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## The Third International Meeting on Genetic Disorders in the RAS/MAPK Pathway: Toward a Therapeutic Approach

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### Abstract

“The Third International Meeting on Genetic Disorders in the RAS/MAPK Pathway: Towards a Therapeutic Approach” was held at the Renaissance Orlando at SeaWorld Hotel (August 2–4, 2013). Seventy-one physicians and scientists attended the meeting, and parallel meetings were held by patient advocacy groups (CFC International, Costello Syndrome Family Network, NF Network and Noonan Syndrome Foundation). Parent and patient advocates opened the meeting with a panel discussion to set the stage regarding their hopes and expectations for therapeutic advances. In keeping with the theme on therapeutic development, the sessions followed a progression from description of the phenotype and definition of therapeutic endpoints, to definition of genomic changes, to identification of therapeutic targets in the RAS/MAPK pathway,

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to preclinical drug development and testing, to clinical trials. These proceedings will review the major points of discussion.

### Keywords

Ras; neurofibromatosis; noonan syndrome; costello syndrome; cardio-facio-cutaneous syndrome

## INTRODUCTION: THE RAS/MAPK PATHWAY DISORDERS

Molecular genetic discoveries have shown that a group of genetic syndromes with phenotypic overlap are caused by germline mutations perturbing RAS/mitogen activated protein kinase (MAPK) signaling [Rauen, 2013]. The disorders currently known to involve germline mutation of genes that encode proteins in the RAS/MAPK pathway are summarized in Table I. Although each disorder presents a distinct constellation of clinical problems, there are significant areas of overlap, including: (i) central nervous system dysfunction, resulting in particular learning difficulties and/or various degrees of intellectual disability; (ii) cardiovascular abnormalities, variously affecting the heart, blood vessels, and lymphatics; (iii) abnormalities of skeletal development; (iv) craniofacial anomalies and dysmorphia; (v) cutaneous lesions, including tumors, pigmentary changes, abnormal skin texture, and vascular malformations; and (vi) risk of benign and/or malignant tumors. The RAS/MAPK pathway plays a central role in the signal transduction of extracellular stimuli, principally through receptor tyrosine kinases, to the intracellular compartment [De Luca et al., 2012]. The pathway is critical for many cellular processes, including cancer biology, as somatic pathway mutations are observed in approximately 30% of malignancies [Bos, 1989]. It is also vital for human development, as evidenced by the craniofacial, cardiac, ectodermal, hematopoietic, musculoskeletal, and central nervous system defects shared by the heritable RAS/MAPK disorders.

### Session 1: RAS/MAPK Pathway Phenotypes and Therapeutic Goals

Defining of the phenotypes of the various RAS/MAPK pathway disorders is a necessary prerequisite to initiating therapeutic trials. Knowledge of the phenotypes and natural history will identify priorities for treatment and inform the identification of endpoints to judge efficacy. The session on RAS/MAPK Phenotypes and Therapeutic Goals was chaired by Drs. Karen W. Gripp (DuPont Hospital for Children) and Judith Allanson (Children's Hospital of Eastern Ontario). Speakers were encouraged to identify major priorities for therapeutic trials in each of the disorders.

Dr. Scott Plotkin (Massachusetts General Hospital) described an international collaboration to define clinical trial endpoints in neurofibromatosis, a program called REiNS (Response Evaluation in Neurofibromatosis and Schwannomatosis). He emphasized the importance of working closely with the FDA to identify endpoints that might be acceptable to approve clinical use of a drug. The REiNS collaboration includes working groups on imaging endpoints, functional endpoints, patient-reported outcomes, whole body MRI, visual endpoints for optic pathway glioma, neurocognitive endpoints, and biomarkers. Aside from working collaboratively with the FDA, he stressed the importance of international

collaboration and identification of intermediate endpoints to permit trial completion in a reasonable time period.

Dr. Emma Burkitt Wright (University of Manchester) described the cardiofaciocutaneous syndrome (CFC) phenotype. This condition is genetically heterogeneous, with mutations in *BRAF*, *MEK1*, *MEK2*, and *KRAS*. Some genotype–phenotype differences have been established, which will be important to take into account in organizing clinical trials. Major priorities for therapy include feeding problems, irritability, seizures, limited mobility and exercise intolerance, and hypertrophic cardiomyopathy.

Dr. Bronwyn Kerr (University of Manchester, UK) spoke on phenotypic features of Costello syndrome. This syndrome includes neurocognitive, cardiovascular and physical changes, including a significantly increased malignancy risk. More than 80% of affected individuals have cardiac problems, including congenital heart defects, cardiomyopathy, and arrhythmia [Lin et al., 2011]. Various activating mutations in *HRAS* have been identified, with some specific mutations predicting severity of manifestations. Therapeutic priorities include cardiomyopathy, adaptive behavior, hypotonia, feeding difficulties, and irritability. It was noted, however, that families tend to find children with Costello syndrome to have a pleasant social manner, and may be concerned about negative changes in behavior as an unintended consequence of treatment.

