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Advancing RAS/RASopathy Therapies (ART): An NCI-sponsored Intramural and Extramural Collaboration for the study of RASopathies

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Abstract

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RASopathies caused by germline pathogenic variants in genes that encode RAS pathway proteins. These disorders include neurofibromatosis type 1 (NF1), Noonan syndrome (NS), cardiofaciocutaneous syndrome (CFC), and Costello syndrome (CS), and others. RASopathies are characterized by heterogenous manifestations, including congenital heart disease, failure to thrive, and increased risk of cancers. Previous work led by the NCI Pediatric Oncology Branch has altered the natural course of one of the key manifestations of the RASopathy NF1. Through the conduct of a longitudinal cohort study and early phase clinical trials, the MEK inhibitor selumetinib was identified as the first active therapy for the NF1-related peripheral nerve sheath tumors called plexiform neurofibromas (PN). As a result, selumetinib was granted breakthrough therapy designation by the FDA for the treatment of PN. Other RASopathy manifestations may also benefit from RAS targeted therapies. The overall goal of Advancing RAS/RASopathy Therapies (ART), a new NCI initiative, is to develop effective therapies and prevention strategies for the clinical manifestations of the non-NF1 RASopathies and for tumors characterized by somatic RAS mutations. This report reflects discussions from a February 2019 initiation meeting for this project, which had broad international collaboration from basic and clinical researchers and patient advocates.

Keywords

RASopathies; Ras/MAP kinase pathway; Costello syndrome; Noonan syndrome; Cardiofaciocutaneous syndrome

INTRODUCTION:

In February 2019, a multidisciplinary group of 38 care providers, basic scientists, and representatives from the patient advocacy group RASopathiesNet, assembled at the National Cancer Institute (NCI) for the Advancing RAS/RASopathies Therapies (ART) initiation meeting. ART is a collaborative effort involving basic, clinical, and translational researchers from NCI-funded extramural institutions, the NCI RAS Initiative, and two intramural divisions of the NCI, the Center for Cancer Research and the Division of Cancer Epidemiology and Genetics; and affected individuals, their families, and representatives from patient advocacy groups. The goal of this two-day meeting was to announce ART as a new NCI-sponsored, multi-institution initiative to identify therapies for individuals with RASopathies or tumors with somatic RAS variants. In addition, the participants aimed to define the RASopathies and RASopathy-associated genes for the purposes of ART, identify the available tools for translational research in the RASopathies, describe the longitudinal cohort study that is the cornerstone of ART, and discuss potential solutions for the various challenges that have hindered investigators from initiating therapeutic clinical trials for individuals with RASopathies to date.

The RASopathies are a clinically and molecularly defined group of disorders caused by germline pathogenic variants in genes that encode components of the Ras/MAPK pathway (Rauen, 2013) (Table 1). The RASopathies collectively are rare diseases but they result in significant clinical morbidity and mortality. Individuals with RASopathies have overlapping but heterogeneous clinical presentations, some of which are life threatening, including

cardiomyopathy and failure to thrive, and others that affect quality of life, such as disordered sleep and developmental delay. To date, few effective treatment strategies have been discovered, making the RASopathies a significant area of unmet medical need.

The important role of the RAS pathway in many, primarily adult, malignancies has led to the development of multiple agents targeting this pathway for oncologic indications. Most of these agents have not been studied clinically in individuals with RASopathies or with pediatric cancers driven by somatic RAS pathway variants, which include solid tumors, such as embryonal rhabdomyosarcoma (Shern et al., 2014), malignant peripheral nerve sheath tumor (Brohl, Kahen, Yoder, Teer, & Reed, 2017), relapsed neuroblastoma (Eleveld et al., 2018), and malignant ectomesenchymoma (S. C. Huang et al., 2016), as well as hematologic malignancies such as juvenile myelomonocytic leukemia (JMML), acute lymphoblastic leukemia (ALL), and acute myelogenous leukemia (AML) (Irving et al., 2014). Some of the challenges that are impeding clinical trials of RAS pathway targeting agents in individuals with RASopathies or pediatric tumors include the rarity of the diseases and the young age of the affected population. However, there is a unique opportunity to use a RAS pathway targeting agent to treat pediatric individuals with either RAS-driven cancers or RASopathies, and to learn more about the pathogenic nature of the RAS pathway in both the germline and somatic setting. This model has been successful with neurofibromatosis type 1 (NF1), a prototypic RASopathy, in which selumetinib, a MEK inhibitor that targets the RAS pathway, induces shrinkage of large plexiform neurofibromas in these individuals (Dombi et al., 2016).

Defining the ART RASopathies

A major focus of the two-day ART initiation meeting was defining the non-NF1 RASopathies that are to be the focus of the initiative. This question sparked a lively discussion among the world-leading RASopathy experts, but no consensus was reached. In general, those in attendance hypothesized that there might be two groups of RASopathy disorders: classical and non-classical. The classical RASopathies, Noonan (NS), Costello (CS), cardiofaciocutaneous (CFC), NF1, and Legius syndrome (LS), share a common phenotype consisting of a combination of features such as craniofacial malformations, congenital heart disease, failure to thrive, neurocognitive impairments and cancer predisposition. Individuals with the non-classical RASopathies, such as capillary arteriovenous malformation syndrome and SYNGAP1 syndrome, do not have failure to thrive and congenital heart disease, and the risk of developing cancer for individuals with these syndromes is incompletely described. To test the hypothesis that the risk of developing cancer is higher for individuals with classical RASopathies than for those with non-classical RASopathies, we will study individuals with classical and non-classical RASopathies in the context of the ART.

Defining the ART RASopathy genes: the genes of the Ras/MAPK pathway

The Ras/MAPK pathway is comprised of the RAS proteins, RAS activators, RAS inactivators, RAS effectors, and other pathway modulators (Figure 1). This pathway regulates diverse cellular processes such as proliferation, differentiation, and survival and is commonly mutated in cancer. RAS proteins function as guanine nucleotide-regulated binary

on-off switches. In normal quiescent cells, RAS is predominantly GDP-bound and inactive. Growth factors activate RAS-selective guanine nucleotide exchange factors (RAS-GEFs; *e.g.*, SOS1, SOS2) to promote nucleotide exchange and formation of active RAS-GTP. Once in the active, GTP-bound conformation, RAS can bind to a variety of effector proteins to transmit its downstream signals, including the RAF kinases and PI3 kinase. RAS-selective GTPase activating proteins (RAS-GAPs; *e.g.*, NF1, RASA1, RASA2, SYNGAP1) normally promote GTP hydrolysis to return RAS to its GDP-bound resting state (Cox & Der, 2002, 2010; Cox, Fesik, Kimmelman, Luo, & Der, 2014).

In sporadic cancer, RAS isoforms are somatically mutated at three main hotspots (Gly^{12}, Gly^{13}, and Gln^{61}); these variants render RAS GAP-insensitive, and thus persistently GTP-bound and active (Cox & Der, 2002, 2010; Cox et al., 2014). The mechanisms by which other cancer-associated RAS variants affect RAS activity are currently being studied. RAS variants at Ala^{146}, for example, increase the fraction of GTP-bound, activated RAS by enabling RAS to spontaneously exchange GDP for GTP in the absence of a RAS-GEF (Poulin et al., 2019). In addition to being identified in cancer, germline RAS pathogenic variants are found in the RASopathies. *HRAS* variants are associated with CS, and variants in *NRAS*, *KRAS,* and the related genes *MRAS*, *RRAS,* and *RIT1*(Flex et al., 2014; Young & Rodriguez-Viciana, 2018) are associated with NS (Bustelo, Crespo, Fernandez-Pisonero, & Rodriguez-Fdez, 2018; Rauen, 2013; Tidyman & Rauen, 2016a). Germline pathogenic variants in RAS-GEFs and RAS-GAPs are also found in RASopathies: for example, *SOS1* and *SOS2* variants are found in NS, *NF1* variants are found in NF1, and *RASA1* variants are found in capillary arteriovenous malformation syndrome (Table 1) (Boon, Mulliken, & Vikkula, 2005; Bustelo et al., 2018; Rauen, 2013; Tidyman & Rauen, 2016a).

