Immune dysregulation in Down syndrome: mechanisms and clinical trials

June 9th, 2025

NICHD Council Meeting

Joaquin Espinosa, PhD

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School of Medicine
UNIVERSITY OF COLORADO
ANSCHUTZ MEDICAL CAMPUS

LINDA CRNIC INSTITUTE for DOWN SYNDROME

University of Colorado Anschutz Medical Campus

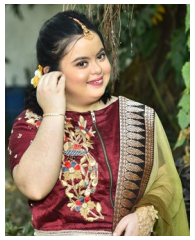
GLOBAL DOWN SYNDROME FOUNDATION

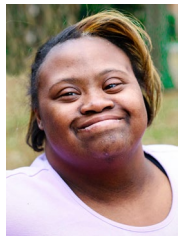
INCLUDE Data Coordinating Center

DS-Connect® The Down Syndrome Registry

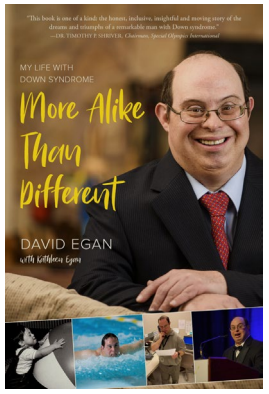
Down syndrome:

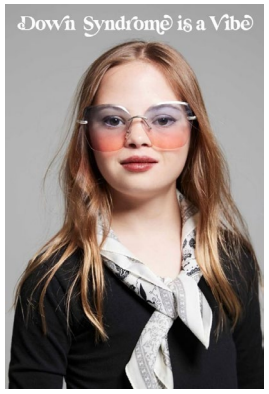
A diverse population surpassing all expectations







There are more persons alive with Down syndrome today than ever before.

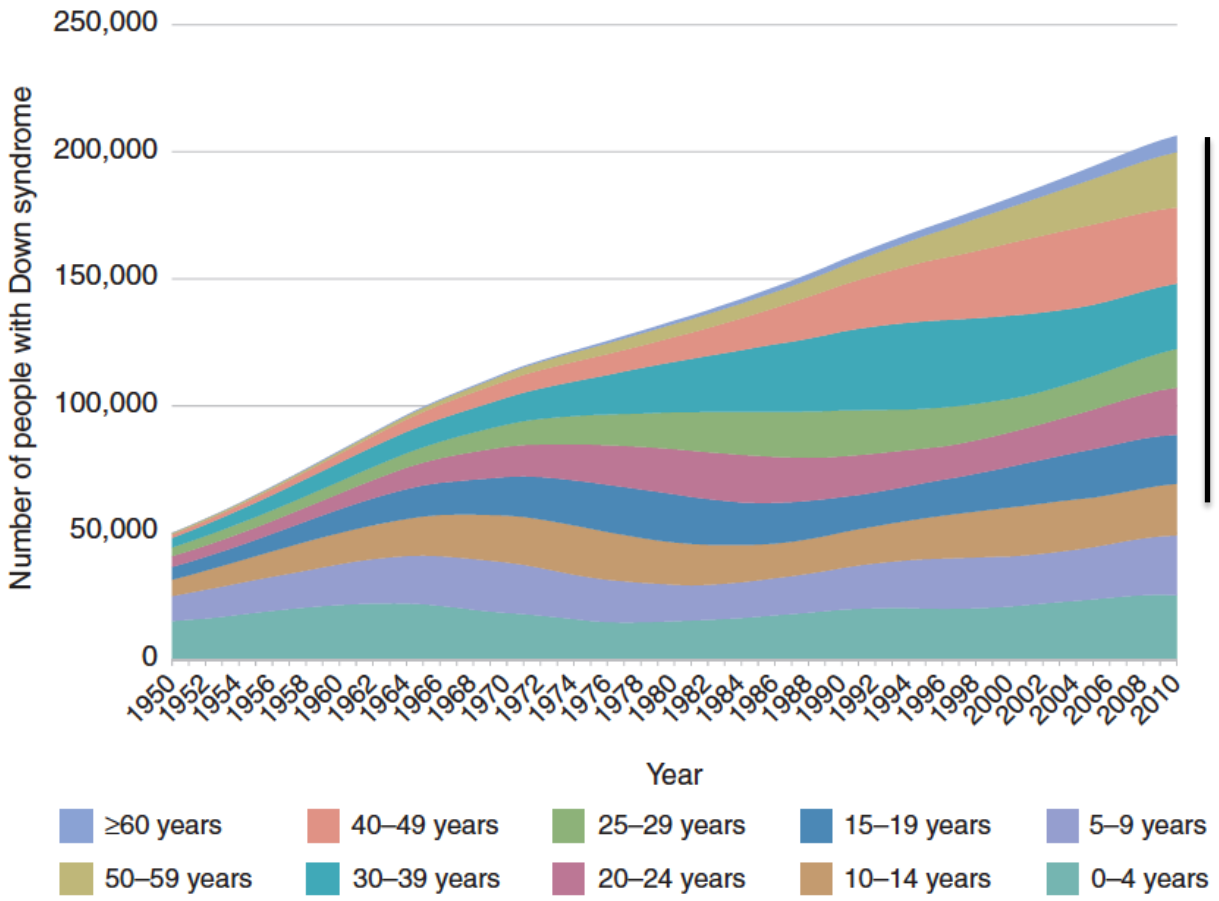
People with Down syndrome are living longer than ever before.

Individuals with Down syndrome are defying outdated notions about what is possible when living with an extra chromosome.

Down syndrome:

A growing population with many unmet needs

Down syndrome **IS NOT** a ‘rare disease’



Adults!

DeGraaf et al, 2017

The birth rate has not decreased.

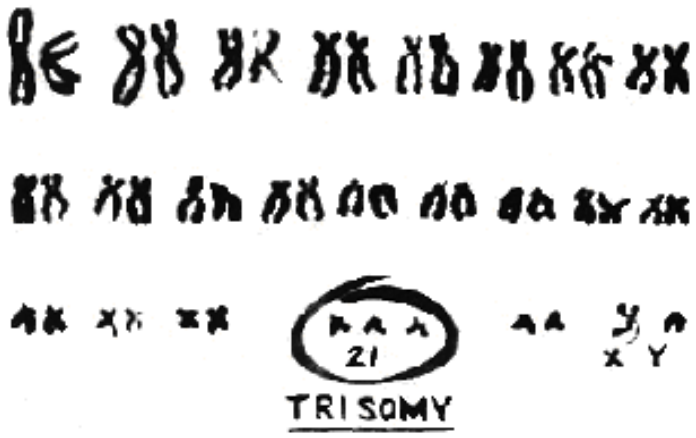
Large gains in life expectancy have led to significant growth of the population with Down syndrome.

Life expectancy is now ~60 years.

People with Down syndrome are here to stay…

Down syndrome:

The ultimate challenge in precision personalized medicine



Gautier

Turpin

Lejeune

The chromosomal abnormality causing Down syndrome (i.e., trisomy 21) has been known since the late 1950's.

Chromosome 21 was sequenced back in 2000, leading to the identification of ~225 genes.

No mutations, simply 1.5x gene dosage.

How does an extra chromosome 21 causes the various hallmarks of Down syndrome?

People with Down syndrome have a different ‘clinical risk profile’

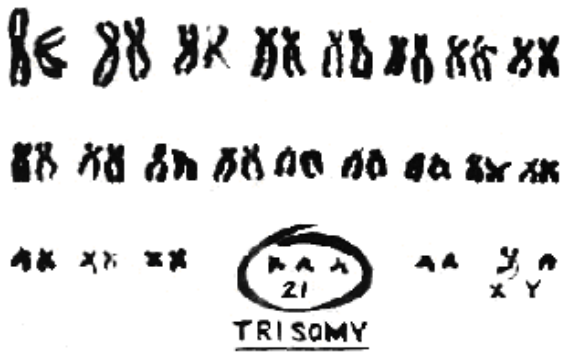
Cancer

Atherosclerosis

Hypertension

Allergies

Common (but variable) traits:
Neurodevelopmental delay
Stunted growth
Early ageing



Alzheimer’s

Autoimmunity

Lung infections

Vision & Hearing

Congenital heart disease, autism spectrum disorders, seizures disorders, and more…

To help people with Down syndrome live longer and healthier lives, we must study the **co-occurring conditions** of Down syndrome

2017: an inflexion point

Congressional hearing on Down syndrome research

U.S.A. House of Representatives

October 25th, 2017

Michelle Sie Whitten, Dr. Joaquin Espinosa, Dr. William. Mobley, Frank Stephens

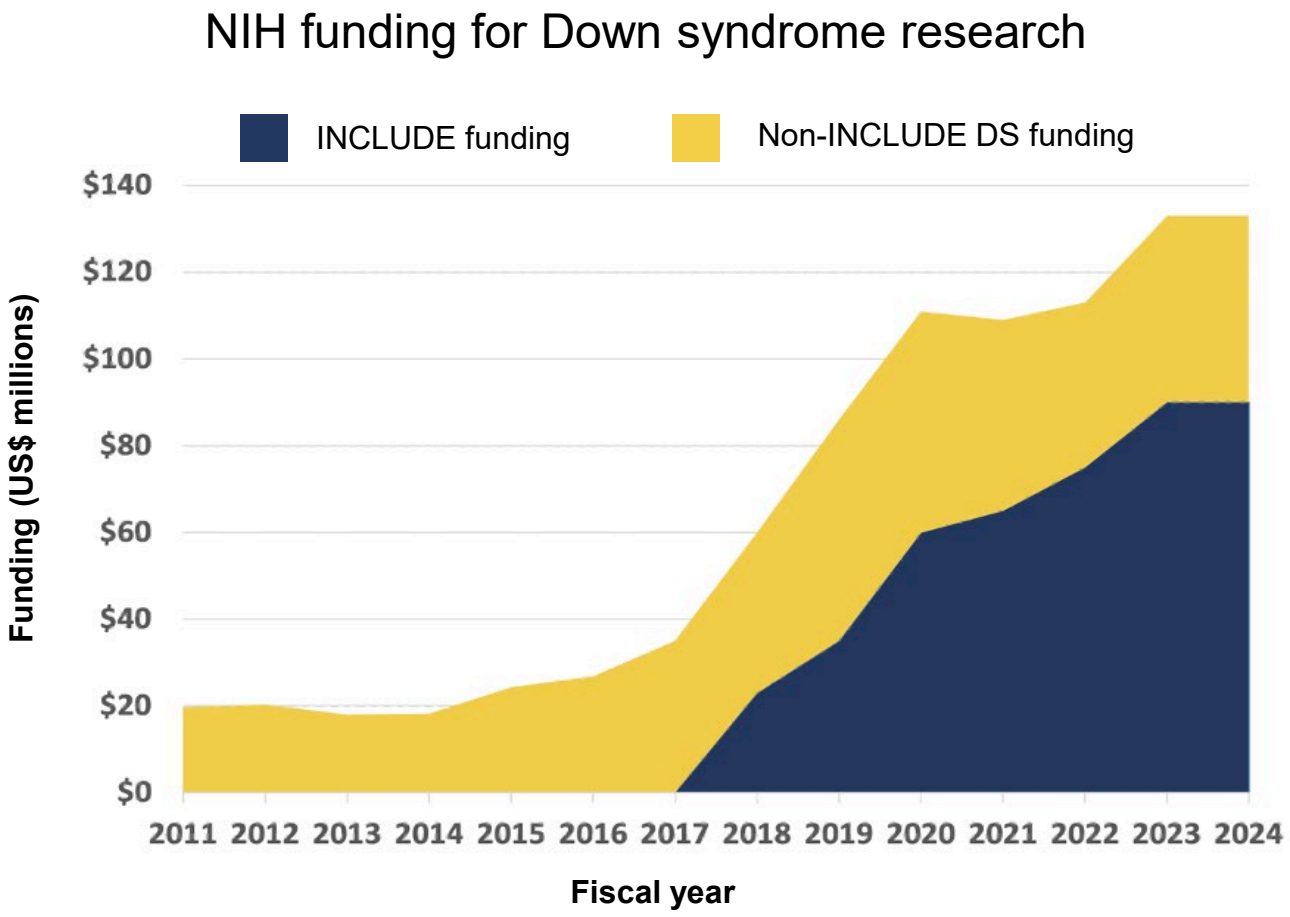
Frank Stephens:
'I am a man with Down syndrome, and my life is worth living'

Dr. Francis Collins, Frank Stephens

A productive collaboration between self-advocates, members of Congress, the GLOBAL Down Syndrome Foundation, scientists, and NIH officers, leading to creation of the NIH INCLUDE Project

