The Path Traveled: Progress in Developing Therapeutics for Cutaneous Neurofibromas

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Response Evaluation In Neurofibromatosis Schwannomatosis
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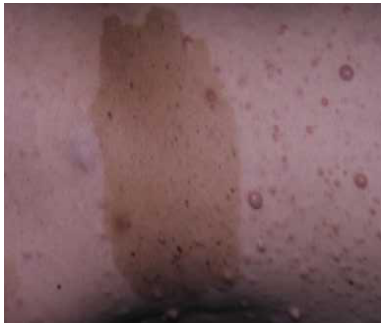
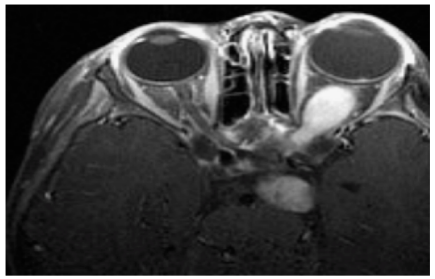
Neurofibromatosis Type 1

- Estimated prevalence 1/3600
- Can affect any organ system of the body.
  - Skin: cutaneous neurofibromas, café au lait spots and skin fold freckling
  - Nervous system tumors: Multiple peripheral and central nervous system tumors including neurofibromas, gliomas, malignant peripheral nerve sheath tumors
  - Multiple other manifestations: cognitive deficits, bone dysplasias, ophthalmologic abnormalities, vascular anomalies, cardiovascular abnormalities and an increased risk of non-nervous system malignancies

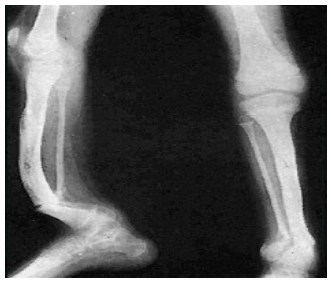
Cutaneous neurofibromas



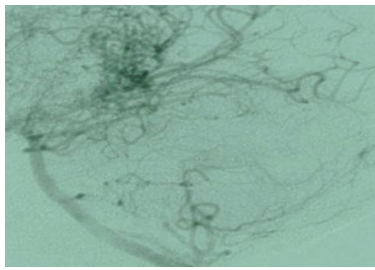
Café-au-lait spots



Optic pathway glioma and brainstem glioma



Tibial pseudoarthrosis



Moyamoya disease

Evans DG, et al,Am J of Med Genet. 2010
Photos courtesy of Kaleb Yohay and Pierre Wolkenstein

Neurofibromatosis Type 1

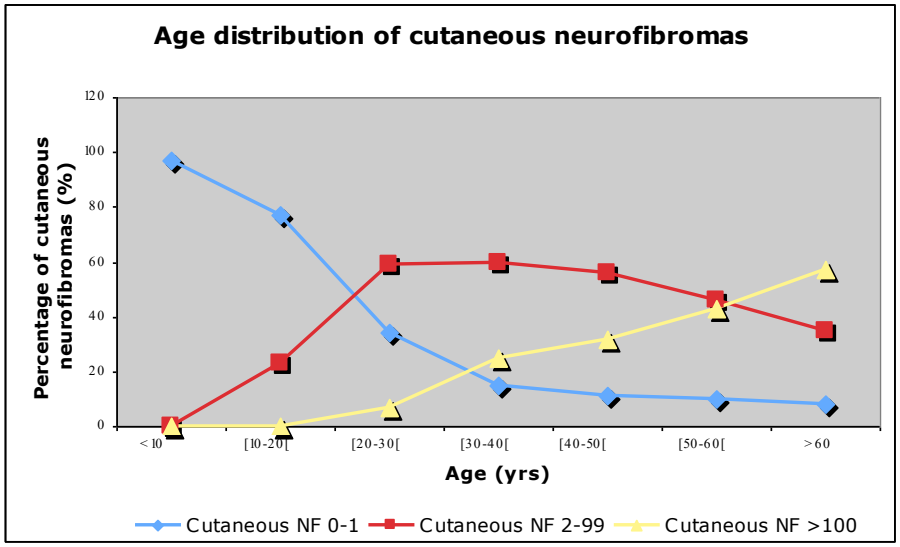
- Autosomal dominant
  - ~50% of cases are *de novo*
  - Symptoms within a family can vary widely
- Gene is neurofibromin on 17q11.2
  - Tumor suppressor gene
  - Protein neurofibromin regulates Ras
- Continuum of peripheral nerve sheath tumors
  - Plexiform neurofibromas
  - Atypical neurofibromatous neoplasms of uncertain biologic potential (ANNUBP)
  - Malignant Peripheral Nerve Sheath Tumors

Among the Most Common Single Gene Inherited Conditions

- Familial combined hyperlipidemia
- Familial hypercholesterolemia
- Dominant otosclerosis
- Adult polycystic kidney disease
- Multiple exostoses
- Huntington's disease
- Fragile X-syndrome
- **Neurofibromatosis**
- Cystic Fibrosis
- Duchenne muscular dystrophy

Huson et al, Brain, 1988
McGaughran et al, J Med Gen, 1999
Evans et al, Journal Med Gen, 2002
Ferner et al. J Med Gen , 2007

Prevalence and Impact of Cutaneous Neurofibromas



Skindex	Score (mean ± SD)		
	Visibility (Ablon's index)		
	Grade 1 (n = 43)	Grade 2 (n = 94)	Grade 3 (n = 19)
Emotion	25.3 ±24.2	43.5 ±24.9	49.1±32.2
Symptoms	17.4 ±15.9	27.5 ±18.3	37.6±23.0
Functioning	9.9 ±11.7	21.6 ±19.0	40.8±30.7

Summary of available data:

- Cutaneous neurofibromas (cNF) can be present in childhood, but increase over time such that the highest burden starts in the second to third decades and increases throughout life
- **>99% of adults with NF1 are afflicted with cNF**
- **Cause symptoms and decrease emotional wellbeing with significant impact on function and QOL**

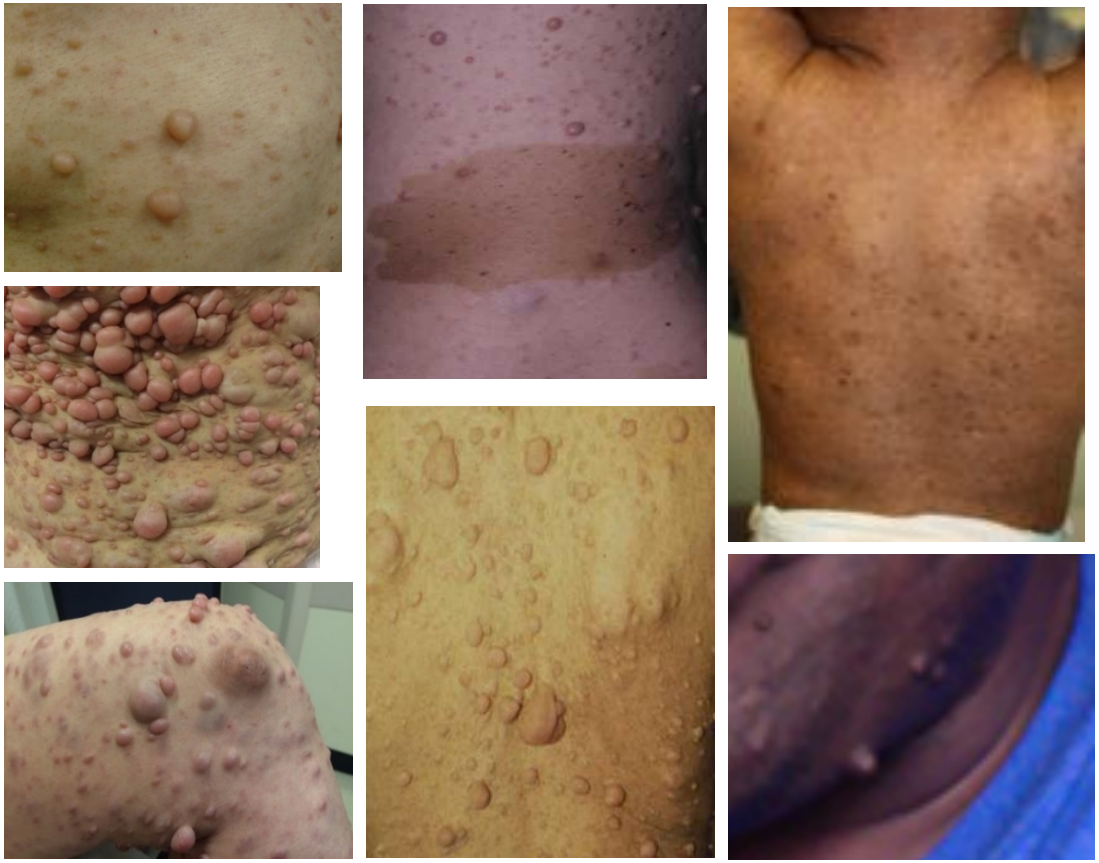
Huson S, et al, Brain, 1988
Wolkenstein P et al, Arch Dermatol, 2001
Page et al, AJMG, 2006
Granstrom S, et al., Dermatology, 2012

