RASopathies

2nd 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

RASopathiesNet
Connect ~ Collaborate ~ Cure

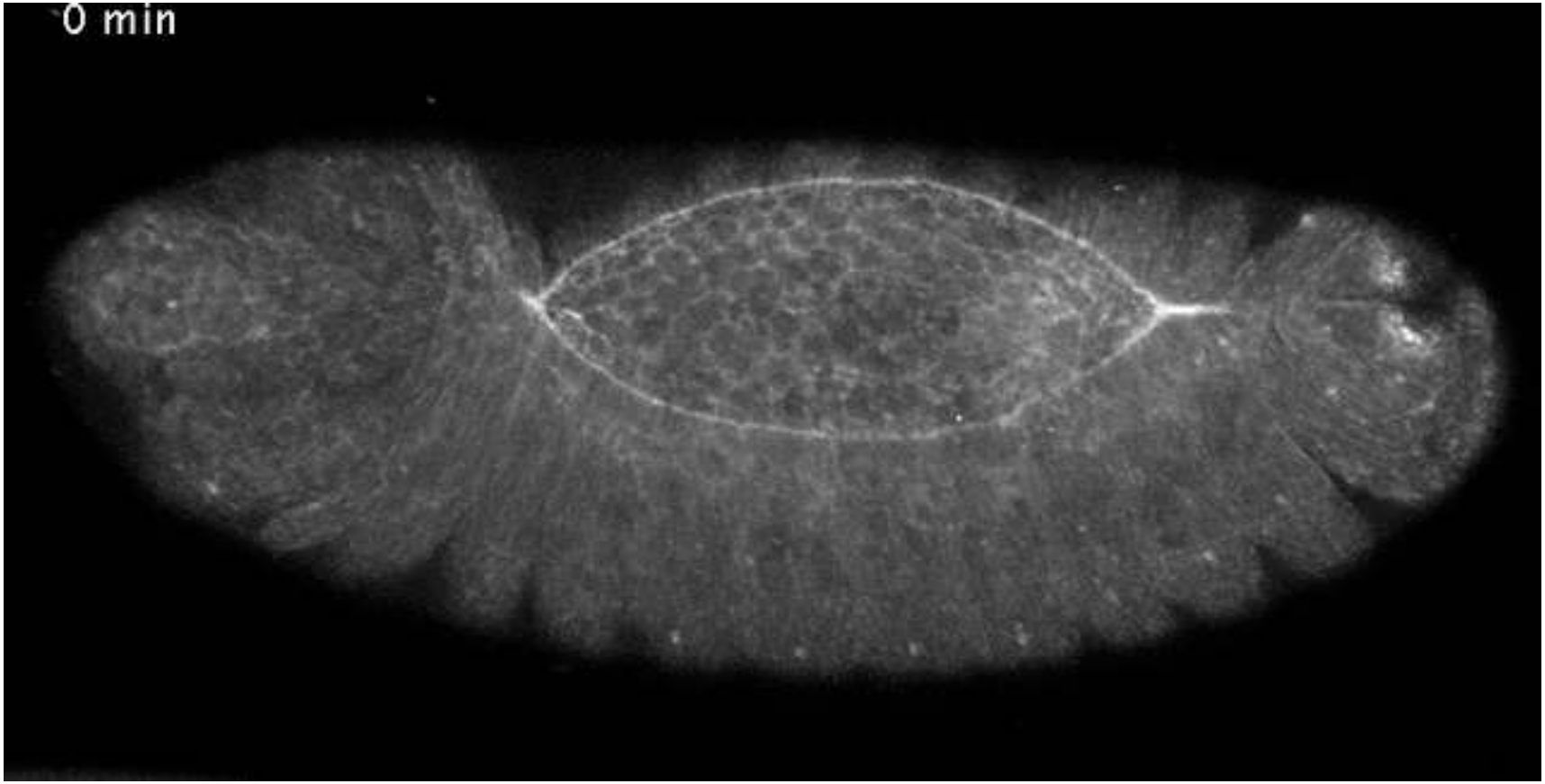
WELCOME

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:

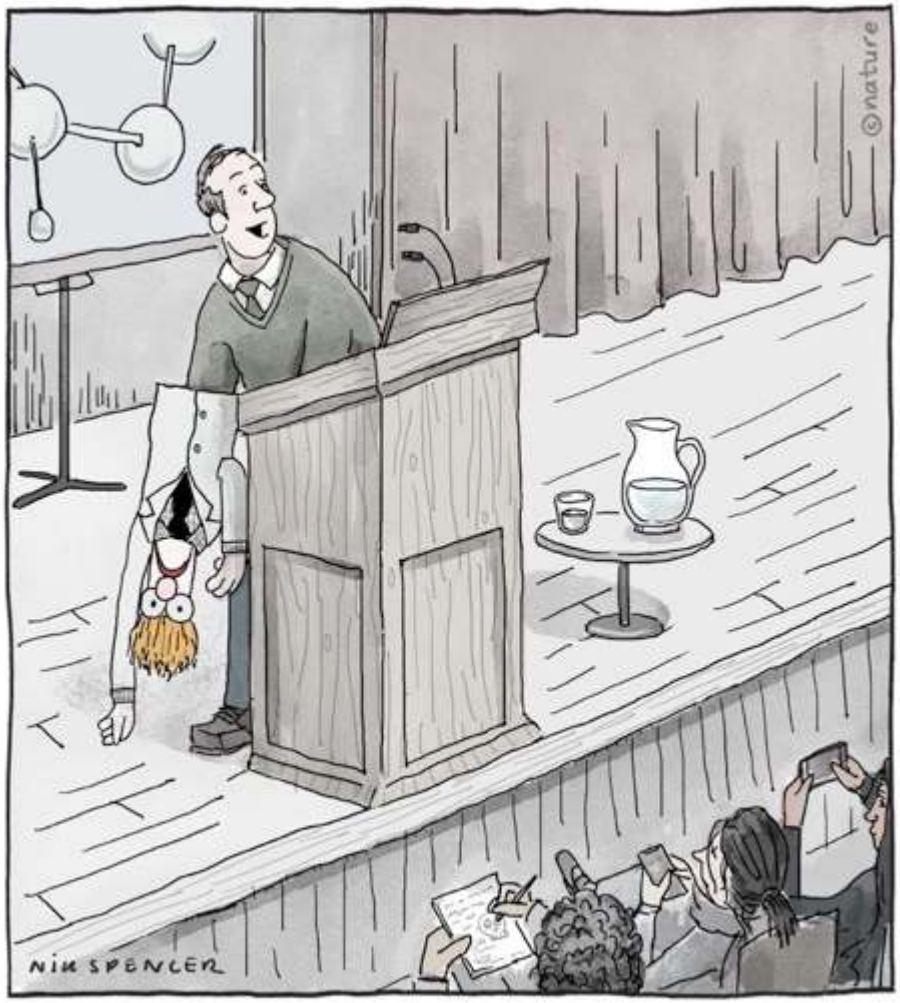
Co-organizing RASopathies symposia

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~~ co-presenter."