2018: NIH launches the INCLUDE Project

A trans-NIH project to increase research in Down syndrome



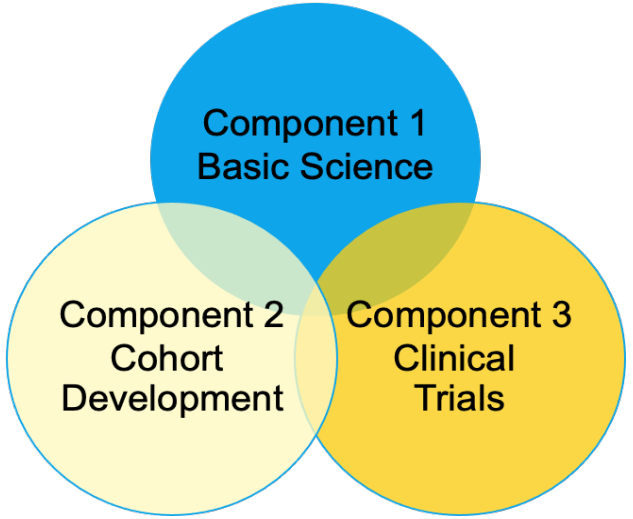
THE INCLUDE PROJECT

21 NIH Institutes participating

389 Projects funded

14 Clinical trials

450+ Scientific publications



An example of translational science supported by INCLUDE: a journey from the petri dish to clinical trials

Idea

Basic Research
- Identify Target

Pre-Clinical Research
- Validate Target
- Develop Therapeutic

Clinical Trials
- Test Safety
- Test Efficacy

Regulatory Approval & Medical Care

2016 → 2020 → 2022 - - - - - - - - →

Trisomy 21 consistently activates the interferon response

Kelly D Sullivan[1,2,3,4*], Hannah C Lewis[1,2], Amanda A Hill[1,2], Ahwan Pandey[1,2,3,4], Leisa P Jackson[1,3,4], Joseph M Cabral[1,3,4], Keith P Smith[1], L Alexander Liggett[1,5], Eliana B Gomez[1,3,4], Matthew D Galbraith[1,2,3,4], James DeGregori[1,5,6,7,8,9], Joaquín M Espinosa[1,2,3,4*]

eLIFE elifesciences.org

☑ NCT04246372 **Completed**

Tofacitinib for Immune Skin Conditions in **Down Syndrome**

Conditions

Down Syndrome | Alopecia Areata | Atopic Dermatitis / Eczema | Hidradenitis Suppurativa | Psoriasis | Vitiligo

☐ NCT05662228 **Recruiting**

Therapies for **Down Syndrome** Regression Disorder

Conditions

Down Syndrome | Regression

Supported by grants from multiple NIH institutes via INCLUDE

NIH THE INCLUDE PROJECT

The Human Trisome Project (HTP)

A large **cohort study** with deep clinical data, a multidimensional biobank, and -omics datasets

>1,400 participants recruited since 2016

LINDA CRNIC INSTITUTE
HUMAN TRISOME PROJECT™
GLOBAL DOWN SYNDROME FOUNDATION

GLOBAL
DOWN SYNDROME FOUNDATION

trisome.org

Thousands of biospecimens collected and 'omics datasets generated

- Clinical histories
- Genomes
- Transcriptomes
- Immune maps
- Metabolomes
- Microbiomes

Data shared through the INCLUDE Data Hub

INCLUDE Data Hub

includedcc.org

50+ research projects supported

30+ publications

Funded by:

NIH *Eunice Kennedy Shriver* National Institute of Child Health and Human Development

NIH National Heart, Lung, and Blood Institute

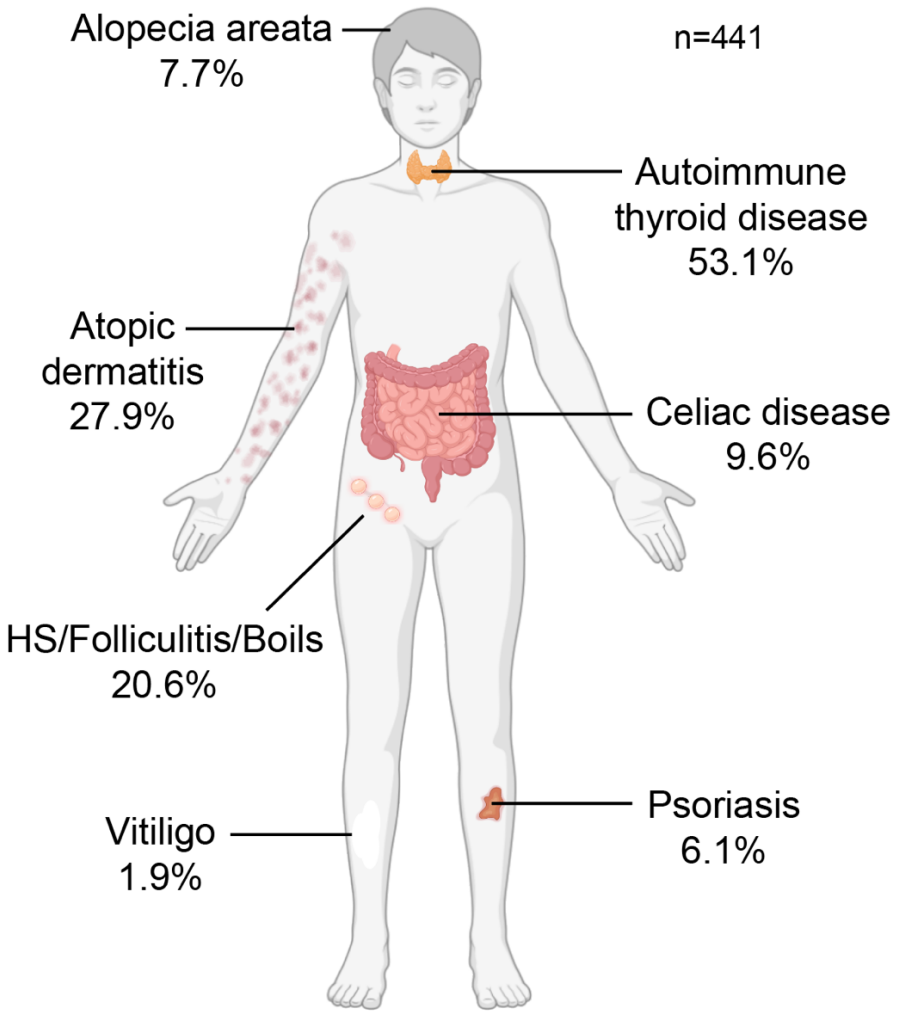
NIH National Institute on Aging

NIH National Institute of Allergy and Infectious Diseases

NIH National Institute on Deafness and Other Communication Disorders

NIH National Center for Advancing Translational Sciences

Key observation: widespread autoimmunity in Down syndrome



Rachubinski et al, eLIFE 2024

~75% of adults with Down syndrome have been diagnosed with at least one autoimmune condition

>50% of people with Down syndrome have autoimmune thyroid disease (AITD), leading to **hypo**thyroidism or **hyper**thyroidism

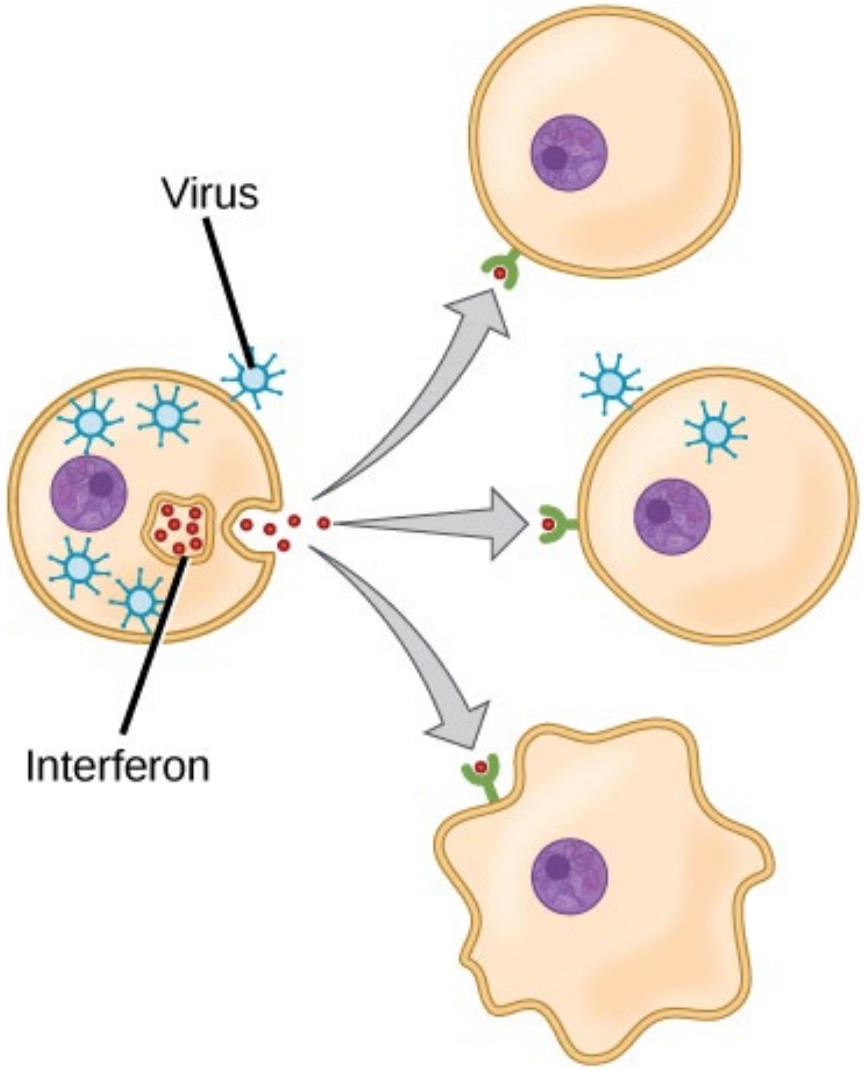
>35% adults with Down syndrome have been diagnosed with one or more autoimmune skin conditions

~10% of adults with Down syndrome have been diagnosed with celiac disease

Type I diabetes, 'Down syndrome arthropathy', and other, more rare autoimmune conditions, are also more common

>80% of these autoimmune conditions are diagnosed during pediatric age.

People with Down syndrome have hyperactive interferon signaling



Interferon (IFN) signaling is an important part of the immune system involved in the anti-viral defense.

Interferons are ‘cytokines’ that activate many different types of immune cells.

Interferon hyperactivity is a known risk factor for autoimmunity.

