RASopathies

2\textsuperscript{nd} Annual RAS-Targeted Drug Development Summit
Workshop F
9/14/2020

Beth Stronach, PhD, RASopathiesNet
Bruce D. Gelb, MD, Icahn School of Medicine at Mount Sinai
Hi, I’m Sam

Photos by Rick Guidotti of POSITIVE EXPOSURE  “Change how you see, See how you change”
Introductions

Beth Stronach, Lisa Schoyer, Elisabeth Parker, Lisa Schill
I used to study tissue closure in fruit flies!
My Roles with RASNet:

Co-organizing RASopathies symposia

Writing content- website, newsletters, grant applications

Curating research publications and opportunities

Tweeting for RASNet
@rasopathiesnet

Advocating for RASopathies research and treatments at meetings

"And now, let me introduce today's keynote speaker.”
More about Bruce

- Physician-Scientist
  - Trained in Pediatric Cardiology
  - Molecular Genetics

- Icahn School of Medicine at Mount Sinai (NYC)
  - Director, Mindich Child Health and Development Institute
  - Co-Direct, Cardiovascular Genetics Program
Topics for today

- RASopathy syndromes, prevalence, manifestations
- RASopathy mutations compared with RAS cancer mutations
- Getting to treatments for RASopathies
  - Targets and treatment considerations
  - Preclinical models
  - Endpoints
  - What have early/small clinical trials told us?
Topic 1
RASopathy syndromes
Prevalence and manifestations
In memory of Dr. Jacqueline Noonan

Pediatric cardiologist who described a pediatric cohort, in the 1960’s, with congenital heart defects and other shared traits

This disorder was later coined “Noonan syndrome”

2021 marks the 20th anniversary of the publication of the first NS gene, PTPN11 (SHP2)

Association of Costello syndrome with HRAS, Cardio-facio-cutaneous syndrome with BRAF, and Neurofibromatosis-1 with a RAS-Gap, led to the insight that germline alteration of RAS-MAPK signaling underlies a group of neurodevelopmental disorders…

The RASopathies
Syndromes

Costello  CS
Cardio-facio-cutaneous  CFC
Legius /NF1-like  LS
Neurofibromatosis 1  NF1
Noonan  and  Noonan-like  NS
NS with multiple lentigines  NSML
NS with loose anagen hair  NSLH
Capillary malformation-arteriovenous malformation  CM-AVM

Aoki et al, 2015, JHumGen
Causal Mechanisms

- Human development
- Germline mutations
- Somatic mosaicism
- Somatic mutations

Developmental stage:
- Fertilization: Oocyte, Sperm
- Zygote: Ectoderm, Mesoderm, Endoderm
- Gastrula
- Adult

Mutation during gametogenesis:
- De novo mutation
- Mutation

Castel, Rauen, McCormick, 2020, Nat Reviews
How do the syndromes manifest?

- Short stature
- Characteristic facial features
- Developmental delay
- Congenital heart and valve defects
- Gastrointestinal dysfunction
- Neurocognitive issues, ADHD
- Bleeding and lymphatic abnormalities
- Low muscle tone
- Pain
- Hypertrophic cardiomyopathy
- Skin and hair anomalies
- Seizures
- Cancers
Hypertrophic Cardiomyopathy Outcomes

Wilkinson et al., Am Heart J 2012
Discussion
Topic 2
RASopathy vs. Cancer mutations
Analysis of cancer and RASopathy genetic databases reveals that
~19% of all cancer cases &
~4% of developmental disorders
contain Ras mutations.

Prior, Hood, Hartley, 2020, Cancer Research
Prior, 2020, in press
RASopathy vs. RAS cancer mutations

Source: NSEuroNet database

Ian A. Prior, Ras Variant Biology and Contributions to Human Disease, in press
RAS family alignment

Rasopathy mutations

- Mg$^{2+}$/N - Mg$^{2+}$/nucleotide binding
- SW1 - Switch 1
- SW2 - Switch 2
- Farnesylation site
- Mutation hot spots
- Phosphorylation sites
- Ubiquitylation/
  Acetylation sites
- Nitrosylation site
- Ca$^{2+}$ binding

