Department of Health and Human Services

**AA Initiative**

NYSTEM NEW YORK STATE STEM CELL SCIENCE

DF

American Skin Association™

NATIONAL ALOPECIA AREATA FOUNDATION

NYSTAR
New York State Foundation for Science, Technology and Innovation