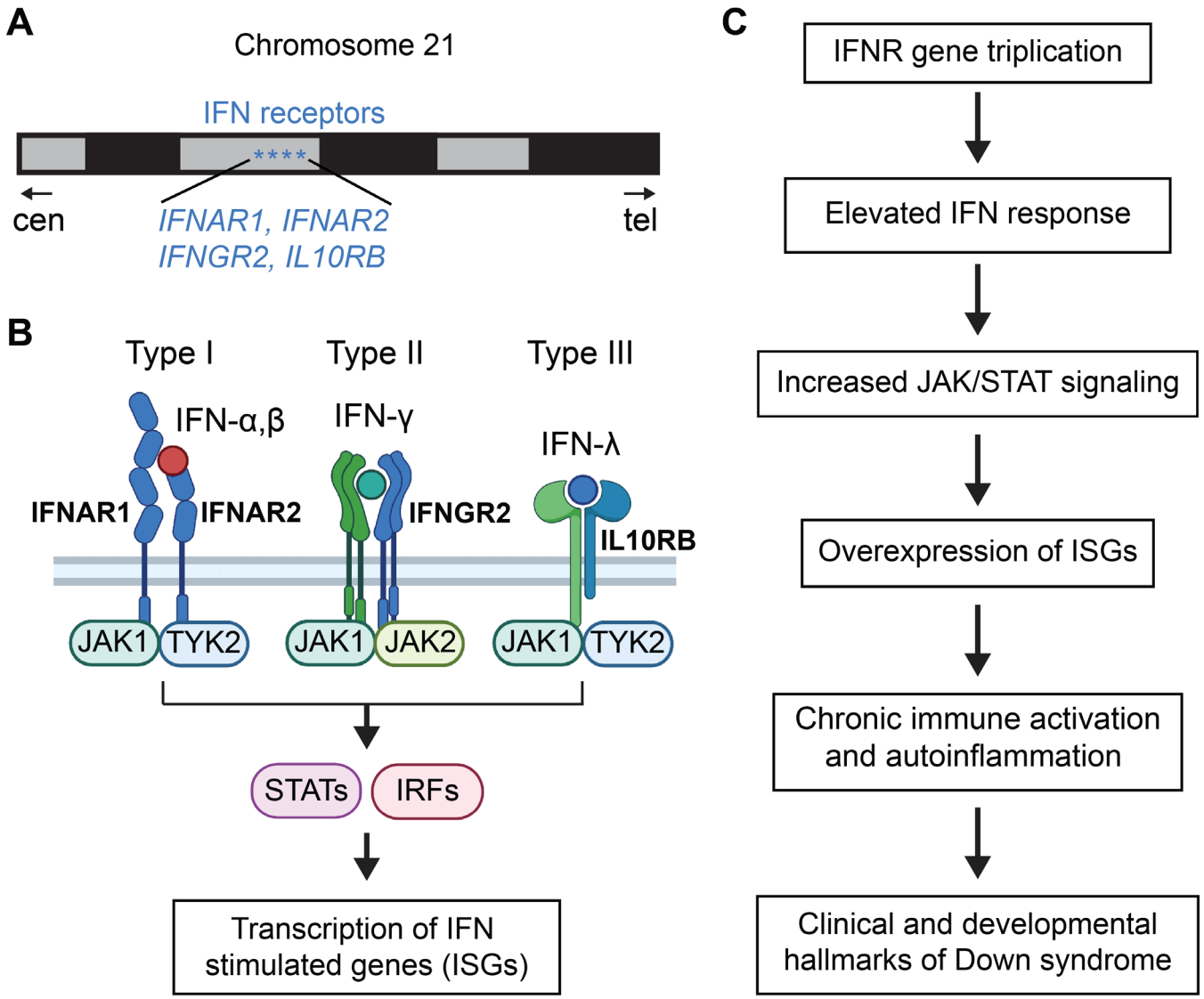
Trisomy 21 consistently activates the interferon response

Kelly D Sullivan[1,2,3,4]*, Hannah C Lewis[1,2], Amanda A Hill[1,2], Ahwan Pandey[1,2,3,4], Leisa P Jackson[1,3,4], Joseph M Cabral[1,3,4], Keith P Smith[1], L Alexander Liggett[1,5], Eliana B Gomez[1,3,4], Matthew D Galbraith[1,2,3,4], James DeGregori[1,5,6,7,8,9], Joaquín M Espinosa[1,2,3,4]*

eLIFE elifesciences.org

Sullivan et al, eLIFE 2016

Why do people with Down syndrome have hyperactive interferon signaling?



Four interferon receptors (IFNRs) are encoded on chromosome 21!!!

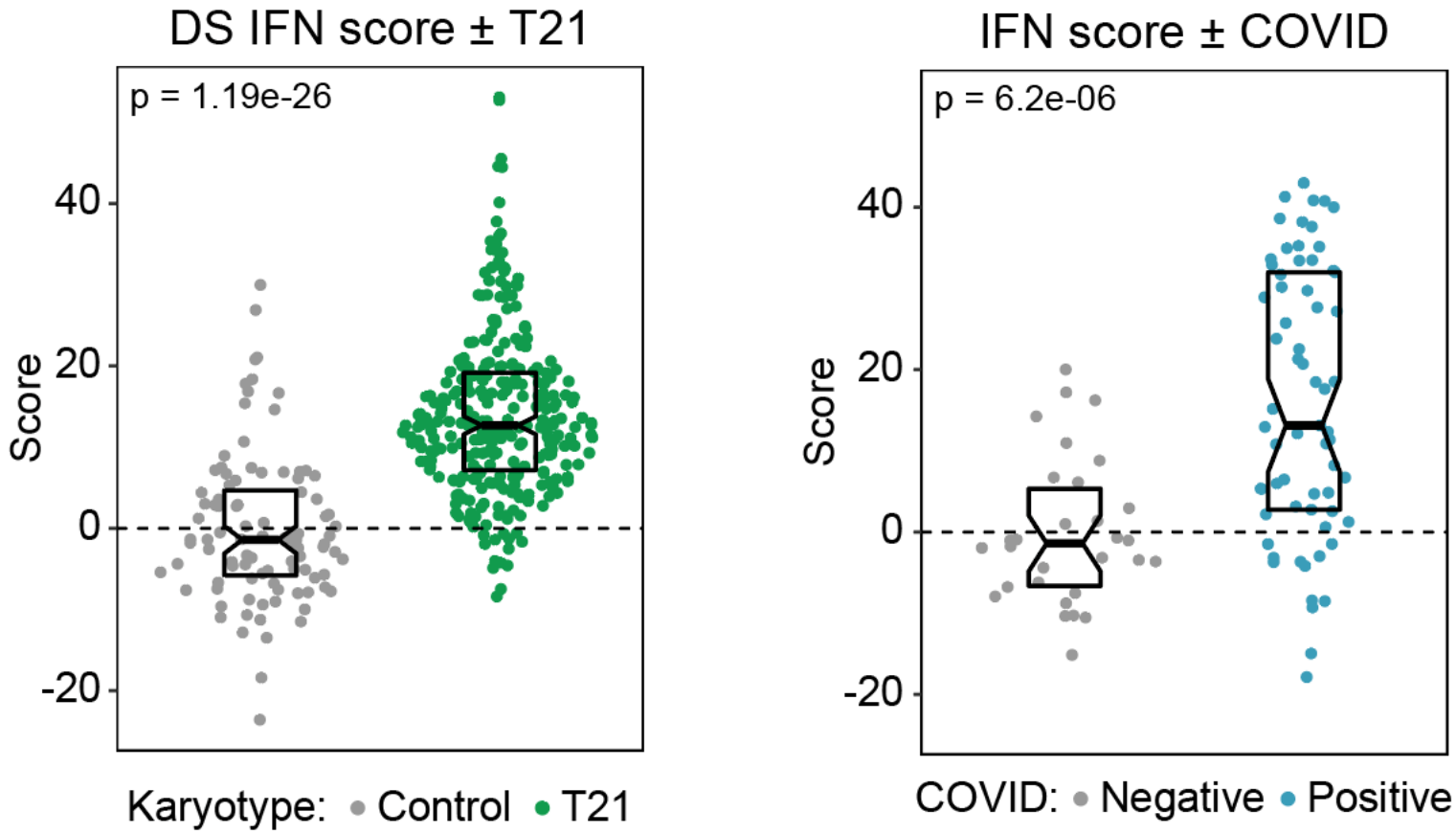
Hypotheses:

IFNR triplication causes elevated interferon responses, increased JAK/STAT signaling, and autoinflammation.

Interferon hyperactivity is a driver of pathology in Down syndrome.

The blood of people with Down syndrome looks like is fighting a viral infection

The hyperinflammation observed in Down syndrome is on par with that observed during a COVID-19 infection



IFN scores are commonly used to monitor the degree of IFN activity

T21: trisomy 21

Galbraith et al, Science Advances 2023

People with Down syndrome display ‘sterile inflammation’

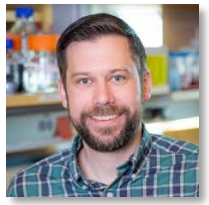
Trisomy 21 causes changes in the circulating proteome indicative of chronic autoinflammation

Kelly D. Sullivan[1,2], Donald Evans[1], Ahwan Pandey[1,2], Thomas H. Hraha[3], Keith P. Smith[1], Neil Markham[1], Angela L. Rachubinski[4], Kristine Wolter-Warmerdam[5], Francis Hickey[5], Joaquin M. Espinosa[1,2,6] & Thomas Blumenthal[1,6,7]

SCIENTIFIC REPORTS

2017

Sullivan



Evans

Smith

Rachubinski

Hickey

Blumenthal

A plasma proteomics analysis revealed widespread elevation of many inflammatory factors in people with Down syndrome.

Many pathogenic cytokines are highly elevated in Down syndrome, such as IL-6 or TNF-alpha.

The blood of individuals with trisomy 21 resembles the blood of those in the general population affected by autoinflammatory conditions…

The immune system of people with Down syndrome is highly dysregulated

Mass Cytometry Reveals Global Immune Remodeling with Multi-lineage Hypersensitivity to Type I Interferon in Down Syndrome

Katherine A. Waugh,[1] Paula Araya,[1] Ahwan Pandey,[1,2,3] Kimberly R. Jordan,[4] Keith P. Smith,[1] Ross E. Granrath,[1] Santosh Khanal,[2] Eric T. Butcher,[1] Belinda Enriquez Estrada,[1] Angela L. Rachubinski,[1,5] Jennifer A. McWilliams,[4] Ross Minter,[1] Tiana Dimasi,[1] Kelley L. Colvin,[1,5,6] Dmitry Baturin,[7] Andrew T. Pham,[1] Matthew D. Galbraith,[2] Kyle W. Bartsch,[1] Michael E. Yeager,[1,5,6] Christopher C. Porter,[8] Kelly D. Sullivan,[1,2,5] Elena W. Hsieh,[1,4,5] and Joaquin M. Espinosa[1,2,3,9,*]

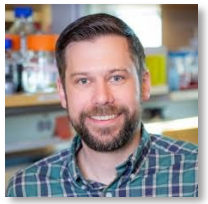
2019

Cell Reports

Waugh

Araya

Sullivan



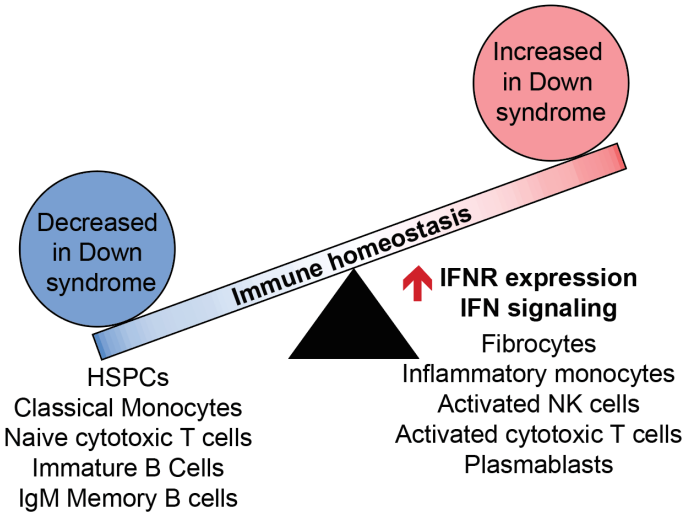
Tuttle

Smith

Hsieh

Immune mapping analyses revealed vast changes in the immune system of people with Down syndrome.

Many important immune cell types are either depleted, elevated, or dysfunctional in Down syndrome.



Immune hyperactivity affects the metabolism of people with Down syndrome

Trisomy 21 activates the kynurenine pathway via increased dosage of interferon receptors

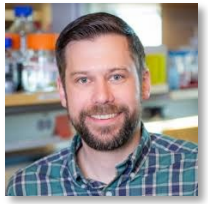
Rani K. Powers[1,2,3], Rachel Culp-Hill[4], Michael P. Ludwig[1,3], Keith P. Smith[1], Katherine A. Waugh[1], Ross Minter[1], Kathryn D. Tuttle[1], Hannah C. Lewis[1], Angela L. Rachubinski[1,5], Ross E. Granrath[1], María Carmona-Iragui[6,7], Rebecca B. Wilkerson[4], Darcy E. Kahn[1], Molishree Joshi[8], Alberto Lleó[6], Rafael Blesa[6], Juan Fortea[6,7], Angelo D'Alessandro[1,4], James C. Costello[2,3], Kelly D. Sullivan[1,3,5,8*] & Joaquin M. Espinosa[1,3,8,9*]

2019

nature COMMUNICATIONS

Powers

Sullivan



Ludwig

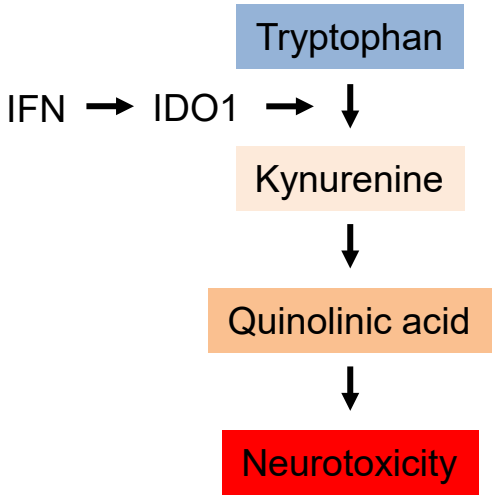
Smith

D'Alessandro

Costello

A plasma metabolomics analysis revealed widespread metabolic dysregulation in Down syndrome.