The RAF family mitogen-activated protein kinase kinase kinases (MAPKKK, *e.g. ARAF, BRAF, RAF1*) are also mutated somatically in cancer tissues and in the germline in RASopathies. Binding to activated RAS recruits RAF to the plasma membrane, disrupts auto-inhibitory binding between the N-terminal regulatory domain and C-terminal kinase domain and induces RAF dimerization, all of which results in activation of RAF kinase activity. Active RAF phosphorylates and activates the MAP kinase kinases, MEK1 and MEK2 (encoded by *MAP2K1* and *MAP2K2,* respectively), which in turn phosphorylate and activate the MAP kinases, ERK1 and ERK2 (encoded by *MAPK3* and *MAPK1,* respectively). The ERK kinases in turn phosphorylate many additional substrates, including those responsible for the forward transmission of the mitogenic signal and those that provide inhibitory feedback regulation of the MAP kinase pathway.

In cancer cells, *BRAF* is commonly mutated at Val^{600}. This hotspot mutation allows for the BRAF kinase to be active in the absence of dimerization and in the absence of active RAS (Freeman, Ritt, & Morrison, 2013). The class I, BRAF Val^{600}, variants are uncommon in RASopathies (Li et al., 2019), but non- Val^{600} class II and class III BRAF variants are observed in CFC. Class II BRAF variants function as RAS-independent constitutively active dimers, while class III BRAF variants increase MAPK signaling due to an increased ability to be activated by RAS (Dankner, Rose, Rajkumar, Siegel, & Watson, 2018). The RAF family member *RAF1* is rarely mutated in cancer, but germline pathogenic variants in *RAF1* are found in individuals with NS (Pandit et al., 2007). Somatic variants in *ARAF* at the

Ser^{214} codon have been found in lung adenocarcinoma and melanoma but have yet to be found in individuals with classical RASopathies; however, this variant has been associated with lymphatic anomaly (Li et al., 2019). Germline and somatic variants in ARAF and RAF1 primarily prevent these kinases from being inhibited through their interactions with 14–3-3 proteins (Molzan et al., 2010). Somatic mutations in *MAP2K1* and *MAP2K2* also occur in cancer, and germline pathogenic variations in these genes cause CFC (Bromberg-White, Andersen, & Duesbery, 2012). The ERK kinase genes, *MAPK3* and *MAPK1*, however, are rarely mutated in either cancer or the RASopathies (Gao et al., 2013).

Other Ras/MAPK pathway modulators, such as *PTPN11*, *CBL*, *SPRED1*, *SHOC2*, *PPP1CB*, and *LZTR1* also have germline pathogenic variants associated with RASopathies (Tidyman & Rauen, 2016b). *PTPN11* encodes SHP2, a protein phosphatase and molecular scaffold that is required for full activation of the Ras/MAPK pathway. Gain-of-function germline pathogenic variants in *PTPN11* are found in individuals with NS, while loss-of-function variants in *PTPN11* occur in Noonan syndrome with multiple lentigines (NSML, OMIM 151100) (W. Q. Huang et al., 2014). *CBL* encodes c-Cbl, an E3 ubiquitin ligase and adaptor protein that negatively regulates the Ras/MAPK pathway in part by catalyzing the ubiquitination and subsequent degradation of EGFR and other tyrosine kinases (Swaminathan & Tsygankov, 2006). Germline pathogenic variants in *CBL* have been identified in NS (Niemeyer et al., 2010). *SPRED1* facilitates the recruitment of NF1 to the plasma membrane, which is critical for NF1 to function as a RAS-GAP. Loss of function germline pathogenic variants in *SPRED1* are found in LS (Dunzendorfer-Matt, Mercado, Maly, McCormick, & Scheffzek, 2016; Hirata et al., 2016; Stowe et al., 2012). *SHOC2* encodes a molecular scaffold that positively regulates the Ras/MAPK pathway by facilitating the interaction between RAS and RAF (Jang & Galperin, 2016). Additionally, a complex of SHOC2, MRAS and PP1C (*PPP1CB*) stimulates RAF activity by dephosphorylating an inhibitory phospho-site (Rodriguez-Viciana, Oses-Prieto, Burlingame, Fried, & McCormick, 2006; Young et al., 2018). Germline pathogenic variants in *SHOC2* (OMIM 607721) and *PPP1CB* (OMIM 617506) are found in Noonan syndrome with loose anagen hair (Tidyman & Rauen, 2016b). LZTR1 was originally thought to function as a transcription factor, but recently has been shown to function as a scaffold that facilitates the mono-ubiquitination of HRAS, NRAS, and KRAS via the E3 ubiquitin ligase, CUL3. This mono-ubiquitination inhibits RAS association with the plasma membrane and thus inhibits RAS activity. Loss of function pathogenic variants in *LZTR1* cause aberrant MAP kinase activity, are found in individuals with NS, and are inherited in both an autosomal dominant and an autosomal recessive manner (Steklov et al., 2018).

Recently, germline pathogenic variants in genes not currently associated with the Ras/MAPK pathway have been identified in individuals with clinically diagnosed RASopathies. These genes include *AZML1*, which encodes a secreted protease inhibitor; and *MYST4*, which encodes a histone acetyl transferase (Tidyman & Rauen, 2016a). The mechanisms by which these variants contribute to the development of RASopathies are currently under investigation. In addition, pathogenic variants in a RAS-related gene *RABL3* were found in one family with hereditary pancreatic cancer and zebrafish with germline variants in *RABL3* exhibited features of a RASopathy (Nissim et al., 2019). Pathogenic variants in other known Ras/MAPK pathway genes, such as *DUSP1–6* and *SPRY1–4*, either have not yet been

identified in individuals with RASopathies or require further validation in their association with RASopathy pathogenesis. While they will not be considered RASopathy genes for the purpose of the ART longitudinal cohort study, these genes will all be included in the population genomics assessment portion of ART, discussed below.

ART longitudinal cohort study

The cornerstone of ART is a prospective longitudinal cohort study for individuals with non-NF1 RASopathies. One aim of this study is to inform cancer screening recommendations for this population by determining the incidence of malignancy in individuals with RASopathies and the underlying differences between individuals with a RASopathy who develop tumors and those who do not. Retrospective studies have put the overall lifetime risk of cancer for individuals with RASopathies at between 1.6 and 4.6% (Kratz et al., 2015; Kratz, Rapisuwon, Reed, Hasle, & Rosenberg, 2011). These studies, however, are subject to publication bias and may inaccurately estimate the frequency of cancer in these individuals. In the current study, we aim to evaluate the risk of cancer for individuals with RASopathies in a prospective manner. The current cancer screening recommendations for individuals with RASopathies are abdominal ultrasound every 3–6 months until 10 years of age and urinalysis for hematuria annually after 10 years of age for individuals with CS (Gripp, Kawame, Viskochil, & Nicholson, 2004; Gripp et al., 2002), and annual complete blood counts for individuals with NS (Villani et al., 2017). We anticipate that, in the aggregate, the results of this study will ultimately allow us to expand the current cancer screening guidelines to additional RASopathies and additional tests.