Dr. Amy Roberts (Boston Children’s Hospital) spoke about the phenotype of Noonan syndrome. Therapeutic priorities are hypertrophic cardiomyopathy, short stature, and learning disability. Twenty to thirty percent of affected individuals have hypertrophic cardiomyopathy, with some specific mutations more likely associated with this complication [Lee et al., 2011]. There have been encouraging preclinical studies in mouse models using MEK inhibitors. Short stature has been treated with growth hormone, leading to significant height gain. Neurocognitive problems exhibit some genotype–phenotype correlations. It was noted that learning improves in patients when stimuli are spaced far apart in time.

## Session 2: Genomic Approaches

Identification of the genes underlying the various disorders has been of key importance in elucidating biological mechanisms. Dr. Ludwine Messiaen (University of Alabama at Birmingham) moderated the session on genomic approaches. She began the session with a description of genetic studies of neurofibromatosis type 1 (NF1). Over 2,500 distinct pathogenic *NF1* variants have been identified; most lead to haploinsufficiency, though a considerable proportion (~16%) are missense variants. The latter present a challenge; family and functional studies are needed to classify them as pathogenic versus benign variants. Some genotype–phenotype correlations have been established, especially a severe phenotype associated with the constitutional 1.4 Mb microdeletion and absence of neurofibromas in those with a three base pair deletion in exon 17 [Upadhyaya et al., 2007]. Efforts are underway to perform more detailed analysis of genotype–phenotype correlations to confirm suggested associations or reveal new ones. She also reviewed studies of Legius syndrome, in which pigmentary features overlap with those of NF1 but tumors do not occur, due to mutation in *SPRED1*, which also encodes a RAS/MAPK protein.

Dr. Reza Ahmadian (Heinrich–Heine University, Düsseldorf, Germany) discussed structure–function relationships in mutations involving RAS, contrasting those associated with cancer with those associated with developmental disorders. Modulation of RAS function is complicated by a number of factors, including the complexity of the signaling pathway, the occurrence of multiple RAS isoforms, interactions with additional new modulatory proteins, diverse activation mechanisms, and various cellular compartments where RAS proteins are found. Dr. Conxi Lázaro (Catalan Institute of Oncology, Barcelona, Spain) described her work on the use of morpholinos (antisense oligonucleotides) as a therapeutic approach to treat cells from NF1 patients harboring a special type of splicing mutation (deep intronic mutation) to restore the correct function of the mutated NF1 protein, neurofibromin. In cells from these NF1 patients her group demonstrated a restoration of the normal splicing and demonstrated reduction of RAS-GTP levels [Pros et al., 2009; Fernández-Rodríguez et al., 2011]. In NF1, more than 30% of pathogenic variants affect the correct splicing of the gene, although a few proportion are deep intronic mutations; it has to be demonstrated if this approach can be used for other splice mutations as well. Challenges include the fact that each variant would require a specific oligonucleotide (morpholino or similar) and that these morpholinos would need to be delivered reliably to the cells. There is also a need for relevant preclinical models. Dr. Yoko Aoki (Tohoku University, Sendai, Japan) discussed efforts to identify pathogenic variants in patients with Noonan syndrome in whom no pathogenic variant had been found in the known genes. Whole exome sequencing revealed four that were noted to pathogenic variants in *RIT1*, which encodes a 25-kD protein in the RAS family. Altogether, pathogenic variants were found in 17/180 affected individuals. Most had congenital heart defects, and this phenotype is replicated in a zebrafish model.

### Session 3: RAS Pathway Biology and Identification of Therapeutic Targets Moderators

Detection of mutations in genes of the RAS/MAPK pathway opened the door to understanding of pathogenesis and identification of therapeutic targets. The session on this topic was led by Drs. Ype Elgersma (ERASmus University) and Alcino Silva (University of California, Los Angeles). A keynote talk on drug discovery was provided by Dr. G. Sitta Sittampalam (NIH), who described the TRND Program (Therapeutics for Rare and Neglected Diseases) within The National Center for Advancing Translational Science (NCATS) at NIH. The program is intended to encourage and speed the development of new drugs for rare and neglected diseases through collaborations with scientists at NIH, academia, nonprofit organizations, and pharmaceutical and biotechnology companies. Through these, collaborative efforts can be advanced to preclinical development and ultimately to clinical trials. A program focusing on the RAS/MAPK pathway is in progress. Other programs at NCATS of interest to the rare disease community include the BrIDGs Program (Bridging Interventional Development Gaps) to overcome obstacles in late stage preclinical development and the Rare Disease Clinical Research Network that supports studies therapeutic endpoints and clinical trials.