People with Down syndrome display activation of the kynurenine pathway, which produces neurotoxic metabolites:



IFN induces the kynurenine pathway

IDO1 is an IFN-inducible gene

Kynurenine and quinolinic acid are neurotoxic metabolites involved in many neurological conditions

Conclusion:

Interferon signaling is hyperactive in Down syndrome

Question:

What are the impacts of hyperactive interferon signaling in Down syndrome?

Could Down syndrome be understood as an **interferonopathy**?

Defining the impacts of IFN hyperactivity in mice

nature genetics

Article

https://doi.org/10.1038/s41588-023-01399-7

Triplication of the interferon receptor locus contributes to hallmarks of Down syndrome in a mouse model

2023

Katherine A. Waugh, et al,
Kelly D. Sullivan & Joaquin M. Espinosa

nature genetics

Waugh

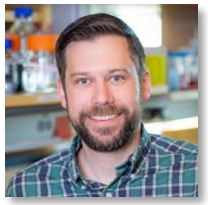
Minter

Tuttle

Araya

Galbraith

Sullivan



Work from the Maroun and Bianchi labs documented interferon hyperactivity in mouse models of Down syndrome carrying three copies of the receptors.

Early work from Maroun pointed to a pathogenic role for the receptors in mice.

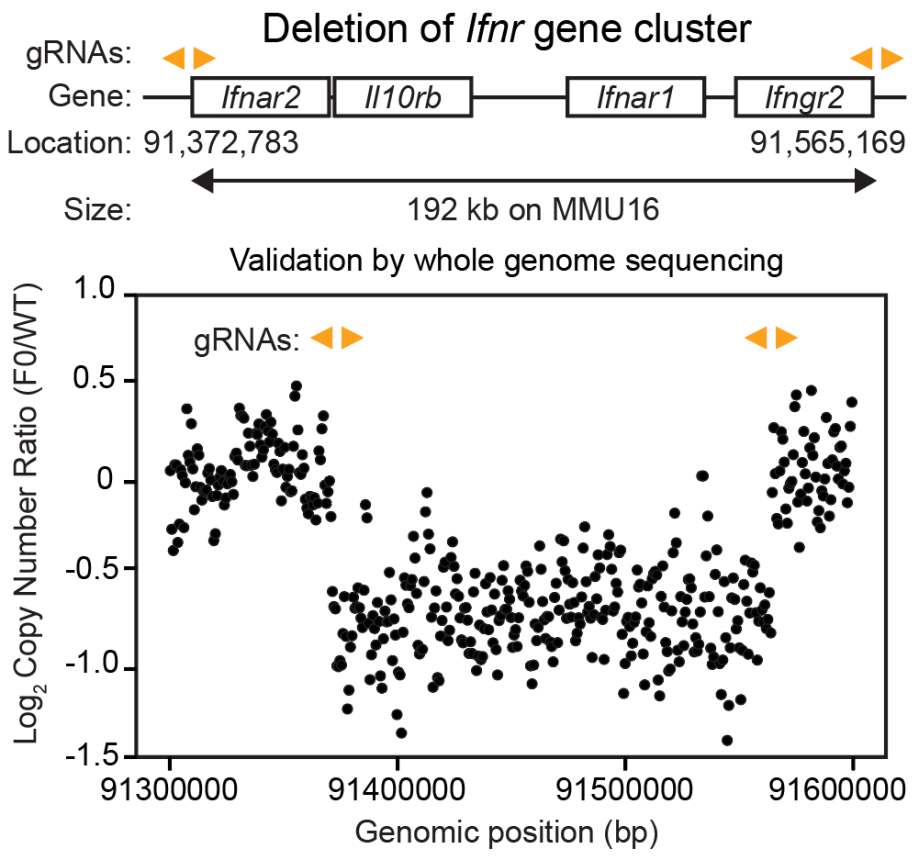
Maroun

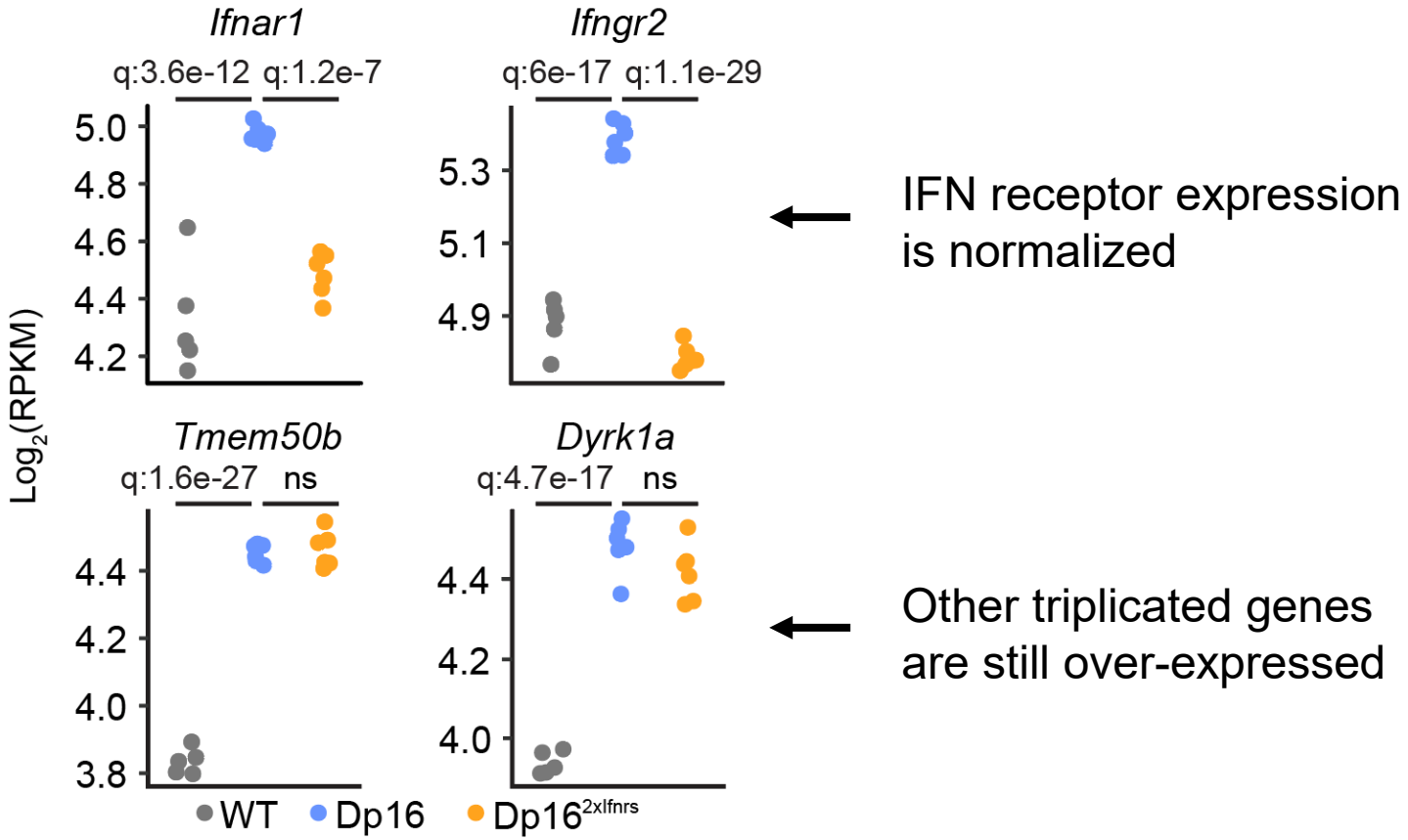
Bianchi

What would happen if we could ‘normalize’ receptor copy number?...

Normalizing interferon receptor gene dosage in mice

Clean deletion of the four interferon receptors using CRISPR technology while preserving triplication of ~120 other genes in this mouse model





WT: wild type (two copies) | Dp16: mouse model of Down syndrome | $Dp16^{2xIFNRs}$: Dp16 with two copies of receptors

Triplication of the interferon receptors contributes to:

- Global dysregulation of gene expression across multiple tissues.
- Lethal immune hypersensitivity.
- Congenital heart malformations.
- Developmental delays and cognitive deficits.
- Craniofacial abnormalities.

Humankind's best friend:

Funded by:

THE INCLUDE PROJECT

NIH National Institute of Allergy and Infectious Diseases

Key observation: the interferonopathy starts in the womb…

What would be the therapeutic benefit of attenuating IFN signaling in individuals with Down syndrome?

Waugh et al, *Nature Genetics* 2023

Would drugs that decrease the interferon response improve the health of persons with Down syndrome?

Approved therapies that decrease the interferon response: JAK inhibitors

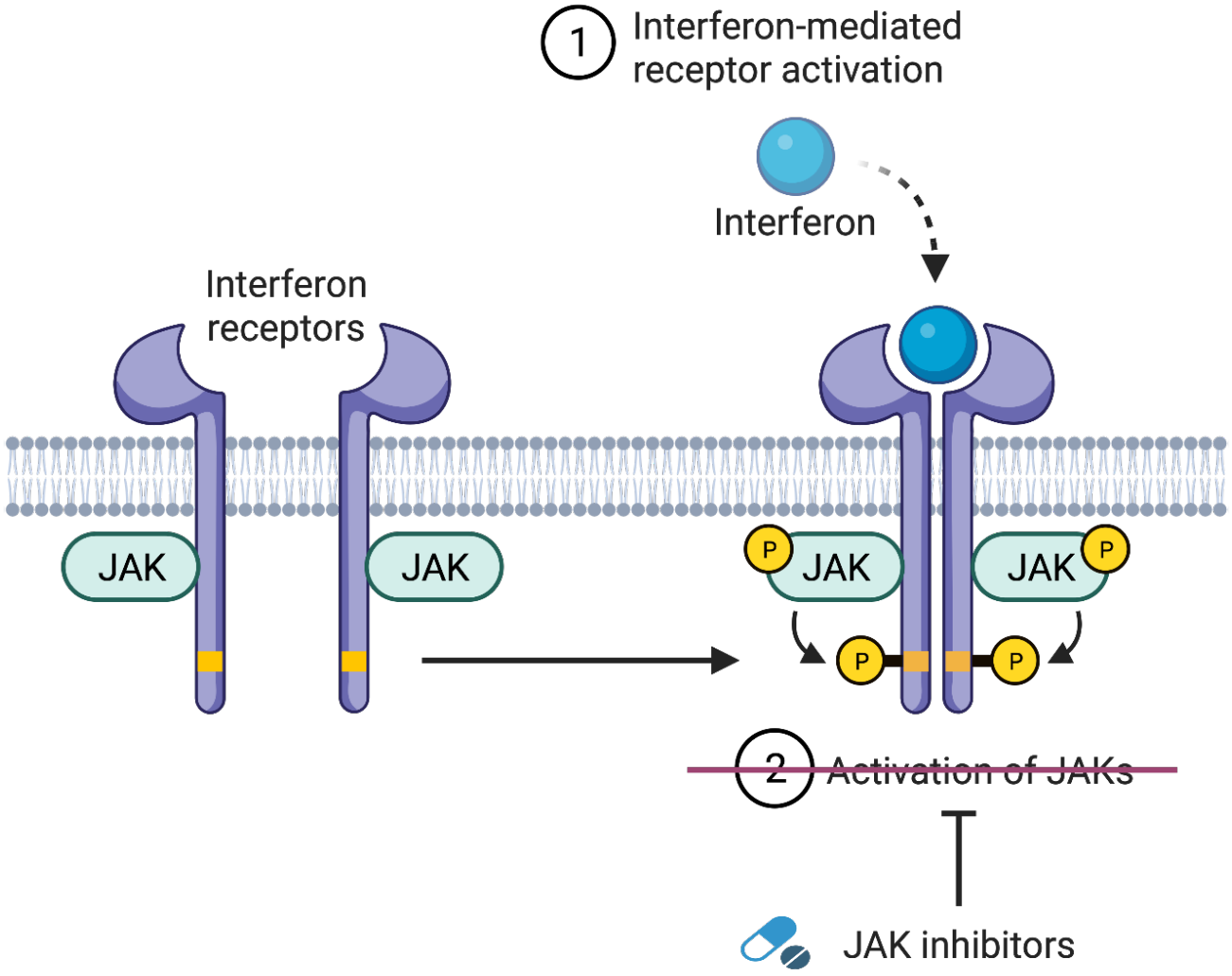
Target	JAK1/3	JAK1/2	JAK1	JAK1/2	JAK1
Rheumatoid arthritis	+	+	+		
Psoriatic arthritis	+		+		
Polyarticular course JIA	+				
Ulcerative colitis	+		+		
Atopic dermatitis			+		+
COVID-19		+			
Alopecia areata		+			
Chron's disease			+		
Polycythemia vera				+	
Ankylosing spondylitis			+		
Myelofibrosis				+	
GVHD				+	
Axial spondylarthritis			+		

There are many JAK inhibitors approved for many different indications.

