THE CENTER FOR RESEARCH IN FOP & RELATED DISORDERS

26TH Annual Report of the Fibrodysplasia Ossificans Progressiva (FOP) Collaborative Research Project

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26th Annual Report of the Fibrodysplasia Ossificans Progressiva (FOP) Collaborative Research Project
Highlights from the 10th Annual Joshua’s Future of Promises Bingo for a Cure! Event in Allentown, PA

Top row, left to right: Joshua Scoble (Emmaus, PA) gives an amazing speech at the opening of the event, while his parents David and Stacy Scoble look on proudly; Dr. Kaplan and A.J. Gonzales (Bellmawr, NJ); Joey Hollywood (Bridgewater, NJ) and Dr. Kaplan; Middle row, left to right: More than 1,500 supporters play bingo; Right: Dr. Kaplan with (from left to right) A.J. Gonzales (Bellmawr, NJ) and Joshua Scoble (Emmaus, PA). Joey Hollywood (Bridgewater, NJ); Kathy Ford (Somers Point, NJ), Lindsay Ruiz (Somers, NY), Jeannette and Lisa Bordeau (Kintnersville, PA); Bottom row, left: Suzanne Hollywood, Meiqi Xu, Amanda Cali and Dr. Qi Shen
The cultural anthropologist Lauren Eiseley said, “We think we learn from teachers, and we sometimes do. But, the teachers are not always to be found in school or in great laboratories. Sometimes what we learn depends upon our own powers of insight.”

Insights often come at the strangest moments – and in the most unexpected places. For example, at a Chinese restaurant at holiday time with an iPhone in one hand deciphering an emergent message from an FOP patient in a faraway land, while with the other hand fumbling with a wrapped fortune cookie trying to liberate the message inside. Finally, during the ambidextrous encounter, the package ruptured, and the fortune was revealed:

Perhaps some baker of fates sealed this encoded message inside a cookie to foreshadow a verdict on the waning year? Or, perhaps some therapeutic gene-whisperer discreetly slipped out of the kitchen to encourage a revelation for the annual report? One can only imagine.

But, there it was - an encrypted message inside a broken cookie at a busy Chinese restaurant on a late December afternoon - while waiters were barking orders and seasonal revelers were sipping hot tea. As philosopher Hilary Mantel said, “We are oblivious of information until we are ready for it.”

“*The odds of hitting your target go up dramatically when you aim at it.*”

*The past year has been dominated by talk of targets. It is no surprise then that the theme of this year’s annual report is targets - transformative targets for the treatment of FOP.*

*Why have we chosen targets rather than therapies? We haven’t. They are two sides of the same coin. When targets are identified, therapies are developed – for polio, degenerative arthritis, ulcers, cataracts, strep throat, gallstones, cystic fibrosis,*
chronic myeloid leukemia, FOP. It all starts with targets. Therapies are based on targets. Without targets, there are no therapies. Transformative therapies are discovered and developed for transformative targets, and transformative targets are identified through research. **Today’s targets define tomorrow’s therapies.**

Worldwide interest in FOP skyrocketed in the wake of the historic discovery of the FOP gene in 2006 - the first hard target for FOP therapy. The FOP gene discovery launched an industry that catapulted the field to clinical trials. FOP research is now a worldwide enterprise. As of January 1, 2017, there were 24 universities actively engaged in FOP research: 14 in the United States, seven in Europe, and three in Asia. As of January 1, 2017, there were 11 pharmaceutical and biotechnology companies actively developing drugs for FOP based on a multitude of hard targets, and over 30 companies that have expressed interest.

Presently, there are over 20 medical experts in FOP on six continents, 13 active FOP support groups worldwide, and eight sites actively involved in clinical trials for FOP. This has been a remarkable sea change in the past few years that is occurring in a rapidly-evolving area of target discovery and drug development.

The concept of orphan drugs for orphan diseases has been widely embraced in just the past five years. This new vision is leading to massive research investment in FOP on the part of academia and industry worldwide - all propelled by the discovery of the FOP gene and by the identification of its robust therapeutic target - the ACVR1 receptor and its interacting molecules and pathways.

The discovery of the FOP gene in 2006 was a transformative event in the history of FOP, and immediately revealed at least four durable targets and approaches to treatment and/or prevention. These include:

1. **Diminishing the activity of the mutant receptor (ACVR1/ALK2) that causes increased BMP pathway signaling** - through inhibitory monoclonal antibodies, ligand traps, signal transduction inhibitors (STIs) or inhibitory RNA

2. **Inhibiting inflammatory triggers of FOP flare-ups**

3. **Directing FOP stem cells away from cartilage or bone to alternate cell fates**

4. **Blocking the body’s response to signals within the tissue microenvironment such as hypoxia that amplify dysregulated BMP signaling and promote the formation of FOP lesions**

A contemporary view of hard targets for FOP places the mutated ACVR1 receptor at the center of the bullseye. But, ACVR1 is not the only target. FOP is a complex disease, and it is unlikely that one magic bullet will effectively and safely neutralize such an enemy.

In the past year, research has identified new targets – and old targets have been probed for new therapies. Without research there would be no targets. Without targets, there would be no therapies. You can’t hit the bullseye if you don’t have a target. And, the odds of hitting your target go up dramatically when you have a target to aim at.

Throughout the history of modern FOP research, transformative ideas have led to the discovery of transformative targets. No targets, no therapies.
THE CENTER

Since its establishment in 1989, the FOP Collaborative Research Project has had a singular mission - to determine the cause of FOP and to use that knowledge to advance treatments and a cure for FOP. During the past 26 years, we have moved from the wastelands of a little understood rare disease to the watershed of clinical trials. We identified the genetic cause of FOP and used that knowledge to spearhead worldwide research efforts to develop therapies that will transform the care, and the lives, of individuals with FOP.

In partnership with our benefactors, we have expanded the frontiers of drug discovery and development in this rare and disabling condition, dismantled physical and perceptual barriers that have impeded progress, and inspired global research into small molecules, antibodies, and gene therapy for FOP.