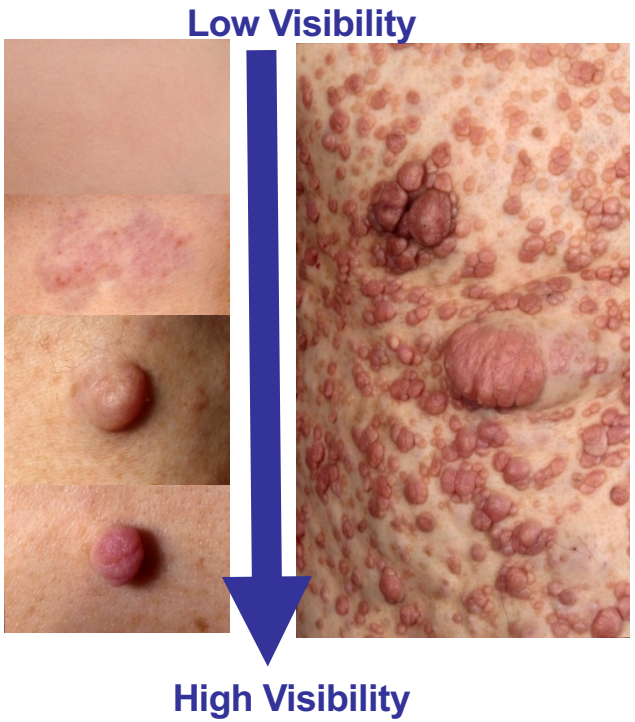
2016: Status of cNF Research

- Agreement by all stakeholders that cNF are a common, important and unmet medical need.
- Awareness that current/previous treatments lacked sufficient detail about the populations treated, well defined and reproducible endpoints, or prospective, regular evaluations to allow independent assessment of efficacy.
- Awareness that there was insufficient natural history data about cNF.
- Recognition of the need for model systems.
- Recognition that there is an opportunity to leverage learnings from plexiform neurofibromas to accelerate therapeutic development for cNF.

Available cNF Natural History Data

Variable Severity of cNF
Burden Within and Across People with NF1





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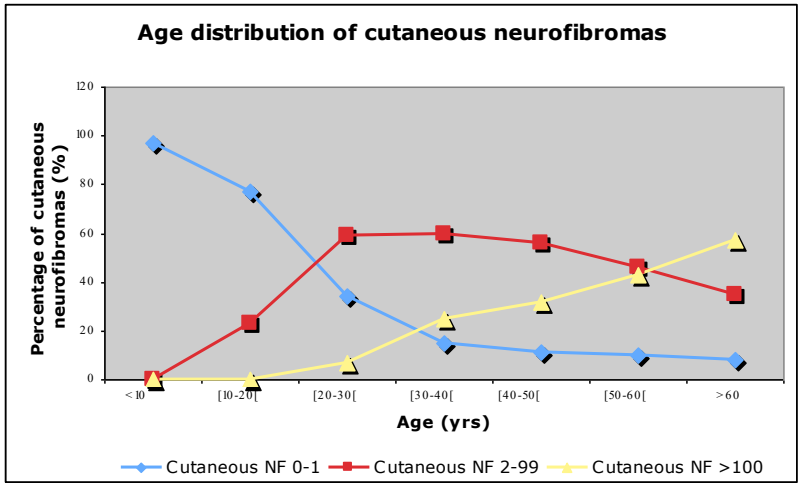
Number of cNF in People with NF1

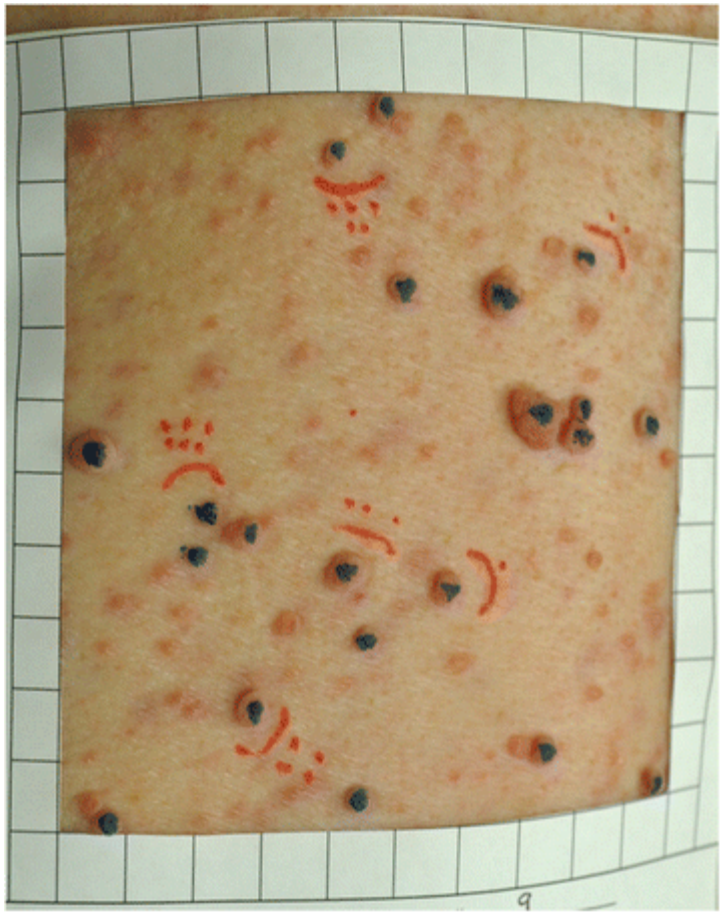
- 155 people with NF1 evaluated (ages 0-85)
- Majority of lesions were on the trunk
  - 17/94 (18%) Welsh patients had cNF on the head and neck.
- Suggests that development of new cNF is maximal after the second decade; but occurs across the life span.