A second important objective of the ART longitudinal cohort study is to provide a better understanding of non-tumor RASopathy manifestations with the goal of developing meaningful outcome measures for interventional trials targeting these manifestations. Signaling through the Ras/MAPK pathway controls the behaviors of many cell types throughout the human body and over the course of human development. A wide variety of organs and physiological systems are affected by altered Ras/MAPK signaling due to RASopathy pathogenic variants, causing congenital clinical phenotypes. Reflecting the systemic nature of the RASopathy syndromes, many areas of medical need were discussed at the meeting. These medical issues involve many clinical specialties, including audiology, cardiology, dentistry, dermatology, genetics, endocrinology, gastroenterology, hematology, immunology, neurology, ophthalmology, orthopedics, psychology, pulmonology, pain/palliative care, and urology.

Working groups comprised of clinicians, other health care providers, and patient advocates were established at the kickoff meeting for several of these domains, including working groups for neurocognitive impairment and pain; cardiac manifestations; gastrointestinal/feeding and speech/swallow manifestations; gross motor manifestations; and skin-related manifestations. In many cases, the natural history of these non-malignant RASopathy manifestations has yet to be formally studied, however, there was agreement among the attendees at the meeting that pain and anxiety/depression appear to be prevalent in adults with RASopathies. Patient/caregiver-reported outcomes and direct performance measures of cognitive functioning are being used to assess the physical, mental, and social well-being of

individuals with NF1, and in the ART study we aim to use these measures to assess the burden of disease in individuals with other RASopathies as well.

Individuals who have been clinically diagnosed with a RASopathy and/or who have a germline pathogenic variant in a Ras/MAPK pathway gene will be eligible for enrollment on the longitudinal cohort trial. There will be no age limit for enrollment. All enrolled individuals will receive a battery of self- or caregiver-administered questionnaires and their medical records will be examined. Some participants will also be invited to the NIH Clinical Center for a detailed in-person assessment and additional clinical and research laboratory tests and imaging studies in the areas detailed below. We plan to focus our assessments on RASopathy manifestations that are understudied and have a significant impact on the lives of individuals with a RASopathy and their caregivers, such as developmental delay and chronic pain.

ART Population Genomics

Electronic health record (EHR)-linked, population-based exome-sequenced cohorts, such as the Genomic Ascertainment Cohort at the NIH (TGAC, NCT03632239), are facilitated by the decrease in cost of whole-exome sequencing and are changing the traditional rare-disease clinical genetics paradigm. The new “genotype-first” approach reverses the traditional “phenotype-first” strategy. In ongoing projects, the NCI Division of Cancer Epidemiology and Genetics (DCEG) Clinical Genetics Branch is first quantifying pathogenic germline variations in known cancer genes such as *DICER1* and then determining the associated phenotype from the EHR. This approach promises to reduce ascertainment bias, characterize the full phenotypic spectrum of genetic diseases, permit more precise cancer incidence and penetrance estimates, facilitate the identification of novel syndromes, and ultimately lead to the development of standard of care guidelines.

For the ART study, we will identify individuals with pathogenic variation in RAS pathway genes (and matched-related controls) in available exome- and genome-sequenced cohorts. We will investigate the associated phenotype in cases and controls using novel statistical methods (*e.g.*, PheWAS) and clinical evaluation (*e.g.*, EHR chart review), and will also invite selected individuals/families to the NIH RASopathy Clinic described above. We anticipate that these analyses will enable the discovery of new RASopathy phenotypes as well as the validation of new RASopathy genes.

Preclinical RASopathy models for translational studies

An important goal of ART is to create a repository for preclinical RASopathy models, a goal that will be achieved in part through collaboration with the NCI RAS Initiative. As discussed below, translational studies in model systems are needed before the initiation of therapeutic clinical trials for individuals with RASopathies. The available models, as well as their potential utility in translational studies, are summarized here.

Single-cell and animal models can be used to study the effect of specific variants on cell biology, embryologic development, organ systems and functional abnormalities. Cell models used to study the RASopathies include skin fibroblasts and induced pluripotent stem cells (iPSCs). Patient-derived skin fibroblast cell lines are ideal for the biochemical

characterization of signal transduction pathways but require a skin biopsy and are generally not the cell type relevant to disease pathogenesis. In contrast, patient-derived iPSCs can be differentiated into a variety of cell types, facilitating studies of RASopathy pathogenesis. However, the disadvantages of iPSCs include their high cost to generate and maintain. In addition, the cells that differentiate from iPSCs have embryonic phenotypes, not adult ones. To date, iPSCs have been used to study RASopathy pathogenesis in cardiomyocytes, neural cells, and blood cell types (Jaffre et al., 2019; Mulero-Navarro et al., 2015; Rooney et al., 2016; Tasian et al., 2019). In the context of ART, we will attempt to create both fibroblast and iPSC lines from each participant who travels to the NIH Clinical Center.

RASopathies have been modeled in two invertebrate animals, *Caenorhabditis elegans* and *Drosophila melanogaster.* Those models have been used to explore disease pathogenesis, including neurocognitive deficits. In addition, *Drosophila* models are used for drug screening in a relatively inexpensive and high-throughput manner (Oishi et al., 2006). Well-established vertebrate models for RASopathies include *Danio rerio* (zebrafish) and *Mus musculus* (mice). Zebrafish models are relatively efficient and economical(Howe et al., 2013) and the consequences of various of RASopathy-associated variants on cardiac structure (Koenighofer et al., 2016), body shape and length, and tumor development (Santoriello et al., 2009) have been studied. Despite the advantages of zebrafish models, mouse models more accurately represent human organ function and the size of a mouse allows for a more detailed analysis of organ structures to be performed. Multiple mouse models with Ras/MAPK activating variants have been established for NS (Altmuller et al., 2017), CS (Oba et al., 2018), and CFC (Aoidi et al., 2018). Selection of a pre-clinical model, then, is guided by ease and cost of use, ability to provide drug exposure at the appropriate time and dosage, and availability of outcome measures for drug effects. As part of ART, we will attempt to collect multiple animal models of each RASopathy for the purpose of conducting preclinical efficacy studies of potential therapeutics.

Therapeutic Clinical Trials for Individuals with RASopathies: challenges and opportunities

The Ras/MAPK pathway is an attractive target for cancer treatment, and multiple small molecule inhibitors directed against components of the pathway are currently being evaluated as cancer therapies. Because the phenotypic signs and symptoms of RASopathies develop over time, Ras/MAPK inhibitors may represent novel therapies for individuals who have a RASopathy variant that is associated with increased Ras/MAPK activity. The use of Ras/MAPK inhibitors after birth might ameliorate disease progression or even reverse disease manifestations. For example, the hypertrophic cardiomyopathy observed in CS and NS is not always diagnosed at birth. In the postnatally diagnosed cases, cardiomyocyte hypertrophy may be due to the accumulation of metabolites over time, which could potentially be prevented by inhibition of aberrant Ras/MAPK activity (Lin et al., 2011).

