Costello and Cardio-Facio-Cutaneous Syndromes: Moving Toward Clinical Trials in RASopathies

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Abstract
The RASopathies, one of the largest groups of multiple congenital anomaly syndromes known, are caused by germline mutations in various genes encoding components of the Ras/mitogen-activated protein kinase (MAPK) pathway. The RASopathies have many overlapping characteristics, including craniofacial manifestations, cardiac malformations, cutaneous, musculoskeletal, gastrointestinal, and ocular abnormalities, neurocognitive impairment, hypotonia, and an increased risk of developing cancer. Costello syndrome (CS) and cardio-facio-cutaneous (CFC) syndrome are two of the more rare RASopathies. CS is caused by activating mutations in HRAS, and CFC is caused by dysregulation of signaling in the Ras/MAPK pathway due to mutations in BRAF, MEK1, or MEK2. The Ras/MAPK pathway, which has been well-studied in cancer, is an attractive target for inhibition in the treatment of various malignancies utilizing small molecule therapeutics that specifically inhibit the pathway. With many inhibitors of the Ras/MAPK pathway in clinical trials, the notion of using these molecules to ameliorate developmental defects in CS and CFC is under consideration. CS and CFC, like other syndromes in their class, have a progressive phenotype and may be amenable to inhibition or normalization of signaling.
INTRODUCTION

The Ras/mitogen-activated protein kinase (MAPK) pathway is essential in the regulation of the cell cycle, differentiation, growth, and senescence, all of which are critical to normal mammalian development. The Ras/MAPK pathway has been extensively studied in the context of cancer since Ras was found to be somatically mutated in approximately 20% of malignancies [Bos, 1989], and hyperactivated ERK is found in approximately 30% of human cancers [Hoshino et al., 1999] due to activating mutations in either Ras or BRAF.

The RASopathies are a group of medical genetic syndromes and may represent one of the largest groups of multiple congenital anomaly syndromes known. These include Noonan syndrome (NS), LEOPARD syndrome, capillary malformation-AV malformation syndrome, neurofibromatosis type 1 (NF1), Costello syndrome (CS), cardiofacio-cutaneous syndrome (CFC), and Legius syndrome [Tidyman and Rauen, 2008]. Caused by germline mutations in various key genes encoding components of the Ras/MAPK pathway, the RASopathies have many overlapping characteristics, including craniofacial manifestations, cardiac malformations, cutaneous, musculoskeletal, and ocular abnormalities, neurocognitive impairment, hypotonia, and an increased cancer risk.

Costello syndrome and CFC are two of the more rare RASopathies with estimates of several hundred individuals for each group world-wide. CS is caused by activating mutations in HRAS whereby approximately 80% of patients with a molecular diagnosis have the common missense mutation pG12S. CFC is caused by dysregulated Ras/MAPK signaling. The mutations that cause CFC are more heterogeneous than the mutations in CS; approximately 75% of patients with a molecular diagnosis have BRAF mutations, and about 25% of CFC individuals have a mutation in either MAP2K1 (MEK1) or MAP2K2 MEK2 (for review see Tidyman and Rauen [2008]). Both CS and CFC have organized and active family advocacy groups. The CS Family Network (CSFN) based in the US works very closely with the International CS Support Group (ICSSG; www.costellokids.com). This group has an active registry and is working toward building a database of registrants. Likewise, CFC International, also based in the US, reaches out worldwide to families and has built a database of registrants that includes a biobank (www.cfcsyndrome.org). These advocacy groups are in the process of uniting to create “The RASopathy Network” (www.ras-pathway-syndromes.com).

The Ras/MAPK pathway is an attractive target in the treatment of cancer utilizing small molecule therapeutics that specifically inhibit the pathway. Many are in development and several are currently undergoing clinical trials, with some already FDA approved [Sebolt-Leopold, 2008]. Ras pathway agents, such as farnesyl transferase inhibitors (FTIs) that prevent posttranslational modification of Ras, are being evaluated for cancer treatment and...
may be of therapeutic use for syndromes in this pathway, especially CS. In addition, BRAF and MEK inhibitors offer the same potential in the possible treatment of CS and CFC. Thus, the same molecular inhibitors of the Ras/MAPK pathway being developed as cancer therapeutics may provide opportunities to therapeutically treat the developmental disorders caused by Ras/MAPK hyperactivation. Because many of the phenotypic signs and symptoms of the RASopathies are not static, the possible use of systemic therapies after birth to reduce MAPK activity holds the potential to ameliorate disease progression of some signs and symptoms. Proof of principle for using small molecule inhibition of an activated Ras pathway has been demonstrated in animal models for Apert syndrome, a craniosynostosis syndrome caused by a germline mutation in fibroblast growth factor receptor 2 (FGFR2) [Shukla et al., 2007] and in NS caused by SOS1 activation [Chen et al., 2010]. In these studies, treatment of mice with a MEK inhibitor or small hairpin RNAs restored normal Ras/MAPK signaling when treatment was used either pre-natally or post-natally. Thus, there is potential for systemic therapeutic options for other Ras pathway-activated syndromes. However, moving toward clinical trials for these rare populations will require a thoughtful approach, including selection of robust endpoints and the optimal small molecule inhibitor for each syndrome. Most importantly, clinical trials for these rare syndromes will require a united front between basic science and clinical translational researchers, private industry and advocacy organizations.