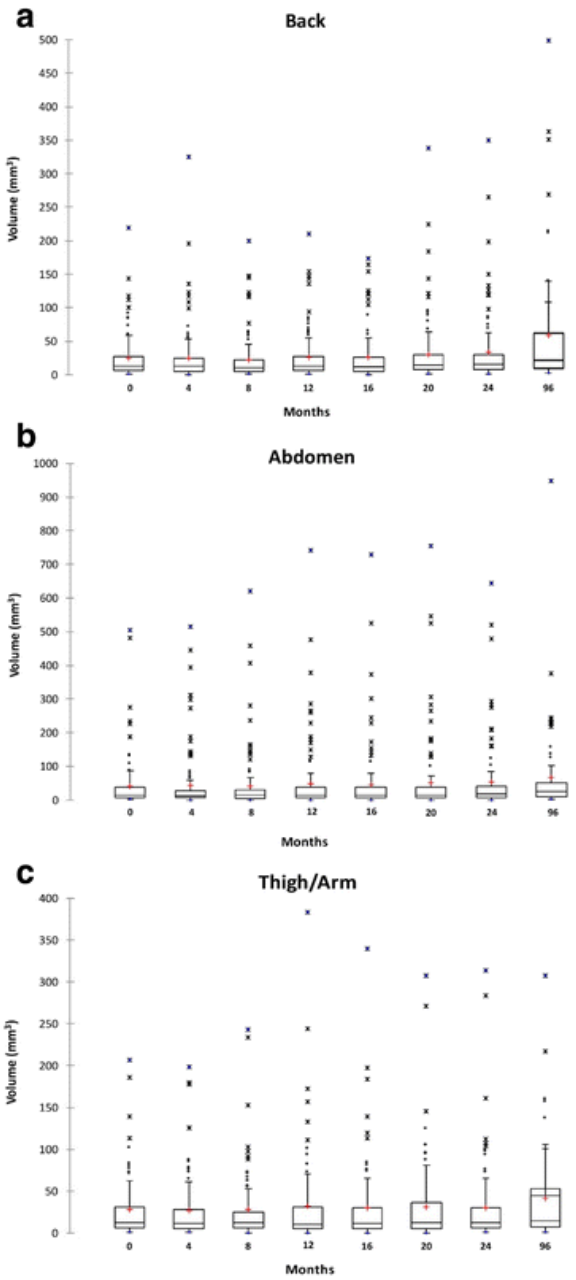
		% of cases with the following		
Age	No of cases	1-10 lesions	11-100 lesions	>100 lesions
0-10	21	10	0	0
11-20	35	40	14	3
21-30	28	25	57	18
31-40	26	8	46	46
41-50	13	0	15	85
51-60	13	0	46	64
61-70	13	0	0	100
71-85	6	0	17	83



Huson et al,1989
Wolkenstein P et al, Arch Dermatol, 2001

Natural History Growth Measurement cNF





Measuring cNF via calipers within 100cm^2 paper frames in adults aged 30-70 yrs over 96 months.

Size: In lesions that changed, average monthly increase in volume was 0.37 mm^3 on the back, 0.28 mm^3 on abdominal and 0.21 mm^3 on extremities.

Number: cNF number increased over 8 years: 3.1 in the back, 1.7 in the abdominal, and 0.4 in the extremities.

Cannon et al, Orphanet J Rare Dis. 2018

Cutaneous Neurofibroma Summit

Nov. 9-11, 2016. Lansdowne Resort. Leesburg, Virginia

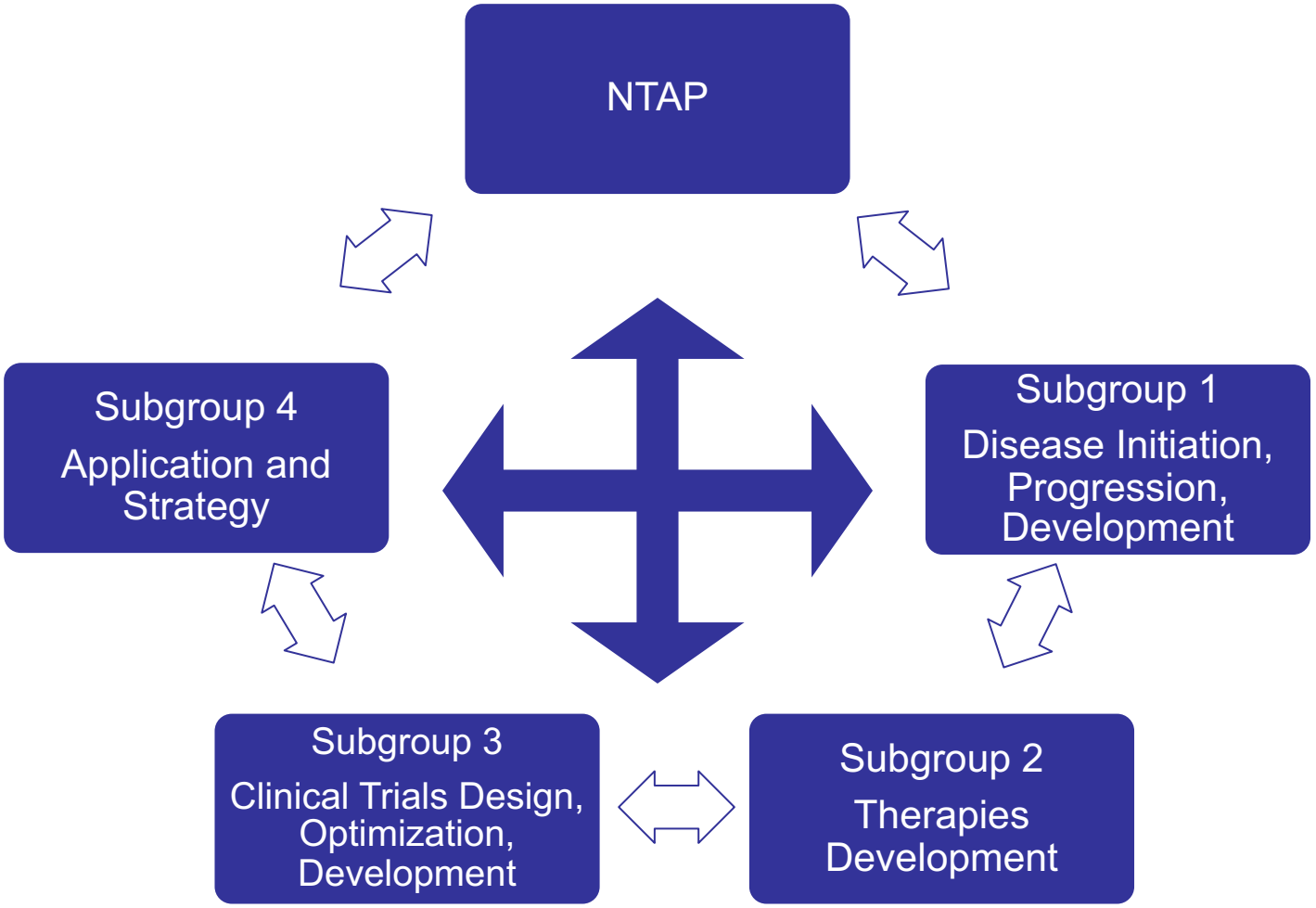
Goals :

- Identify the key questions for treating cNF, such that post-meeting NTAP could launch RFAs aimed at addressing those questions in the next 3yr
- Create a community of invested thinkers and establish connections

Small working group of experts from academia, industry, and regulatory with expertise in:

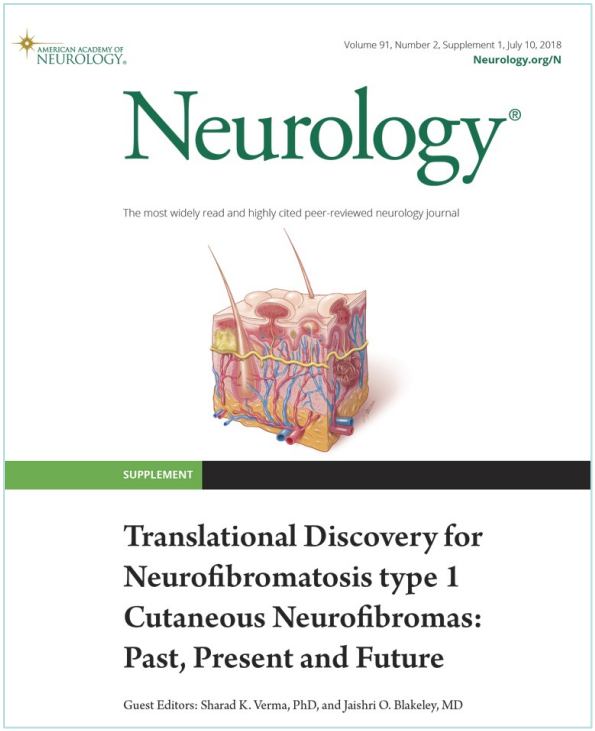
- NF1 biology
- Immunology
- Dermatology
- Genetics
- Skin Cancer
- Surgery
- Regenerative Medicine
- Tissue Repair
- Clinical Development
- Biostatistics