Proof of mechanism that small molecule inhibitors of the Ras/MAPK pathway can prevent RASopathy manifestations has been achieved by studies showing that prenatal administration of MEK inhibitors prevents the development of RASopathy phenotypes (Jindal, Goyal, Burdine, Rauen, & Shvartsman, 2015). However, additional studies demonstrating the effectiveness of postnatal administration of small molecule Ras/MAPK

pathway inhibitors are needed to support the initiation of therapeutic trials of these agents for individuals with RASopathies. One study using the *Raf1*$^{L613V/+}$ mouse model of NS did find that administration of the MEK inhibitor PD0325901 during weeks 4 through 10 of life could prevent the development of cardiomyopathy (Wu et al., 2011). Interestingly, in a different model of NS, *Kras*^{V14I}, prenatal but not postnatal exposure to PD0325901 prevented development of cardiac defects (Hernandez-Porras et al., 2014). Because responsiveness to MEK inhibition is genotype-specific, studies of additional RASopathy models are required. Support for the use of Ras/MAPK inhibitors in non-NF1 RASopathies also comes from the clinical report of two infants with heart failure due to NS-associated hypertrophic cardiomyopathy who were treated with the MEK inhibitor, trametinib. In both of these individuals, trametinib treatment led to partial reversal of myocardial hypertrophy within 4 months of treatment initiation (Andelfinger et al., 2019).

Additionally, proof of concept that inhibitors of the Ras/MAPK pathway are effective treatments for individuals with RASopathies has been achieved by the clinical evaluation of the MEK inhibitor, selumetinib, in the treatment of NF1-related plexiform neurofibromas. Plexiform neurofibromas (PNs) are histologically benign tumors of nerve sheaths that can cause pain, disfigurement, and a variety of functional impairments. PNs have the potential to transform into aggressive cancers called malignant peripheral nerve sheath tumors. A phase II clinical trial evaluating the effectiveness of selumetinib in the treatment of children with inoperable NF1-related PNs demonstrated that most individuals treated with selumetinib experienced tumor shrinkage, a result not typically observed in the natural history of PNs in this population. Importantly, a variety of standardized functional evaluations and patient reported outcome measures were used to demonstrate that PN shrinkage due to selumetinib therapy resulted in improvement of PN-related pain and motor impairment (Dombi et al., 2016). These results led to selumetinib being granted US FDA Breakthrough Therapy designation as the first potentially active therapy for NF1-related PNs and supports the idea that Ras/MAPK inhibitors could beneficially affect individuals with non-NF1 RASopathies.

The design of therapeutic trials for individuals with RASopathies is challenging due to the rarity of the diseases, inter-patient phenotypic variability, lack of knowledge of the natural history of disease manifestations, and the lack of validated therapeutic endpoints. Potential therapeutic endpoints for the non-NF1 RASopathies include growth, neurocognitive function, and hypertrophic cardiomyopathy; these endpoints will be assessed longitudinally in the RASopathy natural history study. The heterogeneity of disease manifestations will necessitate the use of novel trial designs, such as the “N of 1” design, in which cycles of placebo or the experimental treatment are given to a single individual in randomized order with several crossover periods. In this type of trial, the participant acts as their own control (Abrahamyan et al., 2016). Another consideration in the design of therapeutic trials for individuals with RASopathies is the need for long-term administration of drug, which alters the acceptable side effect profile compared to drugs that are administered for a limited time period. Notably, for NF1, PN shrinkage is observed at doses of selumetinib lower than the recommended adult cancer dose, doses that also do not provide complete abrogation of ERK phosphorylation within the PN tissue (Jousma et al., 2015). This suggests that lower and sustained dosing of RAS targeting agents may be a successful strategy for the treatment of the clinical manifestations of other RASopathies. Pharmacodynamic studies are needed to

identify appropriate dosing regimens of RAS targeting agents for use in therapeutic trials for individuals with RASopathies, and could potentially be used as clinical trial endpoints, as was done with miransertib in Proteus syndrome (Keppler-Noreuil et al., 2019).

Other small molecule inhibitors of the Ras/MAPK pathway that may have efficacy in individuals with RASopathies include the farnesyl transferase inhibitors (FTIs), pan-RAF inhibitors, ERK inhibitors, direct RAS inhibitors, SHP2 inhibitors, inhibitors of the RAS: SOS interaction, and copper chelators. FTIs, such as tipifarnib, inhibit farnesylation, a post-translational modification of RAS that is required for RAS membrane localization and thus, activation. NRAS and KRAS bypass the requirement for farnesylation by undergoing an alternative post-translational modification that similarly allows for membrane localization. However, HRAS, the RAS isoform affected in CS, is unable to be alternatively modified, and thus its membrane localization and cellular function are suppressed by tipifarnib (Cox, Der, & Philips, 2015). FTIs, then, may represent a potential therapeutic for CS.

The pan-RAF inhibitors are able to inhibit MAP kinase signaling in the presence of wild type BRAF, while the $BRAF^{V600E}$ specific inhibitors, such as vemurafenib, cause paradoxical activation of the MAP kinase pathway in this context(Peng et al., 2015). ERK inhibitors, such as ulixertinib, were developed in order to combat the acquired resistance to MEK inhibitors that results from rebound MAP kinase reactivation. Ulixertinib is in clinical development in pediatrics and may be of benefit to individuals with RASopathies (Sullivan et al., 2018). Recently, several direct inhibitors of $KRAS^{G12C}$ have been developed, including ARS-1620 (Janes et al., 2018). These drugs have not yet been tested in children and are likely to have limited utility in individuals with RASopathies because the $KRAS^{G12C}$ variant is an uncommon mutation in the RASopathies. SHP2 inhibitors, which stabilize the auto-inhibited confirmation of this phosphatase, are promising RASopathy therapeutics for all RASopathies with aberrant MAP kinase activity except for NS caused by SHP2 (*PTPN11*) pathogenic variants(Nichols et al., 2018). Preclinical tool compounds that inhibit the interaction between RAS and SOS to inhibit RAS activation are currently in development and represent an exciting class of potential novel RASopathy therapeutics (Hillig et al., 2019). Finally, the MEK1/2 kinases require the heavy metal, copper, for their kinase activity. Copper chelation with ammonium tetrathiomolybdate (TTM), which is used clinically for individuals with Wilson disease, might represent a novel method by which to decrease MAP kinase signaling in individuals with RASopathies (Brady, Crowe, Greenberg, & Counter, 2017). Investigators at the NCI have extensive experience with the clinical use of the drugs that target the Ras/MAPK pathway and are thus uniquely positioned to facilitate the design of trials using these drugs in the treatment of individuals with RASopathies.

While most RASopathies are associated with increased Ras/MAPK activity, NSML caused by germline loss-of-function variants in *PTPN11*, is associated with decreased Ras/MAPK activity. Individuals with NSML, then, are unlikely to benefit from the Ras/MAPK directed therapeutics described above. Pathogenic variants of *PTPN11* that are associated with NSML instead induce an increase in PI3 kinase/AKT/mTOR activity. Importantly, hypertrophic cardiomyopathy and other RASopathy manifestations are reversed by post-natal administration of small molecule inhibitors of AKT or mTOR in mouse models of NSML (Marin et al., 2011; Wang et al., 2017), indicating that individuals with NSML may

derive therapeutic benefit from clinical inhibitors of the PI3 kinase pathway. In summary, there are many potential therapeutic options for individuals with RASopathies, and the most effective therapeutic for each individual may be disease- or even genotype-specific.