Here, at The Center for Research in FOP & Related Disorders, our work is broad and comprehensive while focused on seven spheres of FOP activity:

- Clinical care and consultation worldwide
- Clinical research and infrastructure development
- Basic research (identification of therapeutic targets)
- Translational research (preclinical drug testing & biomarker discovery)
- Cali Developmental Research Grants Program
- Clinical trial development and proof-of-principle Investigation in patients
- Education

Right: Tholi Mekiti (Cape Town, South Africa) at the FOP Friends Conference and Family Meeting in Manchester, UK; Far right: Roger Zumfelde (Bad Fallingbostel, Germany) at the FOPeV Annual Medical, Scientific and Patient Meeting in Valbert, Germany
The Center for Research in FOP & Related Disorders is unique. It is the world’s first and only comprehensive center for FOP. Here at The Center, we have had a very busy year. During the past year, we achieved the following milestones in our FOP program:

**Clinical Care and Consultation Worldwide**

› Guided patients, families and doctors worldwide in their daily battles with FOP

› Directed the world’s largest FOP clinic and referral center

› Coordinated medical management of FOP patients worldwide

› Conducted international FOP clinics for patients and families in Livorno, Italy; Manchester, United Kingdom; Valbert, Germany; Paris, France; and St. Louis, Missouri
Clinical Research and Infrastructure Development

› Published the findings of a massive global survey of FOP flare-ups that will inform the design and evaluation of clinical trials.

› Concluded the first patient-reported Longitudinal Natural History Study in FOP. The study will be published in a special issue of BONE in early 2018.

› Advocated for the direct deposit and open access of annotated whole genome sequence data from a sponsored clinical trial into the IFOPA patient registry medical portal.

› Submitted for publication a study describing the FOP Cumulative Analogue Joint Involvement Scale (CAJIS), a scale that validates and establishes a rapidly administered and clinically useful evaluation tool for FOP. The CAJIS score is essentially an APGAR score for FOP.

› Incorporated the CAJIS evaluation into three ongoing clinical trials.

› Consulted on the development and implementation of a patient-reported physical function outcome measure for adults and children with FOP.

› Consulted on the development and implementation of the Clementia Longitudinal Natural History Study.

› Championed and brokered the prospective deposit of data from the Clementia Longitudinal Natural History Study into the IFOPA Registry Database.

› Championed and brokered the deposit of annotated and de-identified genomic data from a pending clinical trial into the IFOPA Registry Database.

› Championed a single unified international registry for FOP by the IFOPA, and owned by the FOP community.
Basic Research (Identification of Therapeutic Targets)

› Published studies in mouse models of classic FOP showing that palovarotene prevents spontaneous heterotopic ossification (HO) as well as injury-induced HO.

› Published a major study showing that palovarotene restores normal structure and function to FOP growth plates, suggesting the possibility of safe and early intervention for HO in children - an observation with major implications for clinical trials.

› Identified the cellular response to tissue hypoxia and inflammation as a major factor in the induction and amplification of FOP flare-ups and a transformative target for therapy.

› Discovered a potential therapeutic target in the hypoxia sensing pathway and identified several drugs that could be repurposed to treat FOP.

› Introduced a medication that targets the cellular response to tissue hypoxia into the clinic on a compassionate off-label basis for the management of FOP in children.

› Examined the role of Activin A in inducing dysregulated bone morphogenetic protein (BMP) pathway signaling in primary human osteoprogenitor cells from FOP patients – thus providing proof-of-principle for anti-Activin A monoclonal antibodies for the first time in human stem cells – a precursor to its use in clinical trials for the prevention and treatment of FOP.

› Investigated a molecular mechanism by which the innate immune system amplifies inductive BMP signaling in FOP stem cells.

› Investigated molecular mechanisms and immunologic triggers of FOP flare-ups in state-of-the-art knock-in mouse models of classic FOP.

› Investigated cellular inflammatory triggers of early FOP lesions using novel triple knock-in FOP mouse models and identified key cellular targets in the innate immune system.

› Expanded collaborative investigations with developmental neurobiologists and pediatric oncologists on mechanisms of disease activity in malignant brainstem gliomas and in FOP, two catastrophic childhood diseases associated with common mutations in ACVR1.

› Investigated the response of the dysregulated ACVR1 signaling pathway in FOP to biomechanical signals in the early FOP lesional microenvironment that can promote bone formation.
Investigated the molecular mechanisms by which the mutant FOP gene alters the response of cells to their physical environment. These studies suggest that FOP cells are predisposed to misinterpret signals from their tissue microenvironment as instructions to form cartilage and bone.

Investigated molecular mechanisms by which ultra-rare FOP variants trigger promiscuous BMP signaling and subsequent HO.

Continued to expand and develop the FOP SHED Cell Tooth Fairy Program. This substantial library of connective tissue progenitor cells is essential for ongoing and future studies in therapeutic target identification and drug discovery in FOP.

Pursued collaborative studies to identify modifier genes/serum factors in a man with the FOP mutation and characteristic toe malformations, but who is relatively asymptomatic for progressive heterotopic ossification. Whole exome sequencing was conducted on DNA samples from the patient, his unaffected brother, and both unaffected parents. The data were analyzed using multiple approaches and data filters, identifying candidate genes, and cellular pathways/processes of interest. To add strength to this analysis, whole exome sequencing from three additional mild FOP patients have been added to the analysis. Potential targets based on serum analysis were also identified. Intense investigation will continue on this project.

Translational Research
(Preclinical Drug Testing & Biomarker Discovery Program)

Screened new categories of compounds for efficacy in preventing HO in FOP mouse models.

Annotated an extensive library of plasma biomarker samples in a large cohort of classically-affected FOP patients and non-FOP age and sex-matched controls.

Conducted a detailed biomarker analysis on these plasma samples.