Dr. Silva described his work on preclinical targeting of neurocognitive deficits associated with RASopathies using mouse models. He showed evidence that RAS signaling can affect both excitatory and inhibitory neurons, and that cognitive phenotypes are not necessarily due to neurodevelopmental abnormalities, but may be caused instead by changes in adult mice.

He presented evidence that increased RAS activity in either inhibitory or excitatory neurons leads to deficits in long-term potentiation, and that these deficits can be reversed with statins or with MEK inhibitors. Dr. Elgersma discussed results with a Costello syndrome mouse model based on an *HRAS* activating mutation. The animals demonstrate neurocognitive deficits by mechanisms that differ from those in *Nf1* mutant mice. He further showed that the NF1 protein interacts with the hyperpolarization-activated cyclic nucleotide-gated (HCN) channel and that attenuation of the HCN current, together with increased MAPK-dependent synapsin phosphorylation, leads to increased inhibition seen in the *Nf1* mutants. Their studies highlight that different disorders in the RAS/MAPK pathway may exhibit neurocognitive defects by distinct mechanisms.

Dr. Sonia Mulero-Navarro (Icahn School of Medicine at Mount Sinai) described work with induced pluripotent stem cell lines from patients with Noonan syndrome to model disorders of myelopoiesis and cardiovascular development. She modeled juvenile myelomonocytic leukemia in this system and demonstrated upregulation of two micro RNAs.

Cardiomyocytes developed from iPS cells from patients with Noonan syndrome with multiple lentigines show an abnormal phenotype that can be rescued with rapamycin.

#### Session 4: Preclinical Drug Development and Testing

The RAS/MAPK pathway has been targeted for drug discovery due to its importance in cancer, and this opened the way to preclinical tests in developmental disorders as well. Dr. Bruce Gelb (Icahn School of Medicine at Mount Sinai) moderated this session. Dr. Gelb described a preclinical model for *RAF1* mutation using *Drosophila*. His group is screening for drugs that rescue pupal lethality in mutant flies. Dr. Maria Kontaridis (Harvard Medical School) described a mouse model based on *PTPN11* mutation that replicates many features of Noonan syndrome with multiple lentigines (NSML, formerly LEOPARD syndrome). Activation of the mTOR signaling pathway was noted, and hypertrophic cardiomyopathy in these animals is reversed by rapamycin treatment. She noted that a child with severe cardiomyopathy and NSML improved with everolimus treatment, at which point a heart transplant could be performed. Dr. Amy Roberts noted a clinical trial initiated based on this approach. Dr. Benjamin Neel (Ontario Institute for Cancer Research) described work with an activating *Raf1* mutation mouse model, which developed hypertrophic cardiomyopathy. The phenotype is rescued with postnatal treatment with a MEK inhibitor. Using a Cre-inducible system that targets different cell types in the heart they showed that involvement of both cardiomyocytes and endothelial cells is required to produce the full cardiomyopathy. They also developed iPS cells that model aspects of the cardiac phenotype, which are rescued by treatment with MEK inhibitor.

#### Session 5: Clinical Trials

Clinical trials began based on progress in identification of therapeutic targets and preclinical studies. This session was led by Dr. Katherine Rauen (UCSF). Dr. Bruce Korf (University of Alabama at Birmingham) described the Neurofibromatosis Clinical Trials Consortium (<http://cdmrp.army.mil/nfrp/consortium/trialDetails.shtml>), which is funded by the Department of Defense. The Consortium includes 17 sites in the US and 1 in Australia and is charged to perform clinical trials in NF1, NF2, and schwannomatosis. The rationale for

the Consortium is that clinical trials for rare disorders are best conducted when multiple centers pool their resources to increase patient enrollment. Several trials have been completed by the Consortium, and additional trials are in progress.

Dr. Reymundo Lozano (UC Davis) discussed his experience in clinical trials for fragile X syndrome. Though this condition is not a member of the RAS/MAPK pathway disorders, his experience may inform efforts to treat other rare disorders. He discussed ongoing and completed trials and related challenges, including dosing, drug safety, and definition of therapeutic endpoints. Dr. Chao Zhang (Plexxikon) spoke about efforts to test drugs targeting BRAF in melanoma. He noted a paradoxical activation of the MAPK pathway in tumors treated with BRAF inhibitors, leading to cutaneous squamous cell carcinomas and keratocanthomas. A new class of BRAF inhibitors does not have this effect. Dr. Calum MacRae (Brigham and Women's Hospital) described an open-label proof of concept trial of a MEK inhibitor in 15 patients with Noonan syndrome. The study targets hypertrophic cardiomyopathy and evaluation takes place after 6 months of treatment.