These medicines are used by rheumatologists, dermatologists, gastroenterologists, hematologists and more!

Could JAK inhibitors 'normalize' the immune system in Down syndrome?

JAK inhibitors could attenuate the ill effects of interferon receptor overdose



JAK inhibitors are small molecules designed to inhibit the JAK enzymes acting ‘downstream’ of the interferon receptors.

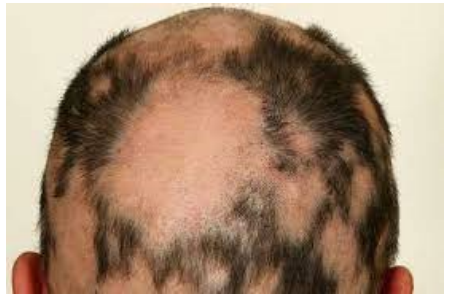
JAK inhibitors are taken daily orally as pills and have a short ‘half-life’ in the body.

The action of JAK inhibitors is fully reversible, as they are rapidly cleared from the human body within hours.

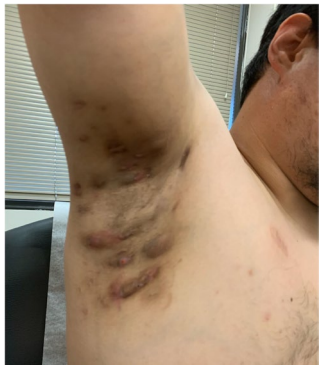
First clinical trial for JAK inhibition in Down syndrome

Treating five immune skin conditions in one trial

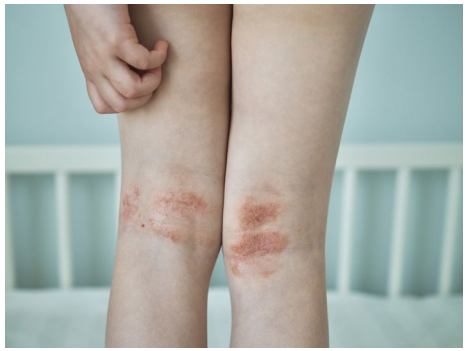
Alopecia areata (patchy hair loss)



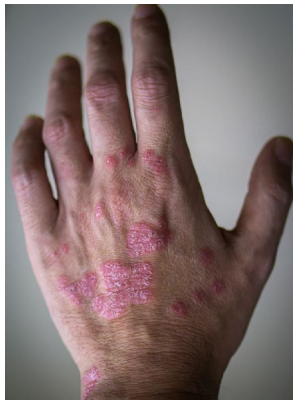
Hidradenitis suppurativa (boils)



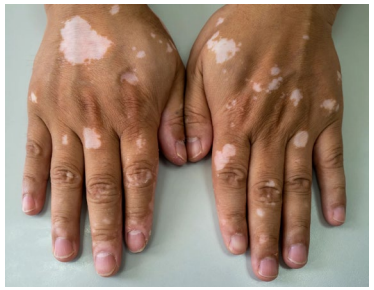
Atopic dermatitis (eczema)



Psoriasis



Vitiligo



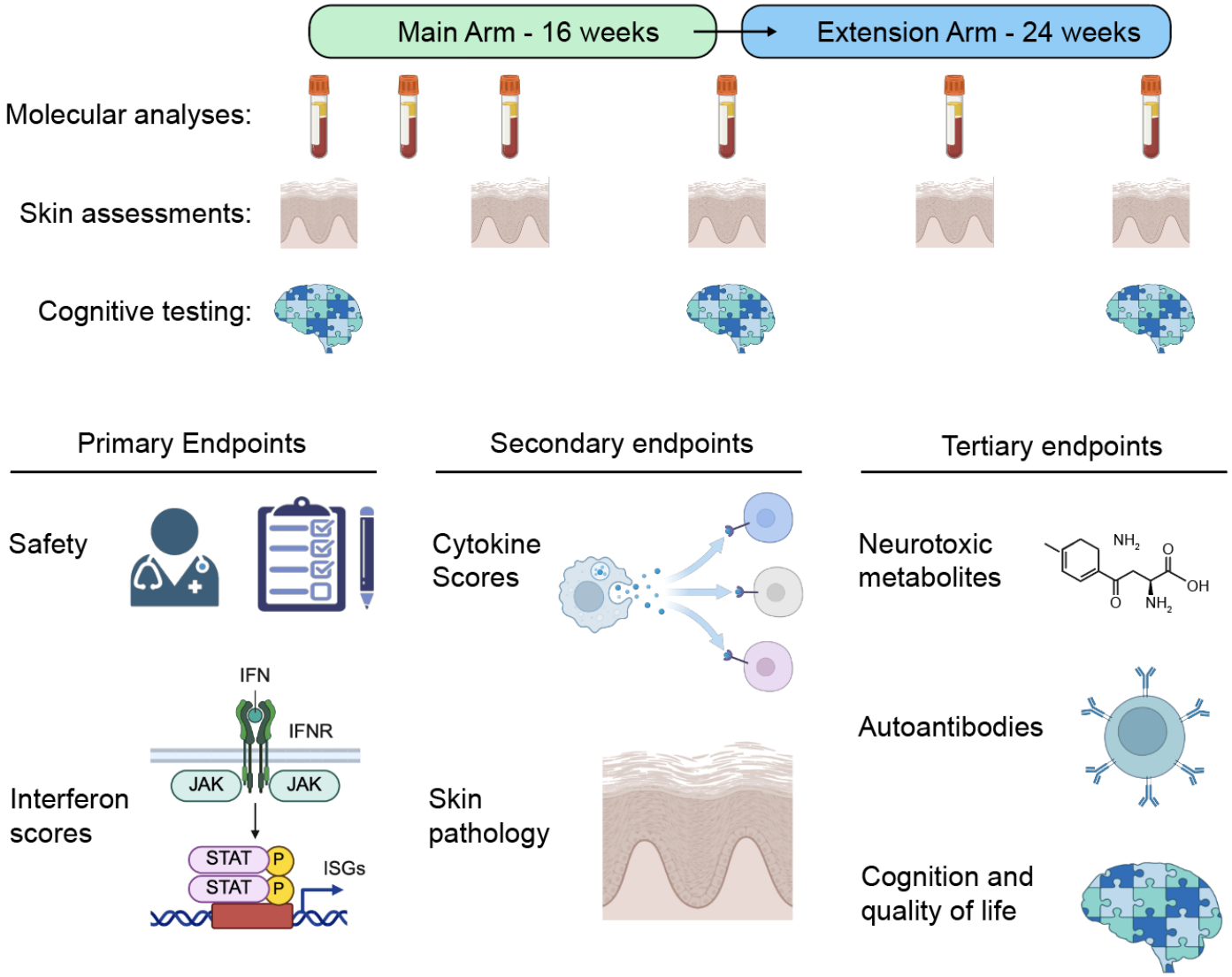
All five conditions are more common in people with Down syndrome

More than 35% of adults with Down syndrome have been affected by one of these conditions

4-9 months of treatment with the FDA-approved JAK inhibitor Tofacitinib (Xeljanz)

Funded by: NIH THE INCLUDE PROJECT NIH National Institute of Arthritis and Musculoskeletal and Skin Diseases

First clinical trial for JAK inhibition in Down syndrome



- ✓ Phase II, open label
- ✓ Ages 12-50
- ✓ 40 participants completing 16 weeks of treatment
- ✓ JAK inhibitor: tofacitinib

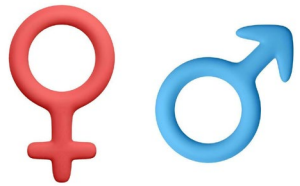
Key endpoints:

- Safety
- Immune markers
- Skin pathology
- Cognition

First clinical trial for JAK inhibition in Down syndrome

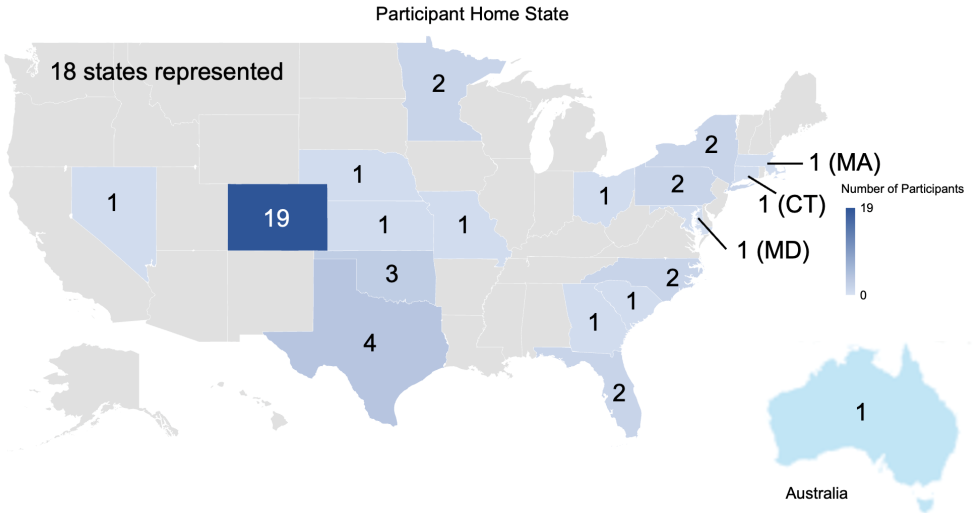
Top metrics:

Half males, half females



25% Hispanics | 15% Black or biracial

60% from outside of Colorado



Most common qualifying conditions:

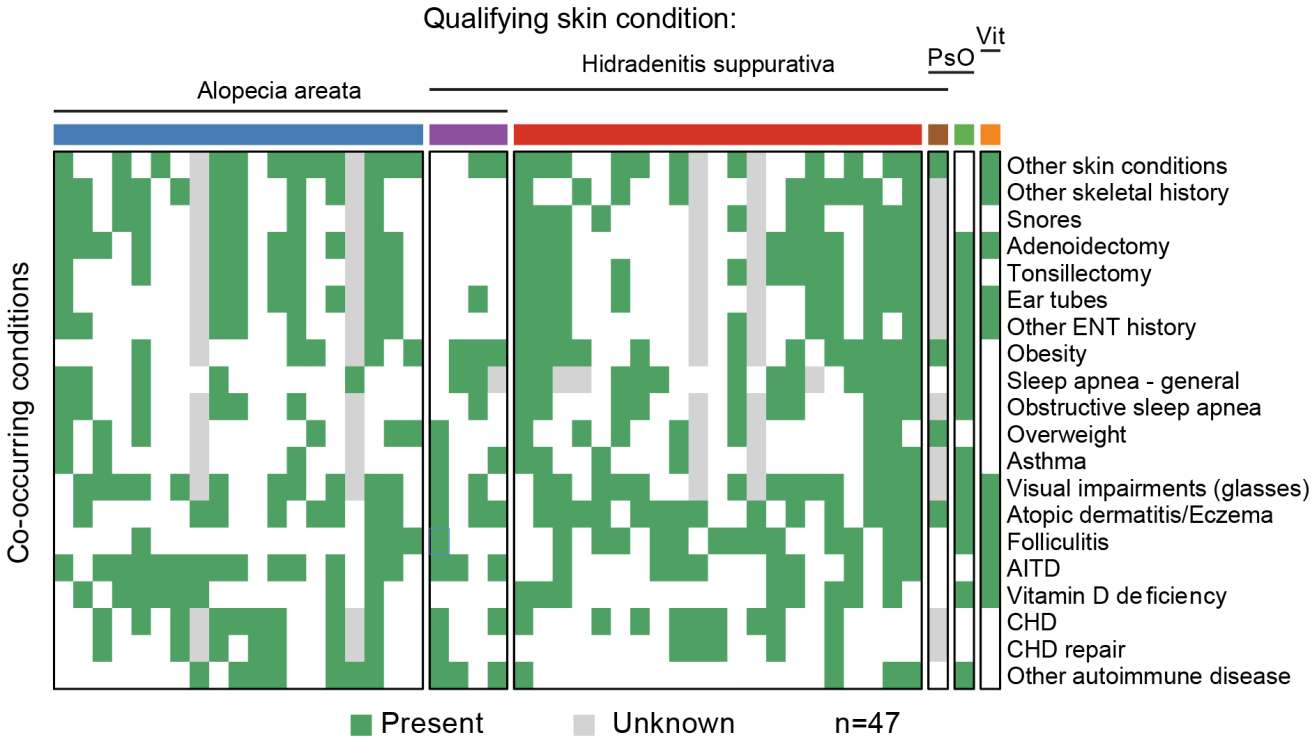
1. Hidradenitis suppurativa
2. Alopecia areata
3. Psoriasis