CONCLUSIONS:

Few interventional clinical trials evaluating potential therapies for individuals with RASopathies other than NF1 have been initiated to date, in part due to the rarity of some of the disorders, the spectrum of clinical manifestations, challenges in diagnosis, limited longitudinal follow-up, and lack of validated outcome measures. ART, particularly the longitudinal cohort study portion of the initiative, is designed to better characterize the natural history of RASopathy clinical manifestations and establish validated outcome measures that can be used to monitor the effectiveness of therapies in future interventional trials. The RASopathy research and patient advocacy communities have laid the foundations required for the development of clinical trials and possible effective therapies. In biennial RASopathy meetings over the past decade, the development of clinical trials and selection of adequate endpoints has been an important part of the agendas (Korf et al., 2015; Rauen et al., 2010; Stevenson et al., 2016). Individuals with a RASopathy are uniquely positioned to benefit from many of the RAS pathway-targeted therapies developed for cancer therapy (Rauen et al., 2011). Outreach activities through RASopathiesNet and the syndrome-specific family groups have indicated that individuals with a RASopathy are eager to engage in the scientific process. These individuals are interested in participating in clinical studies related to natural histories and therapeutic interventions, albeit with the knowledge that their participation may be taxing to them and their caregivers. Importantly, the NCI and NIH Clinical Center can facilitate ART because of the success of previous natural history and treatment studies of rare diseases at the NCI, and the expertise of investigators associated with the NCI RAS Initiative. The success of the ART, however, is dependent upon the continued input from international RASopathy experts and patient advocates who were present at the initiation meeting. Based on the positive experience from this meeting, we are optimistic that ART will benefit the RASopathy research and patient communities.

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REFERENCES:

Abrahamyan L, Feldman BM, Tomlinson G, Faughnan ME, Johnson SR, Diamond IR, & Gupta S (2016). Alternative designs for clinical trials in rare diseases. Am J Med Genet C Semin Med Genet, 172(4), 313–331. doi:10.1002/ajmg.c.31533 [PubMed: 27862920]

Altmuller F, Pothula S, Annamneedi A, Nakhaei-Rad S, Montenegro-Venegas C, Pina-Fernandez E, . . . Fejtova A (2017). Aberrant neuronal activity-induced signaling and gene expression in a mouse model of RASopathy. PLoS Genet, 13(3), e1006684. doi:10.1371/journal.pgen.1006684

Andelfinger G, Marquis C, Raboisson MJ, Theoret Y, Waldmuller S, Wiegand G, . . . Hofbeck M (2019). Hypertrophic Cardiomyopathy in Noonan Syndrome Treated by MEK-Inhibition. J Am Coll Cardiol, 73(17), 2237–2239. doi:10.1016/j.jacc.2019.01.066 [PubMed: 31047013]

Aoidi R, Houde N, Landry-Truchon K, Holter M, Jacquet K, Charron L, . . . Charron J (2018). Mek1(Y130C) mice recapitulate aspects of human cardio-facio-cutaneous syndrome. Dis Model Mech, 11(3). doi:10.1242/dmm.031278

Boon LM, Mulliken JB, & Vikkula M (2005). RASA1: variable phenotype with capillary and arteriovenous malformations. Curr Opin Genet Dev, 15(3), 265–269. doi:10.1016/j.gde.2005.03.004 [PubMed: 15917201]

Brady DC, Crowe MS, Greenberg DN, & Counter CM (2017). Copper Chelation Inhibits BRAF(V600E)-Driven Melanomagenesis and Counters Resistance to BRAF(V600E) and MEK1/2 Inhibitors. Cancer Res, 77(22), 6240–6252. doi:10.1158/0008-5472.CAN-16-1190 [PubMed: 28986383]

Brohl AS, Kahen E, Yoder SJ, Teer JK, & Reed DR (2017). The genomic landscape of malignant peripheral nerve sheath tumors: diverse drivers of Ras pathway activation. Sci Rep, 7(1), 14992. doi:10.1038/s41598-017-15183-1 [PubMed: 29118384]

Bromberg-White JL, Andersen NJ, & Duesbery NS (2012). MEK genomics in development and disease. Brief Funct Genomics, 11(4), 300–310. doi:10.1093/bfgp/els022 [PubMed: 22753777]

Bustelo XR, Crespo P, Fernandez-Pisonero I, & Rodriguez-Fdez S (2018). RAS GTPase-dependent pathways in developmental diseases: old guys, new lads, and current challenges. Curr Opin Cell Biol, 55, 42–51. doi:10.1016/j.ceb.2018.06.007 [PubMed: 30007125]

Cox AD, & Der CJ (2002). Ras family signaling: therapeutic targeting. Cancer Biol Ther, 1(6), 599–606. [PubMed: 12642680]

Cox AD, & Der CJ (2010). Ras history: The saga continues. Small GTPases, 1(1), 2–27. doi:10.4161/sgtp.1.1.12178 [PubMed: 21686117]

Cox AD, Der CJ, & Philips MR (2015). Targeting RAS Membrane Association: Back to the Future for Anti-RAS Drug Discovery? Clin Cancer Res, 21(8), 1819–1827. doi:10.1158/1078-0432.CCR-14-3214 [PubMed: 25878363]

Cox AD, Fesik SW, Kimmelman AC, Luo J, & Der CJ (2014). Drugging the undruggable RAS: Mission possible? Nat Rev Drug Discov, 13(11), 828–851. doi:10.1038/nrd4389 [PubMed: 25323927]

Dankner M, Rose AAN, Rajkumar S, Siegel PM, & Watson IR (2018). Classifying BRAF alterations in cancer: new rational therapeutic strategies for actionable mutations. Oncogene, 37(24), 3183–3199. doi:10.1038/s41388-018-0171-x [PubMed: 29540830]

Dombi E, Baldwin A, Marcus LJ, Fisher MJ, Weiss B, Kim A, . . . Widemann BC (2016). Activity of Selumetinib in Neurofibromatosis Type 1-Related Plexiform Neurofibromas. N Engl J Med, 375(26), 2550–2560. doi:10.1056/NEJMoa1605943 [PubMed: 28029918]

Dunzendorfer-Matt T, Mercado EL, Maly K, McCormick F, & Scheffzek K (2016). The neurofibromin recruitment factor Spred1 binds to the GAP related domain without affecting Ras inactivation. Proc Natl Acad Sci U S A, 113(27), 7497–7502. doi:10.1073/pnas.1607298113 [PubMed: 27313208]

Eleveld TF, Schild L, Koster J, Zwijnenburg DA, Alles LK, Ebus ME, . . . Molenaar JJ (2018). RAS-MAPK Pathway-Driven Tumor Progression Is Associated with Loss of CIC and Other Genomic Aberrations in Neuroblastoma. Cancer Res, 78(21), 6297–6307. doi:10.1158/0008-5472.CAN-18-1045 [PubMed: 30115695]

Flex E, Jaiswal M, Pantaleoni F, Martinelli S, Strullu M, Fansa EK, . . . Tartaglia M (2014). Activating mutations in RRAS underlie a phenotype within the RASopathy spectrum and contribute to leukaemogenesis. Hum Mol Genet, 23(16), 4315–4327. doi:10.1093/hmg/ddu148 [PubMed: 24705357]

Freeman AK, Ritt DA, & Morrison DK (2013). Effects of Raf dimerization and its inhibition on normal and disease-associated Raf signaling. Mol Cell, 49(4), 751–758. doi:10.1016/j.molcel.2012.12.018 [PubMed: 23352452]

Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, . . . Schultz N (2013). Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal, 6(269), pl1. doi:10.1126/scisignal.2004088

Gripp KW, Kawame H, Viskochil DH, & Nicholson L (2004). Elevated catecholamine metabolites in patients with Costello syndrome. Am J Med Genet A, 128A(1), 48–51. doi:10.1002/ajmg.a.30100 [PubMed: 15211656]