IMPORTANT ISSUES IN EARLY TRIAL DESIGN

Implementing a clinical trial for a rare disorder is a daunting task. Introducing a drug that interferes with activation of Ras or the functioning of the MAPK pathway in a non-cancer setting will be challenging on many levels. Understanding the molecular and biological characteristics in cell culture and animal models is the first step in optimally designing the initial clinical trials. From a trial’s aspect, the Phase I trial is the first step in studying a novel agent in a disorder or disease population. Unless the safety and maximally tolerated dose (MTD) of the drug has been defined in a similar population, these two objectives will be the primary endpoints of the Phase I trial. The preliminary efficacy (how well the drug works) is evaluated, but this is usually considered a secondary endpoint. The first dose level of the trial is typically derived from large animal toxicity assessment. Once the starting dose and frequency of administration is defined, three to six patients are enrolled at this dose level and then observed for a pre-specified period for safety (www.ctep.cancer.gov). If no dose-limiting toxicities are seen, the dose is escalated by a modified Fibonacci method to the next higher level until the MTD and recommended Phase II dose is identified [Eisenhauer et al., 2000]. A typical Phase I study enrolls approximately 15–50 patients. Once the MTD is established, 6–12 patients are treated at this dose for thorough safety assessment and preliminary insight into possible efficacy is gained. Once a Phase II dose is established, a Phase II trial will evaluate efficacy in a defined patient population (typically 40–400 patients). Subsequent Phase III trials compare the novel agent in a randomized fashion against the best currently available treatment option or best supportive care. Phase III trials often range from 50 to several hundreds of patients. Because of the rarity of CFC and CS, even the initial trial evaluating safety should include carefully considered secondary endpoints to assess preliminary efficacy. Establishing the optimal dose may involve the vast
The majority of known mutation-positive individuals in the United States alone. Therefore, the secondary endpoints for a Phase I trial should be selected to optimize the likelihood of benefit for the patients.

The objective of a clinical trial for CS and CFC would be to define a measurable change from baseline signs or symptoms and determine what would entail a meaningful measurable difference for the patient. To safeguard against prolonged drug exposure in the absence of benefit, Phase I trials typically evaluate endpoints within 6–8 weeks. In the absence of a detectable benefit, patients are removed from the study. In keeping with the mandate of safety first, endpoints should therefore be considered with short-term measurable benefit in mind. As an example, while changes in cardiac or neurological function may not be seen following a short period of treatment, changes in constitutional signs and symptoms or dermatological changes may be observed. These may, therefore, be more feasible endpoints in the first clinical trial. With the rarity of the patients, secondary endpoints, which may not yield a significant change, should nonetheless be monitored even in initial trials so as not to miss a potential positive impact.

Drug development in a genetic disorder of an activated Ras/MAPK pathway offers a unique opportunity to assess pharmacological and pharmacodynamic markers, ranging from drug levels to changes in pathway activation in tissues such as peripheral blood mononuclear cell, hair follicles, and skin. Much can be learned with minimally invasive tests. The RASopathy patient population is in a unique position; many novel agents exist to potentially alleviate signs and symptoms for CS and CFC patients and their families. Prior to engaging in a clinical trial, defining a clear research question and study outline is critical. In addition, a multidisciplinary team should include the experts in the field, caregivers, and patients to overcome the considerable hurdles in dealing with an entirely novel field of drug development in such a rare and unique patient population.

Choosing Proper Populations and Endpoints for Costello and CFC Syndromes

An important consideration for any clinical intervention is selection of the appropriate population to be recruited and enrolled in a clinical trial. Key considerations include confidence in affected status (use of disease-specific diagnostic criteria), gender, age, ethnicity, ancestral background, and knowledge of the molecular etiology. These considerations are important for both safety and efficacy issues. With respect to safety, patients who are clinically deteriorating may demonstrate changes while on therapy that are due to disease progression rather than effects of the intervention. The application of a functional scale as part of the eligibility criteria enables investigators to recruit patients in the same general health state for a given disorder. For example, use of the Karnofsky and Lansky performance scores (10–100) can discriminate patients who have more severe disease, which could place them in a higher risk category for exposure to an agent that may not have a well-defined safety profile in patients with significant health problems [Lansky et al., 1987].

The proper selection of a subpopulation within a specified condition that shares a common clinical manifestation is crucial to a successful trial, especially in disorders with highly variable clinical expressivity. There are practical contingencies in this selection process,
specifically in recruitment of potential patients. The selection of rare manifestations in rare conditions could lead to significant difficulties in completing enrollment. The appropriate selection of a population is best illustrated in the eligibility and exclusion criteria, which can be quite extensive. Integral in this process is the capability of measuring a specific clinical manifestation and the level of confidence in objectively measuring anticipated changes in this manifestation. In essence, this represents the determination of endpoints that will determine the outcome and feasibility of the clinical trial.