Cutaneous Neurofibroma 2016 Summit Process



NTAP Symposium for Cutaneous Neurofibroma Therapy Manuscripts

- Creating a Comprehensive Research Strategy for Cutaneous Neurofibromas
- Cutaneous neurofibromas: Current clinical and pathological issues
- The Biology of Cutaneous Neurofibromas
- Considerations for Development of Therapies for Cutaneous Neurofibroma
- Clinical Trial Design for Cutaneous Neurofibromas



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Key Summit Outcome Priorities

I. Cutaneous Neurofibroma Terminology

II. cNF Clinical Trial Endpoints

III. Surgical Outcomes

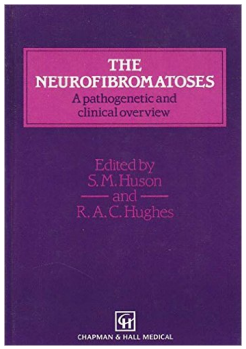
IV. Establishing a scientific framework in which to launch requests for applications to better understand the pathophysiology of CNF

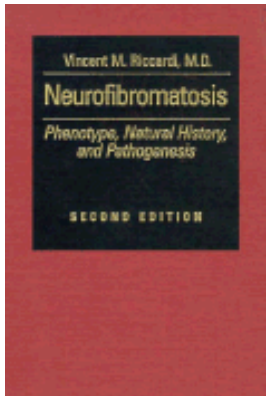
Cutaneous Neurofibroma Terminology

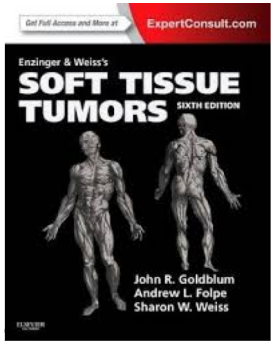
Pathology and Clinical Definition of Cutaneous Neurofibromas

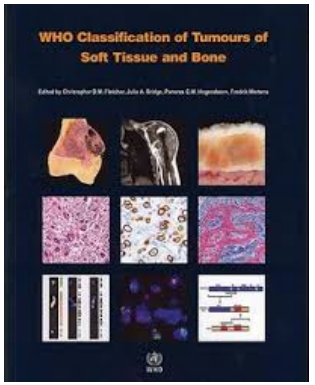
- Simply defined cNF are neurofibromas that occur in the skin.
- However: cutaneous neurofibromas can differ in:
    - Numbers, in shapes, in symptoms
    - Symptoms, pruritus or no pruritus
    - According to the patient
    - According to the dermatologist or other clinician
    - According to the pathologist
    - According to the clinic-pathological overlap

- Multiple existing classification systems used inconsistently







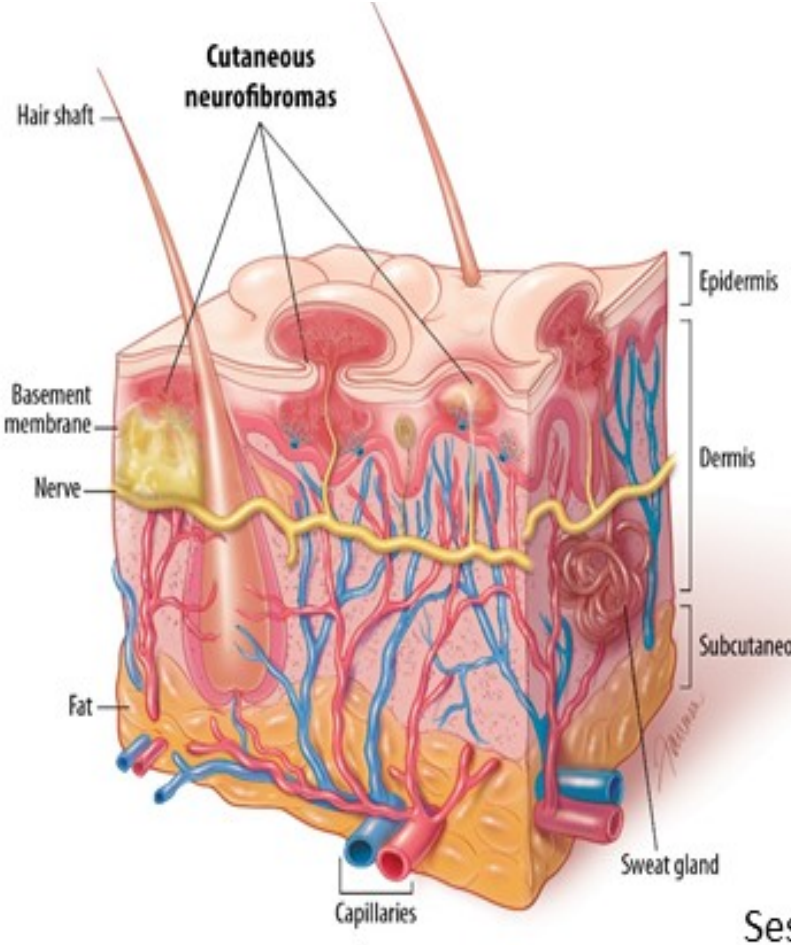


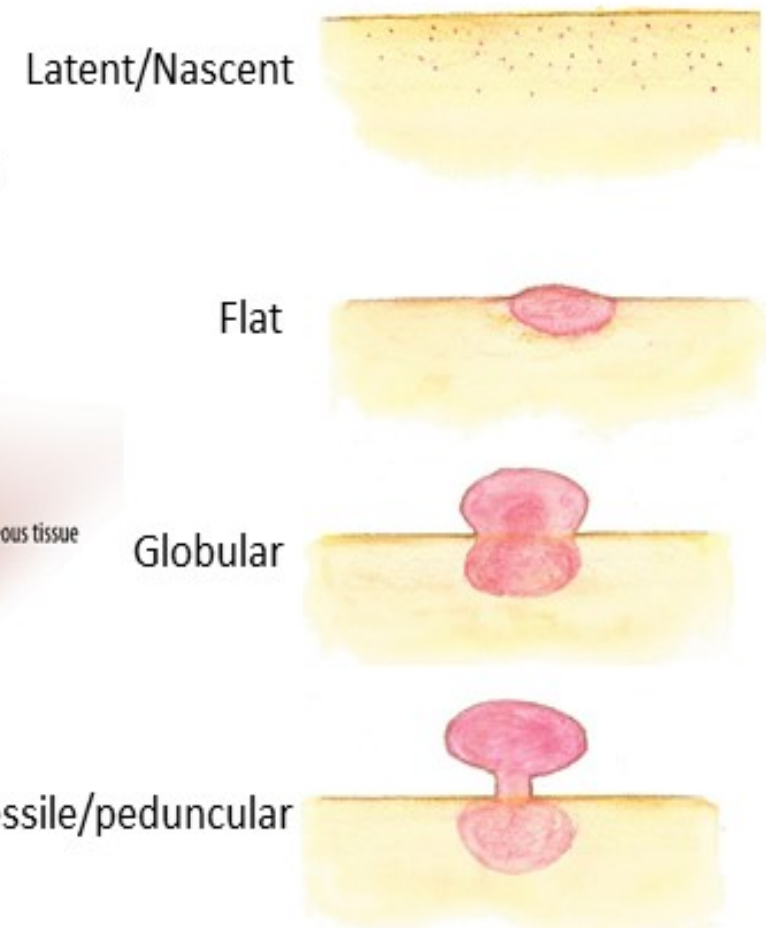
Correlation of Clinical Pathologic Histology Reporting

A unified, agreed upon system of reporting neurofibromas would:

- Allow correlation with clinical and molecular data
- Allow stratification of neurofibromas for therapeutic discovery
    - Allow treatment trials in appropriate subtypes
- Allow comparison across trials and between human tumors and model systems

Need for Common Clinical Terminology





Do we all agree on what we see and how we quantify it?