Developmental Research Grants Program

Supported three highly innovative developmental research projects in the Cali Developmental Research Grants Program:

- “Molecular Basis of Pathogenic Signaling and High Throughput Testing of FOP Therapies in a Zebrafish Model System” (Mary Mullins, Ph.D., The University of Pennsylvania)
- “Identifying Alternative Therapeutic Targets and Genetic Interactors in FOP” (Ed Hsiao, M.D., Ph.D., - University of California, San Francisco)
- “Novel Allosteric Destabilizers as Therapeutics for FOP” (Jay Groppe, Ph.D., Texas A&M University)
Clinical Trial Development and Proof-of-Principle Investigation in Patients

› Consulted on the study design of five clinical trials in development by three pharmaceutical/biotech companies.

› Advised 30 pharmaceutical and biotech companies on the development of novel drugs for clinical trials in children and adults with FOP, based on identified targets.

› Advanced understanding of small molecule inhibitors in physiologic and pathologic chondrogenesis in children - knowledge and approaches that are vital to future clinical trials for FOP.

› Developed, validated, and published an analog method for radiographic assessment of heterotopic ossification in FOP. This method enables practical, quantitative assessment of HO in clinical trials and is incorporated in the ongoing interventional trials and natural history study.

› Enrolled and followed patients in two sponsored interventional clinical trials.

› Enrolled and followed patients in a sponsored longitudinal natural history study.

Education

› Mentored the next generation of physicians and scientists working on FOP in the classroom, clinic, and laboratory.

› Mentored high school, college, medical, and graduate students on research projects to expand vital knowledge and scientific and public awareness of FOP.

› Educated physicians, scientists, researchers, and regulators at medical and scientific forums, meetings, and conferences worldwide.

› Organized and edited a special issue of the journal BONE on heterotopic ossification (scheduled for publication in early 2018).

Our work at The Center is continually evolving as we cross the bridge daily between the clinic and the laboratory and back again in a process that builds knowledge and deep understanding of FOP to help us accomplish our ultimate mission.

The scope of research in the FOP laboratory covers a range of investigations that are focused on identifying and characterizing transformative targets for therapy.
The collaborative activities of the FOP Laboratory focus on six major research areas:

1. Identifying and characterizing central signaling targets in the induction and amplification of FOP lesions. These studies are conducted by Meiqi Xu, Salin Chakkalakal, Michael Convente, John Fong, Haitao Wang, Alexandra Stanley, Robyn Allen, Will Towler, and Niambi Brewer. This vital research enables the development of drugs that target these pathways.

2. Identifying and characterizing immunologic and microenvironmental targets that amplify FOP flare-ups. These studies are conducted by graduate students, Michael Convente, Niambi Brewer, and Alexandra Stanley, and research scientists Haitao Wang and Vitali Lounev. Their projects investigate the cellular response to the immunologic, biochemical and biomechanical microenvironments of early (pre-cartilage/bone) FOP lesions. Stunning new therapeutic targets are emerging from their work, and it is possible that one or more such targets will become the basis for clinical trials with re-purposed drugs.

3. Identifying cell and tissue targets in FOP lesions. These studies are conducted by Vitali Lounev, Michael Convente, Salin Chakkalakal, John Fong, Haitao Wang, Will Towler, Alexandra Stanley, Ruth McCarrick-Walmsley, and Robert Caron. These studies identify the specific cells and mechanisms that can be targeted to block heterotopic ossification.

4. Identifying and characterizing developmental targets in FOP that impact joint development and degenerative joint disease. These studies are conducted by Will Towler, Salin Chakkalakal, and collaborators Adam Resnick and medical student Harry Han. These projects include two research areas:
   1) Effects of the FOP mutation on skeletal development, joint formation, and degeneration, and
   2) Shared disease mechanisms of mACVR1 in DIPG brain tumors and FOP

5. Developing in vitro and in vivo FOP models for drug “target testing.” These studies are conducted by post-doctoral fellows Salin Chakkalakal and Girish Ramaswamy, by graduate students Michael Convente, Robyn Allen, and Alexandra Stanley, and by research scientists Vitali Lounev, Deyu Zhang, Meiqi Xu, and Ruth McGarrick-Walmsley. Their projects are centered on developing new resources for FOP research that will be used in multiple other projects as well as used for in vivo screening of drug treatment candidates. This work is a vital part of the infrastructure for drug discovery and development – the infrastructure for a cure.

6. Pre-clinical drug testing in FOP mouse models is conducted by Haitao Wang, Vitali Lounev, Deyu Zhang, and Salin Chakkalakal.
Despite remarkable advances in FOP research over the past several years, we remain far from understanding some of the most basic and fundamental mysteries of FOP:

- What are the cellular and molecular triggers of FOP flare-ups?
- How does FOP progress in the absence of flare-ups?
- How do the immune system and the lesional tissue microenvironment influence the progression of FOP?
- What is the relationship between the innate immune system and the skeletal progenitor cells that initiate FOP flare-ups?
- What insights do the ultra-rare genetic variants of FOP (which affect only 2-3% of FOP patients worldwide) teach us about the function of the genetic switch that drives heterotopic ossification in FOP, and how do these ultra-rare insights inform the identification of new targets for drug development?

These questions and more continue under intense investigation at the FOP Center, and their answers will help identify and confirm novel targets for drug discovery and development.

In the next section of the annual report, we will review the present status of FOP target identification and drug development.
During war, targets are identified based on their strategic value. Weapons are designed and selected to destroy those targets and minimize collateral damage. We are at war with FOP - a complex enemy with many moving targets. Various strategies for attacking classic and newly discovered targets are coming into focus. Some of the targets and weapons (drugs) are relevant during disease quiescence, some during disease activity; some during childhood, some during adulthood; some during flare-ups, some during non-flare-up activity.

2016 was a year in the bunkers. The three translational advances based on the previous identification of targets and described in detail in the 25th annual report - palovarotene, Activin A antibodies, and silencers of the hypoxia sensing alarm - are in clinical trials, poised to enter clinical trials, or are being used compassionately in patient care, respectively.