Khan, Rhett, O’Bryan, 2020, BBA Mol Cell Res
<table>
<thead>
<tr>
<th>RASopathy associated cancers</th>
<th>Somatic RAS cancers</th>
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<tr>
<td>Rhabdomyosarcoma</td>
<td>Head and neck SCC</td>
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<td>Hematologic JMML, AML, ALL</td>
<td>Lung adenocarcinoma</td>
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<td>Neuroblastoma</td>
<td>Cutaneous melanoma</td>
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<td>Glioma</td>
<td>Thyroid carcinoma</td>
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<td>Neurofibroma</td>
<td>Pancreatic ductal adenocarcinoma</td>
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<td>Malignant peripheral nerve sheath tumor</td>
<td>Colorectal adenocarcinoma</td>
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<td>Bladder cancer</td>
<td>Bladder urothelial adenocarcinoma</td>
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</table>
Substitutions in Noonan syndrome
Noonan Syndrome vs. Cancer

**JMML/MDS/AML (N = 27)**

- D61Y, V (26%)
- E69K (15%)
- G60V (4%)
- F71L, K (7%); A72T, V (11%)
- E76K, V, G, A (33%)
- G503A (4%)

**N-SH2**

- T42A (2%)
- Y63C (7%)
- A72S, G (6%); T73I (2%); E76D (1%)
- G60A (4%); D61N, G (4%); Y62D (2%)

**C-SH2**

- D106A (5%)
- Q79R (8%)

**PTP**

- G268S, Y279C, I282V, F285L, S (10%)
- E139D (5%)
- M504V (4%)
- P491S, R501K, S502L (3%)
- N308D, S, T (36%)

**NOONAN SYNDROME (N = 91)**
Noonan Syndrome vs. Cancer

Immunocomplex Phosphatase Assays

[Bar graph showing pmoles of phosphate for WT, N308D, D61Y, E76K, C459G]
 Noonan Syndrome vs. Cancer

Noonan Syndrome

Growth Factor

RTK

D308 SHP-2

K76 SHP-2

Ras-GDP

Ras-GTP

Phenotype

Embryonic Lethal

Cancer

GM-CFS

GMRα

GMRβ

D308 SHP-2

K76 SHP-2

Ras-GDP

Ras-GTP

Proliferation
RASopathy vs. cancer mutations

Jindal et al, 2017, PNAS
The Happle hypothesis

![Diagram showing germline and somatic mutations]

Castel, Rauen, McCormick, 2020, Nat Reviews
RASopathy vs. Cancer mutations

Ras: Beating heart of cancer by Darryl McConnell
Discussion

Break
Topic 3
Getting to Treatments:
Targets and treatment considerations
Targets and Treatment

- Many molecular targets
- Common feature: Hyperactive RAS/MAPK signaling (autosomal dominant GOF, haploinsufficient or LOF)
- Multi-system pathology
- No current treatments or cures beyond symptom management
Treatment considerations

- Turn volume down, not off
- Multi-system, multi-organ
- Timing and duration
- Minimal chance of escape through mutation selection
- Some potential indications not life-threatening
- Applicability of side effect profile from patients with cancer?
Treatment considerations

Goyal, Nat Genetics 2017
Treatment considerations

Goyal, Nat Genetics 2017
Treatment options

- Small molecule inhibitors [RAS, RAF, MEK, ERK, SHP2, SOS1]
- RNA silencing
- Degradation
- Gene therapy [NF]
- Gene editing / Base editing
- Other cellular vulnerabilities
Topic 3
Getting to Treatments:
Preclinical Models
Preclinical Models

- Cell-based
  - Induced pluripotent stem cell-derived cells (e.g., cardiomyocytes)

- Animal
  - Fruit fly
  - Zebrafish
  - Mouse
  - Worm
Preclinical Raf1 Mouse

Wu et al., J Clin Invest 2011
Preclinical Raf1 Mouse

- MAPK signaling
  - Increased Erk activation
  - No change in p38 or Jnk

- Mek inhibitor (PD0325901)
  - 6-week treatments from 4 weeks of age
  - Rescued the hypertrophic cardiomyopathy

Wu et al., J Clin Invest 2011
Swimming toward solutions: Using fish and frogs as models for understanding RASopathies

Victoria L. Patterson | Rebecca D. Burdine

Methods:
RNA or morpholino injection
Nf1 KO, transgene insertion
High throughput screening