Dr. Bruce Gelb

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

Molecular Genetics and Prevalence

Syndromes

Costello CS

Cardio-facio-cutaneous CFC

Legius /NF1-like LS

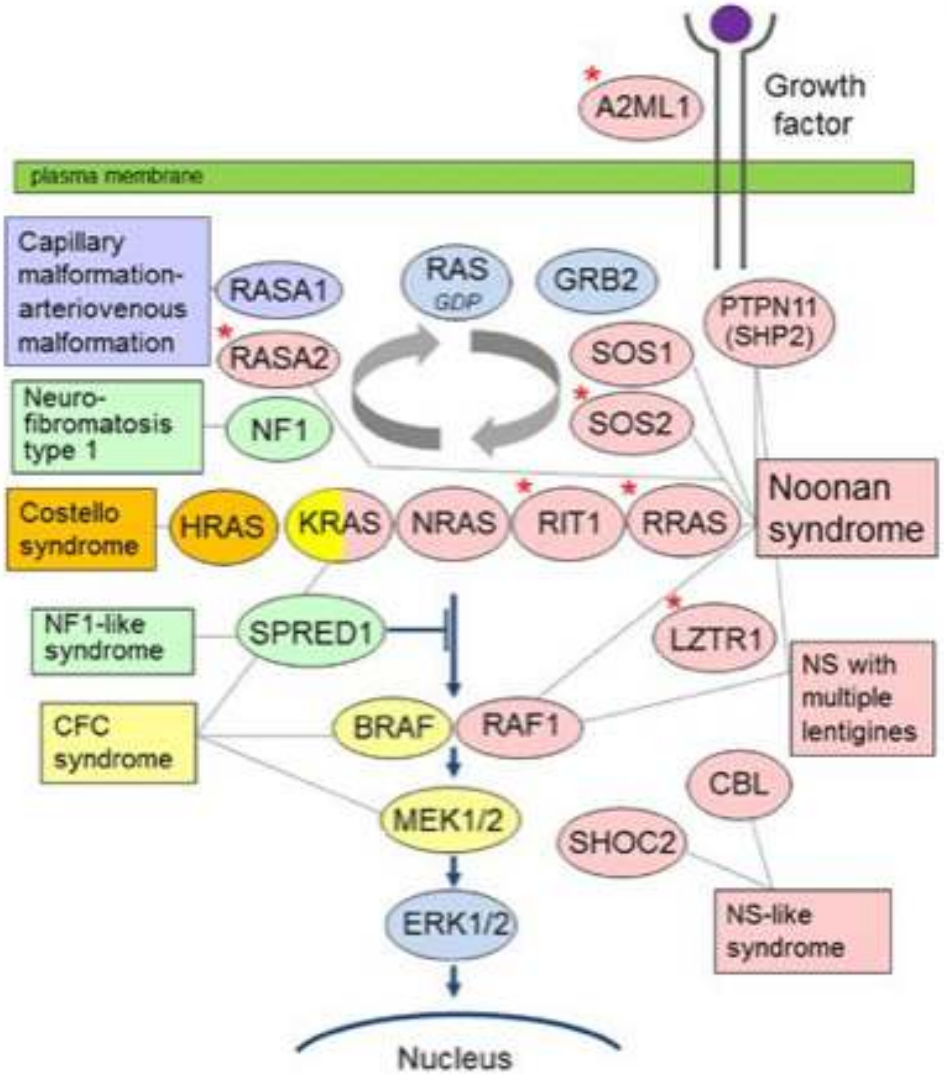
Neurofibromatosis 1 NF1

Noonan and Noonan-like NS

NS with multiple lentigines NSML

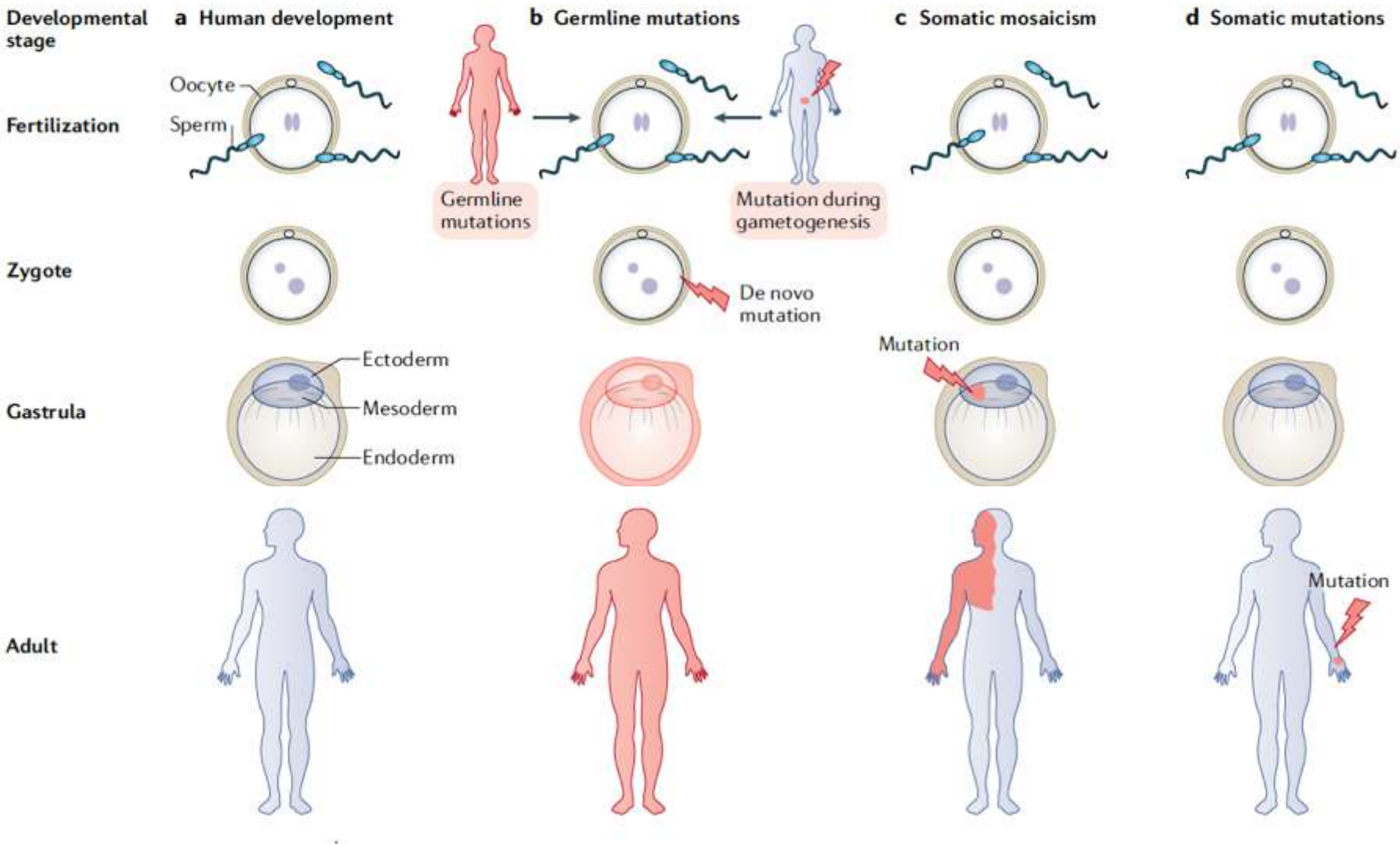
NS with loose anagen hair NSLH

Capillary malformation-arteriovenous malformation CM-AVM



Aoki et al, 2015, *JHumGen*

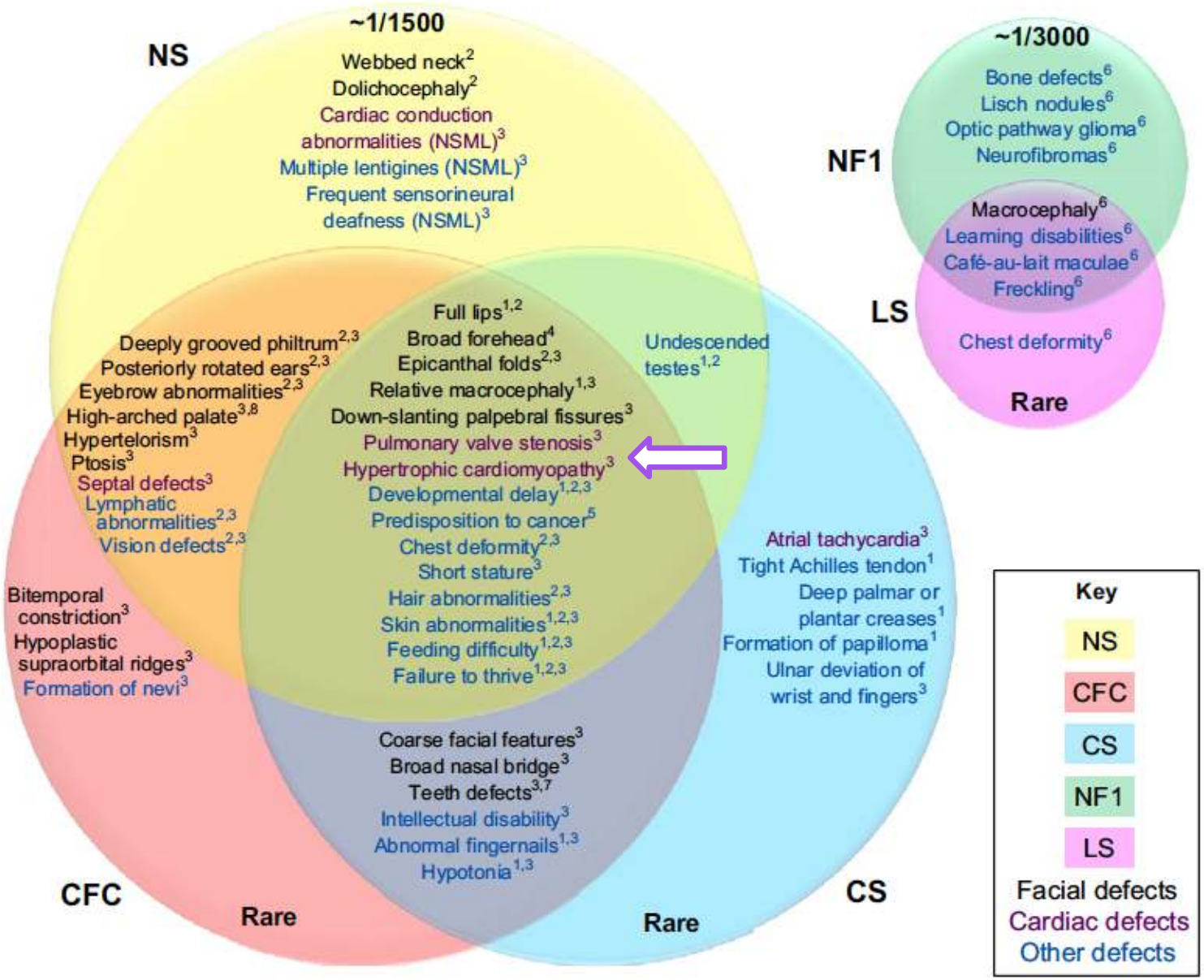
Causal Mechanisms



Castel, Rauen, McCormick, 2020, *Nat Reviews*

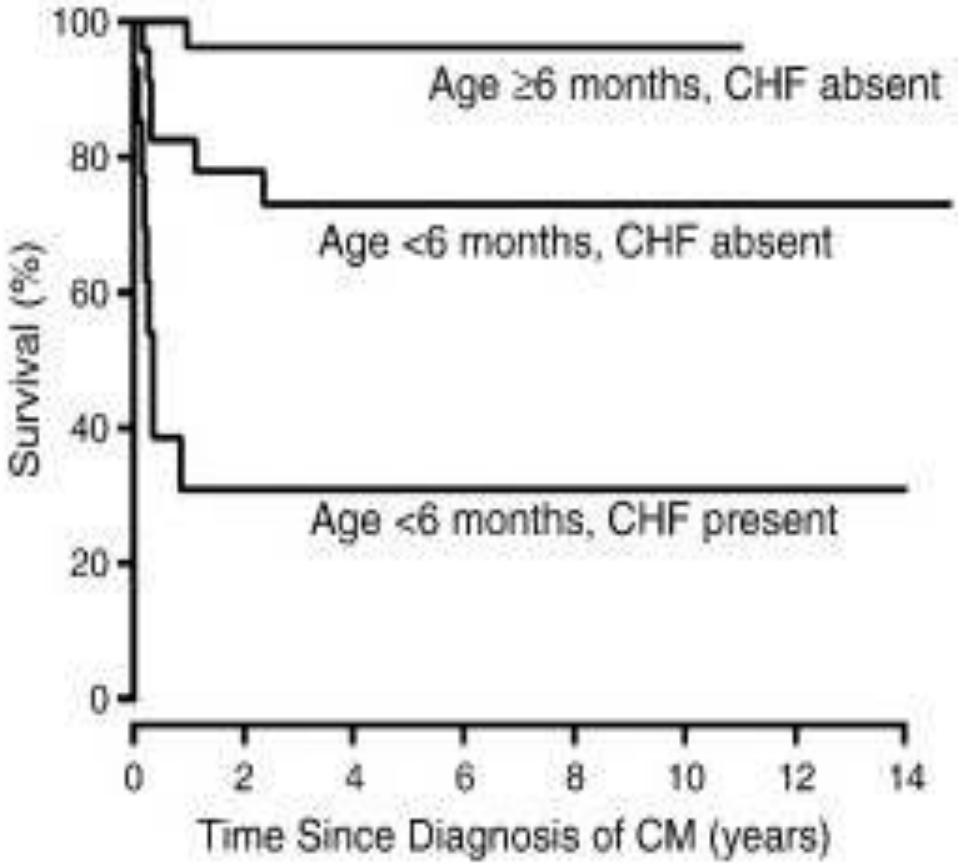
How do the syndromes manifest?