THE INCLUDE PROJECT

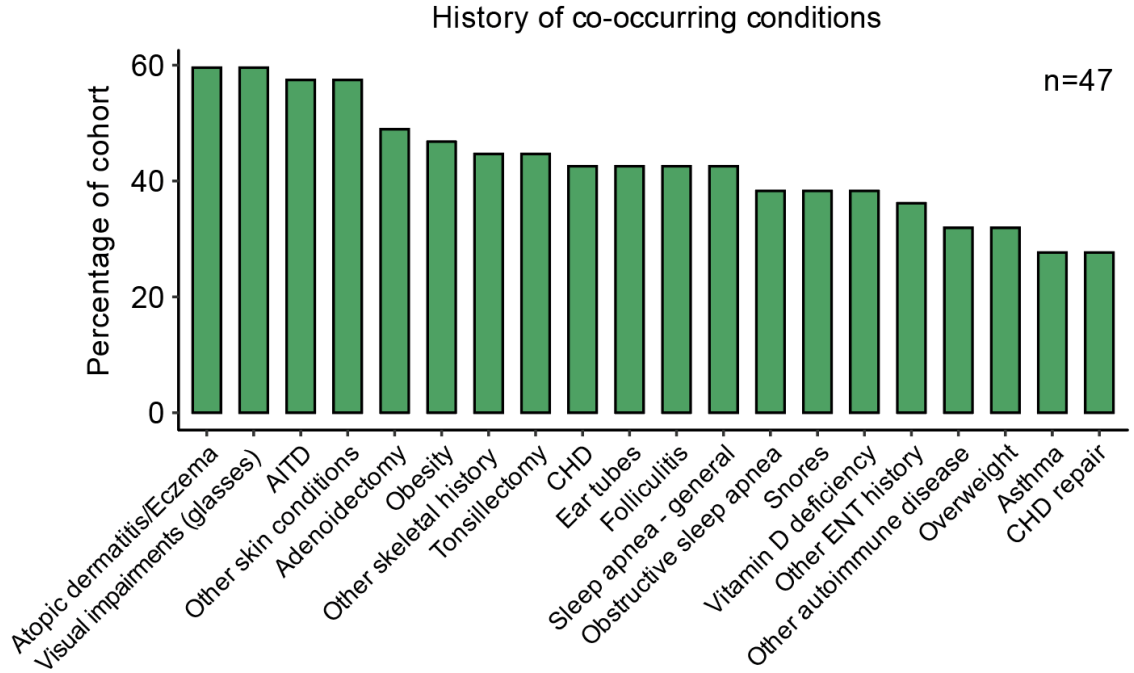
NIH National Institute of Arthritis and Musculoskeletal and Skin Diseases

Clinical trials in Down syndrome: a labor of love

A population with high medical complexity



PsO: psoriasis Vit: vitiligo



AITD: autoimmune thyroid disease CHD: congenital heart defect

The importance of tailored exclusion / inclusion criteria and monitoring protocols

Study doctors:

Norris

Dunnick

Bruckner

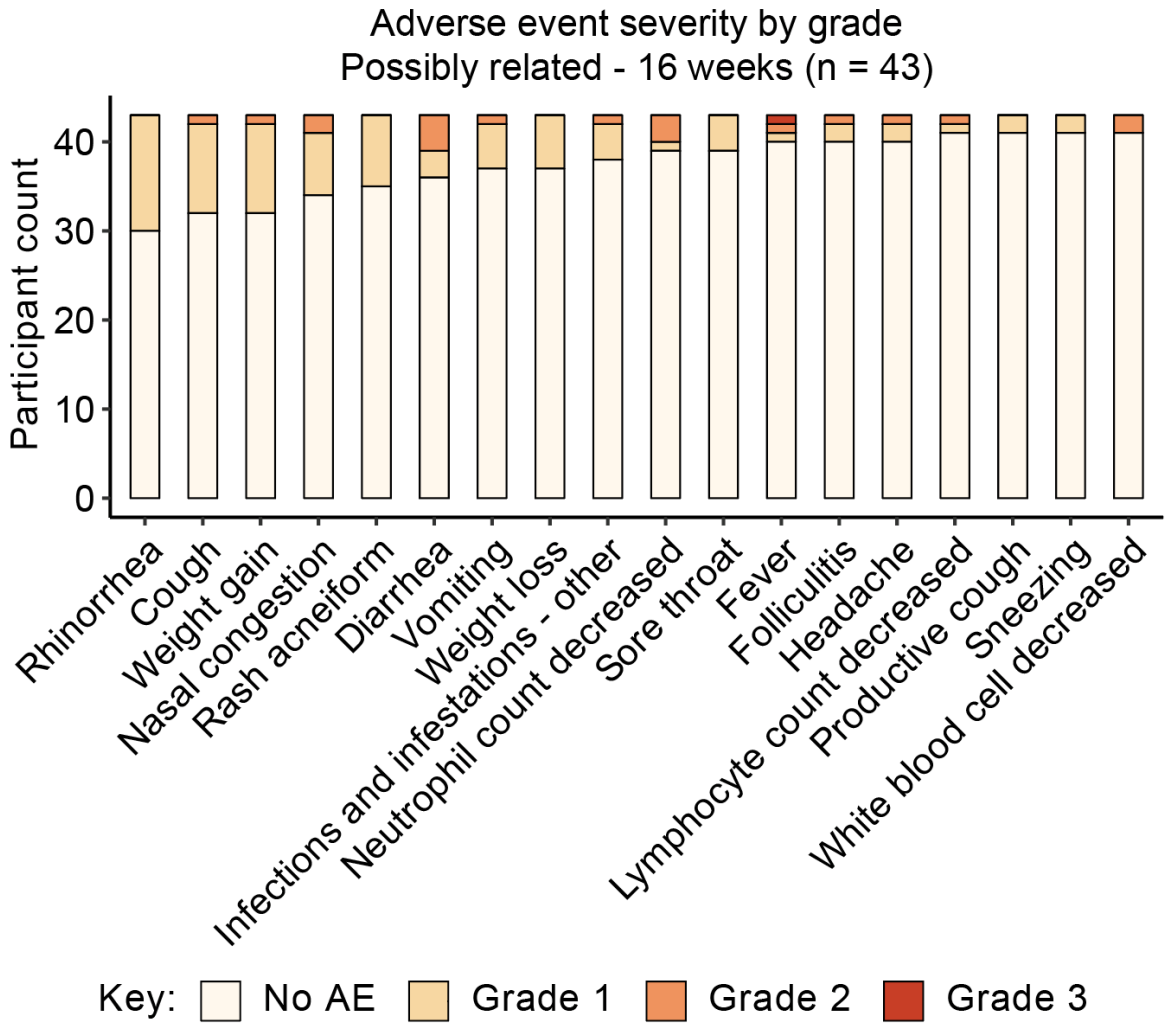
Gurnee

Martin

Wallace

JAK inhibition is safe in Down syndrome

Safety endpoint was met!



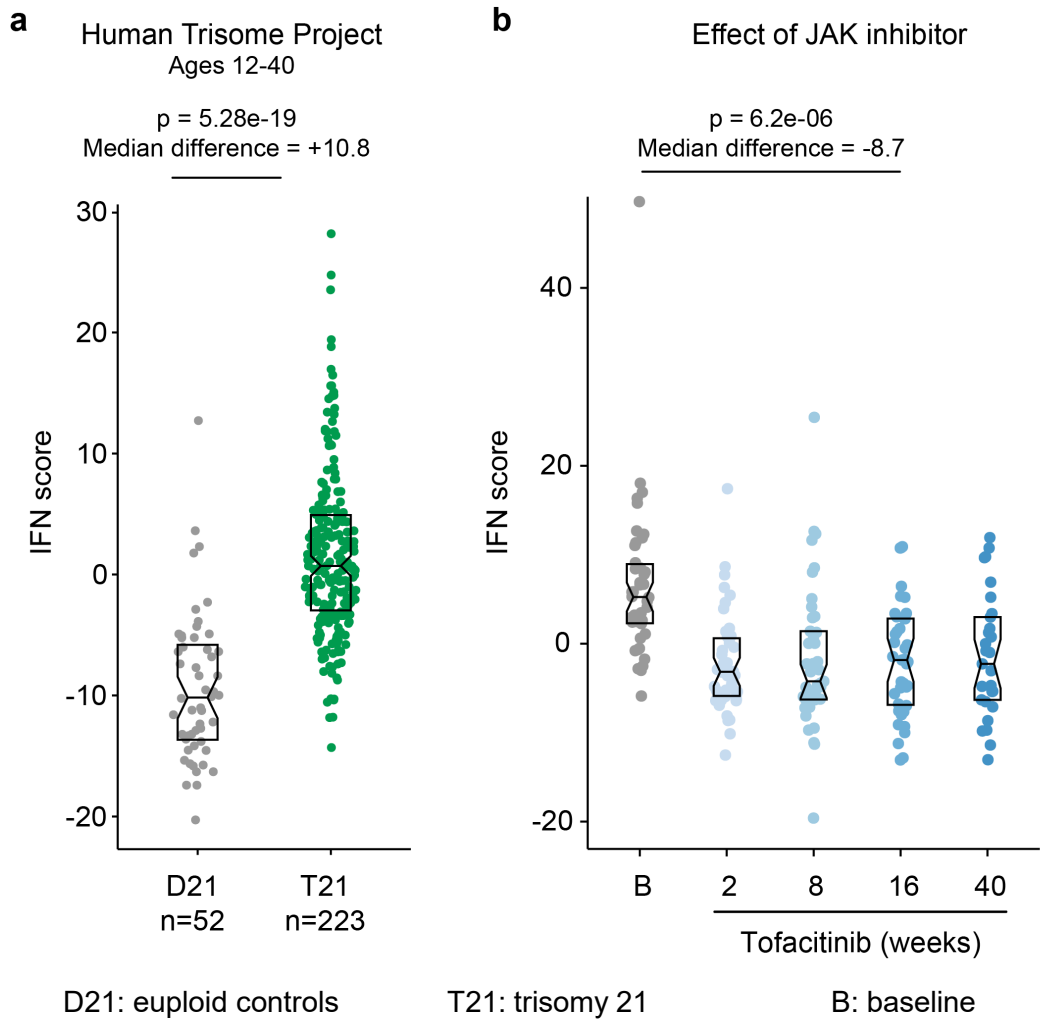
Safety profile reminiscent of that observed in the general population:

- Symptoms of upper respiratory infection
- Weight change
- Skin rash
- Mild clinical lab abnormalities

A single serious adverse event documented over 27.7 years of observation: an episode of thromboembolism in a participant taking oral contraceptive pills, which are known to increase risk of thromboembolism. Participant recovered favorably.

JAK inhibition reduces interferon scores and other biomarkers of autoinflammation

Normalization of IFN scores without overt immune suppression



D21: euploid controls T21: trisomy 21 B: baseline

The JAK inhibitor reduces IFN scores down to the range observed in the general population, not any lower.