Gripp KW, Scott CI Jr., Nicholson L, McDonald-McGinn DM, Ozeran JD, Jones MC, . . . Zackai EH (2002). Five additional Costello syndrome patients with rhabdomyosarcoma: proposal for a tumor screening protocol. Am J Med Genet, 108(1), 80–87. doi:10.1002/ajmg.10241 [PubMed: 11857556]

Hernandez-Porras I, Fabbiano S, Schuhmacher AJ, Aicher A, Canamero M, Camara JA, . . . Barbacid, M. (2014). K-RasV14I recapitulates Noonan syndrome in mice. Proc Natl Acad Sci U S A, 111(46), 16395–16400. doi:10.1073/pnas.1418126111 [PubMed: 25359213]

Hillig RC, Sautier B, Schroeder J, Moosmayer D, Hilpmann A, Stegmann CM, . . . Bader B (2019). Discovery of potent SOS1 inhibitors that block RAS activation via disruption of the RAS-SOS1 interaction. Proc Natl Acad Sci U S A, 116(7), 2551–2560. doi:10.1073/pnas.1812963116 [PubMed: 30683722]

Hirata Y, Brems H, Suzuki M, Kanamori M, Okada M, Morita R, . . . Yoshimura A (2016). Interaction between a Domain of the Negative Regulator of the Ras-ERK Pathway, SPRED1 Protein, and the GTPase-activating Protein-related Domain of Neurofibromin Is Implicated in Legius Syndrome and Neurofibromatosis Type 1. J Biol Chem, 291(7), 3124–3134. doi:10.1074/jbc.M115.703710 [PubMed: 26635368]

Howe K, Clark MD, Torroja CF, Torrance J, Berthelot C, Muffato M, . . . Stemple DL (2013). The zebrafish reference genome sequence and its relationship to the human genome. Nature, 496(7446), 498–503. doi:10.1038/nature12111 [PubMed: 23594743]

Huang SC, Alaggio R, Sung YS, Chen CL, Zhang L, Kao YC, . . . Antonescu CR (2016). Frequent HRAS Mutations in Malignant Ectomesenchymoma: Overlapping Genetic Abnormalities With Embryonal Rhabdomyosarcoma. Am J Surg Pathol, 40(7), 876–885. doi:10.1097/PAS.0000000000000612 [PubMed: 26872011]

Huang WQ, Lin Q, Zhuang X, Cai LL, Ruan RS, Lu ZX, & Tzeng CM (2014). Structure, function, and pathogenesis of SHP2 in developmental disorders and tumorigenesis. Curr Cancer Drug Targets, 14(6), 567–588. [PubMed: 25039348]

Irving J, Matheson E, Minto L, Blair H, Case M, Halsey C, . . . Eckert C (2014). Ras pathway mutations are prevalent in relapsed childhood acute lymphoblastic leukemia and confer sensitivity to MEK inhibition. Blood, 124(23), 3420–3430. doi:10.1182/blood-2014-04-531871 [PubMed: 25253770]

Jaffre F, Miller CL, Schanzer A, Evans T, Roberts AE, Hahn A, & Kontaridis MI (2019). Inducible Pluripotent Stem Cell-Derived Cardiomyocytes Reveal Aberrant Extracellular Regulated Kinase 5 and Mitogen-Activated Protein Kinase Kinase 1/2 Signaling Concomitantly Promote Hypertrophic Cardiomyopathy in RAF1-Associated Noonan Syndrome. Circulation, 140(3), 207–224. doi:10.1161/CIRCULATIONAHA.118.037227 [PubMed: 31163979]

Janes MR, Zhang J, Li LS, Hansen R, Peters U, Guo X, . . . Liu Y (2018). Targeting KRAS Mutant Cancers with a Covalent G12C-Specific Inhibitor. Cell, 172(3), 578–589 e517. doi:10.1016/j.cell.2018.01.006 [PubMed: 29373830]

Jang ER, & Galperin E (2016). The function of Shoc2: A scaffold and beyond. Commun Integr Biol, 9(4), e1188241. doi:10.1080/19420889.2016.1188241

Jindal GA, Goyal Y, Burdine RD, Rauen KA, & Shvartsman SY (2015). RASopathies: unraveling mechanisms with animal models. Dis Model Mech, 8(9), 1167. doi:10.1242/dmm.022442 [PubMed: 26398161]

Jousma E, Rizvi TA, Wu J, Janhofer D, Dombi E, Dunn RS, . . . Ratner N (2015). Preclinical assessments of the MEK inhibitor PD-0325901 in a mouse model of Neurofibromatosis type 1. Pediatr Blood Cancer, 62(10), 1709–1716. doi:10.1002/pbc.25546 [PubMed: 25907661]

Keppler-Noreuil KM, Sapp JC, Lindhurst MJ, Darling TN, Burton-Akright J, Bagheri M, . . . Biesecker LG (2019). Pharmacodynamic Study of Miransertib in Individuals with Proteus

Syndrome. Am J Hum Genet, 104(3), 484–491. doi:10.1016/j.ajhg.2019.01.015 [PubMed: 30803705]

Koenighofer M, Hung CY, McCauley JL, Dallman J, Back EJ, Mihalek I, . . . Bodamer OA (2016). Mutations in RIT1 cause Noonan syndrome - additional functional evidence and expanding the clinical phenotype. Clin Genet, 89(3), 359–366. doi:10.1111/cge.12608 [PubMed: 25959749]

Korf B, Ahmadian R, Allanson J, Aoki Y, Bakker A, Wright EB, . . . Schoyer L (2015). The third international meeting on genetic disorders in the RAS/MAPK pathway: towards a therapeutic approach. Am J Med Genet A, 167A(8), 1741–1746. doi:10.1002/ajmg.a.37089 [PubMed: 25900621]

Kratz CP, Franke L, Peters H, Kohlschmidt N, Kazmierczak B, Finckh U, . . . Zenker M (2015). Cancer spectrum and frequency among children with Noonan, Costello, and cardio-facio-cutaneous syndromes. Br J Cancer, 112(8), 1392–1397. doi:10.1038/bjc.2015.75 [PubMed: 25742478]

Kratz CP, Rapisuwon S, Reed H, Hasle H, & Rosenberg PS (2011). Cancer in Noonan, Costello, cardiofaciocutaneous and LEOPARD syndromes. Am J Med Genet C Semin Med Genet, 157C(2), 83–89. doi:10.1002/ajmg.c.30300 [PubMed: 21500339]

Li D, March ME, Gutierrez-Uzquiza A, Kao C, Seiler C, Pinto E, . . . Hakonarson H (2019). ARAF recurrent mutation causes central conducting lymphatic anomaly treatable with a MEK inhibitor. Nat Med, 25(7), 1116–1122. doi:10.1038/s41591-019-0479-2 [PubMed: 31263281]

Lin AE, Alexander ME, Colan SD, Kerr B, Rauen KA, Noonan J, . . . Gripp KW (2011). Clinical, pathological, and molecular analyses of cardiovascular abnormalities in Costello syndrome: a Ras/MAPK pathway syndrome. Am J Med Genet A, 155A(3), 486–507. doi:10.1002/ajmg.a.33857 [PubMed: 21344638]

Marin TM, Keith K, Davies B, Conner DA, Guha P, Kalaitzidis D, . . . Kontaridis MI (2011). Rapamycin reverses hypertrophic cardiomyopathy in a mouse model of LEOPARD syndrome-associated PTPN11 mutation. J Clin Invest, 121(3), 1026–1043. doi:10.1172/JCI44972 [PubMed: 21339643]