These notable advances occurred because hard targets have been identified. This knowledge has transformed our understanding of FOP and, along with the insights from the gene discovery, are poised to transform the therapeutic landscape of FOP. In a nutshell, these discoveries showed that 1) inhibition of the cartilage scaffold of endochondral bone formation inhibits the formation of heterotopic bone in a genetic animal model of classic FOP; 2) the pathological activity of the mutant FOP receptor (mtACVR1) is sensitive to an unsuspected extracellular hormone-like protein and immunological mediator, Activin A (Act A) and that blocking the activity of Act A blocks heterotopic ossification (HO) in a mouse model of FOP; and 3) early FOP lesions are profoundly hypoxic (oxygen starved). The intracellular ‘alarm’ protein HIF1-α, which regulates the cellular response to hypoxia, keeps mtACVR1 active when it should be destroyed, thus dramatically amplifying BMP pathway signaling and stimulating HO. Blocking the activity of HIF1-α substantially diminishes HO in mouse models of FOP.

In 2016, scientists at The Center for Research in FOP and Related Disorders verified the findings of Hatsell, Hino and colleagues in human osteoprogenitor
stem cells (SHED cells; see The Tooth Ferry Program at the FOP Lab: SHEDding Light on FOP) from baby teeth of FOP patients and controls, showing that these human FOP cells are responsive to Activin A. These investigations were reported at the IFOPA Drug Development Forum by Dr. Haitao Wang in October 2016 and will be published in a special upcoming edition of the journal BONE.

TARGET: THE PRE-CARTILAGE SCAFFOLD

In FOP, heterotopic bone forms by a mechanism called endochondral ossification – in other words, through an obligate cartilage scaffold. This is how most normal bones of the skeleton grow in length and how fractures heal – by forming a cartilage template and then transforming it into bone. Palovarotene, a drug now in clinical trials for FOP, selectively targets a pathway involved in cartilage formation. Pacifici and Iwamoto showed in 2011 that palovarotene inhibited extra bone growth in mice genetically engineered to form heterotopic bone. The extra bone that appears in FOP flare-ups progresses through a cartilage stage before replacement with mature bone. If the cartilage scaffold can be inhibited, bone will not form. Thus, the pre-cartilage scaffold is a target for FOP.

In September, 2016, scientists from The Center for Research in FOP and Related Disorders at the University of Pennsylvania, reporting in The Journal of Bone and Mineral Research, announced a major breakthrough in understanding the role of palovarotene in FOP. In their paper, “Palovarotene inhibits heterotopic ossification and maintains limb mobility and growth in mice with the human ACVR1R206H FOP fibrodysplasia ossificans progressive (FOP) mutation,” lead author Dr. Salin Chakkalakal, senior author Dr. Eileen Shore together with their colleagues Masahiro Iwamoto, Maurizio Pacifici and Fred Kaplan tested palovarotene in a mouse model carrying the same human gene mutation that causes FOP for the first time. They reported that palovarotene prevented spontaneous heterotopic ossification in FOP mice. In addition, palovarotene protected growing newborn FOP mice when given to lactating mothers. Importantly, palovarotene maintained joint, limb and body motion, providing clear evidence for its encompassing therapeutic potential as a treatment for FOP.

Palovarotene activates the turn-off signal for cartilage formation. This does not target ACVR1, the mutated protein encoded by the FOP mutation, a receptor in the BMP signaling pathway, but targets other molecules interacting with BMP signaling pathways in pre-cartilage cells that are downstream of ACVR1 action.

“Palovarotene works by stopping the overall process of extra bone formation,” Shore said. “We knew that the drug affects the cartilage stage of ectopic ossification, but also recognized that this drug may impair cartilage formation in growth plates leading to reduced skeletal growth. The FOP mutation and resulting increased BMP signaling also mildly reduce skeletal bone growth. Our concern was that treating children with FOP with palovarotene would synergize to further impact their overall growth, potentially leading to additional health complications. Instead, we found that palovarotene appears to balance the effects of the FOP mutation, effectively restoring near normal growth. This was a wonderful surprise.”

These results mean that palovarotene is likely safe to use in children with FOP without concerns about affecting normal skeletal bone growth. “This changes how we think about treating children with FOP. We may be able to give the drug to kids for a longer, more chronic approach,” Shore added.

When the scientists gave palovarotene to nursing female mice, they passed along the drug’s benefit to their mutant offspring. “If the drug’s benefits translate to humans, it could mean that newborn babies diagnosed with FOP could benefit from early treatment,” Pacifici said. “This is especially important, because once an abnormal bone growth occurs, it is permanent.”
One consequence of FOP is that surgeons cannot remove the excess bone tissue caused by the disease because tissue damage from surgery triggers even more bone growth. In this study, palovarotene not only inhibited spontaneous heterotopic ossification, but also prevented it when mice were experimentally injured. This finding also indicates potential benefits for treating or preventing heterotopic ossification that results from trauma, head injuries, war wounds, or joint replacement.

In 2014, Clementia Pharmaceuticals, after several years of consultation with The Center for Research in FOP & Related Disorders, began a Phase-2 clinical trial as well as an open label extension trial of palovarotene in adults with FOP. Clementia’s Phase-2 clinical trial was designed to determine whether palovarotene would have the same effect in patients with FOP.

In 2016, Clementia Pharmaceuticals announced top line results from its Phase-2 clinical trial investigating palovarotene for the treatment of FOP. Several positive trends were detected in this 40-subject placebo-controlled trial, including reductions in the proportion of subjects who developed new HO, reductions in volume of new HO, improvement in patient-reported pain associated with flare-ups, and a decrease in the duration of flare-ups - although none of these outcomes reached statistical significance. Subjects tolerated palovarotene well: all subjects completed the 12-week trial and enrolled in the Phase-2 open-label extension trial. The results of this landmark clinical trial are encouraging and closely mirror what was observed in previously reported animal studies with palovarotene.

Clementia continues to gather important additional data in the Phase-2 extension trial and in the ongoing observational Natural History Study. Data from these studies will inform the design of a Phase-3 registration trial, which is expected to start in 2017. The Center for Research in FOP & Related Disorders has been a major contributor through basic, translational, and clinical research on this novel target and potential therapy.