- Establishing the degree of interobserver variability

- Methodology
  - 6 pathologists (neuropathology or dermatopathology)
  - 100 scanned slides
  - First analysis of a pilot number (28) to come to an agreement on terminology for histological features
  - Analysis of the remaning samples for reproducibility

- Outcomes
  - Analysis of concordance and identification of problem areas
  - Consensual terminology
  - Consensual description
  - Consensual diagnoses

cNF Classification Project Schema

Qualitative review of Test set (28 H&E slides) Aim 1

- Pathologists describe what they see in ≤ 200 words
- Count # times term used: review answers and tally terms used (known, and new terms when >3 times)
- Summarize frequency of terms used across samples
- Assess concordance across several

Quantitative review of validation set (>50 H&E slides)

Aim 2

- Build reporting platform (a drop down menu) using terms from qualitative study in aim 1
- Tally concurrence of terms for each slide from the validation set
- Pathologists review validation set using the on line drop down menu form
- Determine if there are patterns of terms that identify subsets of cNF by H&E

Aim 3

- Create revised criteria based on aims 1&2
- Determine if there are pathology patterns associated with each visual subtype of cNF.
- Provision of revised data; look at concordance
- All reviewers view digital photographs of cNF and using pull down form define them as: nascent/latent; flat, sessile, globular, pedunculated, for each individual sample

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Clinical and Pathological Terminology for Cutaneous Neurofibroma

Clinical classification	Adjective	Pathology	Histological	Molecular
Cutaneous neurofibroma	Flat Globular Peduncular	Diffuse neurofibroma	TBD	TBD
Cutaneous and subcutaneous NF	Diffuse	Diffuse spreading and plexiform neurofibroma	TBD	TBD
Subcutuaneous NF	Deep	Nodular/Plexiform neurofibroma	TBD	TBD

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Establishing Cutaneous Neurofibroma Clinical Trial Endpoints

Establishing Cutaneous Neurofibroma Clinical Trial Endpoints

- High-Resolution Ultrasonography for Measurement and Characterization of Cutaneous Neurofibromas in children, adolescents, and adults with NF1
- Comparison of caliper versus digital photography versus ultrasound versus other tumor measurement techniques
- Validation of existing or development of disease specific PROs
- Development of novel techniques: smart phone apps, global assessment scale, etc.

Quantifying Surgical and Device Based Interventions

Standard Treatment for Cutaneous Neurofibromas

- Surgery (blade based or electrodessication) or laser treatments are the current mainstay of therapy for cNF
- Gaps:
  - Details of methodology
  - Systematic outcome data
- Aims:
  - Collect outcomes data in a systematic fashion
    - Demographics (including skin type and location)
    - Short and long term photographs
    - PRO and patient satisfaction

Surgical Outcomes

Coordinate with surgeons and dermatologists performing procedures for cutaneous neurofibromas

Retrospective analysis:

- Define endpoints
- Create dataset
- Evaluate long term outcomes

Prospective analysis:

- Common methodology
- Confirmed tumor volume treated
- Skin type and region
- Short and long term outcomes

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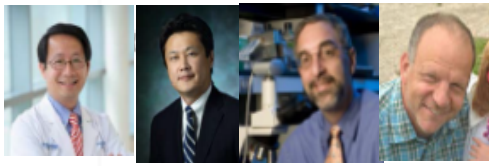
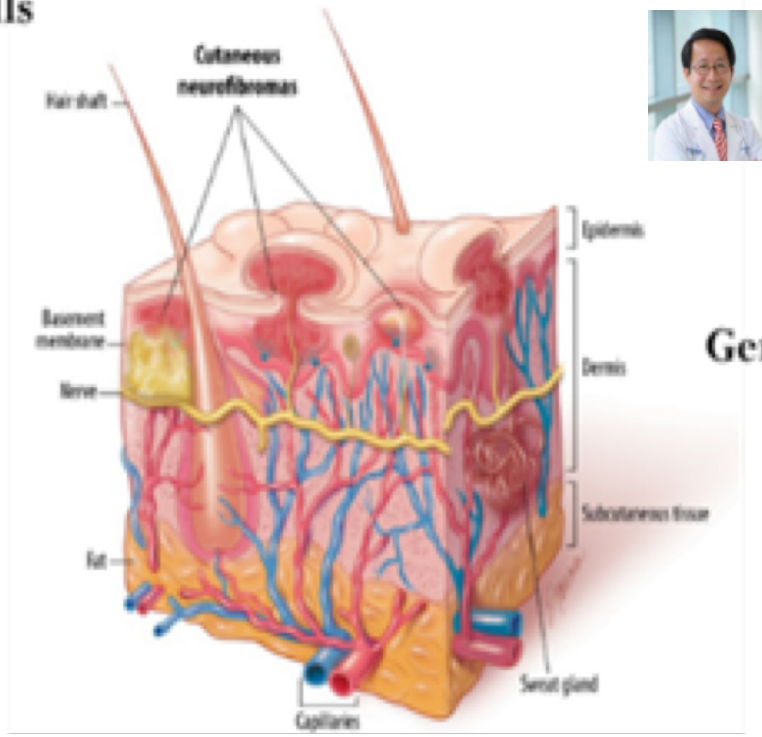
Addressing Critical Questions of cNF Pathophysiology and Generating Model Systems

Nine Projects Selected for the first cNF Biology RFA

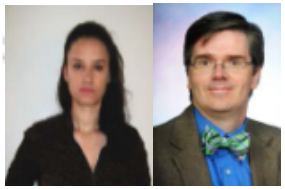
Nerve Microenvironment and Non-Schwann cells

Cells of Origin





Preclinical Models



Genetic and Molecular factors

Cells of Origin

Spatiotemporal loss of *NF1* in Schwann cell lineage leads to different types of cutaneous neurofibroma susceptible to modification by the Hippo pathway

Zhiguo Chen, Juan Mo, Jean-Philippe Brosseau, Tracey Shipman, Yong Wang, Chung-Ping Liao, Jonathan M. Cooper1, Robert J. Allaway, Sara J.C. Gosline, Justin Guinney, Thomas J. Carroll and **Lu Q. Le**

Cellular origin, tumour progression and pathogenic mechanisms of cutaneous neurofibromas revealed by mice with *Nf1* knockout in boundary cap cells

Katarzyna J Radomska, Fanny Coulpier, Aurelie Gresset, Alain Schmitt, Amal Debbiche, Sophie Lemoine, Pierre Wolkenstein, Jean-Michel Vallat, Patrick Charnay and **Piotr Topilko**