Phenotypic assays:
<table>
<thead>
<tr>
<th>Diagram Part</th>
<th>Phenotypes</th>
<th>RASopathy</th>
<th>Reference</th>
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<th>Phenotypes</th>
<th>RASopathy</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Mice Only</td>
<td>Bone defects</td>
<td>NF1</td>
<td>(Kolaczek et al., 2000)</td>
<td>Drosophila Only</td>
<td>Mitochondrial defects</td>
<td>NF1</td>
<td>(Williams et al., 2007)</td>
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<td>Neurofibromas</td>
<td>NF1</td>
<td>(Rosenbaum et al., 1997)</td>
<td>Synaptic overgrowth defects</td>
<td>NF1</td>
<td>(Walker et al., 2013)</td>
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<td>Sex-linked effects</td>
<td>NF1</td>
<td>(Diggins-Andrews et al., 2014)</td>
<td>Sustained escape response</td>
<td>NF1</td>
<td>(The et al., 1997)</td>
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<td>Muscle Abnormalities</td>
<td>NF1</td>
<td>(Sullivan et al., 2014)</td>
<td>Ectopic veins</td>
<td>NS</td>
<td>(Oishi et al., 2006)</td>
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<td></td>
<td>Attention deficits</td>
<td>NF1</td>
<td>(Brown et al., 2010)</td>
<td>Photoreceptor defects</td>
<td>NS</td>
<td>(Oishi et al., 2006)</td>
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<td>Working memory deficits</td>
<td>NF1</td>
<td>(Shilovsky et al., 2010)</td>
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<td>Leaner metabolic profile</td>
<td>NS</td>
<td>(Tajan et al., 2014)</td>
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<td></td>
<td>Hematologic disease</td>
<td>NS</td>
<td>(Araki et al., 2009)</td>
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<td>Triangular face</td>
<td>NS, CS</td>
<td>(Araki et al., 2004; Schuhmacher et al., 2008)</td>
<td>Schwann cell hyperplasia</td>
<td>NF1</td>
<td>(Shin et al., 2012)</td>
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<td></td>
<td>Enlarged spleen</td>
<td>NS</td>
<td>(Araki et al., 2004)</td>
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<td>Liver defects</td>
<td>NF1, NS, CFC, CS</td>
<td>(Araki et al., 2004; Figueiredo et al., 2012; Hegedus et al., 2007; Inoue et al., 2014)</td>
<td>Kupffer's vesicle malformation</td>
<td>NS</td>
<td>(Bonetti et al., 2014)</td>
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<td>Lymphatic system defects</td>
<td>CFC</td>
<td>(Inoue et al., 2014)</td>
<td>Precocious ossification</td>
<td>CS</td>
<td>(Santoriello et al., 2009)</td>
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<td>Epileptic seizures</td>
<td>CFC</td>
<td>(Urosevic et al., 2011)</td>
<td>Reduced blood oxygenation</td>
<td>CS</td>
<td>(Santoriello et al., 2009)</td>
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<td>Nasal septal deviation</td>
<td>CS</td>
<td>(Chen et al., 2009)</td>
<td>Scoliotic spine</td>
<td>CS</td>
<td>(Santoriello et al., 2009)</td>
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<td>Papilloma formation</td>
<td>CS</td>
<td>(Chen et al., 2009)</td>
<td>Sterility</td>
<td>CS</td>
<td>(Santoriello et al., 2009)</td>
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<td></td>
<td>Hypermotility</td>
<td>CS</td>
<td>(Viosca et al., 2009)</td>
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<td>Teeth defects</td>
<td>CS</td>
<td>(Goodwin et al., 2014)</td>
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<td>Mice and Drosophila</td>
<td>Myeloproliferative disease</td>
<td>NF1, NS</td>
<td>(Gitler et al., 2004; Mohi et al., 2005)</td>
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<td>Mice and Zebrafish</td>
<td>Neural crest cell defects</td>
<td>NF1</td>
<td>(Isamat et al., 2006)</td>
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<td>Myelin sheath defects</td>
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<td>(Cichowski et al., 1999)</td>
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<td>OPC hyperplasia</td>
<td>NF1, NS</td>
<td>(Bennett et al., 2003; Ehrman et al., 2014)</td>
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<td>Hypertelorism</td>
<td>NS</td>
<td>(Araki et al., 2004)</td>
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<td>Glionas</td>
<td>NF1</td>
<td>(Hagedus et al., 2009)</td>
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<tr>
<td>Mice, Zebrafish, and Drosophila</td>
<td>Learning/cognitive defects</td>
<td>NF1, NS, CS</td>
<td>(Costa et al., 2002; Lee et al., 2014; Viosca et al., 2009)</td>
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<td>Reduced life span</td>
<td>NS, CFC</td>
<td>(Hernández-Porras et al., 2014; Urosevic et al., 2011)</td>
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<td>Growth defects</td>
<td>NS, CFC</td>
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<td>Cardiac defects</td>
<td>NF1, NS, CFC, CS</td>
<td>(Araki et al., 2009; Inoue et al., 2014; Isamat et al., 2006; Schuhmacher et al., 2008)</td>
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Topic 3
Getting to Treatments:
Endpoints
Advancing RAS/RASopathy therapies: An NCI-sponsored intramural and extramural collaboration for the study of RASopathies.