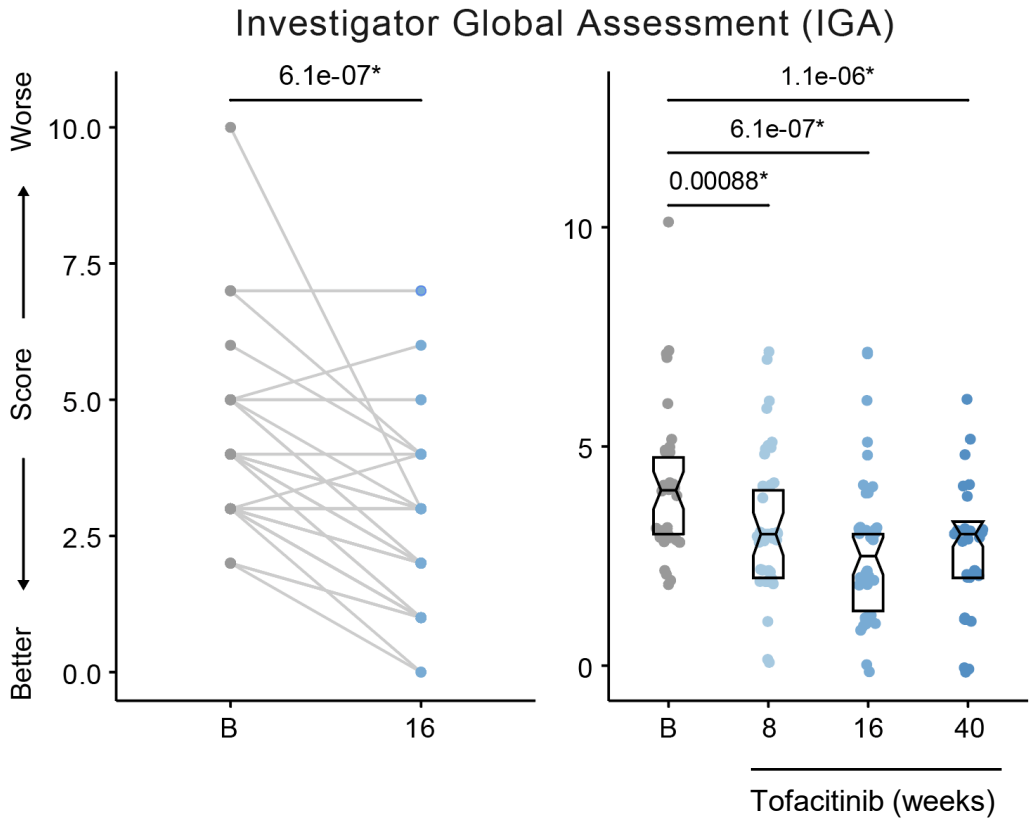
The JAK inhibitor also reduces cytokine scores and kynurenine pathway metabolites.

Endpoints met!

Galbraith et al, *Science Advances* 2023
Rachubinski et al, *eLIFE* 2024

JAK inhibition can be safely used to treat diverse immune skin conditions in Down syndrome

Improvement in skin pathology across multiple conditions



p-values from paired Student's t-test

The JAK inhibitor significantly reduced skin pathology as measured by:

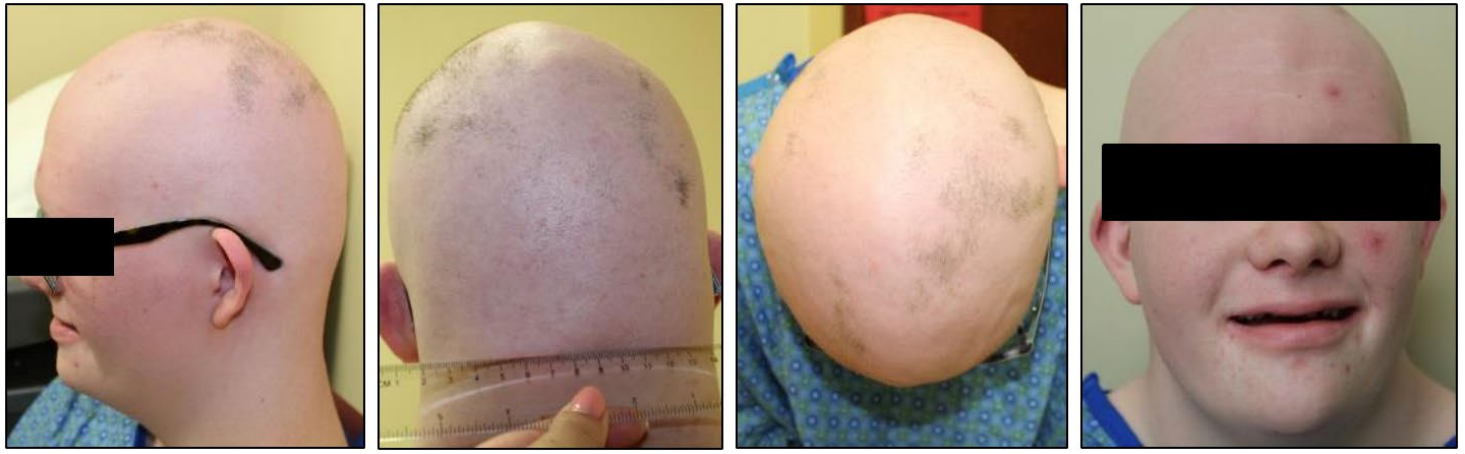
- ✓ Investigator Global Assessment
- ✓ Dermatology Life Quality Index
- ✓ Modified Sartorius Score (for hidradenitis)
- ✓ Severity of Alopecia Tool (for alopecia)

Half of the participants secured prescriptions for the medicine after completing the trial…

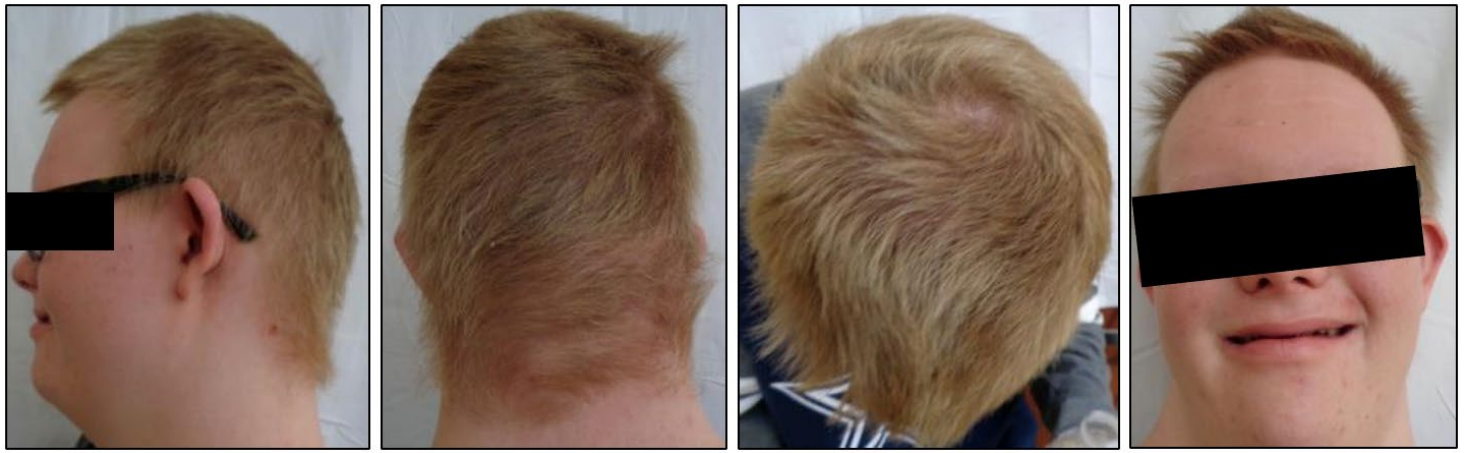
JAK inhibition can be safely used for alopecia areata

When a picture is worth a thousand words

Baseline
SALT = 86



Week 16
SALT = 4

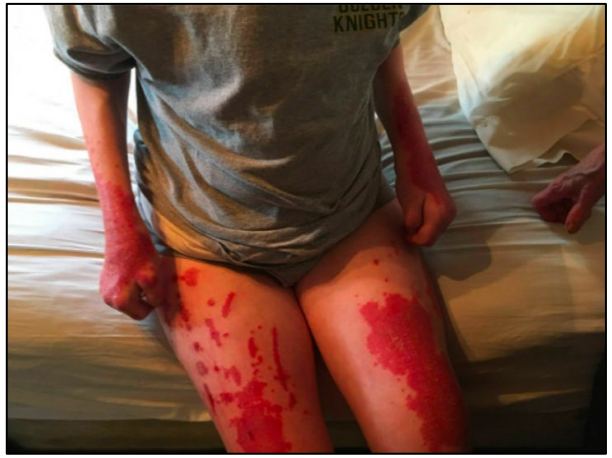


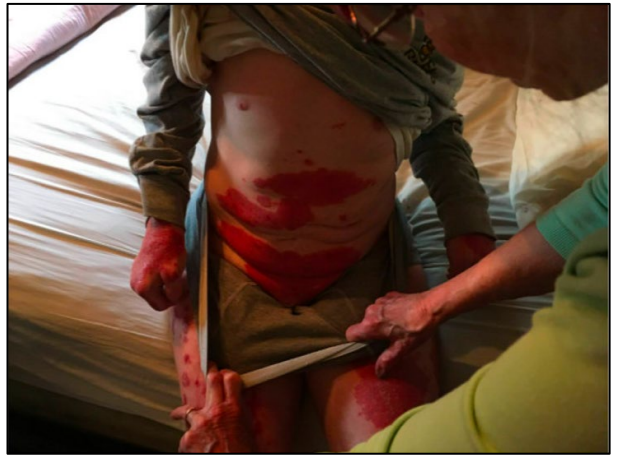
Participant referred known as ‘Ed Sheeran’ to the research team

JAK inhibition can be safely used for psoriasis

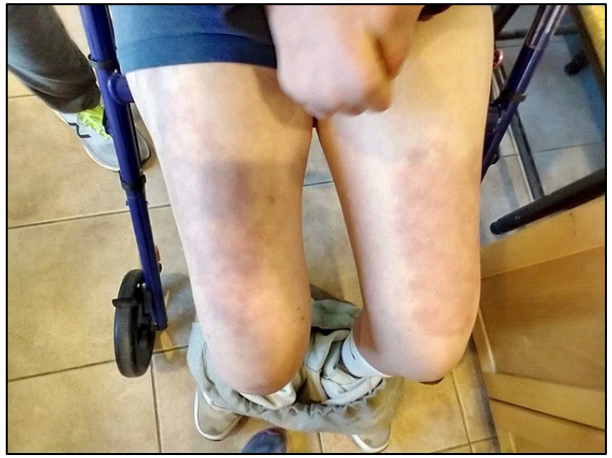
When a picture is worth a thousand words

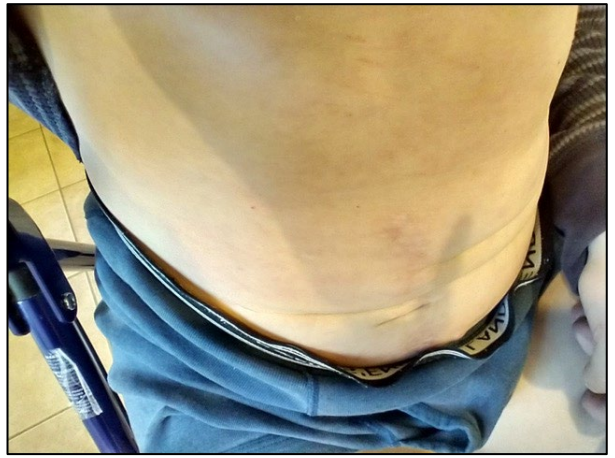
Before





After





Participant monitored outside of the trial at the University of Vermont Medical Center, pictures courtesy of Dr. Ralph Budd

What are the effects of JAK inhibition on cognitive function?