In general, multiple endpoints are selected to best illustrate efficacy of intervention. They are usually measurable objective values that are determined by a team of experts, and consist of primary and secondary endpoints. The identification of a primary endpoint is required to establish the optimal number of subjects to be enrolled as determined by a power analysis using a hypothetical anticipated change in the manifestation associated with the intervention. The use of surrogate markers is an important consideration in situations where biologic endpoints are difficult to measure. A surrogate marker is typically a laboratory measurement or a physical sign used as a substitute for a clinically meaningful endpoint.

In CS and CFC, a number of issues can be considered in devising a clinical trial. First and foremost is the consideration to either lump or split the two conditions. Given each condition’s rarity and known convergence in the Ras pathway, there is good reason to consider pooling the patient population providing there is a common manifestation to be treated with a specific agent. Potential endpoints under consideration for the combined recruitment approach include: cardiomyopathy, short stature, neurocognition, and hypotonia (Table I). However, given the genetic distinction of each syndrome’s genetic cause, with CS being caused by activating mutations in \(HRAS\) and CFC caused by mutations in \(BRAF\), \(MEK1\), or \(MEK2\), separate clinical trials may prove to be a better approach. One final thought of which to be mindful, especially for rare disorders, is that quality of life surveys are now recognized as an important tool for clinical trials, especially in those instances where a patient reports improvement in overall function or well-being that is not captured by the primary or key secondary endpoints [Stevenson and Carey, 2009].

The Ras/MAPK Pathway Function and Feedback Loops

The Ras/MAPK pathway is essential in cellular homeostasis and, therefore, critical to normal mammalian development. The germline mutations associated with RASopathies are in genes that encode proteins of this pathway and in vitro studies indicate that the vast majority of these mutations result in increased signal transduction. Due to the vital functions of the Ras pathway, it is not surprising that germline mutations that alter its activity have profound effects on development. However, much that we have learned about this pathway has come from the study of cancer.

The hierarchical relationship between Ras, Raf, MEK, ERK (MAPK) has been well established in model systems and in mammalian cells (Fig. 1). However, the linear nature of the pathway leads to the false expectation that drugs blocking Raf kinase would be an effective way of reversing transformation by oncogenic Ras, and that Raf kinase inhibitors would have similar properties to MEK inhibitors in terms of clinical efficacy. Neither of these expectations is correct. In cells expressing active mutant Ras, BRAF kinase inhibitors
have been shown to paradoxically activate MEK and ERK, and have so far failed to show efficacy in tumors with mutant Ras (for review see McCormick, 2011). Given that BRAF inhibitors activate ERK, one might expect MEK inhibitors to do the same; however, this is not the case. MEK inhibitors decrease MAPK signaling as expected and do not appear to paradoxically activate the MAPK pathway. Unfortunately, MEK inhibitors have also made little impact in clinical trials to date. These compounds can activate the Akt pathway (another downstream cascade of Ras) possibly accounting for or contributing to their lack of clinical efficacy in cancer.

The mechanism underlying paradoxical activation of downstream effectors by BRAF kinase inhibitors is not fully understood, but involves dimerization of Raf proteins, induced by activated Ras. Dimerization promotes cross phosphorylation reactions between individual kinase proteins; one of these events inhibits kinase activity. Relief of this inhibitory autophosphorylation increases Raf kinase activity and allows MEK activation. At increasing concentrations of Raf kinase inhibitors, MEK phosphorylation is inhibited, but this may require levels of Raf inhibition that are hard to achieve in vivo.

MEK regulation does not involve this type of cross talk. However, MEK and possibly ERK inhibition leads to activation of upstream signaling through relief of negative feedback loops to receptors. This leads to activation of alternate downstream pathways, of which phosphatidylinositol 3′-kinase (PI3’K) appears to be of greatest concern, as this can protect cells from apoptosis and promote cell division. Therefore, for cancer treatment, combinations of MEK inhibitors and receptor inhibitors may be necessary to shut down signaling in cells with a hyperactive MAPK pathway (Fig. 1). These concepts are important when considering small molecule modulators for clinical trials in patients with a RASopathy, being mindful that ideally one would like to “dial down” MAPK activity in these patients and perhaps bypass unwanted paradoxical pathway effects, as opposed to fully abrogating its activity as may be needed in cancer treatment.