NEW TARGETS FOR OLD DRUGS

In an article in *Nature Medicine*, entitled, “Bedside to Bench: Old Drugs Learn New Tricks?” Stephen Strittmatter commented, “Drug repurposing commonly starts with compounds that have already been tested in humans and have demonstrated an acceptable level of safety and tolerability. Such compounds are then used for a medical condition other than originally intended. In this way, the development track avoids unexpected derailment due to toxicity that is not predicted by pre-clinical work. The issue of efficacy can then be evaluated by clinical trials.”

While new potential therapeutics are being developed for many of the targets identified in FOP, an alternate and very attractive approach is to identify currently approved medications that attack these targets and that could be repurposed to address the urgent unmet medical needs of FOP patients.
We recently identified one such drug - imatinib, a powerful inhibitor for a specific form of chronic leukemia. Imatinib emerged as a therapeutic drug in animal model tests against the cellular hypoxia target in FOP. Imatinib has the particularly desirable effects of attacking multiple targets relevant to the pathophysiology of FOP.

Recent published data from The Center for Research in FOP & Related Disorders suggests that imatinib decreases HIF1-α activity and mutant ACVR1 activity in FOP stem cells as well as heterotopic ossification (HO) in a genetic mouse model of FOP. Similar findings were recently found by Ben Levi and his colleagues at The University of Michigan for the immunosuppressant rapamycin which also inactivates the hypoxia-sensing switch and turns-off heterotopic ossification in FOP animal models through a related but slightly different pathway.

In a major commentary in the September 2016 issue of the *The Journal of Bone and Mineral Research* Eric Hess, Professor of Molecular and Skeletal Biology at The University Medical Center in Hamburg, Germany, wrote “In an elegant study using patient material, in vitro assays, and in vivo models, Wang and colleagues (from the University of Pennsylvania) report profound novel insights into the altered molecular mechanism by which cellular hypoxia promotes heterotopic ossification in FOP. The observations made by Wang and colleagues attribute a large part of the disease mechanisms in FOP to deregulated signaling in response to tissue hypoxia. Importantly, using a transgenic mouse model of FOP in which a constitutively active and mutant FOP receptor is ubiquitously expressed in heterotopic ossification, hypoxia was induced by intramuscular injection of cardiotoxin. Treating with imatinib greatly reduced the heterotopic bone formation, thereby maintaining the locomotion of the animals. These findings are consistent with recent reports describing the use of HIF1-α inhibitors to prevent heterotopic ossification in mouse models of FOP, and trauma-induced heterotopic ossification by inhibition of HIF1-α signaling. Although pharmacological inhibition of HIF 1-α to prevent heterotopic ossification is very successful in mice, the applicability in humans remains to be investigated.”

Based on compelling biologic rationale, strong preclinical data, and a favorable safety profile, imatinib is being used on a compassionate basis in several children with uncontrollable flare-ups of FOP. Will imatinib and other repurposed drugs such as rapamycin eventually have a role in the management of FOP? Time will tell.

*Above, left to right:* Joey Hollywood (Bridgewater, NJ) with Drs. Kaplan and Al Mukaddam at The Center for Research in FOP and Related Disorders at The University of Pennsylvania; Katherine Toder, Renee Jurek and Dr. Kaplan meet Carli Henrotay (St. Louis, MO) at the FOP Center at Penn

*Right:* Dr. Frederick Kaplan (University of Pennsylvania) and Dr. Ben Levi (University of Michigan)
TARGET: THE MUTANT ACVR1 RECEPTOR

The discovery of the FOP gene identified ACVR1, the mutated BMP receptor, as the prime pharmacologic target for the treatment of FOP. Multiple therapeutic strategies for inhibiting dysregulated BMP signaling in FOP are being pursued.

Signal Transduction Inhibitors (STIs)

STIs are important molecular tools for studying BMP signaling in FOP, and have great potential for development into powerful therapeutic drugs for FOP.

STIs are small molecules, generally well-absorbed into the blood stream through the GI track, that work by blocking the ‘mouth’ of ACVR1 so that it cannot shout its damaging message to make more bone. Selective STIs for FOP will inhibit ACVR1 (also known as ALK2) rather than ALK1, ALK3 or ALK6 (bone forming receptors in the same family). Such highly-selective molecules are being developed by pharmaceutical companies for eventual use in FOP clinical trials. Broad spectrum STIs that are currently available and that target ACVR1 (among other receptors) are also being considered for repurposing in FOP clinical trials.

Blocking Antibodies against ACVR1

Mutant ACVR1 (in FOP) signals at low levels all the time (even when it should be switched off). Mutant ACVR1 is also hyper-responsive to locally produced hormone-like molecules (also known as ligands) such as BMP4 and Activin A; thus the rationale for using blocking antibodies to ACVR1 in the prevention and treatment of FOP. Therapeutic monoclonal antibodies that target ACVR1 are under development by several pharmaceutical companies.

Blocking Antibodies against Activin A

Activin A (Act A) potently stimulates the mutant FOP receptor (mACVR1) with little or no activation of wild-type (wt) ACVR1. The molecular, physiologic and structural basis for the sensitivity of mACVR1 to Act A is still unknown. Act A has previously been recognized as a potent hormone-like molecule that is a key regulator of the immune system.

Importantl, Act A induces heterotopic ossification in FOP mice. Inhibition of Act A with a fully humanized monoclonal antibody blocks spontaneous and trauma-induced heterotopic ossification in FOP mice. The unexpected discovery of Act A in the pathogenesis of FOP identifies a therapeutic target for FOP and excavates a foundation for clinical development.
Ligand Traps

Ligand traps, magnet-like molecules that attract molecules like BMP4 and Act A and prevent them from activating ACVR1, have been proposed as a therapeutic strategy for FOP. Such molecules have been produced and been shown experimentally to inhibit dysregulated BMP signaling in FOP cells and suppress cartilage and bone formation in vitro. A paper describing this development was published in the journal BONE in 2016 by Dr. Zhang (Shanghai, China) and colleagues. The molecular magnet has not yet been tested in the FOP mice.