- 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**



Jindal *et al.*, 2015, *Disease Models & Mechanisms*

Hypertrophic Cardiomyopathy Outcomes



Wilkinson *et al.*, *Am Heart J* 2012

Discussion

Topic 2
RASopathy vs. Cancer mutations

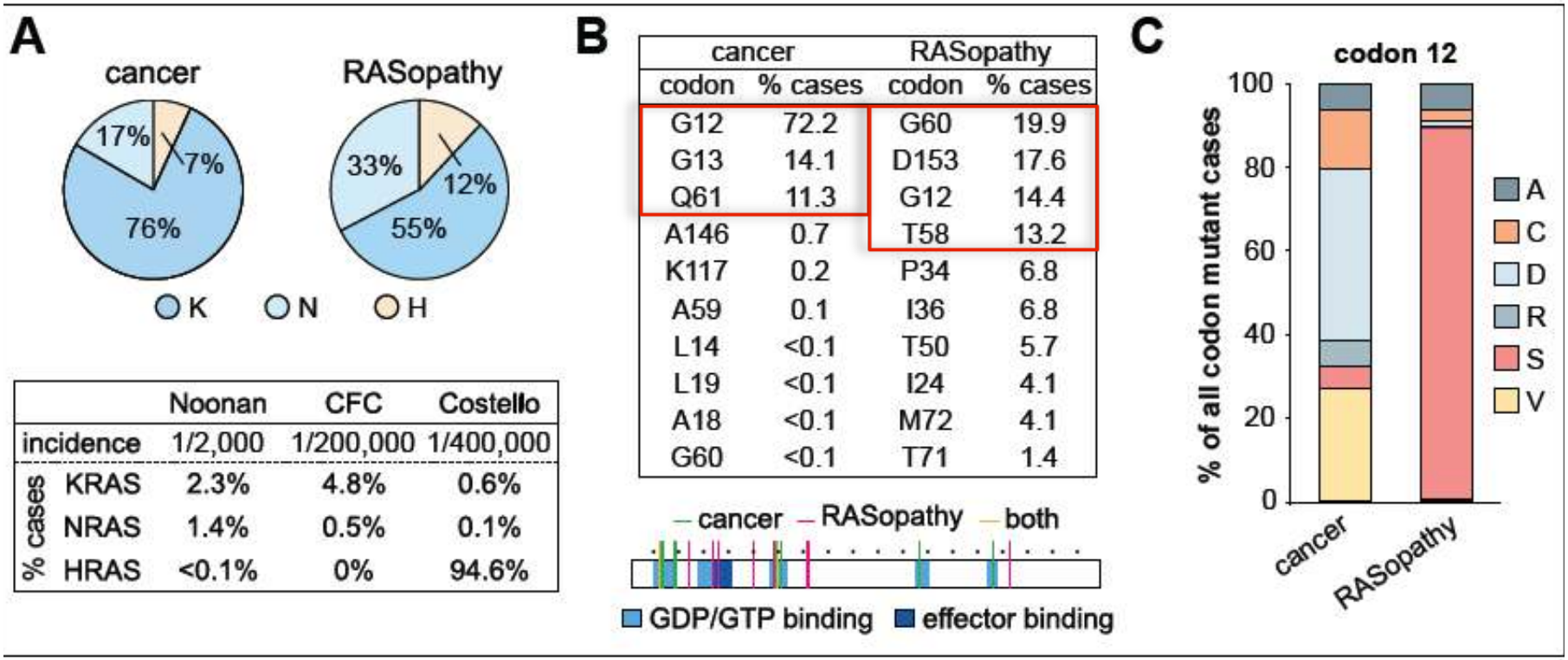
Estimating RAS mutation frequency

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

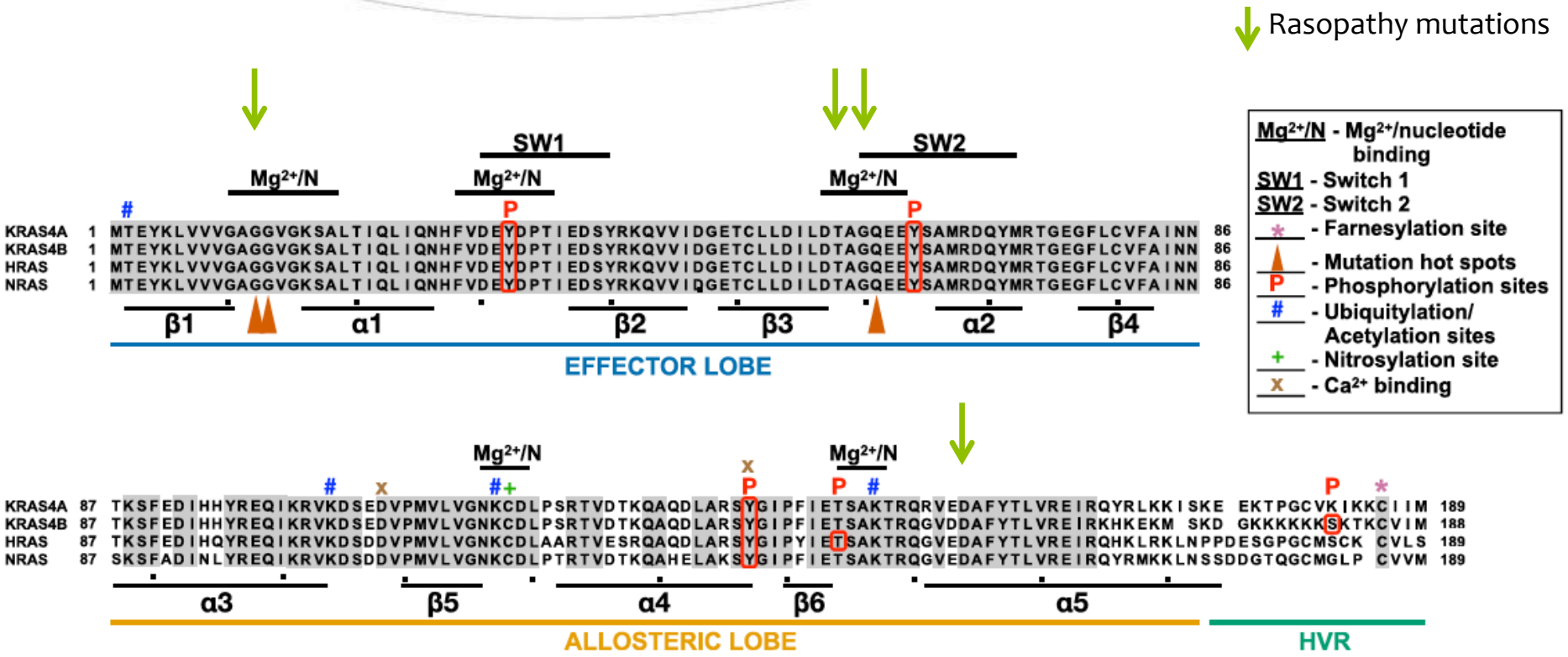


	cancer		RASopathy	
	codon	% cases	codon	% cases
	G12	72.2	G60	19.9
	G13	14.1	D153	17.6
	Q61	11.3	G12	14.4
	A146	0.7	T58	13.2
	K117	0.2	P34	6.8
	A59	0.1	I36	6.8
	L14	<0.1	T50	5.7
	L19	<0.1	I24	4.1
	A18	<0.1	M72	4.1
	G60	<0.1	T71	1.4

		Noonan	CFC	Costello
	incidence	1/2,000	1/200,000	1/400,000
% cases	KRAS	2.3%	4.8%	0.6%
	NRAS	1.4%	0.5%	0.1%
	HRAS	<0.1%	0%	94.6%

Ian A. Prior, Ras Variant Biology and Contributions to Human Disease, *in press*
Source: NSEuroNet database

RAS family alignment



Khan, Rhett, O'Bryan, 2020, BBA Mol Cell Res

RASopathy vs. cancer mutations

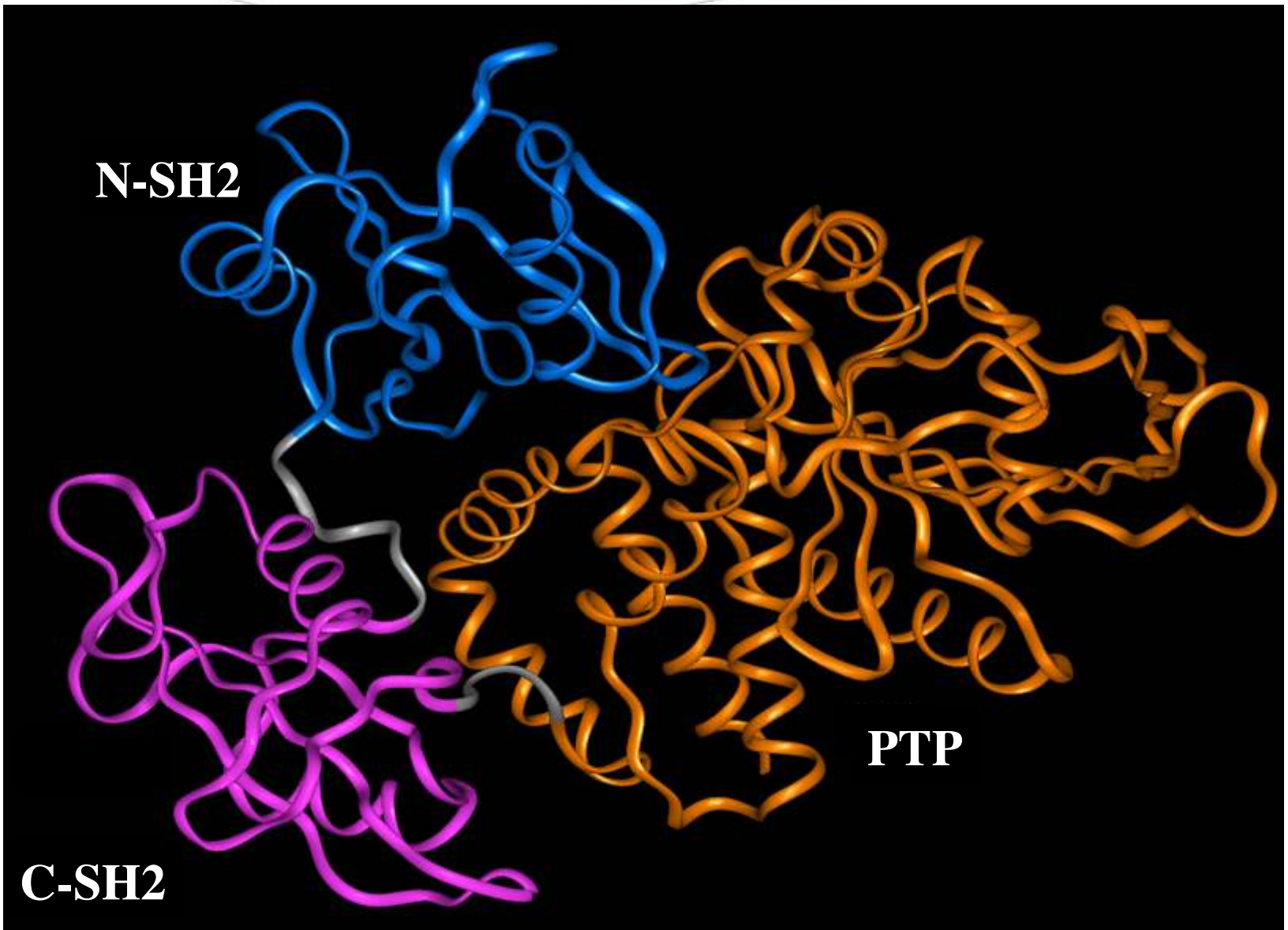
RASopathy associated cancers

- Rhabdomyosarcoma
- Hematologic JMML, AML, ALL
- Neuroblastoma
- Glioma
- Neurofibroma
- Malignant peripheral nerve sheath tumor
- Bladder cancer

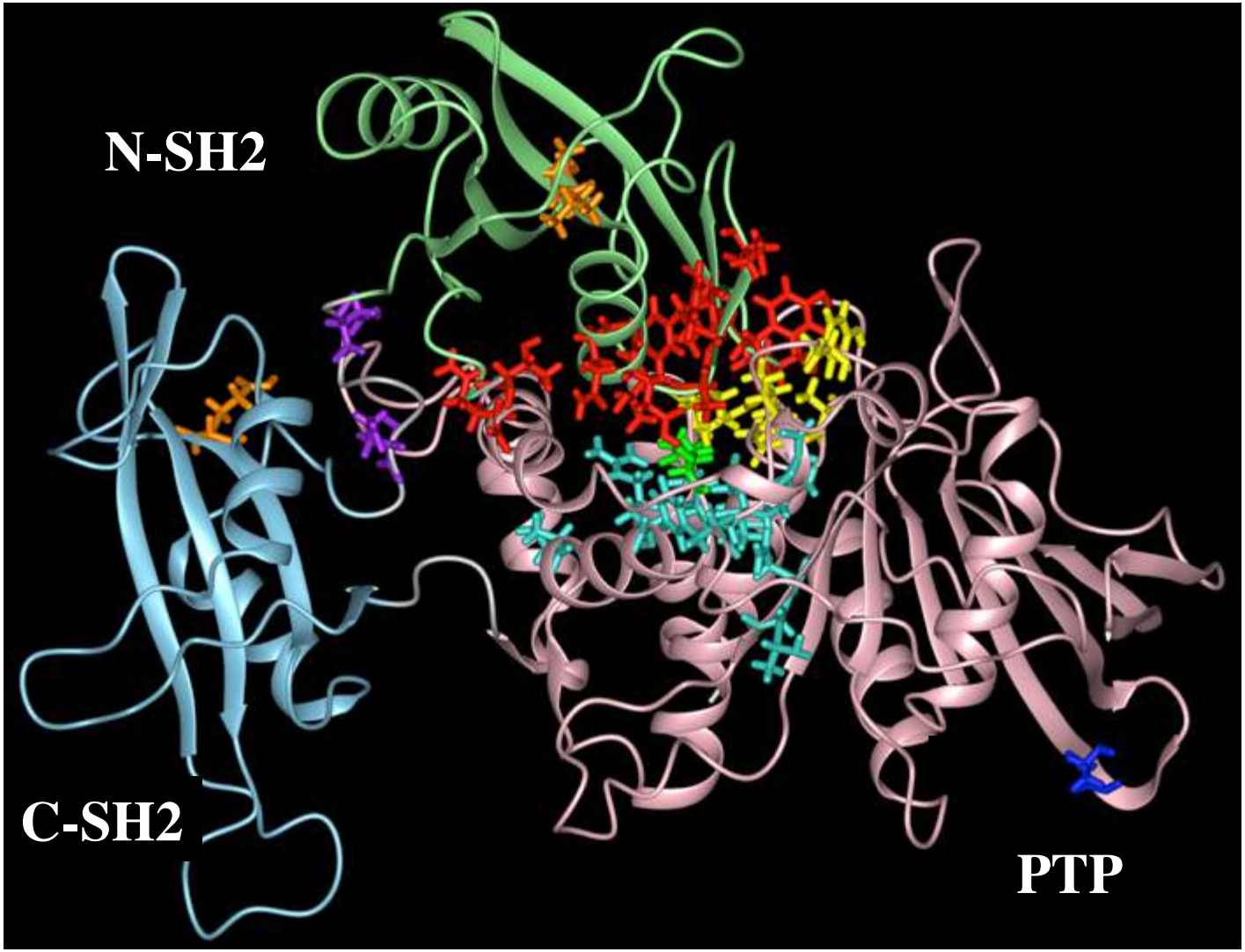
Somatic RAS cancers

- Head and neck SCC
- Lung adenocarcinoma
- Cutaneous melanoma
- Thyroid carcinoma
- Pancreatic ductal adenocarcinoma
- Colorectal adenocarcinoma
- Bladder urothelial adenocarcinoma