POTENTIAL COMPOUNDS FOR TREATING CS AND CFC

Farnesyl Transferase Inhibitors

Farnesyl transferase inhibitors (FTIs) inhibit the enzyme that transfers the farnesyl group onto Ras and other proteins. They were initially developed as potential anti-cancer agents targeting Ras function (for review see Basso et al., 2006}). Several FTIs entered human clinical trials for cancer, including tipifarnib (Johnson & Johnson Pharmaceuticals) and lonafarnib (Schering-Plough Research Institute, now part of Merck Research Labs). Both compounds potently block HRAS function in cells (Table II). In contrast, the KRAS isoform can be post-translationally modified by a distinct prenyl transferase when farnesyl transferase is inhibited, providing an escape mechanism by which KRAS remains associated with the plasma membrane. In Phase I studies in adult cancer patients, the MTD for lonafarnib was 200 mg when dosed orally, twice daily, on a continuous schedule. The primary dose-limiting toxicities were gastrointestinal [Eskens et al., 2001]. The MTD for tipifarnib was 300 mg when dosed twice daily for 21 consecutive days followed by 1 weekoff. Dose-limiting toxicity was my elosuppression [Punt et al., 2001]. Despite promising activity in early trials, neither compound demonstrated sufficient activity in Phase
III studies to warrant regulatory approval for cancer treatment. The success of these studies was limited by an incomplete understanding of which farnesylated proteins account for the observed anti-tumor activity of FTIs and a subsequent inability to select responsive patient subpopulations.

Although FTIs were deemed disappointing in cancer treatment, a potential success story exists in the use of FTIs for the treatment of Hutchinson–Gilford Progeria syndrome (HGPS). HGPS is a rare, fatal genetic syndrome characterized by accelerated aging and caused by a missense point mutation in the lamin A. In healthy individuals, lamin A is farnesylated post-translationally followed by a proteolytic cleavage event which removes the farnesylated carboxy-terminus. Mature lamin A lacks the farnesyl group. In HGPS patients, this proteolytic cleavage step fails to occur, leading to accumulation of an abnormal farnesylated form of lamin A. In vitro and in vivo studies demonstrated that FTIs can correct the abnormal structure of the nuclear envelope caused by mutations in lamin A [Fong et al., 2006]. Importantly, tipifarnib treatment was shown to prevent progression of existing cardiovascular disease in a transgenic mouse model of HGPS [Capell et al., 2008]. Based on this strong preclinical data, the Progeria Research Foundation collaborated with investigators at Children’s Hospital, Boston and Schering-Plough Research Institute to initiate an open-label, clinical trial with a single agent, historically controlled study where all children received lonafarnib for 2 years. The primary endpoint was the change in the rate of weight gain over baseline for each patient with multiple secondary endpoints including changes in cardiovascular function. This trial was fully enrolled by October 2007 (n = 27), and final data analysis is ongoing. These children are now participating in a follow-on study where they are receiving lonafarnib in combination with a statin and a bisphosphonate (www.progeriaresearch.org).

The identification of HRAS mutations as the molecular cause of CS raises the possibility that FTIs may provide clinical benefit to patients. There is extensive clinical experience in both adult and pediatric populations with both tipifarnib and lonafarnib. This experience would prove valuable in guiding dose selection in Costello patients. Another consideration for CS is the ability of the FTI to penetrate into the brain and potentially address neurocognitive aspects of this syndrome. A number of practical considerations in selecting novel agents in a rare, pediatric disorder have been learned from the HGPS experience. These include the potential need to adjust dosing to mg/m² (from flat mg dose), the potential need to reformulate (liquid suspension vs. capsule/tablet), and the importance of assessing pharmacokinetic/pharmacodynamic relationships in preclinical efficacy models and in patient populations. These considerations are in addition to more complex issues including insuring availability of long-term drug supply and interactions with regulatory agencies if positive clinical data should emerge from these trials.

**Raf Inhibitors and MEK Inhibitors**

A growing number of small molecule inhibitors of BRAF and MEK have now entered clinical testing (Table II). Not only does a unique set of clinical agents exist for each target class, but each class also exhibits a different spectrum of activities and safety profiles. Agents targeting Raf are generally ATP competitive. Nexavar (sorafenib) is the first MAPK
pathway inhibitor to win regulatory approval, and it is active against renal cell and hepatocellular carcinomas [Abou-Alfa, 2009]. This agent was originally identified as a potent inhibitor of both CRAF and BRAF but is now known to be a multi-targeted kinase inhibitor. Subsequently, the discovery of oncogenic BRAF mutations in human tumors fueled efforts to design selective BRAF inhibitors. One such agent, PLX4032, recently entered Phase III testing based on its promising clinical activity in melanoma patients [Bollag et al., 2010]. Interestingly, BRAF-selective agents appear to be active only in BRAF-mutated tumors and not in Ras-activated, BRAF wild-type tumors. As described above, paradoxical activation of ERK signaling has been observed in tumors with wild-type BRAF and Ras, and in tumors with mutant Ras. This finding is consistent with observed clinical activity for this agent being restricted to patients with BRAF-mutated tumors. From a safety perspective, PLX4032 has not been reported to be brain-penetrant and has not elicited CNS-related toxicity concerns. Toxicities such as fatigue, rash, and joint pain have been noted [Flaherty et al., 2010]. Furthermore, roughly one-third of patients treated at the MTD developed keratoacanthoma type skin lesions, a class of cutaneous squamous cell carcinomas [Bollag et al., 2010].