Mutant Allele-Specific Inhibitory RNA

Inhibitory RNA capable of suppressing the expression of mtACVR1 in connective tissue progenitor cells from FOP patients restores dysregulated BMP signaling to levels observed in control cells and blocks cartilage and bone formation in vitro. While providing proof-of-principle for allele-specific inhibition of ACVR1 in the prevention of heterotopic ossification in FOP, the in vivo utility of this approach must be confirmed in mouse models of FOP. A hurdle to human application is safe, effective and durable delivery of small inhibitory RNA molecules to relevant progenitor cells. While small molecule inhibitors or biologics may dominate near-term therapeutic options, opportunities on the distant horizon using inhibitory RNA are appreciable.

TARGET: INFLAMMATORY & IMMUNE TRIGGERS

The search for immunological triggers continues to dominate the frontier of FOP research. In all affected individuals, FOP is caused by a gain-of-function mutation in ACVR1 which results in dysregulated BMP pathway signaling. The mutant receptor appears to cause the myriad developmental features of FOP (such as the malformed toes, osteochondromas and cervical spine fusions), but does not appear sufficient to continuously induce the episodic flare-ups that lead to disabling heterotopic ossification. FOP flare-ups strongly implicate the participation of an underlying immunological trigger:

- Flare-ups are triggered by soft tissue injury, muscle fatigue, viruses, and immunizations.
- Activation of flare-ups follows antigenic re-challenge by intramuscular immunizations.
- Flare-ups are exacerbated by intercurrent immunizations.
- Trauma induced by surgical removal of heterotopic bone leads to new bone formation.
- Sudden and massive soft tissue edema and inflammation occurs at the clinical onset of spontaneous flare-ups.
- Perivascular accumulation of lymphocytes, mast cells, and macrophages occurs in affected skeletal muscle during the earliest phase of flare-ups in FOP patients and in mouse models of FOP.
- Infiltration of lymphocytes, mast cells and macrophages occurs between the fascicles of skeletal muscle during the early phases of disease flare-ups in patients and in mouse models of FOP.
- Targeted ablation of macrophages and mast cells impairs heterotopic ossification in mouse models of FOP.
- Early use of high-dose corticosteroids during flare-ups improves symptoms in FOP patients.
- Prophylactic use of high-dose corticosteroids abrogates the formation of heterotopic bone in a mouse model of FOP (but due to side effects, is not recommended for FOP in humans).
- Long periods of disease quiescence can occur following immunoablation/immunosuppression.
- Increased sensitivity of mutant ACVR1 to auto-inflammatory ligands (BMP4 and Activin A) is observed in mouse models of FOP.
- There is a notable absence of heterotopic ossification prenatally.
- Sensory nerves regulate the innate immune system and amplify the formation of heterotopic bone in FOP mice.
- Blocking any major signaling hub in the neuro-inflammatory pathway profoundly inhibits heterotopic ossification in an FOP-like mouse model.

These myriad epidemiologic, clinical, pathological and molecular features of FOP support that the innate immune system plays a prominent and provocative role in the pathophysiology of FOP.

It is intriguing to speculate that perhaps all flare-ups, even those that appear spontaneous, are promoted by the innate immune system through damage-associated molecular patterns (DAMPs), pathogen associated molecular patterns (PAMPs), and attendant stimulation of receptors of the innate immune system. Robust investigation in this area is underway and is likely to identify specific targets amenable for therapy as a new frontier in FOP research.

**TARGET: THE UNEXPECTED**

Several adults have been identified with the classic FOP mutation and congenital features of FOP but a paucity of postnatal heterotopic ossification. These resilient individuals hold the key to understanding factors that trigger FOP flare-ups and amplify progression of the disease. Robust investigation is being conducted to decipher the genetic, epigenetic, environmental, and immunologic factors involved. Whole exome sequencing has been performed to screen for second site modifier mutations, and additional biochemical and molecular studies are being conducted to identify factors that may prevent flare-ups and heterotopic ossification. If distinct factors can be identified in these few individuals, robust targets for therapy are likely to emerge.

This work is a highly collaborative effort between physicians and scientists at The Center for Research in FOP and Related Disorders at The University of Pennsylvania, Vanderbilt University, University of California, San Francisco, and The Mayo Clinic.

**TARGETS: EMERGING STRATEGIES FOR CLINICAL TRIALS**

Successful therapies for FOP will be based on blocking key genetic, molecular, cellular, and tissue targets.

There are several plausible scenarios for clinical trials in which to test possible therapies for FOP:

- Short-term prevention of heterotopic ossification from acute flare-ups
- Long-term prevention of heterotopic ossification
- Combinatorial approach
- Surgical liberation of ankylosed joints
Different medications and strategies may lend themselves to different clinical trial designs. Therapeutic approaches might consider partial blockade of a signaling pathway with a rescue approach for breakthrough flare-ups, should they occur. Finally, due to the tremendous risk to FOP patients, surgical liberation of ankylosed joints should not be undertaken until proven treatment options are established.

The main goal for FOP is preventing progressive heterotopic ossification. Thus, the most important battleground for FOP is childhood. Recent identification of agents such as imatinib and rapamycin that target inflammation and hypoxia-sensing pathways might be repurposed compassionately, or in clinical trials in children, while other novel therapeutics are being developed. STIs currently in non-FOP-related clinical trials that also target ACVR1 might be repurposed for early entry into FOP clinical trials.
In order for the FOP community to fulfill the promise of better treatments and eventually a cure, we will need an **infrastructure for a cure** – similar in concept to the highway networks, mass transit systems, power grids, and communication networks that allow societies to function.

Great scientific and medical enterprises, like great nations, need a stable and robust infrastructure. An infrastructure for a cure is a vital priority of The Center and will facilitate the discovery and development of drugs and the conduct of clinical trials that will allow the entire FOP community to navigate better treatments and a cure. Like a large public works project, it will take a committed effort of the entire international FOP community (patients, patient-organizations, scientists, pharmaceutical and biotechnology companies) to accomplish this goal and we fully embrace that concept.