### **Session 6: Infrastructure to Support Therapeutic Development**

The RAS/MAPK disorders are rare individually, which creates a challenge in conducting clinical research and clinical trials. This session, chaired by Dr. Amy Roberts (Children's Hospital, Boston) was intended to explore the infrastructure needs to facilitate research. Dr. Annette Bakker (Children's Tumor Foundation) described the therapeutic pipeline put in place to facilitate development of treatments for neurofibromatosis. CTF provides funding to basic science and clinical investigators, as well as for patient and professional education. It has been proactive in facilitating preclinical drug testing, partnering with the pharmaceutical industry, and developing a patient registry and biobank. Dr. Corinne Linardic (Duke University School of Medicine) described the tissue bank set up as a central facility to support research with the Children's Oncology Group. Tissues are accessioned centrally under IRB-approved protocols, processed, and then available to investigators for research. Brian Denger (Parent Project Muscular Dystrophy) described their patient registry - DuchenneConnect. This is a structured database into which parents, rather than physicians, enter data. The database is intended to serve as a resource both for clinicians and researchers, and is maintained on a platform provided by Patient Crossroads. The registry also serves as an educational resource for families to include Fact Sheets about aspects of the condition and treatment options as well as regular webinars concerning current care and research issues.

## **CONCLUSIONS**

The RAS signaling pathway first came to light through studies of cancer, but it has become clear that germline mutations that lead to activation of the MAPK pathway lead to a group of developmental disorders; some, but not all, include a predisposition to cancer. The conditions have overlapping phenotypes, but each also has distinct features. The possibility of therapy aimed at reduction of activity of RAS signaling is now within reach, but will require careful consideration of the individual phenotypes and therapeutic goals, definition of clear endpoints, and collaborative efforts to conduct both preclinical studies and clinical



trials. This meeting not only provided a scientific overview of the current state of the field, but represents in itself a collaborative effort among clinicians and scientists who deal with these disorders, and active engagement with patients and families who are directly affected.

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**TABLE I**  
**Summary of Disorders Associated With Germ Line Mutations in Genes Encoding Proteins in the RAS/MAPK Pathway**

Disorder	Genetics	Features	Malignancy	References
Capillary malformation-AV malformation syndrome	Inactivating RASA1 mutations [Bos, 1989]	Capillary and AV malformations	No clear association reported	Eerola et al. [2003]
Cardio-facio-cutaneous (CFC) syndrome	Activating mutations in BRAF, MEK1, MEK2, KRAS	Cardiovascular (pulmonic stenosis, septal defects, hypertrophic cardiomyopathy) Craniofacial abnormalities Dermatologic xerosis, hyperkeratosis	Unclear but rare: Acute lymphoblastic leukemia, lymphoma, neuroblastoma, meningioma	Niihori et al. [2006]; Rodriguez-Viciana et al. [2006]
Costello syndrome	Activating mutations in HRAS	Failure to thrive Intellectual disability Coarse facies Papillomata Abnormal hair Hypertrophic cardiomyopathy Macrocephaly	Rhabdomyo-sarcoma, transition cell carcinoma, neuroblastoma	Aoki et al. [2005]
Hereditary gingival fibromatosis type 1	Activating mutations in SOS1 (frameshift mutations)	Gingival overgrowth	None	Jang et al. [2007]
Legius syndrome	Inactivating SPRED1 mutations	Cafe-au-lait macules Skin fold freckling Macrocephaly	No clear association at this time (if present they are rare)	Brems et al. [2007]; Messiaen et al. [2009]
Noonan syndrome with multiple lentigines	Inactivating mutations in PTPN11, RAF1	Lentigines Cardiac conduction abnormalities Ocular hypertelorism, Genital anomalies Abnormal growth, Sensorineural deafness	No clear association at this time but reports of acute myelogenous leukemia, neuroblastoma, ALL, melanoma	Pandit et al. [2007]
Neurofibroma-tosis type 1	Inactivating NF1 mutations	Cafe-au-lait macules Skin fold freckling Macrocephaly Neurofibromas Skeletal dysplasia Learning disabilities	Glioma, malignant peripheral nerve sheath tumor, juvenile myelomonocytic leukemia, gastrointestinal stromal tumor, glomus tumor	Boyd et al. [2009]
Noonan syndrome	Activating mutations in PTPN11, SOS1, KRAS, NRAS, RAF1, SHOC2, CBL, RIT1	Short stature Cardiovascular defects Intellectual disability Skeletal anomalies Genital anomalies Lymphatic dysplasias Ocular anomalies	Myeloproliferative disorder, juvenile myelomonocytic leukemia, neuroblastoma, rhabdomyo-sarcoma; estimated 3.5 fold increased risk of cancer in PTPN11 associated NS	Jongmans et al. [2011]; Tartaglia et al. [2010]