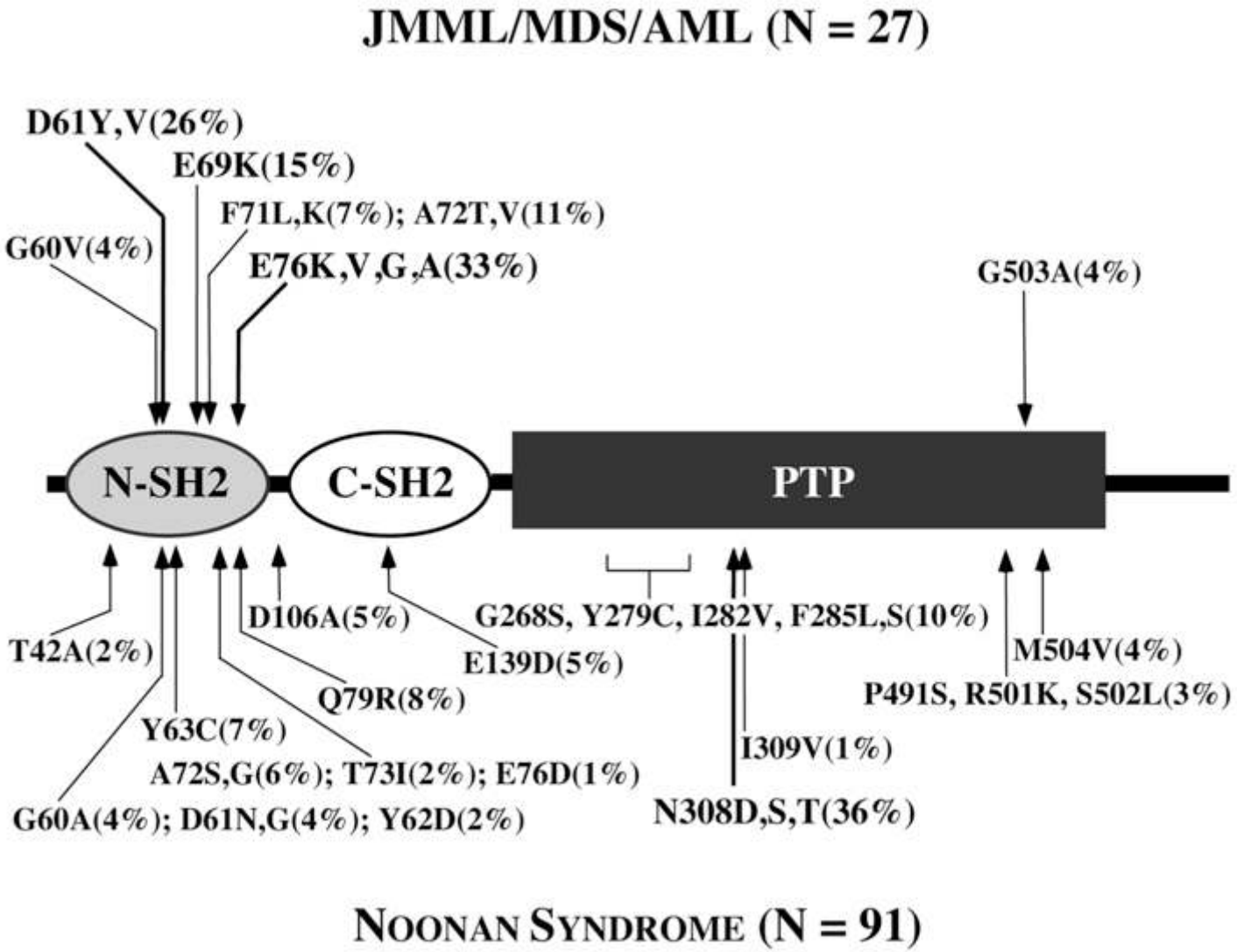
SHP2



Substitutions in Noonan syndrome

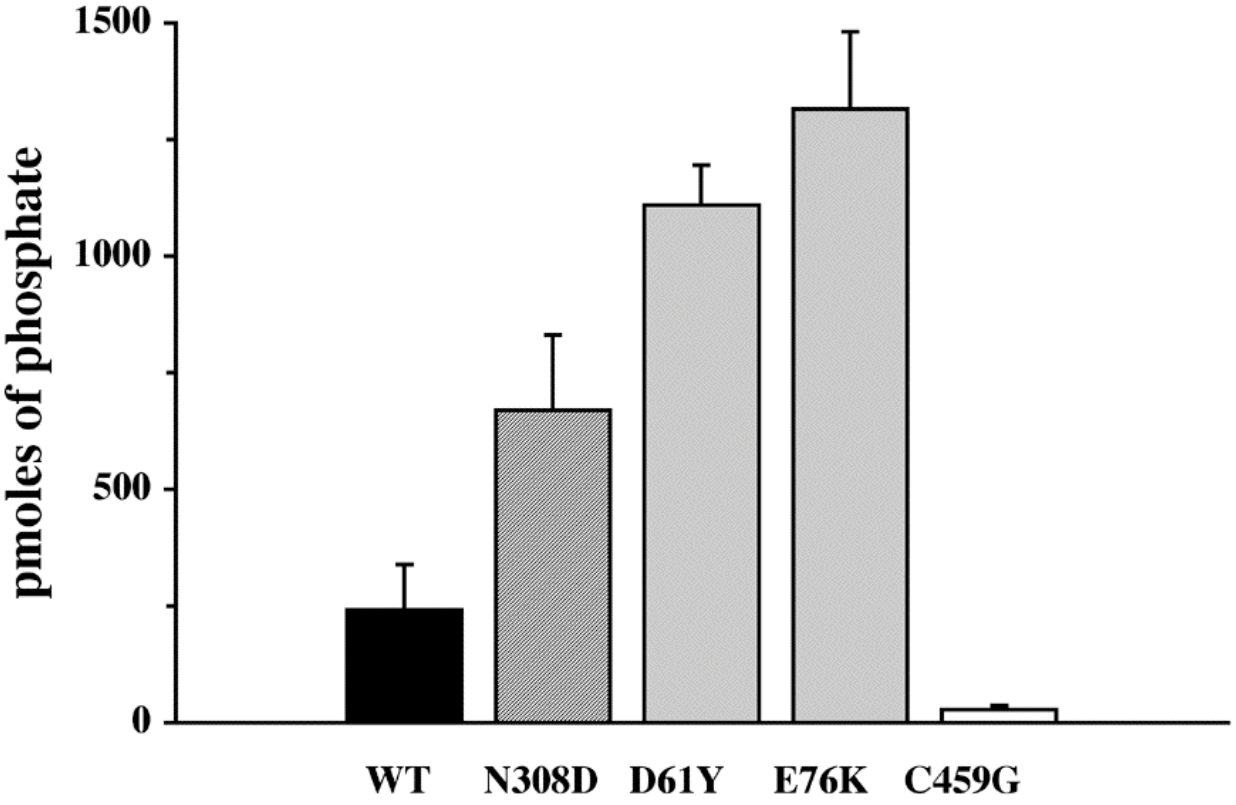


Noonan Syndrome vs. Cancer

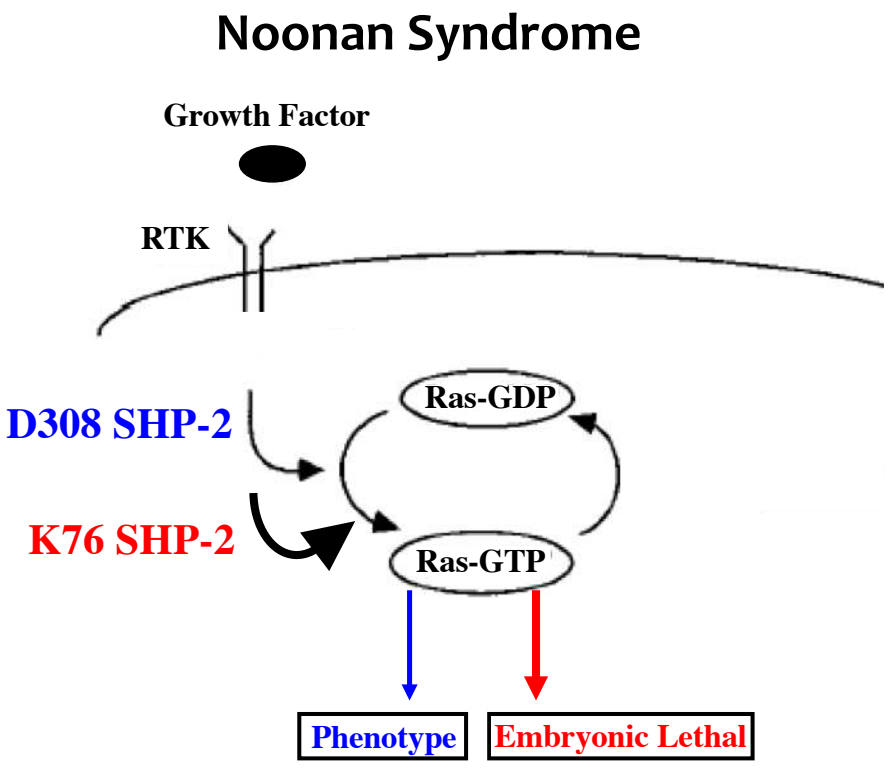


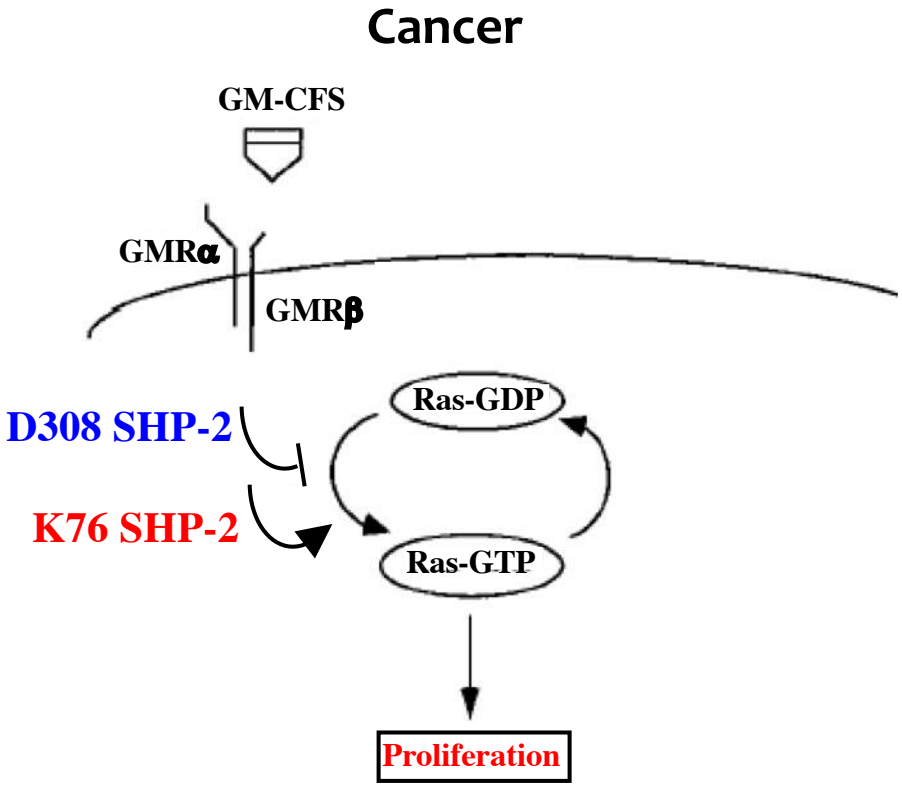
Noonan Syndrome vs. Cancer

Immunocomplex Phosphatase Assays