In this section of the annual report, we will focus on two major ongoing projects in the infrastructure for a cure and what we have done at The Center for Research in FOP & Related Disorders during the past year to facilitate their progress:

- Natural History Infrastructure
- Biomarker Infrastructure

### 1. NATURAL HISTORY INFRASTRUCTURE

The Natural History of Flare-ups in Fibrodysplasia Ossificans Progressiva (FOP): A Comprehensive Global Assessment

Comprehensive knowledge of the natural history of flare-ups and progressive disability in FOP is of paramount importance in the design of clinical trials. While robust cross-sectional natural history studies have been conducted, knowledge of the longitudinal natural history of FOP is still sparse. An annotated natural history and biomarker study has currently enrolled more than 100 patients and will follow them for over three years. Information can be found at clinicaltrials.gov.

Comprehensive natural history studies in FOP are needed to determine outcome measures for the design of informative clinical trials. Measures must include clinically meaningful milestones and predictors of outcome, such as the occurrence of HO and change in clinical function.
We are proud to report the results of an extensive worldwide, prospective, cross-sectional survey of flare-ups in FOP patients - episodic exacerbations that over time result in disabling HO. The study by Pignolo and colleagues from The Center for Research in FOP & Related Disorders was recently published in *The Journal of Bone and Mineral Research*. The entire article can be viewed on the IFOPA website.

In summary, the results from this first world-wide survey of flare-ups in patients with FOP provided a comprehensive perspective on the presentation, initiating factors, anatomic location, and resolution of flare-ups. These results will inform the design of future clinical trials of drugs for episodic and chronic treatment of FOP flare-ups.

Additionally, the first longitudinal natural history study was completed at The Center for Research in FOP & Related Disorders and is being prepared for publication. Importantly, The Center has been one of the major sites for the three-year annotated longitudinal natural history study sponsored by Clementia Pharmaceuticals.

### 2. BIOMARKER INFRASTRUCTURE

Biomarker discovery and assessment is a vital component of the infrastructure for a cure. There are three compelling reasons why biomarkers are needed for successful clinical trial design and evaluation in FOP:

1. To measure and monitor the variability and progression of FOP in each individual and between individuals.

2. To measure and monitor the stages of disease activity during and between flare-ups and the absence of flare-ups.

3. To measure and monitor each individual’s response to the drug being studied.

The Center for Research in FOP & Related Disorders has a large and valuable biobank of plasma samples on FOP patients from various stages of FOP flare-ups. In 2015, we completed a comprehensive annotation of these samples and began a detailed analysis of biomarkers using these samples under a sponsored research agreement. The study has been completed. The results of this study are being prepared for publication and will be widely available to researchers worldwide. **Biomarker infrastructure** is a vital component of the **infrastructure for a cure**.

*Left to right:* The Hickmott Family - Lannett, Caroline, Cody and Peggy - (Abilene, TX) during a visit in Philadelphia; Nicolas Santillan (Durham, NC) displays his teenage ninja mutant turtles for Dr. Kaplan; Holly LaPrade (North Haven, CT) with Dr. Kaplan, Renee Jurek and Katherine Toder at Penn
1. THE FOP MUTATION PROMOTES THE GENERATION OF INDUCED PLURIPOTENT STEM CELLS

“In a study published in the Proceedings of the National Academy of Sciences, Shinya Yamanaka, M.D., Ph.D., (Nobel laureate from Kyoto University who first created induced pluripotent stem cells [iPSCs]), Edward Hsiao from University of California, San Francisco and their colleagues found a way to increase the efficiency of stem cell reprogramming through research on FOP. iPSCs – stem cells created from skin cells that can be transformed into any type of cell in the body – have revolutionized biomedical science. iPSCs have contributed to breakthroughs in regenerative medicine and drug discovery. However, using existing techniques, fewer than one per cent of adult skin cells are reprogrammed into iPSCs.

Although the original goal of the scientists was to create a cellular model to study FOP, they discovered that they could create more iPSCs from cells obtained from FOP patients than those from healthy individuals. But why? They believe this is because BMP signaling – which is over-active in FOP cells enhances a cell’s ability to proliferate – one of the key characteristics of stem cells. To test their hypothesis, the researchers blocked BMP signaling, which resulted in fewer iPSCs being generated from FOP patients’ cells. Conversely, activating the signaling pathway yielded more iPSCs. This is the first report of a naturally occurring genetic mutation improving the efficiency of stem cell generation.

2. PLATELET-DERIVED GROWTH FACTOR RECEPTOR ALPHA (PDGFRα) IS EXPRESSED ON FOP PROGENITOR CELLS

Paul Yu and colleagues from Harvard University reported in a paper in Science Translational Medicine that two distinct tissue-resident progenitor cell lineages drive muscle versus tendon and ligament heterotopic ossification in an animal model of FOP. Importantly, both cell populations expressed platelet derived growth factor alpha (PDGFRα) on their cell membranes. These appear to be the same resident progenitor cells previously identified by multiple investigators. The study confirms previous observations that a population of PDGFRα-expressing cells is sufficient to initiate heterotopic ossification in vivo in the presence of the FOP gene. The authors further showed that both injury-dependent intramuscular and spontaneous ligament heterotopic ossification in FOP mice were effectively controlled by a selective ACVR1 signal transduction inhibitor.
In a related study published in the journal *Stem Cells*, Dr. Ben Levi (The University of Michigan) and colleagues showed that a special population of connective tissue cells resident in tendon and muscle contribute to both trauma-induced and genetic heterotopic ossification.

Levi and his colleagues also showed, as did David Goldhamer and colleagues before, that these cells express PDGFRα. Thus, there seems to be a consistent theme in studies from all laboratories to date that populations of cells that give rise to heterotopic ossification in FOP express PDGFRα. There are several important implications for FOP: 1) that targeting therapy to these cell lineages may help enhance therapeutic benefit and limit collateral damage to other cell types, and 2) directly ablating these cells in connective tissues, if possible, may be therapeutic.