Unlike Raf inhibitors, MEK inhibitors are generally non-ATP competitive and bind in an interior hydrophobic pocket that is adjacent to, but distinct from MEK’s ATP binding site. The uniqueness of the MEK enzyme structure and the allosteric binding mode of MEK inhibitors confer upon these compounds a high degree of selectivity. CI-1040 was the first MEK inhibitor clinical candidate [Sebolt-Leopold et al., 1999] and has subsequently spurred a wave of structurally related analogues with a wide range of target potency and pharmacological properties (Table II). Currently, there are roughly a dozen MEK inhibitor clinical candidates being evaluated in Phase I/II clinical trials. Preclinical data suggest that, as with BRAF inhibitors, MEK inhibitors would prove efficacious in melanoma patients. This prediction has been borne out by the observance of objective clinical responses in melanoma patients treated with the MEK inhibitors PD0325901 [Sebolt-Leopold, 2008] or GSK1120212 [Infante et al., 2010]. With respect to their safety profiles, as a target class, MEK inhibitors are generally associated with rash and diarrhea, toxicities that are likely to be mechanism-related. Early MEK inhibitors (CI-1040, PD0325901, AZD6244) have also been reported to elicit visual disturbances, as evidenced by transient and reversible blurred vision. In the case of PD0325901, retinal vein occlusions were observed in three patients and were associated with delayed onset, i.e., after 3–4 months of continuous daily dosing. Neurologic toxicities were also occasionally observed at the highest doses tested of PD0325901. The newest wave of MEK inhibitors is not thought to be brain-penetrant and, thus far, problematic neurologic and ocular toxicities have been avoided.

The use of Raf or MEK inhibitors in individuals with CS or CFC to treat phenotypic manifestations has sparked much discussion. Two questions are central to the consideration of their use in CS or CFC patients. First, would they be efficacious, and very importantly, are they safe, especially since therapy would more than likely be long term? With respect to BRAF inhibitors, the observation that BRAF-selective agents activate ERK signaling in non-BRAF-mutated tumors raises the concern that not only would these agents not be effective in CS or CFC, but they may be contraindicated in non-oncology settings by
promoting the growth of keratoacanthoma skin lesions. The rationale for efficacy in CS or CFC appears to be stronger for MEK inhibitors. However, the risk-to-benefit ratio needs to be carefully considered for this target class. There exist brain-penetrant MEK inhibitors that conceptually possess potential to remedy the neurocognitive developmental problems encountered in the treatment of CS or CFC patients. However, the critical question remains whether efficacious doses can be reached at sufficiently low levels to avoid the neurologic and ocular toxicities which can occur in cancer patients. A highly conservative clinical dosing scheme accompanied by frequent ocular monitoring appears warranted to address a potential therapeutic niche for MEK inhibitors in the treatment of CS and CFC.

Taking MEK Inhibitors Into Pediatric Clinical Trials

The first clinical trial using a MEK inhibitor in a pediatric population is currently underway. The trial is a Phase I and pharmacokinetic study using the AstraZeneca compound AZD6244 for recurrent or refractory low grade glioma. The trial is being conducted by the Pediatric Brain Tumor Consortium (www.pbtc.org; www.clinicaltrials.gov). Low-grade astrocytoma, one of the most common primary brain tumors of childhood, is associated with high rates of morbidity from radiation therapy. Thus, new treatment strategies are needed. Previous studies have determined that the vast majority of low-grade glioma have Ras/MAPK pathway activation primarily through activation of BRAF, either by a novel genetic fusion of BRAF or by point mutations resulting in the common p.V600E mutation [Schiffman et al., 2010]. Because of this, the use of a downstream MEK inhibitor may have anti-tumor effects in BRAF-activated tumors. The primary objective of trial PBTC-029 is to estimate the MTD and/or recommend a Phase II dose of AZD6244 in children with recurrent or refractory low-grade glioma. In addition, this trial will describe the toxicity profile and define the dose-limiting toxicity of AZD6244. The secondary objectives of this trial are to describe patient variability in AZD6244 pharmacokinetics, describe MRI characteristics and diffusion changes in tumors before and after treatment, determine if brain tumor samples demonstrate a functional biochemical activation of the MAPK pathway, explore pharmacogenetic polymorphisms in AZD6244 metabolizing enzymes and document anti-tumor activity of treatment. PBTC-029 is a dose-escalation trial of AZD6244; the compound will be administered orally, twice daily, for 28 consecutive days. Courses will be repeated every 28 days and continue for 13 courses, or until one of the off treatment criteria has been met. The starting dose for patients will be 33 mg/m²/dose, twice daily, for a total daily dose of 66 mg/m²/day. This dose will be escalated up to 95 mg/m²/dose, twice daily, until the maximum tolerated dose or the recommended Phase II dose is identified.