Molzan M, Schumacher B, Ottmann C, Baljuls A, Polzien L, Weyand M, . . . Ottmann C (2010). Impaired binding of 14–3-3 to C-RAF in Noonan syndrome suggests new approaches in diseases with increased Ras signaling. Mol Cell Biol, 30(19), 4698–4711. doi:10.1128/MCB.01636-09 [PubMed: 20679480]

Mulero-Navarro S, Sevilla A, Roman AC, Lee DF, D'Souza SL, Pardo S, . . . Gelb BD (2015). Myeloid Dysregulation in a Human Induced Pluripotent Stem Cell Model of PTPN11-Associated Juvenile Myelomonocytic Leukemia. Cell Rep, 13(3), 504–515. doi:10.1016/j.celrep.2015.09.019 [PubMed: 26456833]

Nichols RJ, Haderk F, Stahlhut C, Schulze CJ, Hemmati G, Wildes D, . . . Bivona TG (2018). RAS nucleotide cycling underlies the SHP2 phosphatase dependence of mutant BRAF-, NF1- and RAS-driven cancers. Nat Cell Biol, 20(9), 1064–1073. doi:10.1038/s41556-018-0169-1 [PubMed: 30104724]

Niemeyer CM, Kang MW, Shin DH, Furlan I, Erlacher M, Bunin NJ, . . . Loh ML (2010). Germline CBL mutations cause developmental abnormalities and predispose to juvenile myelomonocytic leukemia. Nat Genet, 42(9), 794–800. doi:10.1038/ng.641 [PubMed: 20694012]

Nissim S, Leshchiner I, Mancias JD, Greenblatt MB, Maertens O, Cassa CA, . . . Goessling W (2019). Mutations in RABL3 alter KRAS prenylation and are associated with hereditary pancreatic cancer. Nat Genet, 51(9), 1308–1314. doi:10.1038/s41588-019-0475-y [PubMed: 31406347]

Oba D, Inoue SI, Miyagawa-Tomita S, Nakashima Y, Niihori T, Yamaguchi S, . . . Aoki Y (2018). Mice with an Oncogenic HRAS Mutation are Resistant to High-Fat Diet-Induced Obesity and Exhibit Impaired Hepatic Energy Homeostasis. EBioMedicine, 27, 138–150. doi:10.1016/j.ebiom.2017.11.029 [PubMed: 29254681]

Oishi K, Gaengel K, Krishnamoorthy S, Kamiya K, Kim IK, Ying H, . . . Gelb BD (2006). Transgenic Drosophila models of Noonan syndrome causing PTPN11 gain-of-function mutations. Hum Mol Genet, 15(4), 543–553. doi:10.1093/hmg/ddi471 [PubMed: 16399795]

Pandit B, Sarkozy A, Pennacchio LA, Carta C, Oishi K, Martinelli S, . . . Gelb BD (2007). Gain-of-function RAF1 mutations cause Noonan and LEOPARD syndromes with hypertrophic cardiomyopathy. Nat Genet, 39(8), 1007–1012. doi:10.1038/ng2073 [PubMed: 17603483]

Peng SB, Henry JR, Kaufman MD, Lu WP, Smith BD, Vogeti S, . . . Flynn DL (2015). Inhibition of RAF Isoforms and Active Dimers by LY3009120 Leads to Anti-tumor Activities in RAS or BRAF Mutant Cancers. Cancer Cell, 28(3), 384–398. doi:10.1016/j.ccell.2015.08.002 [PubMed: 26343583]

Poulin EJ, Bera AK, Lu J, Lin YJ, Strasser SD, Paulo JA, . . . Haigis KM (2019). Tissue-Specific Oncogenic Activity of KRAS(A146T). Cancer Discov, 9(6), 738–755. doi:10.1158/2159-8290.CD-18-1220 [PubMed: 30952657]

Rauen KA (2013). The RASopathies. Annu Rev Genomics Hum Genet, 14, 355–369. doi:10.1146/annurev-genom-091212-153523 [PubMed: 23875798]

Rauen KA, Banerjee A, Bishop WR, Lauchle JO, McCormick F, McMahon M, . . . Viskochil DH (2011). Costello and cardio-facio-cutaneous syndromes: Moving toward clinical trials in RASopathies. Am J Med Genet C Semin Med Genet, 157C(2), 136–146. doi:10.1002/ajmg.c.30294 [PubMed: 21495172]

Rauen KA, Schoyer L, McCormick F, Lin AE, Allanson JE, Stevenson DA, . . . Melese T (2010). Proceedings from the 2009 genetic syndromes of the Ras/MAPK pathway: From bedside to bench and back. Am J Med Genet A, 152A(1), 4–24. doi:10.1002/ajmg.a.33183 [PubMed: 20014119]

Rodriguez-Viciana P, Oses-Prieto J, Burlingame A, Fried M, & McCormick F (2006). A phosphatase holoenzyme comprised of Shoc2/Sur8 and the catalytic subunit of PP1 functions as an M-Ras effector to modulate Raf activity. Mol Cell, 22(2), 217–230. doi:10.1016/j.molcel.2006.03.027 [PubMed: 16630891]

Rooney GE, Goodwin AF, Depeille P, Sharir A, Schofield CM, Yeh E, . . . Ullian EM (2016). Human iPS Cell-Derived Neurons Uncover the Impact of Increased Ras Signaling in Costello Syndrome. J Neurosci, 36(1), 142–152. doi:10.1523/JNEUROSCI.1547-15.2016 [PubMed: 26740656]

Santoriello C, Deflorian G, Pezzimenti F, Kawakami K, Lanfrancone L, d'Adda di Fagagna F, & Mione M (2009). Expression of H-RASV12 in a zebrafish model of Costello syndrome causes cellular senescence in adult proliferating cells. Dis Model Mech, 2(1–2), 56–67. doi:10.1242/dmm.001016 [PubMed: 19132118]

Shern JF, Chen L, Chmielecki J, Wei JS, Patidar R, Rosenberg M, . . . Khan J (2014). Comprehensive genomic analysis of rhabdomyosarcoma reveals a landscape of alterations affecting a common genetic axis in fusion-positive and fusion-negative tumors. Cancer Discov, 4(2), 216–231. doi:10.1158/2159-8290.CD-13-0639 [PubMed: 24436047]

Steklov M, Pandolfi S, Baietti MF, Batiuk A, Carai P, Najm P, . . . Sablina AA (2018). Mutations in LZTR1 drive human disease by dysregulating RAS ubiquitination. Science, 362(6419), 1177–1182. doi:10.1126/science.aap7607 [PubMed: 30442762]

Stevenson DA, Schill L, Schoyer L, Andresen BS, Bakker A, Bayrak-Toydemir P, . . . Widemann BC (2016). The Fourth International Symposium on Genetic Disorders of the Ras/MAPK pathway. Am J Med Genet A, 170(8), 1959–1966. doi:10.1002/ajmg.a.37723 [PubMed: 27155140]

Stowe IB, Mercado EL, Stowe TR, Bell EL, Oses-Prieto JA, Hernandez H, . . . McCormick F (2012). A shared molecular mechanism underlies the human rasopathies Legius syndrome and Neurofibromatosis-1. Genes Dev, 26(13), 1421–1426. doi:10.1101/gad.190876.112 [PubMed: 22751498]