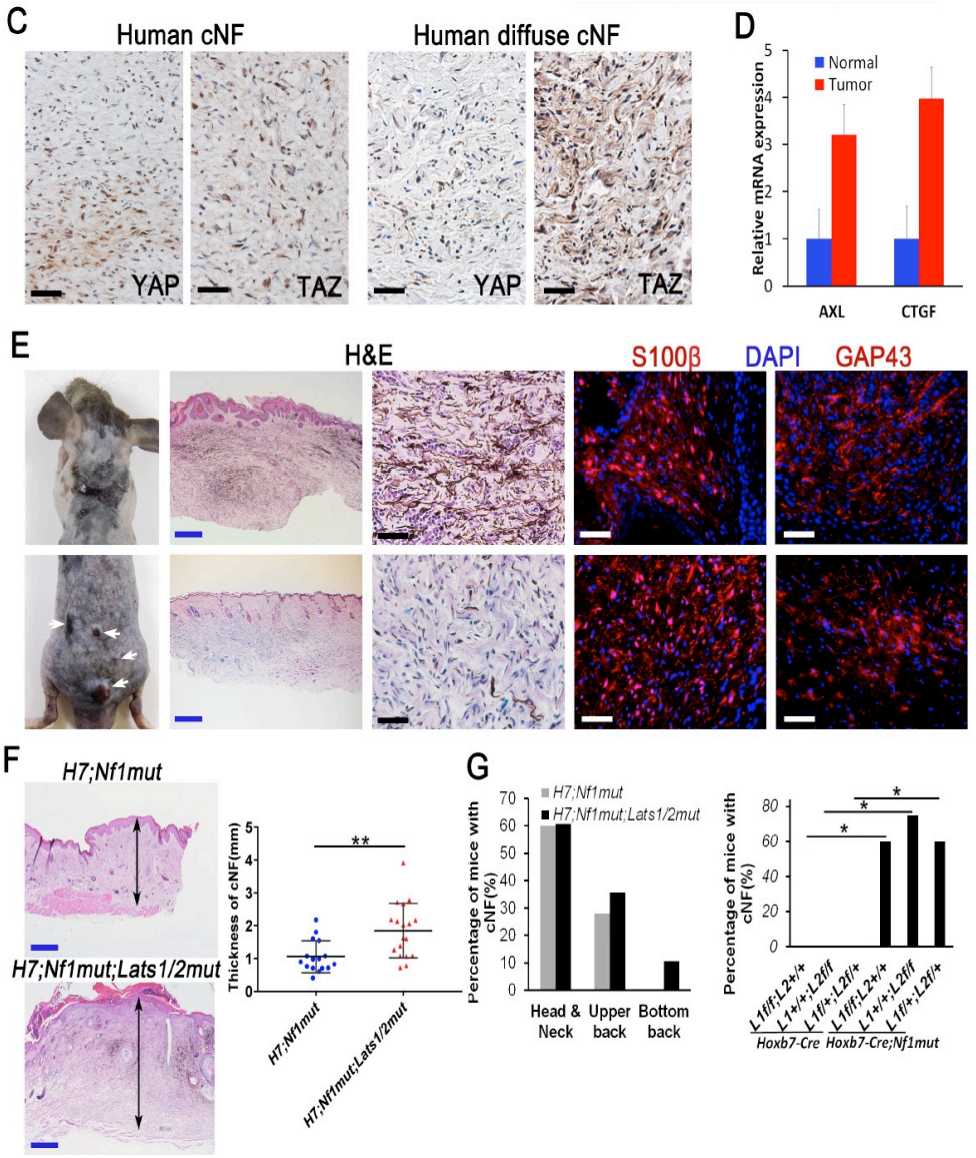
Cell of Origin

1. Hoxb7 is a lineage marker to trace the developmental origin of cutaneous neurofibroma

2. Nf1 KO in Hoxb7 lineage recapitulates both human cutaneous and plexiform neurofibroma

3. Spatio-temporal *Nf1* LOH gives rise to different types of cutaneous neurofibroma

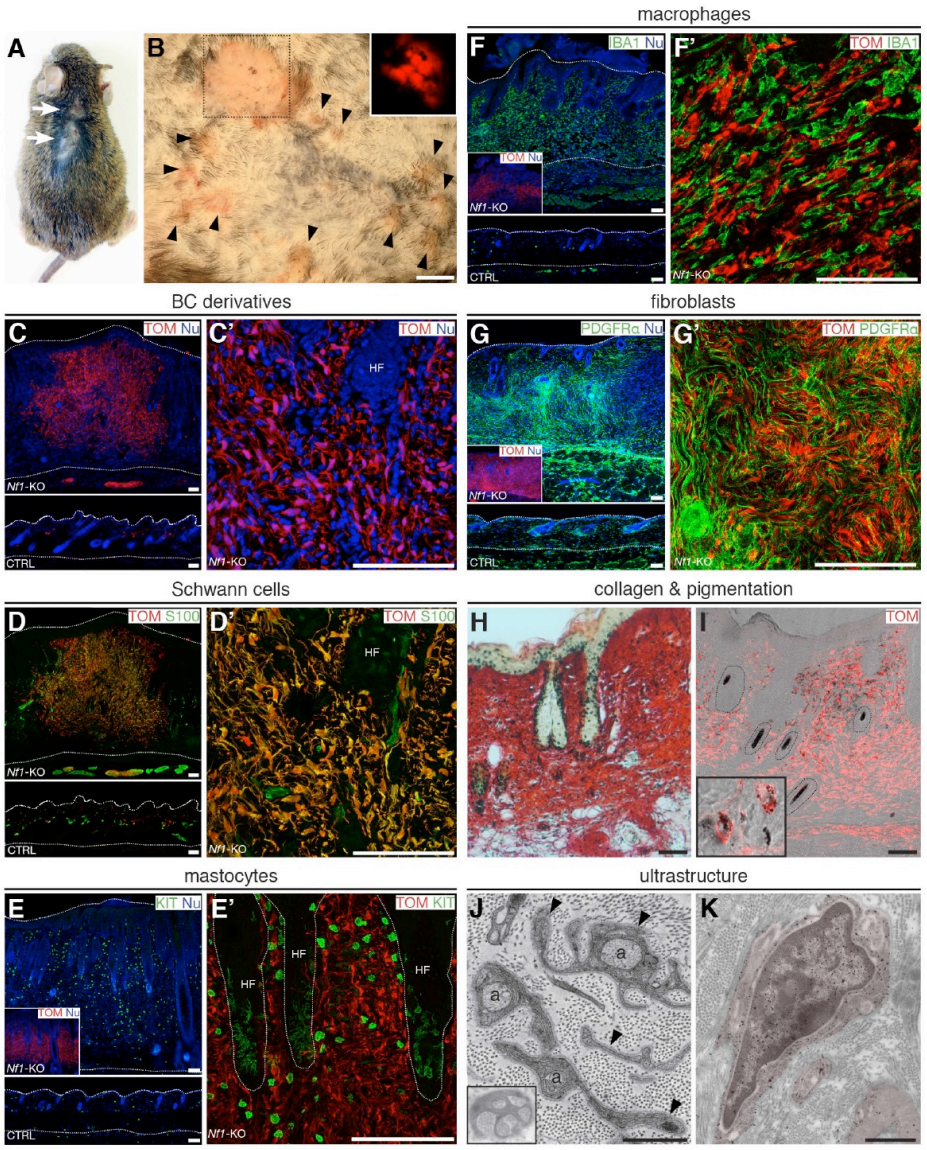
4. Hippo pathway acts as a modifier for neurofibromagenesis



Chen et al, Cancer Discovery, 2018

Cell of Origin

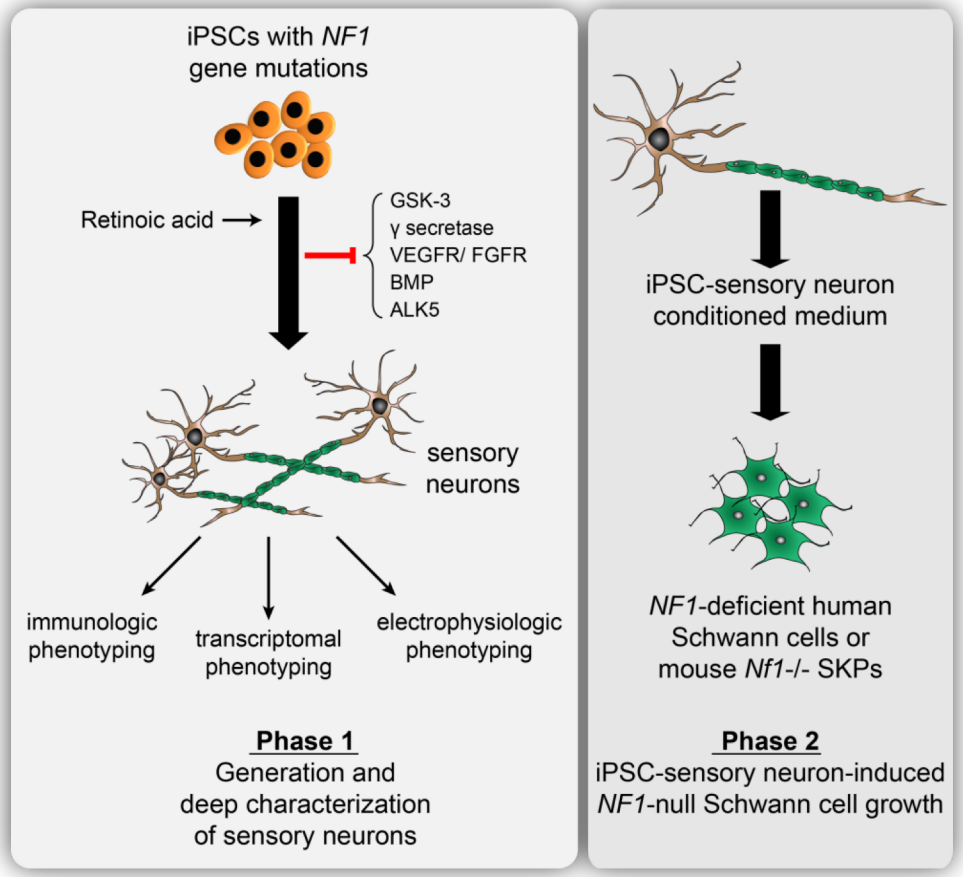
- Mice with *Nf1* loss-of-function in Boundry Cap cell derivatives develop diffuse cNFs
- *Nf1 KO* mice spontaneously develop cNFs around 12 months on both *wt* and *Nf1* heterozygous background
- Often accompanied by pruritis
- Cellular composition is typical of cNFs is similar to human NF1 tumors including mastocytes, macrophages and fibroblasts