This trial will provide preliminary data about dose, safety, tolerability, and pharmacokinetics of a MEK inhibitor in a pediatric population. Ideally, it will serve as a launching point for further investigations of MEK inhibitors in nontumor populations. Pharmacodynamic studies to investigate the impact of drug dose on systemic MAPK signaling will be imperative in the study of non-tumor populations, and the exploration of dose levels below the Phase II dose established in this early clinical trial may be warranted.
LESSONS LEARNED FROM NEUROFIBROMATOSIS TYPE 1: THE FIRST RASOPATHY

Over the past decade, the development and performance of clinical trials in NF1 has rapidly increased. Many of these trials have been biologically based, aimed at modifying signaling through the NF-related aberrantly activated Ras/MAPK pathway. However, as studies have progressed, it has become evident that the complexities of the signaling pathway and feedback mechanisms make such therapy more challenging than originally thought. The development of clinical trials was facilitated by comprehensive, extensive phenotypic studies which had characterized the clinical manifestations of NF1, their incidence and, in part, their course, if untreated. Since NF1, as is the case for the other RASopathies, has variable clinical manifestations, it became clear that early in the development of such trials those eligibility criteria needed to be strict, so as to: (i) enrich the patient population who might benefit from the trials, (ii) not expose patients who would likely not benefit from the trial to potentially toxic agents, and (iii) develop trials which could be interpretable.

One example of this is the ongoing trial utilizing the HMG CoA reductase inhibitor, lovastatin (based on pre-clinical work that the drug could reverse learning deficits in a mouse model) for children with NF1 and neurocognitive disabilities. In the mouse model, lovastatin only reversed specific types of learning disabilities [Li et al., 2005], and a decision was made to target only those children with a similar type of cognitive profile in the study. This increased the likelihood, at least theoretically, that if lovastatin was efficacious, that this could be determined by the randomized study design. For safety reasons, patient entry was also restricted to children of a specific age. These decisions made accrual more difficult than initially thought. After approximately 1 year of patient entry, it was decided to modify the eligibility criteria, so as to be able to complete the study within a reasonable period of time. In studies to be undertaken in the RASopathies, the types of considerations involved in the lovastatin neurocognition study for children with NF1 will likely be informative. A decision needs to be made early in study development of whether the phenotypic characterization of the patients is adequate to define the subset of patients to be studied and if there are adequate number of patients available to answer the study question in a “reasonable” time-frame.

Another challenge faced by the NF community in developing clinical trials was to prioritize the clinical studies to be done. In a disorder with many different clinical manifestations, priorities had to be established based on not only their incidence and severity, but also on having the capabilities to deal with the disease manifestation. Thus, it was critical to mount a study that would significantly impact the disease treatment and serve as a building block for further improvements in outcome. The feasibility of performing such studies was greatly enhanced by the development, through Department of Defense funding, of a clinical trials consortium. This consortium has been in operation for approximately 3 years and initially prioritized studies in plexiform neurofibromas and neurocognitive disabilities. The presence of a reproducible outcome measure (volumetric analysis) made studies for plexiform neurofibromatosis more appealing, and this was the first study opened by the consortium. The neurocognition study soon followed. More recently, the consortium has been able to
open another study targeting progressive low-grade gliomas and is soon to open clinical trials for patients with malignant peripheral nerve sheath tumors and vestibular schwannomas (for those patients with NF2).

The NF experience highlights several critical aspects that have become clear for clinical trial development for RASopathies: (i) the formation of an ongoing consortium can facilitate development, although the consortium may have to reach out to other centers to quickly complete trials; (ii) trials are facilitated in conditions where the incidence and natural history of the condition are best characterized; (iii) trials need to have strict eligibility criteria, although such criteria may limit patient accruals; (iv) for trials to be interpretable, they need to have well considered, standardized objective outcome measures; and (v) in disorders such as the RASopathies, there needs to be a thoughtful prioritization of what studies should be initially undertaken.

PUBLIC/PRIVATE PARTNERSHIPS ARE CRITICAL

New initiatives that will catalyze the development of treatments for rare and neglected disease that collectively affect millions of people throughout the world are needed. Of the more than 6000 rare and neglected diseases, fewer than 200 have any therapy available, and there is limited likelihood of future treatments being developed within pharmaceutical or biotechnology companies. Companies who develop treatments for rare and neglected diseases have a difficult time recovering their costs for research, development, and manufacturing, either due to small patient numbers or diseases occurring in countries that do not have the ability to cover costs. Thus, the approach to therapy development for rare and neglected diseases must be transformed. Building a network of public/private collaborations holds tremendous promise.

The government has initiated a new program, TRND (Therapeutics for Rare and Neglected Diseases Program) that is part of a congressionally mandated program to encourage and accelerate the development of new drugs for rare and neglected diseases. This unique program will create a drug development pipeline within the NIH and is specifically intended to stimulate research collaborations with academic scientists, nonprofit organizations, and pharmaceutical and biotechnology companies working on rare and neglected illnesses. However, finding a way to enlist the capabilities and expertise of the pharmaceutical industry will ultimately be critical.

Biotechnology and pharmaceutical companies are an important source of compounds and biologics that will ultimately target key components of pathways involved in rare disorders. However, companies face numerous challenges when asked to split indications for a specific compound. As an example, there are concerns about price structures; especially when pricing for the two indications is different. There is the potential for unacceptable side effects in a particular population that might not be relevant in the disease setting for which the drug is primarily intended, and might affect the label and thus curtail commercial prospects for a compound. In addition, there are difficulties involved in adapting to a pediatric cohort. However, on the positive side, there is an advantage in obtaining accelerated development in a population with no proven therapies. A consideration is that
companies might take on the clinical development in a public-private partnership for a marketed drug, or a drug in clinical development and, either donate compounds for a very rare condition, or price at the level for the marketed indication.