Sullivan RJ, Infante JR, Janku F, Wong DJL, Sosman JA, Keedy V, . . . Li BT (2018). First-in-Class ERK1/2 Inhibitor Ulixertinib (BVD-523) in Patients with MAPK Mutant Advanced Solid Tumors: Results of a Phase I Dose-Escalation and Expansion Study. Cancer Discov, 8(2), 184–195. doi:10.1158/2159-8290.CD-17-1119 [PubMed: 29247021]

Swaminathan G, & Tsygankov AY (2006). The Cbl family proteins: ring leaders in regulation of cell signaling. J Cell Physiol, 209(1), 21–43. doi:10.1002/jcp.20694 [PubMed: 16741904]

Tasian SK, Casas JA, Posocco D, Gandre-Babbe S, Gagne AL, Liang G, . . . Chou ST (2019). Mutation-specific signaling profiles and kinase inhibitor sensitivities of juvenile myelomonocytic leukemia revealed by induced pluripotent stem cells. Leukemia, 33(1), 181–190. doi:10.1038/s41375-018-0169-y [PubMed: 29884903]

Tidyman WE, & Rauen KA (2016a). Expansion of the RASopathies. Curr Genet Med Rep, 4(3), 57–64. doi:10.1007/s40142-016-0100-7 [PubMed: 27942422]

Tidyman WE, & Rauen KA (2016b). Pathogenetics of the RASopathies. Hum Mol Genet, 25(R2), R123–R132. doi:10.1093/hmg/ddw191 [PubMed: 27412009]

Villani A, Greer MC, Kalish JM, Nakagawara A, Nathanson KL, Pajtler KW, . . . Kratz CP (2017). Recommendations for Cancer Surveillance in Individuals with RASopathies and Other Rare Genetic Conditions with Increased Cancer Risk. Clin Cancer Res, 23(12), e83–e90. doi:10.1158/1078-0432.CCR-17-0631 [PubMed: 28620009]

Wang J, Chandrasekhar V, Abbadessa G, Yu Y, Schwartz B, & Kontaridis MI (2017). In vivo efficacy of the AKT inhibitor ARQ 092 in Noonan Syndrome with multiple lentigines-associated hypertrophic cardiomyopathy. PLoS One, 12(6), e0178905. doi:10.1371/journal.pone.0178905

Wu X, Simpson J, Hong JH, Kim KH, Thavarajah NK, Backx PH, . . . Araki T (2011). MEK-ERK pathway modulation ameliorates disease phenotypes in a mouse model of Noonan syndrome associated with the Raf1(L613V) mutation. J Clin Invest, 121(3), 1009–1025. doi:10.1172/JCI44929 [PubMed: 21339642]

Young LC, Hartig N, Boned Del Rio I, Sari S, Ringham-Terry B, Wainwright JR, . . . Rodriguez-Viciana P (2018). SHOC2-MRAS-PP1 complex positively regulates RAF activity and contributes to Noonan syndrome pathogenesis. Proc Natl Acad Sci U S A, 115(45), E10576–E10585. doi:10.1073/pnas.1720352115 [PubMed: 30348783]

Young LC, & Rodriguez-Viciana P (2018). MRAS: A Close but Understudied Member of the RAS Family. Cold Spring Harb Perspect Med. doi:10.1101/cshperspect.a033621

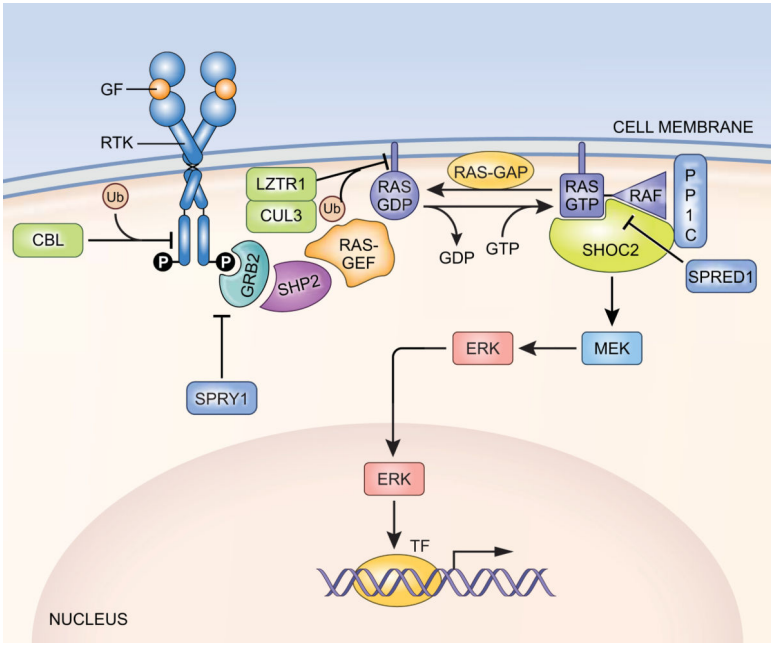

Figure 1:

RASopathy-associated genes are components of the Ras/MAPK pathway. In this figure, RAS GEF corresponds to SOS1 or SOS2, RAS GAP corresponds to NF1, RASA1, RASA2 or SYNGAP1, RAS corresponds to HRAS, NRAS, KRAS, RRAS, MRAS or RIT1, and RAF corresponds to ARAF, BRAF, or RAF1).

Table 1:

Clinical characteristics of Recognized RASopathies (excluding NF1)

Classical RASopathies

Non-Classical RASopathies

Syndrome	GENE	Syndrome Prevalence	Clinical Phenotype	Neoplasms
Noonan Syndrome; Noonan syndrome with multiple lentigines	*CBL, KRAS, MRAS, NRAS, PPP1CB, PTPN11, RAF1, SOS1*	1:2000 to 1:2500	Craniofacial dysmorphic features, congenital heart defects; short stature; undescended testicles; ophthalmologic abnormalities; bleeding disorders; normal neurocognitive function or mild impairment; multiple lentigines; ocular hypertelorism; pectus excavatum or carinatum; hearing loss	Neuroblastoma, acute lymphoblastic leukemia, acute myelogenous leukemia, juvenile myelomonocytic leukemia, low grade glioma, rhabdomyosarcoma
Costello Syndrome	*HRAS*	1:300,000 to 1:1.25 million	Craniofacial dysmorphic features; congenital heart defects; failure to thrive; short stature; ophthalmologic abnormalities; multiple skin manifestations, including papilloma; profound cognitive deficits; hypotonia	Rhabdomyosarcoma, neuroblastoma, transitional cell carcinoma of the bladder
Cardiofacio-cutaneous syndrome (CFC)	*BRAF, KRAS, MAP2K1, MAP2K2*	1:810,000	Craniofacial dysmorphic features; congenital heart defects; failure to thrive; short stature; ophthalmologic abnormalities; multiple skin manifestations, including progressive formation of nevi; cognitive impairment, seizure disorders	Potential increased risk of malignancy; not well described
Legius syndrome	*SPRED1*	Unknown (rare)	Café-au-lait maculae; intertriginous freckling; macrocephaly; normal neurocognitive function or mild impairment	Lipomas, acute myeloid leukemia, mucosal melanoma

Syndrome	GENE	Syndrome Prevalence	Clinical Phenotype	Neoplasms
Capillary arteriovenous malformation syndrome	*RASA1*	Unknown (rare)	Multifocal cutaneous capillary malformations, congenital heart defects	Potential increased risk of malignancy; not well described
SYNGAP1 syndrome	*SYNGAP1*	Unknown (rare)	Global developmental delay, hypotonia, seizures, bony abnormalities, strabismus, failure to thrive, autism spectrum disorders	Not known