Radomska et al, Cancer Discovery, 2018

Cells of Origin

- **Anastasaki and Gutmann**
  - **Washington University**
- Testing the hypothesis that *NF1*-mutant sensory neurons increase *NF1*-deficient Schwann cell proliferation through the elaboration of mitogens.
- Currently characterizing hiPSC-derived *NF1*-mutant sensory neurons, including qPCR marker validation and neurofibromin protein expression.



Preclinical Models cNF

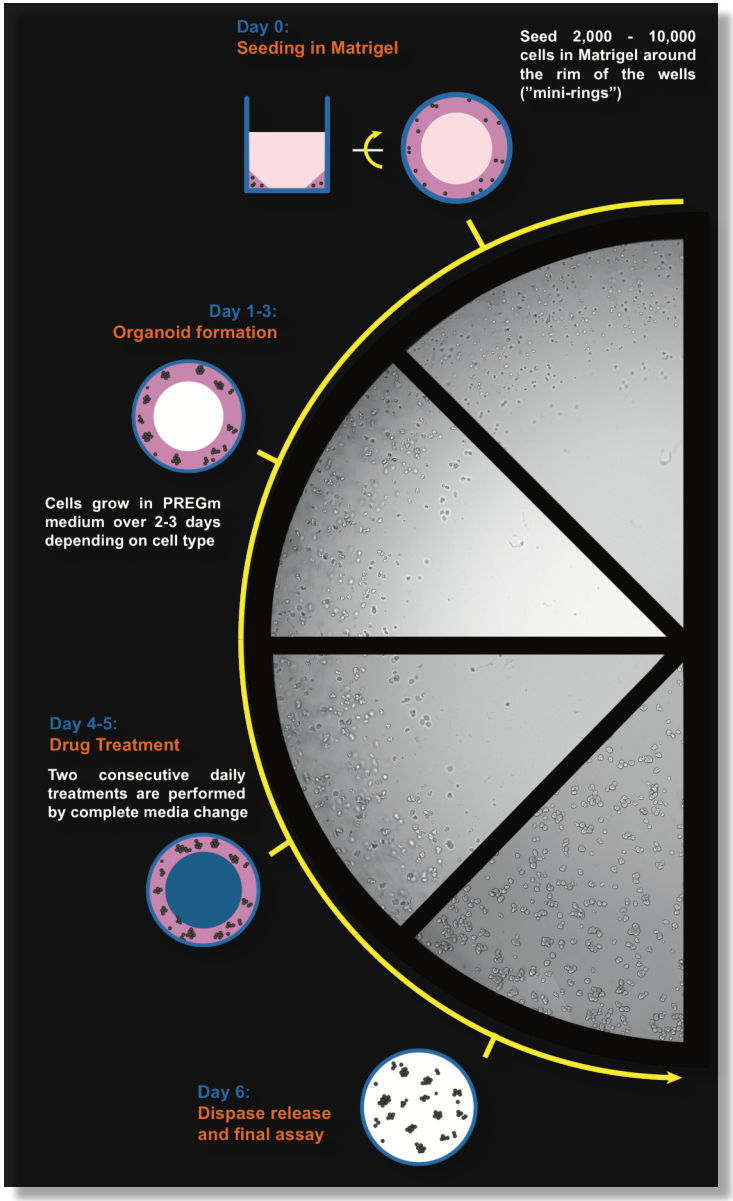
Alice Soragni

- UCLA

3D Organoid Models:

- Miniaturized-ring approach
- Uses fewer cells with no need for expansion in vitro
- Due to the geometry can sustain complete automation
- Adaptability of protocol and final assay
- Can go from tissue procurement to drug testing rapidly

- Have set up a sample procurement pipeline and are performing histologic analysis -> staining for SOX10, S100b, NGFR, NCAM1, SOX2, cytokeratin, CD34; *NF1* sequencing; Methylome; Transcriptome
- Now establishing organoids; optimization of culture conditions: ATP assay + histology
- Have created organoids from cNF from 6 patients.



Exploration of Genetic and Molecular Factors

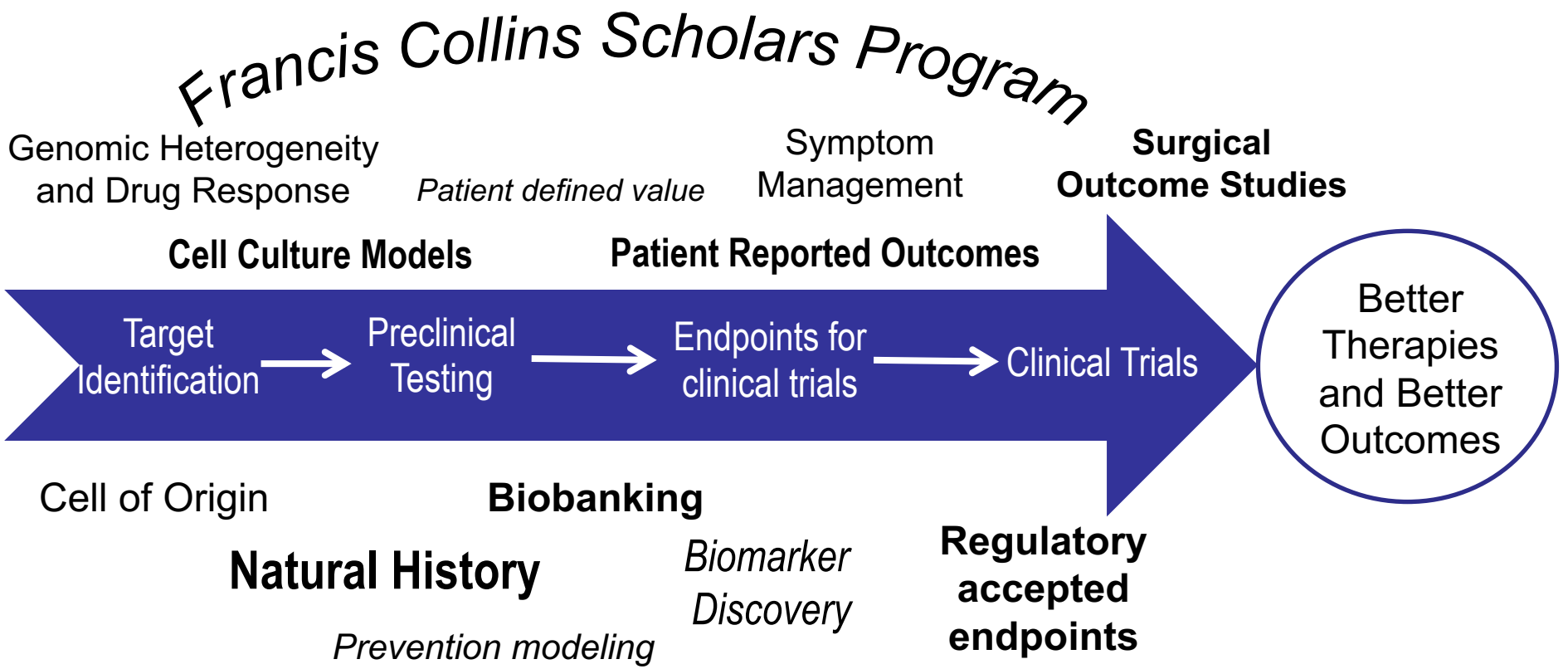
- Edu Serra
  - Identify an expression profile in cNFs produced by heterotypic interactions between SCs and FBs and further dissect it into cell type-specific expression
  - Translate cell type-specific expression due to SC-FB interaction in cNFs into signaling pathways and test functionally their role in cell viability and proliferation
- Peggy Wallace
  - Develop and characterize Schwann cell cultures/cell lines from human cNFs via *TERT+CDK4 immortalization*
  - Laser-capture microdissection to determine the transcript signature in the tumor microenvironment
  - Single-cell authentication and mRNA sequencing in cNF (semi) immortalized cell lines versus pNF (semi) immortalized cell lines
- Gabsang Lee
  - Generation of NF1-/SMASh SOX10::GFP hESC clones. The cloned NF1 Exon1 targeting gRNA-Cas9 plasmid and donor vector were transfected into SOX10::GFP H9 cells (at passage 39) using the AMAXA Nucleofection system (via Lonza). Selection and confirmation studies of SMASh KI/NF1 KO were conducted two weeks post transfection.