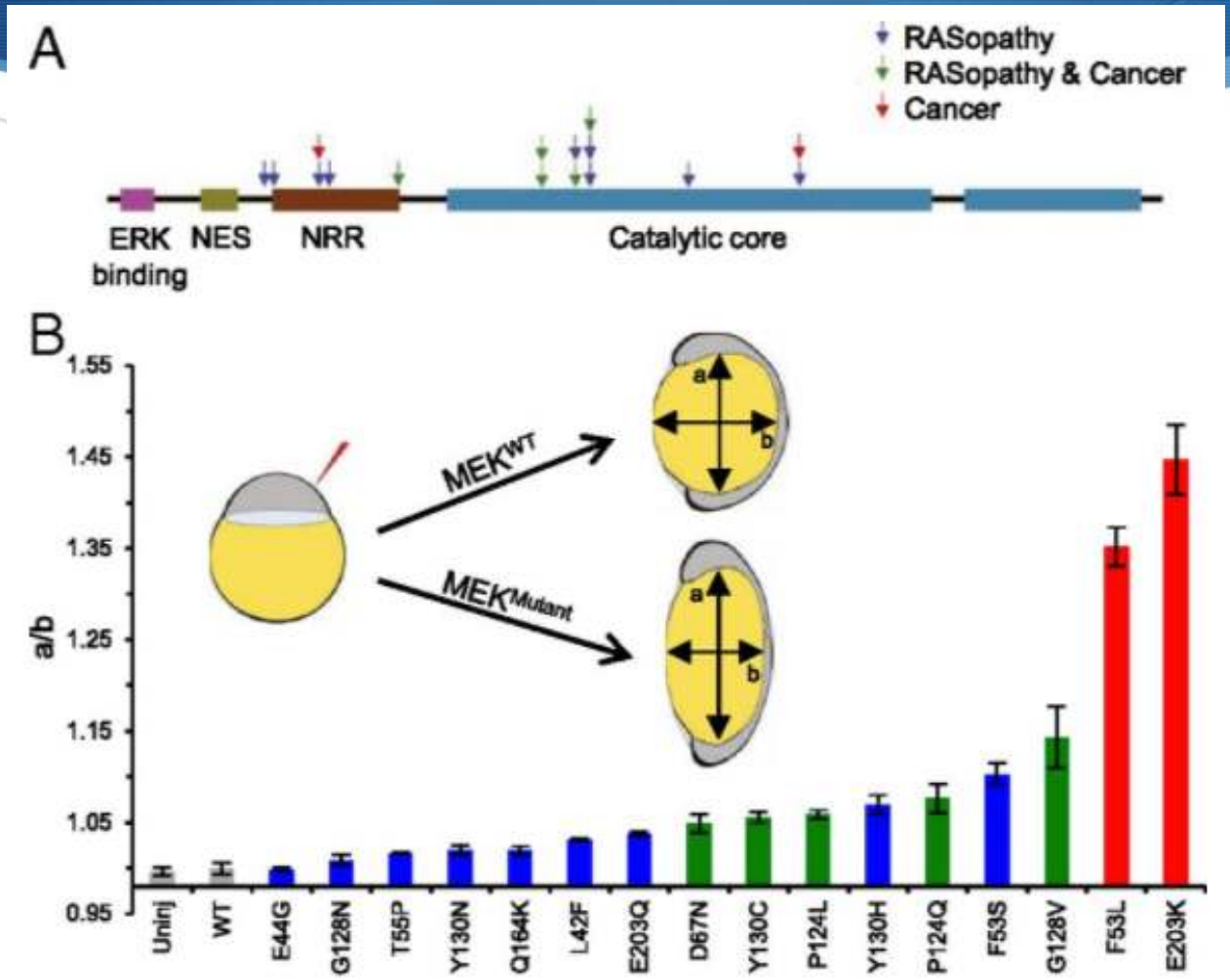


Noonan Syndrome vs. Cancer



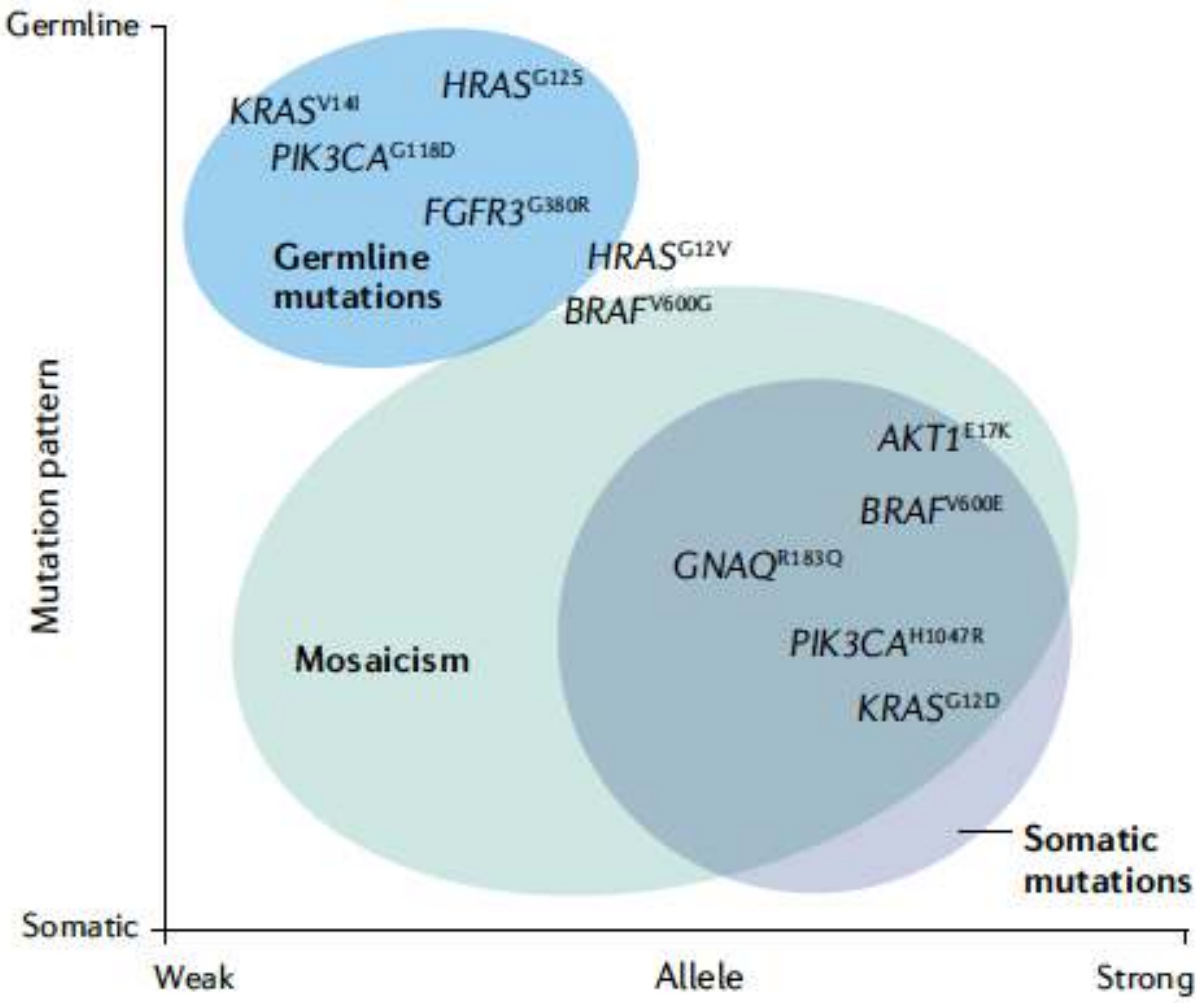


RASopathy vs. cancer mutations



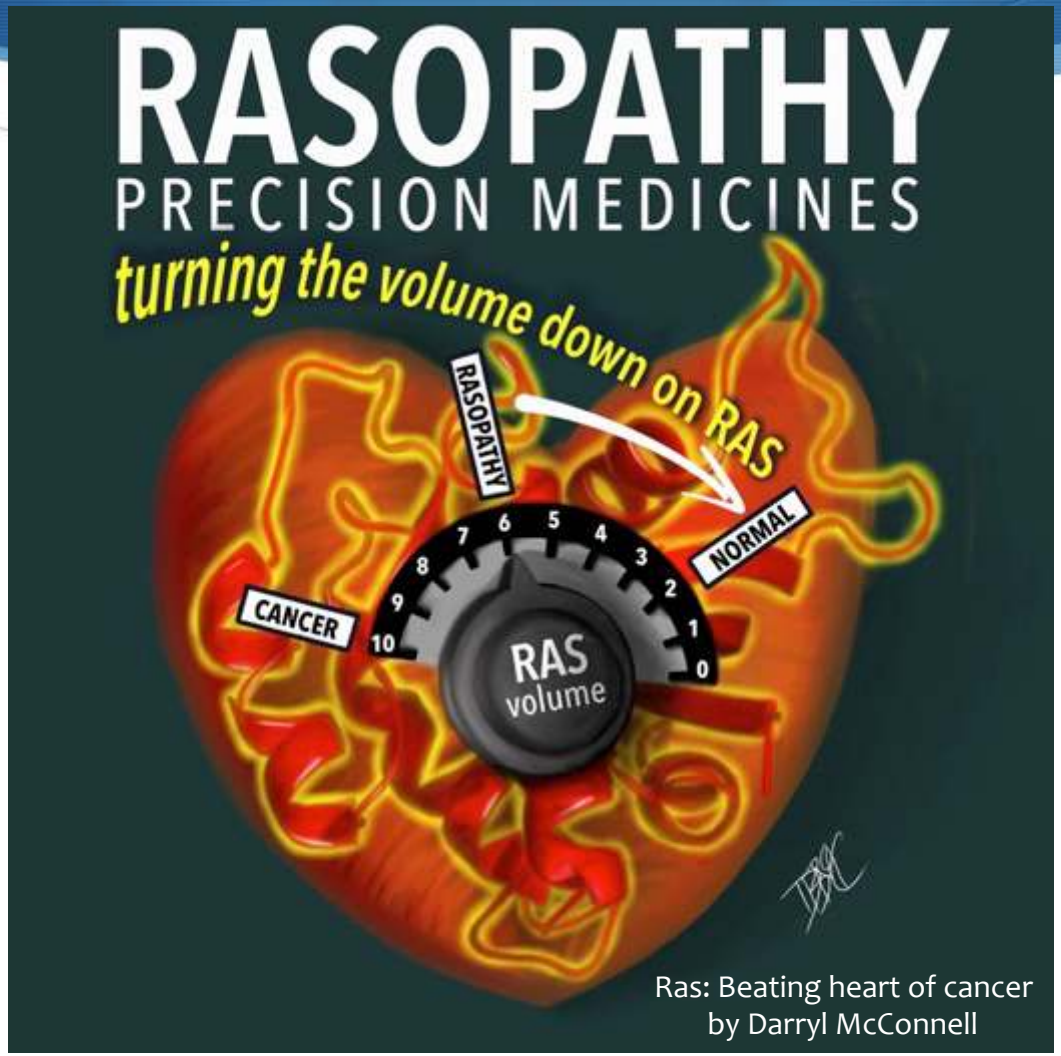
Jindal *et al*, 2017, *PNAS*

The Happle hypothesis



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:

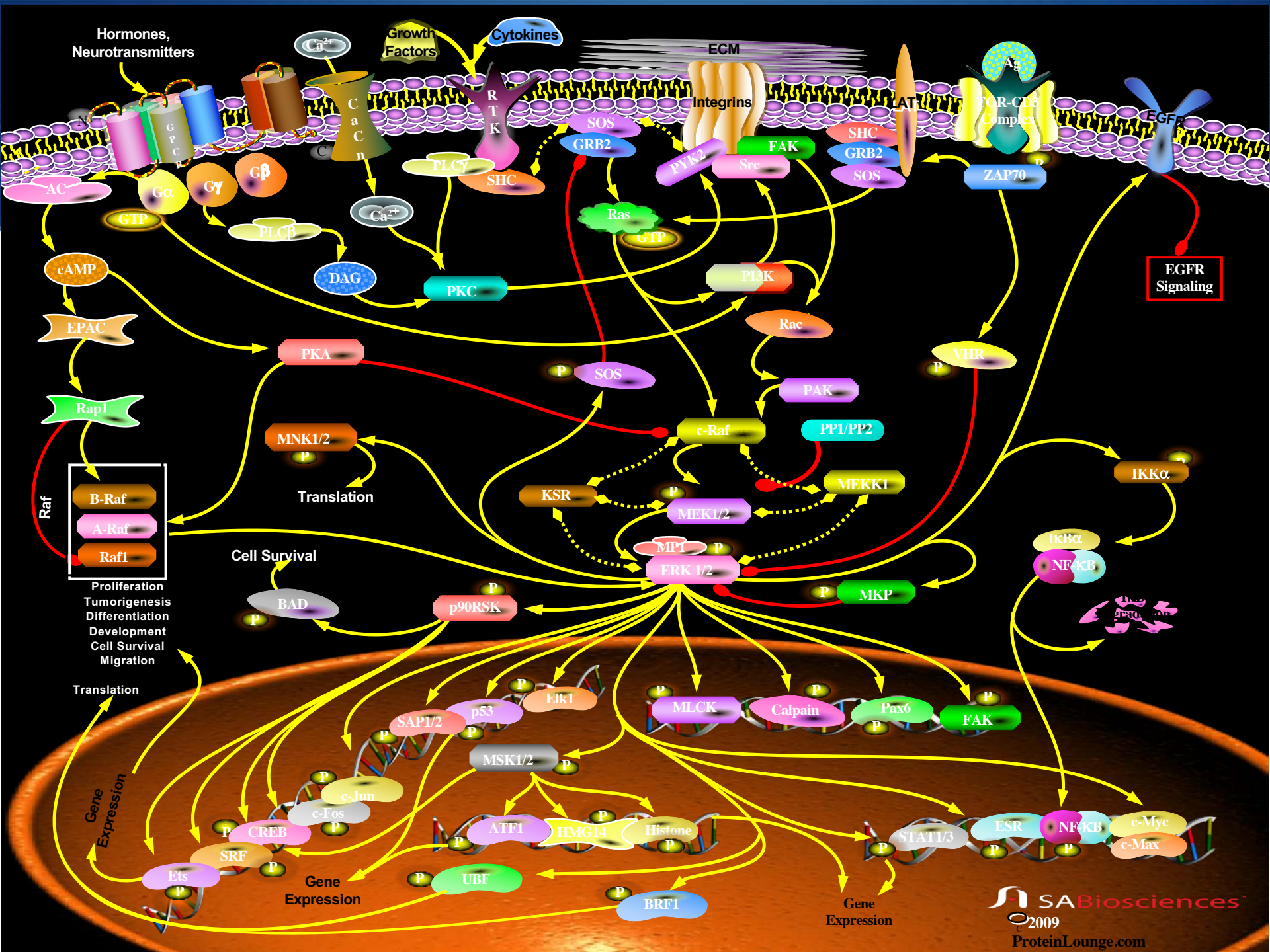
Targets and treatment considerations

Targets and Treatment

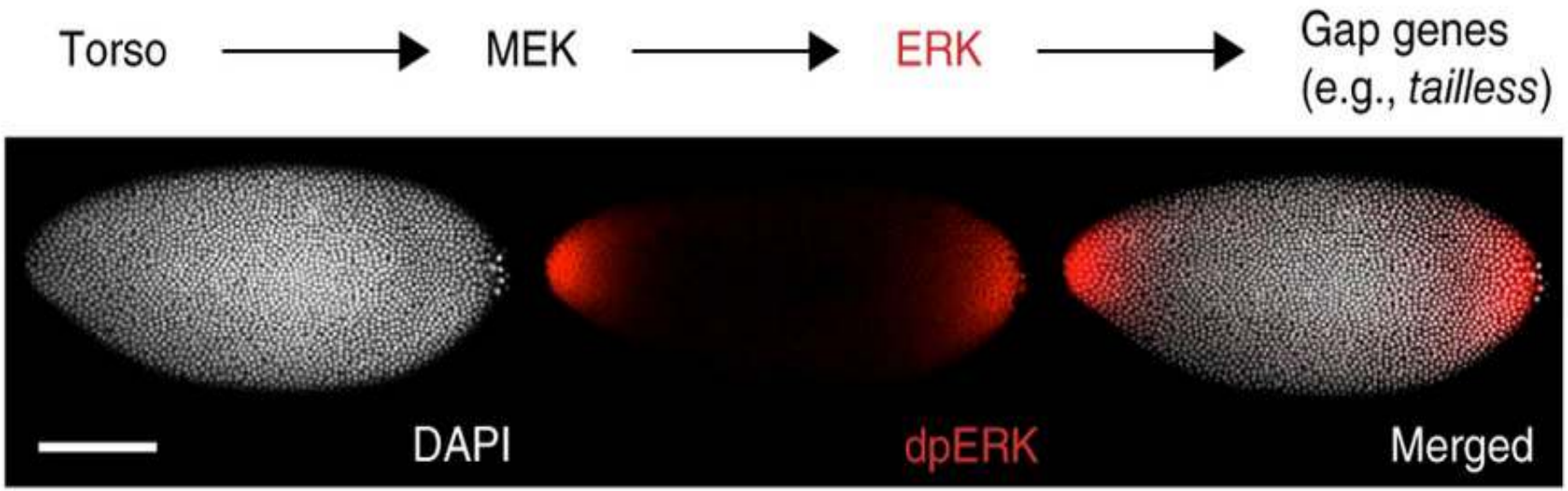
- Many molecular targets
- Common feature: Hyperactive RAS/MAPK signaling (autosomal dominant GOF, haploinsufficient or LOF)
- Multi-system pathology
- No current treatments or cures beyond symptom management

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?

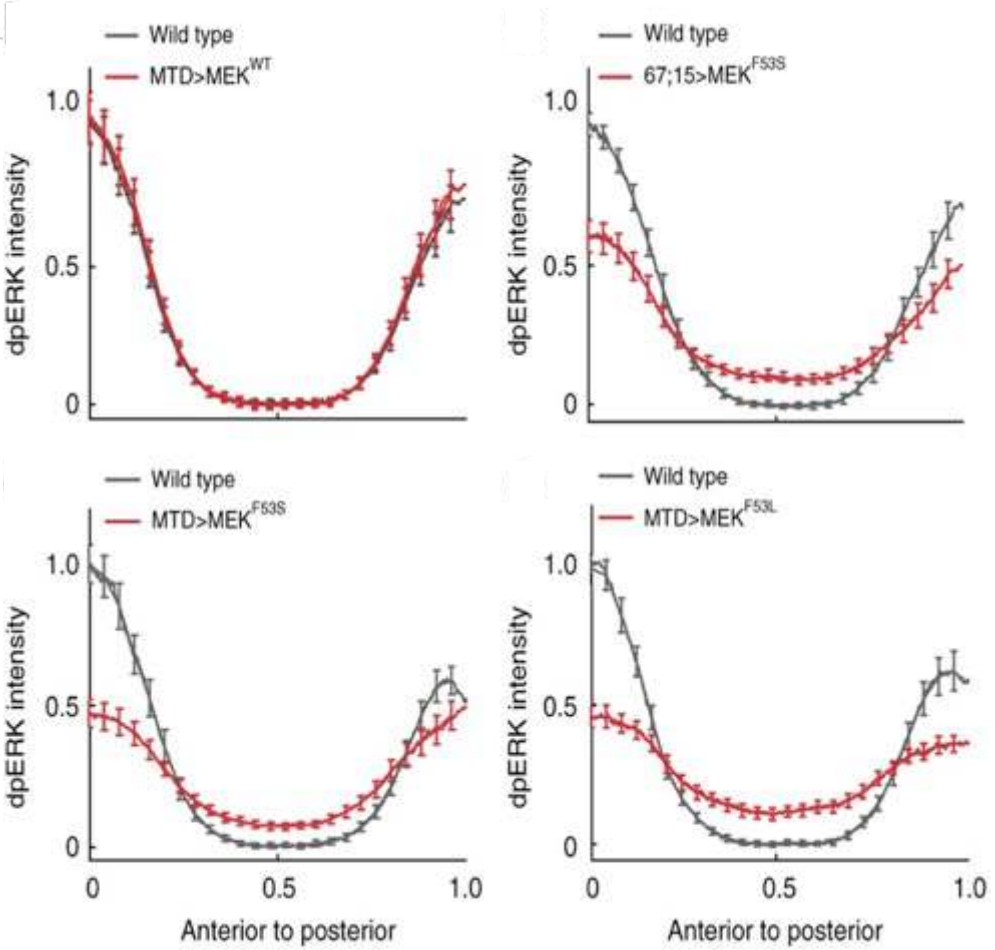

Hormones, Neurotransmitters
Ca2+
Growth Factors
Cytokines
ECM
Integrins
LAT
Ag
TCR-CD3 Complex
EGFR
GPCR
CaCn
RTK
SOS
GRB2
SHC
FAK
Src
PYK2
ZAP70
AC
Gα
Gγ
Gβ
GTP
PLCβ
PLCγ
Ca2+
Ras
GTP
cAMP
DAG
PKC
PI3K
Rac
EPAC
PKA
P
SOS
VHR
EGFR Signaling
Rap1
MNK1/2
Translation
PAK
c-Raf
PP1/PP2
IKKα
Raf
B-Raf
A-Raf
Raf1
KSR
MEKK1
MEK1/2
IκBα
NFκB
MP1
ERK 1/2
MKP
Cell Survival
BAD
p90RSK
Proliferation
Tumorigenesis
Differentiation
Development
Cell Survival
Migration
Translation
Elk1
SAP1/2
p53
MLCK
Calpain
Pax6
FAK
MSK1/2
c-Jun
c-Fos
CREB
SRF
Ets
ATF1
HMG14
Histone
UBF
BRF1
STAT1/3
ESR
NFκB
c-Myc
c-Max
Gene Expression
Gene Expression
Gene Expression
SABiosciences
©2009 ProteinLounge.com

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

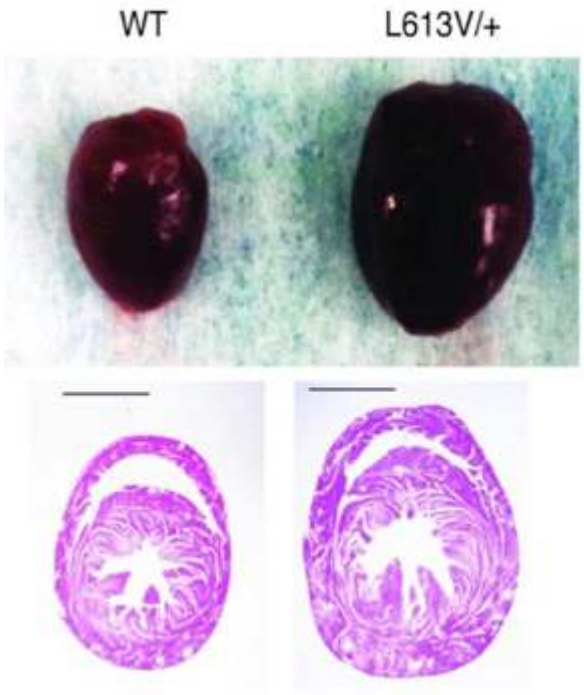
Topic 3
Getting to Treatments:

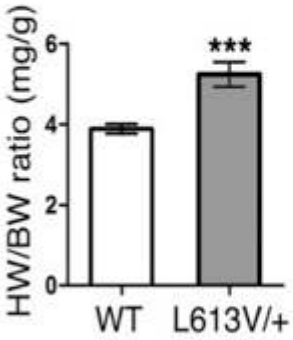
Preclinical Models

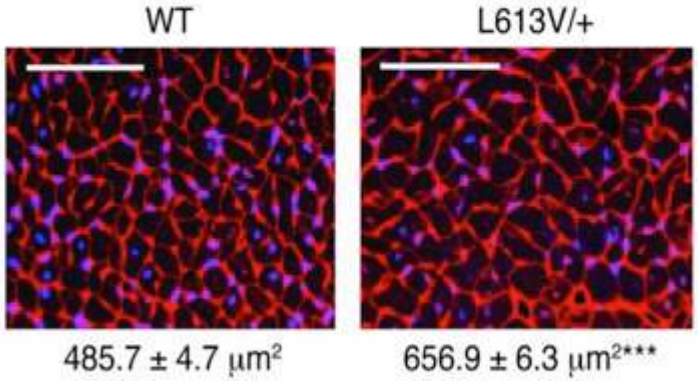
Preclinical Models

- 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

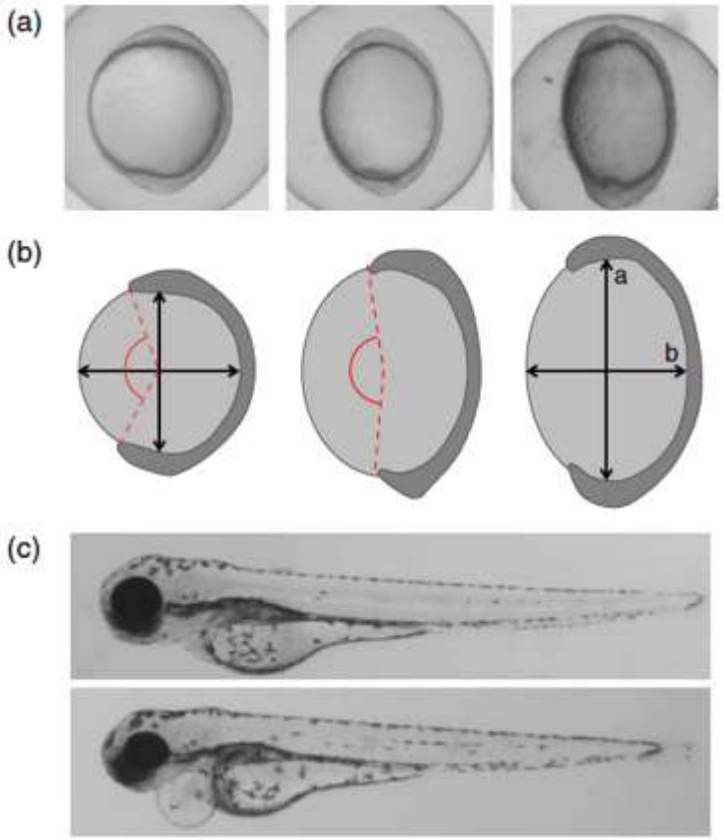
Wu *et al.*, *J Clin Invest* 2011

Swimming toward solutions: Using fish and frogs as models for understanding RASopathies

Victoria L. Patterson | Rebecca D. Burdine

Birth Defects Research, 2020

Phenotypic assays:



Methods:

RNA or morpholino injection

Nf1 KO, transgene insertion

High throughput screening

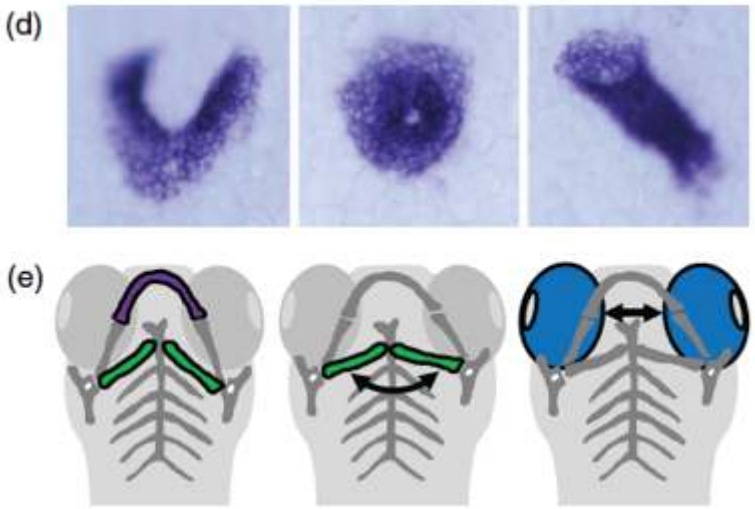


Table S6: Phenotypes of Animal Models of RASopathies. For each phenotype that occurs in each RASopathy, a reference of an animal model is given.

Diagram Part	Phenotypes	RASopathy	Reference
Mice Only	Bone defects	NF1	(Kolanczyk et al., 2007)
	Neurofibromas	NF1	(Rosenbaum et al., 1997)
	Sex-linked effects	NF1	(Diggs-Andrews et al., 2014)
	Muscle Abnormalities	NF1	(Sullivan et al., 2014)
	Attention deficits	NF1	(Brown et al., 2010)
	Working memory deficits	NF1	(Shilyansky et al., 2010)
	Leaner metabolic profile	NS	(Tajan et al., 2014)
	Hematologic disease	NS	(Araki et al., 2009)
	Triangular face	NS, CS	(Araki et al., 2004; Schuhmacher et al., 2008)
	Enlarged spleen	NS	(Araki et al., 2004)
	Liver defects	NF1,NS,CFC,CS	(Araki et al., 2004; Figueiredo et al., 2012; Hegedus et al., 2007; Inoue et al., 2014)
	Lymphatic system defects	CFC	(Inoue et al., 2014)
	Epileptic seizures	CFC	(Urosevic et al., 2011)
	Nasal septal deviation	CS	(Chen et al., 2009)
	Papilloma formation	CS	(Chen et al., 2009)
	Hyperemotivity	CS	(Viosca et al., 2009)
	Teeth defects	CS	(Goodwin et al., 2014)
***Drosophila* Only**	Mitochondrial defects	NF1	(Tong et al., 2007)
	Circadian rhythm defects	NF1	(Williams et al., 2001)
	Synaptic overgrowth	NF1	(Walker et al., 2013)
	Slower escape response	NF1	(The et al., 1997)
	Ectopic veins	NS	(Oishi et al., 2006)
	Photoreceptor defects	NS	(Oishi et al., 2006)
Zebrafish Only	Pigmentation Defects	NF1	(Shin et al., 2012)
	Motor Defects	NF1	(Shin et al., 2012)
	Schwann cell hyperplasia	NF1	(Shin et al., 2012)
	Kupffer's vesicle malformation	NS	(Bonetti et al., 2014)
	C&E defects	NS, CFC	(Runtuwene et al., 2011)
	Precocious ossification	CS	(Santoriello et al., 2009)
	Reduced blood oxygenation	CS	(Santoriello et al., 2009)
	Scoliotic spine	CS	(Santoriello et al., 2009)
	Sterility	CS	(Santoriello et al., 2009)

Mice and *Drosophila*	Mice		*Drosophila*	
Myeloproliferative disease	NF1, NS	(Gitler et al., 2004; Mohi et al., 2005)	NS	(Mohi et al., 2005)

Mice and Zebrafish	Mice		Zebrafish	
Neural crest cell defects	NF1	(Ismat et al., 2006)	NF1, NS	(Shin et al., 2012; Stewart et al., 2010)
Myelin sheath defects	NF1	(Cichowski et al., 1999)	NF1	(Shin et al., 2012)
OPC hyperplasia	NF1, NS	(Bennett et al., 2003; Ehrman et al., 2014)	NF1	(Shin et al., 2012)
Hypertelorism	NS	(Araki et al., 2004)	NS, CFC, CS	(Anastasaki et al., 2012; Runtuwene et al., 2011; Santoriello et al., 2009)
Gliomas	NF1	(Hegedus et al., 2009)	NF1	(Shin et al., 2012)

Mice, Zebrafish, and *Drosophila*	Mouse		Zebrafish		*Drosophila*	
Learning/cognitive defects	NF1, NS, CS	(Costa et al., 2002; Lee et al., 2014; Viosca et al., 2009)	NF1	(Wolman et al., 2014)	NF1, NS	(Buchanan and Davis, 2010; Pagani et al., 2009)
Reduced life span	NS, CFC	(Hernández-Porras et al., 2014; Urosevic et al., 2011)	CS	(Santoriello et al., 2009)	NF1	(Tong et al., 2007)
Growth defects	NS, CFC	(Araki et al., 2004; Urosevic et al., 2011)	NS, CFC, CS	(Anastasaki et al., 2009; Jopling et al., 2007; Santoriello et al., 2009)	NF1	(Walker et al., 2006)
Cardiac defects	NF1, NS, CFC, CS	(Araki et al., 2009; Inoue et al., 2014; Ismat et al., 2006; Schuhmacher et al., 2008)	NF1, NS, CS	(Bonetti et al., 2014; Padmanabhan et al., 2009; Santoriello et al., 2009)	NS	(Yu et al., 2013)

Topic 3
Getting to Treatments:

Endpoints

Natural history study

Am J Med Genet A. 2020 Jan 8. doi: 10.1002/ajmg.a.61485. [Epub ahead of print]

Advancing RAS/RASopathy therapies: An NCI-sponsored intramural and extramural collaboration for the study of RASopathies.

Gross AM[1], Frone M[2], Gripp KW[3], Gelb BD[4,5], Schoyer L[6], Schill L[6], Stronach B[6], Biesecker LG[7], Esposito D[8], Hernandez ER[1], Legius E[9], Loh ML[10], Martin S[1], Morrison DK[11], Rauen KA[12], Wolters PL[1], Zand D[13], McCormick F[7], Savage SA[2], Stewart DR[2], Widemann BC[1], Yohe ME[1].