The FDA will also play a critical role in developing incentives for industry to participate in the development of therapeutics for neglected and rare disease. It is encouraging that the FDA released a report called Advancing Regulatory Science in 2010 in which it designated regulatory science initiatives as the research areas to which it plans to apply “the bulk” of its $25 million FY 2011 budget increase. Under the regulatory science initiative, the FDA plans to form external collaborations with industry, academia, and other government entities, to “transform product development” in the area of toxicology and personalized medicine. Among the various regulatory science collaborations, an NIH/FDA leadership council will work together to advance the initiatives under the regulatory science framework. As discussed earlier, post-marketing studies to investigate drugs in smaller, mutation-defined subpopulations of well-phenotyped patients are precisely the types of trials that industry might be unable to do since they are costly, or may be unwilling to conduct since such analysis might constrict the market for a profitable drug.

Public/Private teams will be able to facilitate advances in rare disease research by bringing together key complementary resources [Melese et al., 2009]. Academic Health Science Centers and Foundations bring experts in the biology and genetics of the disorders, experts in patient care, and the biological samples that are the basis for molecular studies. In addition, forming alliances with disease specific advocacy organizations, in this case, CFC International and the CSFN/ICSSG will be critical. Advocacy organizations for genetic disorders are becoming involved in biomedical and translational research to meet the needs of the individuals within the group [Terry et al., 2007]. Some rare disease research is currently being advanced in such consortiums or networks; examples of which include PXE International, Progeria Research Foundation and Fragile X. The CSFN/ICSSG and CFC International are very supportive of research and have partnered with academic clinical researchers to advance research in these rare genetic syndromes [Rodriguez-Viciana et al., 2006]. Because CS and CFC are such rare genetic disorders, having CSFN/ICSSG and CFC International involved early in the clinical trial process will be critical for patient recruitment and its overall success.

**CONCLUSIONS**

Moving toward clinical trials in the RASopathies represents an exciting time for the field of medical genetics. This group of syndromes is an ideal multiple congenital anomaly model system for the consideration of modifying signal transduction in genetic disorders. The RASopathies have a common pathogenetic mechanism which results in dysregulation of the Ras/MAPK pathway. Since this pathway has targets for inhibition in cancer treatment, there are many small molecule therapeutics that are in development or undergoing clinical trials, with some already FDA approved. Emerging data from mouse models with an activated Ras pathway have supported the notion that phenotypes can be successfully normalized in both the prenatal and postnatal period by manipulating Ras/MAPK activity. Therefore, the possible use of systemic therapies after birth to reduce Ras/MAPK activity in both CS and
CFC holds the potential to ameliorate the progression of signs and symptoms associated with these disorders. Implementing clinical trials for these rare populations will require a thoughtful approach, defining the best target population, choosing robust endpoints, and selecting the optimal small molecule inhibitor for each syndrome. Most importantly, clinical trials for CS and CFC will require a united front between basic science and clinical translational researchers, private industry, and advocacy organizations.

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REFERENCES


Figure 1.
The Ras/MAPK signaling cascade and small molecule inhibition. Ras genes exist as a multigene family that encodes small GTPases. Ras is a critical signaling hub in the cell that is activated by receptor tyrosine kinases (RTK), G-protein-coupled receptors, cytokine receptors, and extracellular matrix receptors. Receptor activation recruits Growth factor receptor-bound protein 2 (Grb2), an adaptor protein, which recruits Son-of-sevenless homolog (SOS1) and Shp2 (the protein product of \( PTPN11 \), which encodes a non-receptor tyrosine phosphatase). Ras activation results in the activation of Raf (BRAF, CRAF, ARAF), the first MAPK kinase of the cascade. Raf phosphorylates and activates MEK (MEK1 and MEK2), which in turn phosphorylates and activates ERK (ERK1 and ERK2). Phosphorylated ERK is the ultimate effector of the MAPK cascade and exerts its function on several downstream molecules. Feedback loops (solid lines) exist within the pathway whereby activated ERK can inhibit at the level of the receptor, SOS and Raf. Potential therapeutics for CS (caused by heterozygous activating missense HRAS mutations) and CFC (caused by heterozygous mutations in BRAF, MEK1, or MEK2) include FTIs, BRAF inhibitors, and MEK inhibitors (dashed lines; Table II).
**TABLE I**