Participants experienced gains in most of the tests administered

Disclaimer: without a placebo control arm, it is hard to interpret these results

Prefrontal Cortex

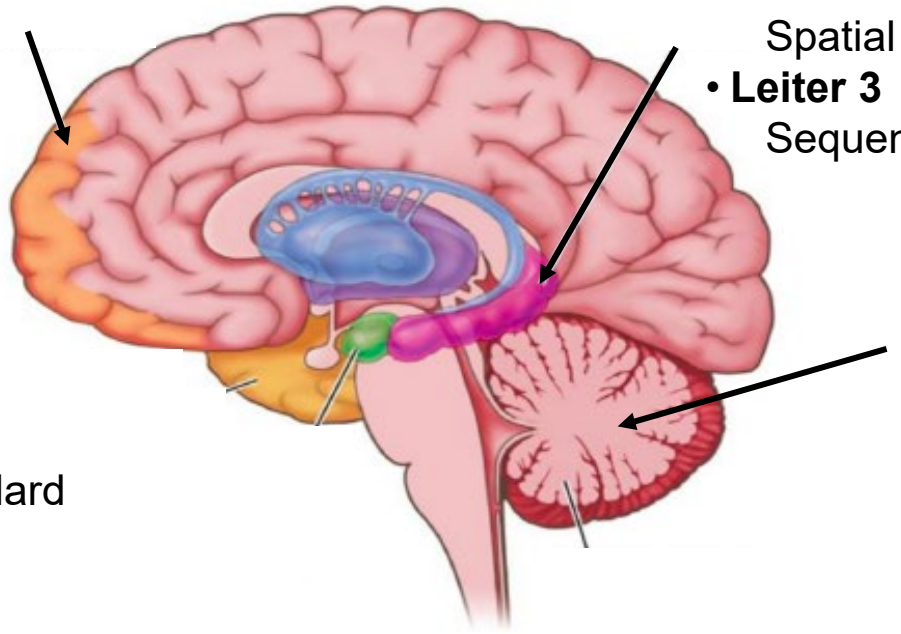
Working memory and executive function

- **Leiter 3**
  - Forward & Reverse Memory
  - Attention Sustained
  - Nonverbal Stroop

Hippocampus

Episodic and spatial memory

- **CANTAB**
  - Paired Associate Learning
  - Spatial Span
- **Leiter 3**
  - Sequential Order



Cerebellum

Motor Control

- **NEPSY II**
  - Total time & total errors
- **CANTAB**
  - Reaction Time Interval
- **SOBC**
  - Simple Reaction Time

Quality of life

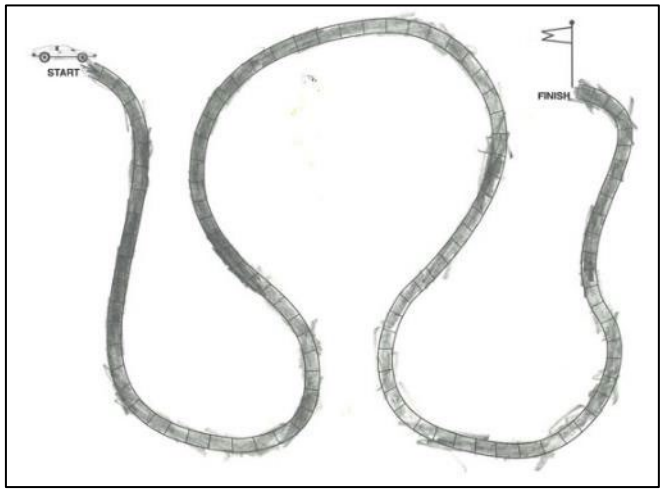
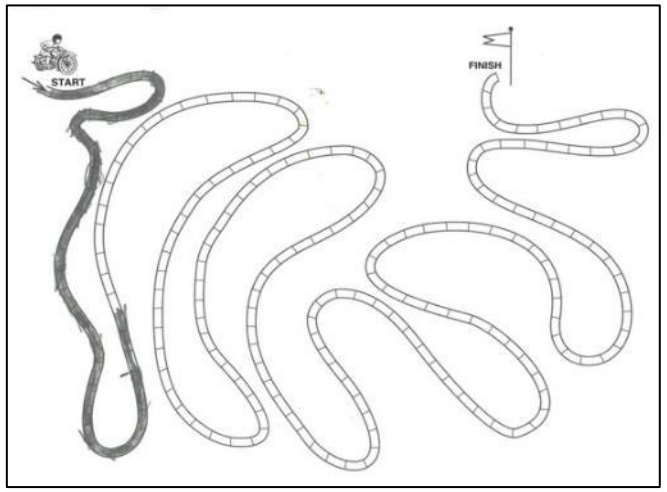
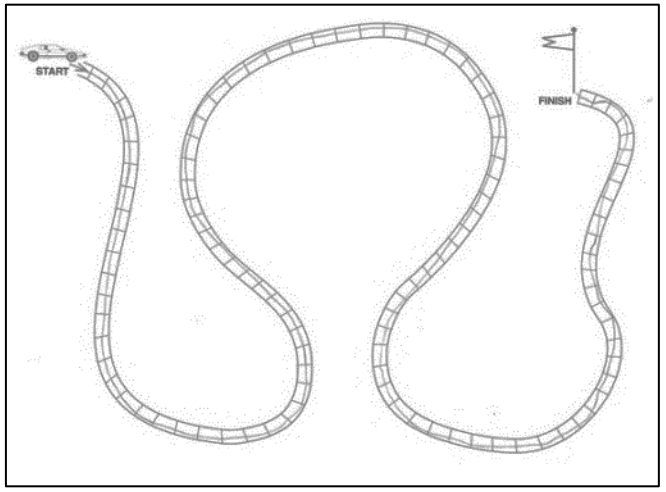
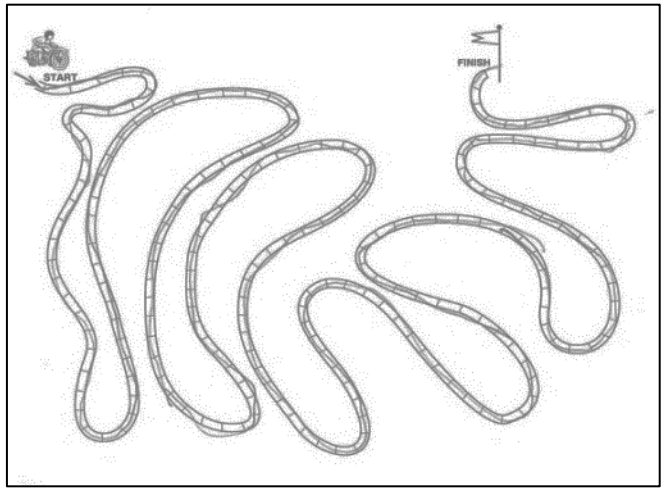
- **PROMIS**
  - Anxiety
  - Depression
  - Positive Affect
  - General Concerns

Overall Development

- **KBIT-2**
  - Verbal & Nonverbal
  - IQ Composite Standard
- **PPVT-5**
  - Raw scores

Sometimes, one participant is all it takes…

Female, 28 years old, history of Down syndrome Regression Disorder (DSRD)

	NEPSY II (car)	NEPSY II (motorcycle)
Baseline		
Week 16		

- Participant experienced DSRD for eight years before the trial.
- Before treatment, the participant was receiving electroconvulsive therapy (ECT) three times a week
- The benefits were so obvious that participant was prescribed tofacitinib ‘off-label’ by her neuroimmunologist, and both Pfizer and Medicaid agreed to pay for it…

Down Syndrome Regression Disorder (DSRD)

- A devastating condition characterized by rapid onset of catatonia, mutism, depersonalization, loss of ability to perform activities of daily living, hallucinations, delusions, and aggression.
- A subset of DSRD cases are associated with signs of immune dysregulation affecting the central nervous system (CNS).
- Is DSRD an autoimmune condition, akin to autoimmune encephalitis?

JAK inhibition in Down Syndrome Regression Disorder

Angela L. Rachubinski[a,b,*], Lina R. Patel[a], Elise M. Sannar[c], Ryan M. Kammeyer[d], Jessica Sanders[b], Belinda A. Enriquez-Estrada[a], Kayleigh R. Worek[a], Deborah J. Fidler[e], Jonathan D. Santoro[f,g], Joaquin M. Espinosa[a,h,*]

2024

Journal of Neuroimmunology

Evidence of blood–brain barrier dysfunction and CSF immunoglobulin synthesis in Down Syndrome Regression Disorder

Jonathan D. Santoro[1,2], Neetha Paul Eduthan[3], Mellad M. Khoshnood[1,2], Saba Jafarpour[1,2], Natalie K. Boyd[1], Benjamin N. Vogel[1], Lina Nguyen[1], Lilia Kazerooni[1], Eleanor Britton[3], Hannah R. Lyford[3], Matthew D. Galbraith[3,4], Angela L. Rachubinski[3,5] & Joaquin M. Espinosa[3,4]

2025

ANNALS of Clinical and Translational Neurology

When the families drive the research

Story behind the design of the first randomized clinical trial for Down Syndrome Regression Disorder

Well+Being

A mystery illness stole their kids' personalities. These moms fought for answers.

Their children's decline was dramatic, with patients losing function in days or weeks, including the ability to talk, move or take care of themselves.

May 12, 2024

The Washington Post

Clinical trial for therapies for Down Syndrome Regression Disorder

Goal: To compare the safety and efficacy of two ‘immune therapies’ (tofacitinib and Intravenous Immunoglobulin - IVIG) versus a psychiatric medicine (lorazepam, a benzodiazepine).

Multi-site collaboration between the Crnic Institute, Children's Hospital Colorado, and Children’s Hospital Los Angeles.

Santoro

Sannar

Kammeyer

Patel

Rachubinski

Children's Hospital LOS ANGELES

Children's Hospital Colorado

LINDA CRNIC INSTITUTE for DOWN SYNDROME

Funded by:

THE INCLUDE PROJECT

NIH Eunice Kennedy Shriver National Institute of Child Health and Human Development

Recruiting now!

A Phase II, *three-arm, open-label*, research-intensive trial

Lorazepam
Brand name: Ativan
Benzodiazepine

IVIG
Brand name: Gammagard
Intravenous Immune Globulin

Tofacitinib
Brand name: Xeljanz
JAK inhibitor

All three medicines studied in this trial are already FDA-approved for **other** medical conditions

The power of ‘drug repurposing’: this study benefits from extensive available data for all three drugs

Open label: participants will know which medicine they are taking

No placebo arm, but instead ‘a delayed treatment group’ for comparison

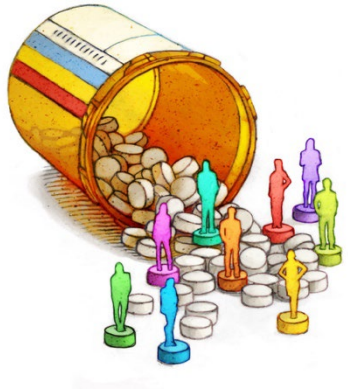
Why compare these three medicines?

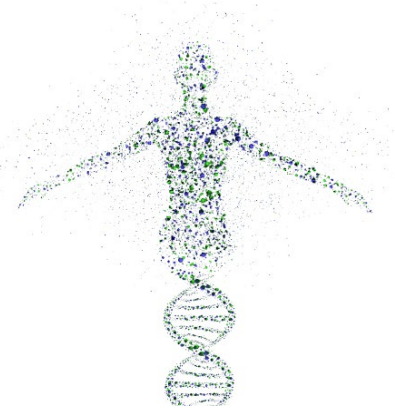
Who would benefit the most from which medicine?

What are the diagnostic characteristics that could predict a good response?

Are there ‘biomarkers’ in the blood or cerebrospinal fluid that could match each participant to their best therapeutic option?

Developing a personalized medicine approach for the treatment of DSRD





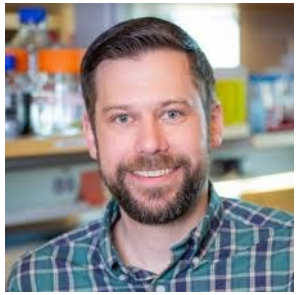
Conclusions

- Interferon hyperactivity could cause many health issues in individuals with Down syndrome, such as autoimmune disorders, severe complications from lung viral infections (e.g., COVID19), and neurological disorders.
- Research into immune system dysregulation in Down syndrome has illuminated therapeutic strategies being tested in first-in-kind clinical trials (e.g., JAK inhibition).
- Restoring immune balance could have multidimensional benefits in Down syndrome, even perhaps from early development.

Outstanding questions

- What is the long-term safety profile of JAK inhibition in people with Down syndrome?
- What are all the possible benefits of immunomodulation in Down syndrome?
- How early could treatment start? Is pre-natal treatment even possible?
- Should everyone with Down syndrome be treated or only those with clinically evident autoimmunity?

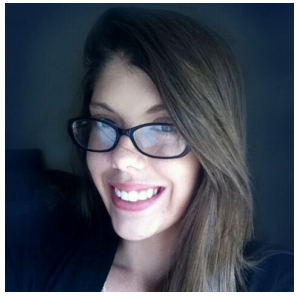
Credits



Kelly Sullivan
Experimental Models Program

Angela Rachubinski
Clinical and Translational Sciences Program

Matthew Galbraith
Data Sciences Program



Lyndy Bush
Administrative and Outreach Program

THE INCLUDE PROJECT

NIH *Eunice Kennedy Shriver* National Institute of Child Health and Human Development

NIH National Heart, Lung, and Blood Institute

NIH National Institute of Allergy and Infectious Diseases

NIH National Institute of Arthritis and Musculoskeletal and Skin Diseases

NIH National Institute on Aging

NIH National Institute on Deafness and Other Communication Disorders

NIH National Center for Advancing Translational Sciences

GLOBAL DOWN SYNDROME FOUNDATION®

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All research participants and their families
Many many wonderful collaborators, all research participants and their families
Michelle Sie Whitten and the amazing team at the Global Down Syndrome Foundation

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