How do the syndromes manifest?

- Short stature
- Developmental delay
- Neurocognitive issues, ADHD
- Congenital heart and valve defects
- Gastrointestinal dysfunction
- Cancers
- Bleeding and lymphatic abnormalities
- Low muscle tone
- Pain
- Hypertrophic cardiomyopathy
- Skin and hair anomalies
- Seizures
Neurocognitive Impairment

- Treatable?

- Animal *PTPN11* models
  - Fruit flies
    - Not developmental
    - Normalized with SHP-2 inhibitor
  - Mouse
    - Neurobehavioral deficits
    - Ameliorated with MEK inhibition

- Human genetic data
  - *SOS1*
    - Noonan syndrome with normal neurodevelopment
    - Only expressed in fetal brain
Topic 3
Getting to Treatments:
Proof of Concept Trials
Proof of concept trials

- MEK inhibitor Selumetinib for NF1–associated inoperable plexiform neurofibromas
  - Most had durable tumor shrinkage and clinical benefit
  - Recent FDA approval for pediatric cases
  - A. Gross et al. 2020 NEJM [ph 2 trial]

- Off-label use of MEK inhibitor Trametinib for RIT1-associated HCM in 2 infants (13 and 14 wks of age)
  - Associated with reversal of HCM and valvular obstruction over 17 months of therapy
  - G. Andelfinger et al. 2019 JACC
Trametinib for HCM

LV Mass

Pro-BNP
Trametinib for Lymphatic Disease

- Li et al., *Nature Medicine* 2019
- Central conducting lymphatic anomaly (CCLA)
  - ARAF gain-of-function missense variant
    - Comparable alleles in *RAF1* for Noonan syndrome
- Zebrafish model
  - Recapitulated lymphatic phenotype
- Treated with cobemitinib
Trametinib for Lymphatic Disease

Before

After
Trametinib for Lymphatic Disease

Before

After
Engaged advocacy groups

HOW CAN WE HELP?

Social media groups
Discussion

Moving Forward-
What is feasible for the RASopathies?
RASopathy Syndrome Genes

- Noonan syndrome (NS) \([PTPN11, SOS1, RAF1, BRAF, KRAS, NRAS, (SHOC2), CBL, RRAS, RIT1, (RASA2), SOS2, MAP3K8, SPRY1, MYST4, LZTR1, (A2ML1)]\)
- Noonan syndrome with multiple lentigines (NSML) \([PTPN11, RAF1]\)
  >formerly LEOPARD syndrome
- Noonan-like syndrome with loose anagen hair (NS-LH) \([SHOC2, PPP1CB]\)
  >SHOC2 (NS-LH1)
  >PPP1CB (NS-LH2)
- Cardio-facio-cutaneous syndrome (CFC) \([BRAF, MAP2K1, MAP2K2, KRAS]\)
- Costello syndrome (CS) \([HRAS]\)
- Neurofibromatosis type 1 (NF1) \([NF1-neurofibromin]\)
- Legius syndrome/ NF1-like (LS) \([SPRED1]\)
- Capillary malformation-arteriovenous malformation syndrome (CM-AVM1) \([RASA1]\)
- Central conducting lymphatic anomaly (CCLA) \([ARAF]\)