**Consideration of Endpoints for Costello and CFC Syndromes**

<table>
<thead>
<tr>
<th>Endpoint considerations</th>
<th>Phenotypic characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer</td>
<td>CS: Cancer is seen in up to ~17% of individuals. Embryonal rhabdomyosarcoma is the most common</td>
</tr>
<tr>
<td></td>
<td>CFC: May have a predisposition to acute lymphocytic leukemia though this is still unclear</td>
</tr>
<tr>
<td>Cardiac</td>
<td>CS and CFC: A cardiac phenotype is seen in about 80% of individuals and are somewhat similar in both groups with hypertrophic cardiomyopathy, structure defects, and arrhythmias present</td>
</tr>
<tr>
<td>Cutaneous</td>
<td>CS: Cutaneous papilloma, redundant skin with deep creases, hyperkeratoses, generalized hyperpigmentation, curly brittle hair</td>
</tr>
<tr>
<td></td>
<td>CFC: Acquired melanocytic nevi, keratosis pilaris, ulerythema ophryogenes, infantile hemangiomas, hyperkeratoses</td>
</tr>
<tr>
<td>Growth</td>
<td>CS and CFC: The vast majority have linear growth delays, feeding, and weight gain issues, gastroesophageal dysmotility and reflux with many requiring gastrostomy tubes for feeding; some with growth hormone deficiency</td>
</tr>
<tr>
<td>Musculoskeletal</td>
<td>CS and CFC: Generalized hypotonia, pectus deformities, joint laxity, kyphosis and/or scoliosis, abnormal bone metabolism, osteopenia</td>
</tr>
<tr>
<td>Neurocognition</td>
<td>CS and CFC: Most with cognitive delay and/or learning disabilities ranging from mild to severe</td>
</tr>
<tr>
<td>Ocular</td>
<td>CS and CFC: Ocular abnormalities are seen in the majority of individuals with strabismus, nystagmus, astigmatism, myopia, or hyperopia being common</td>
</tr>
<tr>
<td>Quality of life (QOL)</td>
<td>CS and CFC: QOL endpoints can be a challenge because of its subjective nature. However, both CS and CFC have multi-system organ involvement which can severely affect activities of daily living both in the family as a whole and in the individual. Thus, QOL may be a valuable endpoint.</td>
</tr>
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</table>
### TABLE II
Current Status of Ras/MAPK Small Molecules for Clinical Trials

<table>
<thead>
<tr>
<th>Agent</th>
<th>Target</th>
<th>Company/partner</th>
<th>Status</th>
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</thead>
<tbody>
<tr>
<td><strong>RAS modulation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lonafarnib (Sarasar)</td>
<td>Farnesyl transferase</td>
<td>Merck/Schering-Plough</td>
<td>Phase II</td>
</tr>
<tr>
<td>Tipifarnib (Zarnestra)</td>
<td>Farnesyl transferase</td>
<td>Johnson &amp; Johnson</td>
<td>Phase I/II</td>
</tr>
<tr>
<td>Salirasib</td>
<td>RAS antagonist</td>
<td>Concordia</td>
<td>Phase I/II</td>
</tr>
<tr>
<td><strong>RAF inhibition</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sorafenib (Nexavar)</td>
<td>RAF/multi-targeted</td>
<td>Onyx/Bayer Pharmaceuticals</td>
<td>Approved</td>
</tr>
<tr>
<td>RAF265</td>
<td>RAF/multi-targeted</td>
<td>Novartis</td>
<td>Phase I/II</td>
</tr>
<tr>
<td>PLX4032</td>
<td>BRAF</td>
<td>Plexxikon/Roche</td>
<td>Phase III</td>
</tr>
<tr>
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<td>BRAF</td>
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<td>Phase I/II</td>
</tr>
<tr>
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<td>BRAF</td>
<td>GlaxoSmithKline</td>
<td>Phase I/II</td>
</tr>
<tr>
<td>XL281 (BMS98662)</td>
<td>BRAF</td>
<td>Exelixis/Bristol-Myers Squibb</td>
<td>Phase I/II</td>
</tr>
<tr>
<td><strong>MEK inhibition</strong></td>
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<td></td>
<td></td>
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<tr>
<td>CI-1040</td>
<td>MEK</td>
<td>Pfizer</td>
<td>Terminated</td>
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<tr>
<td>PD0325901</td>
<td>MEK</td>
<td>Pfizer</td>
<td>On Hold</td>
</tr>
<tr>
<td>AZD6244 (ARRY-886)</td>
<td>MEK</td>
<td>Array/AstraZeneca</td>
<td>Phase I/II</td>
</tr>
<tr>
<td>AZD8330 (ARRY-704)</td>
<td>MEK</td>
<td>Array/AstraZeneca</td>
<td>Phase I/II</td>
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<tr>
<td>ARYR-162</td>
<td>MEK</td>
<td>Array/Novartis</td>
<td>Phase I/II</td>
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<td>ARYR-300</td>
<td>MEK</td>
<td>Array/Novartis</td>
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<tr>
<td>RDEA119</td>
<td>MEK</td>
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<td>Phase I/II</td>
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<tr>
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<td>MEK</td>
<td>Ardea/Bayer</td>
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<td>MEK</td>
<td>Wilex AG</td>
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<td>MEK</td>
<td>Genentech</td>
<td>Phase I/II</td>
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<td>AS703026</td>
<td>MEK</td>
<td>EMD Serono</td>
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<tr>
<td>TAK-733</td>
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<td>Takeda/Millenium</td>
<td>Phase I/II</td>
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