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

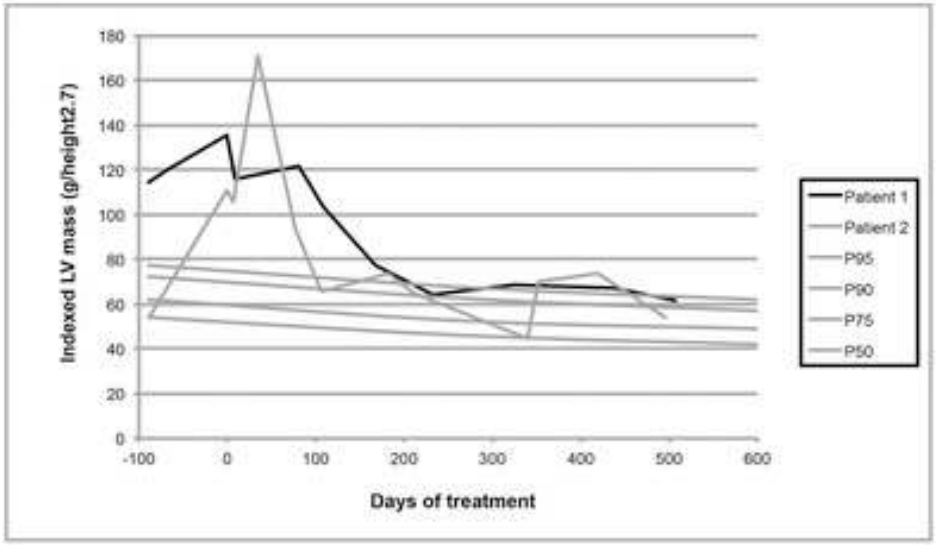
Proof of concept trials

- 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]

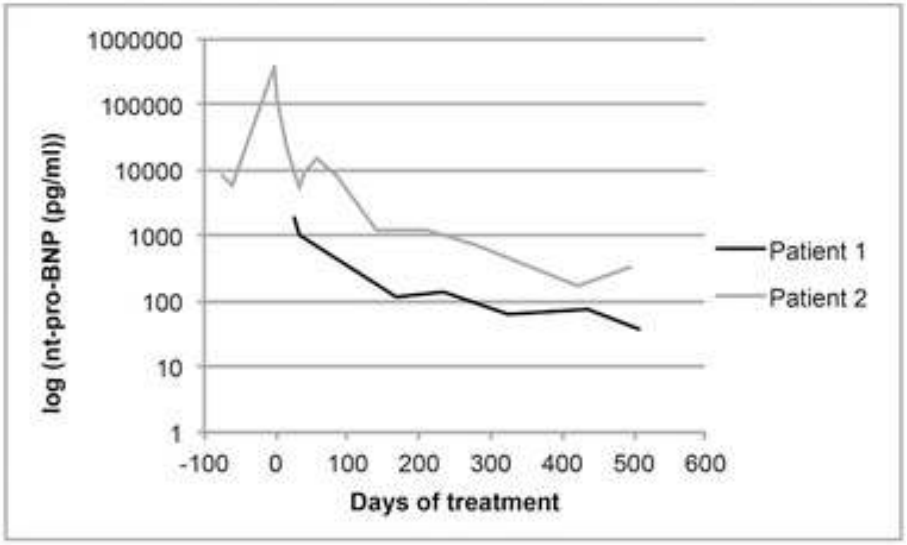
- Off-label use of MEK inhibitor Trametinib for RIT1-associated HCM in 2 infants (13 and 14 wks of age)
  - Associated with reversal of HCM and valvular obstruction over 17 months of therapy
  - G. Andelfinger *et al*. 2019 *JACC*

Trametinib for HCM

LV Mass



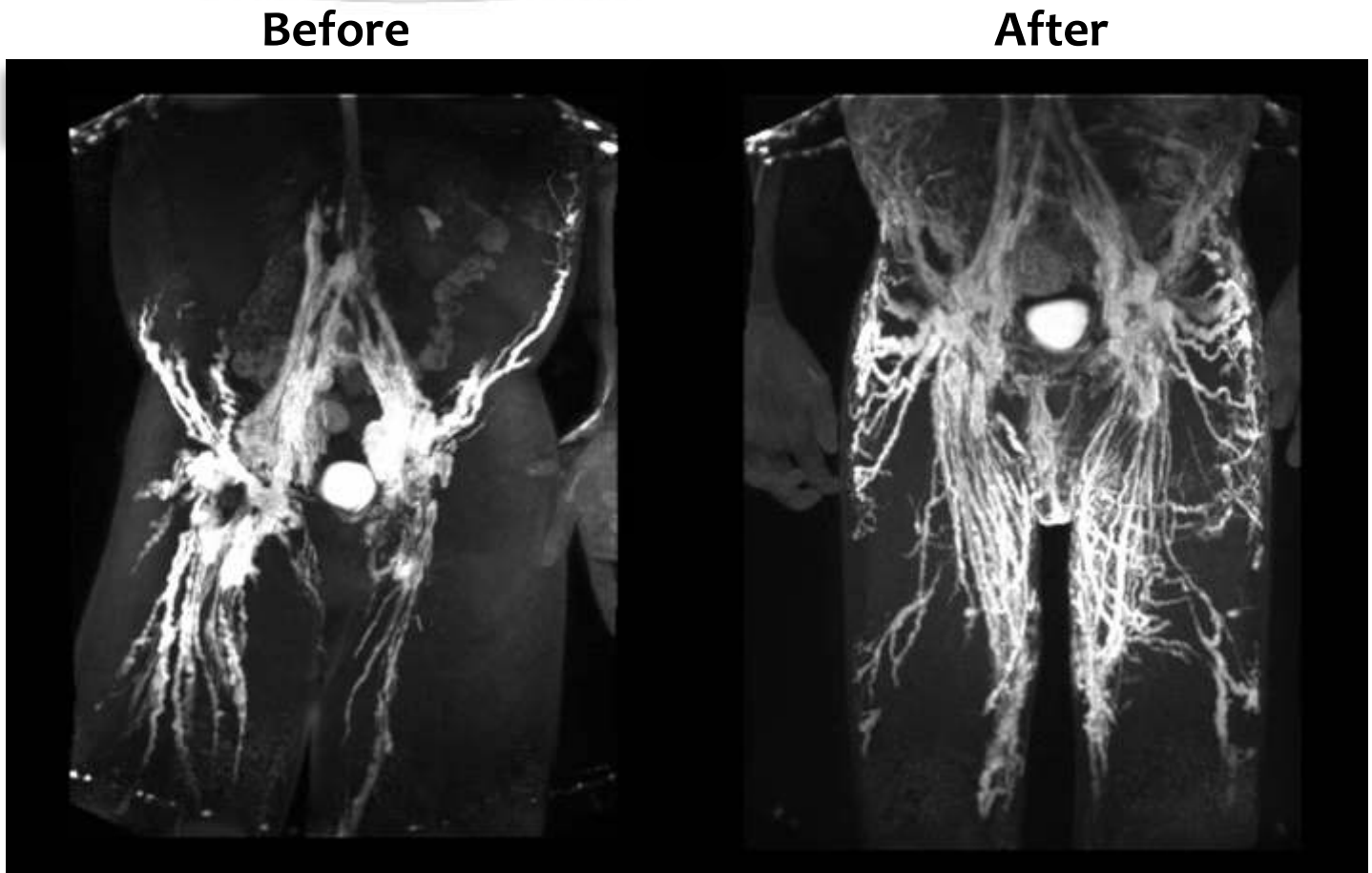
Pro-BNP



Trametinib for Lymphatic Disease

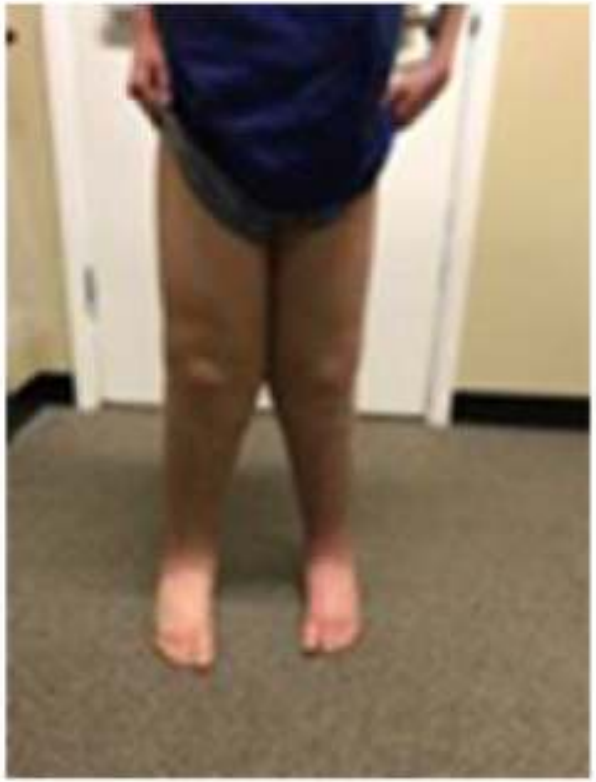
- Li *et al.*, *Nature Medicine* 2019
- Central conducting lymphatic anomaly (CCLA)
  - *ARAF* gain-of-function missense variant
    - Comparable alleles in *RAF1* for Noonan syndrome
  - Zebrafish model
    - Recapitulated lymphatic phenotype
    - Treated with cobemitinib

Trametinib for Lymphatic Disease



Trametinib for Lymphatic Disease

Before



After

Engaged advocacy groups

HOW CAN WE HELP?

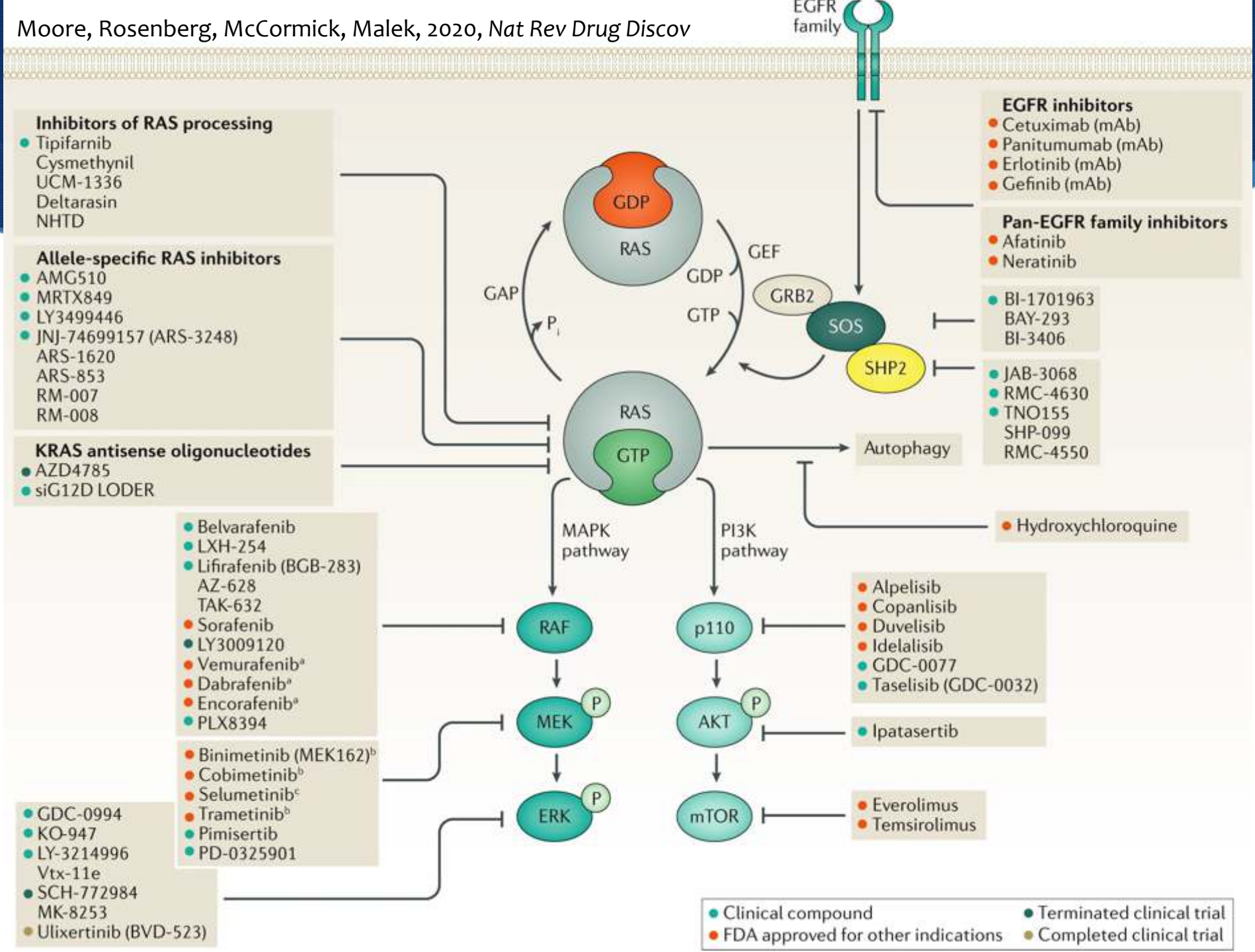
Social media groups

Discussion

Moving Forward-
What is feasible for the RASopathies?

END

Moore, Rosenberg, McCormick, Malek, 2020, *Nat Rev Drug Discov*



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*]