Role of the Microenvironment

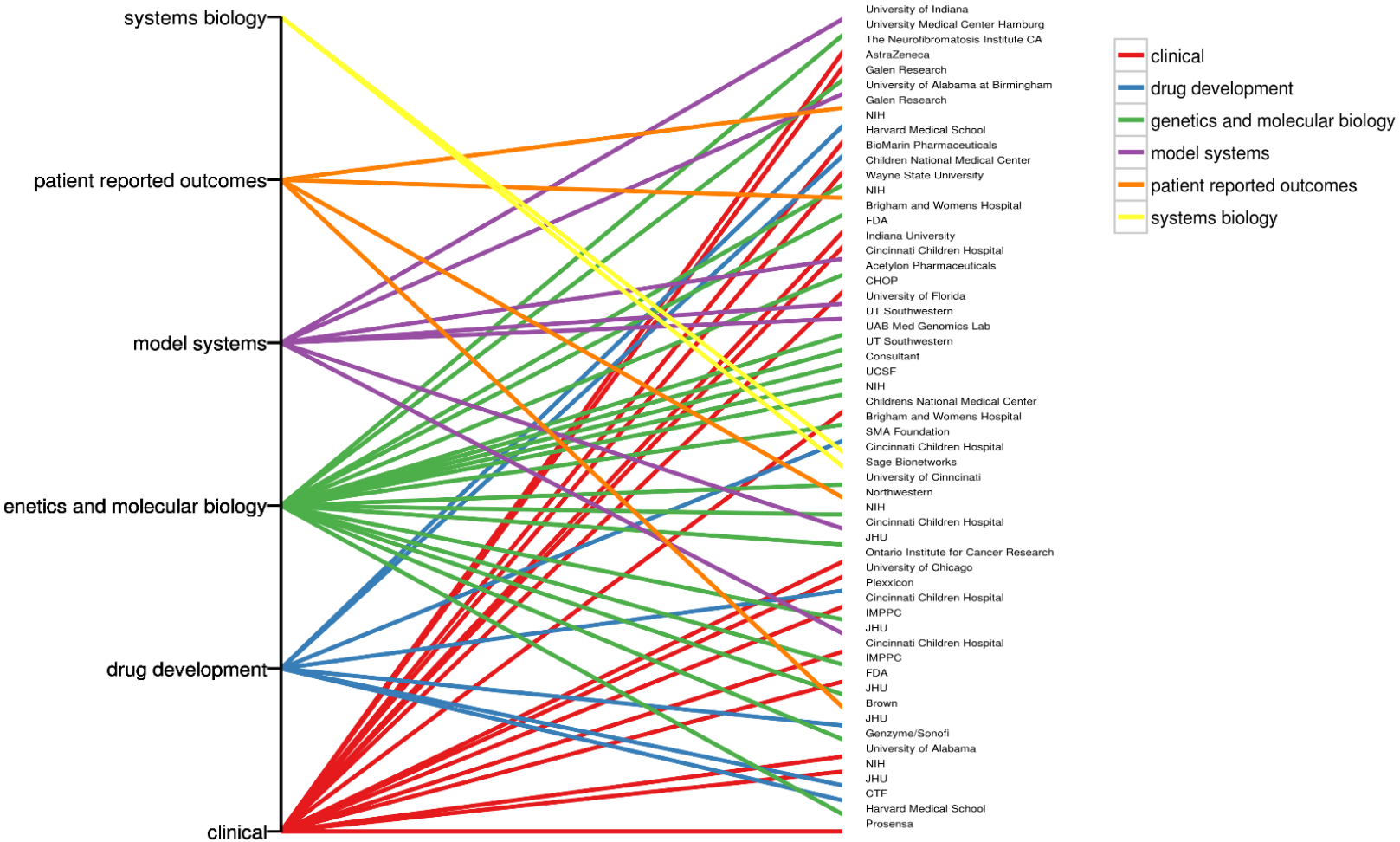
- Juha Peltonen and Sirkku Peltonen
  - Characterization and quantification of T cells residing in cNFs with regard to major T cell markers will be carried out. Clonality of T cells will be evaluated to reveal the diversity of T lymphocytes residing in cNFs.
  - Characterization and quantification of cNF mast cells using immunolabeling for selected mast cell receptors and mediators will be performed.

- Ray Mattingly
  - scRNA-Seq to identify the specific cell populations and sub-populations that make up cNF
  - reconstitute 3D co-cultures of the cell types that form cNF

NTAP's Activities Toward Developing Therapeutics for Cutaneous Neurofibromas



Collaborations



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Thank You

- NTAP Team
  - Jackie Eubanks-Rudd
  - Rhonda Jackson
  - Sharad Verma
- REiNS cNF Collaborators
  - Ashley Cannon
  - Dominique Pichard
  - Andrea Gross
  - Kavita Sarin
  - cNF committee members

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cNF Trial Outcome Measures and Endpoints

Outcome measure	Trial Endpoint	Method of Measurement	Comment
Size	Reduction in cNF size	Caliper, digital and volume photography, US, MRI	Likely substantial reduction in size required to be clinically meaningful
Number	Decrease in appearance of new cNF	Counting development of new cNF	Requires long time to document and control group due to unknown NH
Appearance	Visible improvement in appearance	Digital photography	Difficult to quantitate meaningful visible improvement
cNF related morbidity	Improvement in pruritus, emotional distress, quality of life	PRO	Difficult to attribute morbidity and improvement with certainty to cNF

- Need for validated methods for measurement of cNF and validated PRO
- PRO challenges: Bias, missing data, defining meaningful score change for improvement, concomitant meds

Key Suggestions

- Defining clinical phenotypes
  - Biology of mature versus evolving lesion
  - Responses to treatment: one size fits all?
- Development of guidelines on surgical vs device vs drug therapies
  - Prospective surgical studies
  - Evaluation of existing devices and drugs in POC studies
- Selection and standardization of endpoints, scales, and measurement tools
- Expansion and training of collaborative clinical trial network

Key suggestions

- Study biology and heterogeneity of cNF as part of natural history studies
  - In parallel preclinical models
- Evaluate new technologies for more sensitive measurement of cNF
- Surgery is only modality, which to date results in benefit:
  - Document outcomes prospectively and longitudinally carefully with medical photography, PRO
  - Prospective trial
  - Incorporate biopsies for research questions