3. **PLATELET-DERIVED GROWTH FACTOR RECEPTOR ALPHA (PDGFRα) MAY BE A TARGET FOR FOP**

Tissue fibrosis (fibroproliferation) is a transient phase in muscle and tendon regeneration following injury and is overactive in FOP.

As noted above, a common molecular signature of tissue-resident stem cells that give rise to heterotopic ossification in FOP is platelet-derived growth receptor alpha (PDGFRα).

A recent paper in the journal *Nature* by Alisa Mueller and colleagues defines PDGFRα as a new target for all forms of skeletal fibrosis - and by implication (although not explicitly inferred), the explosive fibroproliferation that precedes heterotopic ossification in FOP. The authors state that “PDGFRα exhibits divergent effects in skeletal muscle. At physiological levels, signaling through this receptor promotes muscle development in growing embryos and regeneration in adult muscle. However, both increased PDGF ligand and enhanced PDGFRα pathway activity cause pathological fibrosis.” The authors show that PDGFRα signaling regulates a population of stem cells that play a supportive role in muscle regeneration but may cause fibrosis and fibroproliferation when aberrantly regulated.

The authors found that these stem cells produce a variant of PDGFRα during regeneration that acts as a decoy to inhibit PDGF signaling and prevent stem cell over-activation. Increasing the expression of this decoy receptor limits fibroproliferation in mice suggesting biological relevance for this receptor and its role in muscle fibroproliferation and fibrosis.

What may this mean for FOP? In an important paper in *The Journal of Clinical Investigation*, Dr. Benjamin Levi and colleagues (from The University of Michigan) reported that the drug rapamycin (which inhibits the response to hypoxia signaling) also eliminates muscle fibrosis in a mouse model of FOP, in part by inhibiting PDGFRα. The authors showed that hyperactive BMP signaling causes intramuscular fibrosis in addition to heterotopic bone, that rapamycin decreases PDGFRα activity in treated muscles and that rapamycin eliminates fibroproliferation associated with hyperactive BMP signaling in an early FOP mouse model.

The findings from the study indicate that rapamycin can reduce fibroproliferation associated with muscle injury in the setting of increased BMP signaling. Rapamycin appears to reduce the presence of PDGFRα expressing progenitor cells at an injury site in a mouse model of FOP which may influence the fibroproliferative scaffold of heterotopic bone formation.

Further testing in state-of-the-art FOP animal models and with newly-available monoclonal antibodies to PDGFRα will help determine if PDGFRα can be added to the list of targets for FOP.
THE TOOTH FERRY PROGRAM AT THE FOP LAB: SHEDDING LIGHT ON FOP

The participation of so many patients and families who contribute blood/DNA samples to advance FOP research has been invaluable and is enormously appreciated. These samples were critical for discovering the FOP gene and for identifying the specific DNA sequence changes that occur in classic and variant forms of FOP. Although much FOP research can now be conducted using mouse models of FOP, FOP patient cells and tissues will always be essential in order to confirm that the information that we learn from mice holds true in humans.

We relied on blood samples from patients for many years since blood can be safely obtained without risk of triggering an FOP flare-up. However, blood cells provide limited information about FOP lesion formation. Fortunately, recent advances have identified additional types of human cell and tissue samples that can be obtained safely and are vitally important to our work. One of these cell types is “SHED cells.”

SHED stands for Stem cells from Human Exfoliated Deciduous teeth – a long name that describes the stem-like progenitor cells that are inside primary or baby teeth. When a baby tooth falls out naturally, we can recover the cells from inside the tooth. We have used baby teeth from FOP patients to show that these cells can be grown in our lab and treated in special ways to form cartilage and bone cells, providing us with an informative system to examine how the FOP mutation affects the differentiation potential of cells involved in an FOP lesion.

A few years ago, The FOP Center started a “Tooth Ferry” program to encourage families to send FOP baby teeth to us so that cells from these teeth could be used for FOP research. These cells have already given us bountiful information about the effects of the FOP mutation on cartilage and bone cell formation. These cells were used in our recent studies to down-regulate the mutant (damaged) copy of the FOP gene by siRNA and are being used in our ongoing studies on the effects of microenvironment factors on FOP flare-ups and lesion formation. Thus, SHED cells continue to be extremely vital for many of our laboratory experiments. Because the cells have a limited lifespan and since multiple samples from a person are very informative, we continually need additional “donations” to continue to conduct our studies with SHED cells.

Anyone with a child who is losing teeth can participate in “The Tooth Ferry Program.” When your child loses a tooth or needs to have one pulled at the dentist’s office, you can send it to us in a preassembled kit that we will provide to you. Teeth from siblings and non-family members are also welcome for comparison. In addition to baby teeth, we are also happy to receive wisdom and other permanent teeth from people with FOP. Permanent teeth also contain stem cells and we are currently investigating their use and applicability in FOP research.
Ruth McCarrick-Walmsley is heading up our effort to collect the teeth and study SHED cells. There is a brief window of opportunity for receiving the teeth with still-healthy cells, so we have developed specific instructions for their handling and shipping. If you decide to participate, we will send you a kit including all of the necessary return packaging (for several teeth), return FedEx labels, Ruth’s contact information, a tooth diagram to fill out and return, and a copy of instructions. We are also providing information about the program on the IFOPA website, however it is very important that you contact us before sending a tooth – if teeth arrive by surprise at the lab, we may not be able to prepare them optimally.

The Tooth Terry Kit is very simple to use. This is an IFOPA-supported program and there is no cost to you. If you have children with teeth still to lose or are being pulled, please contact Ruth at 610-513-4470 or rwalmsle@mail.med.upenn.edu and a “Tooth Ferry Kit” will be on its way to you soon!

THE CALI DEVELOPMENTAL GRANTS PROGRAM

In 1997, the Cali Family, in consultation with Dr. William N. Kelley, M.D., then Dean of The University of Pennsylvania School of Medicine, established The Center for Research in FOP & Related Disorders at The University of Pennsylvania. This was and still remains the only such Center of its kind in the world. Simultaneously, the Cali Family inaugurated the vanguard Extramural Developmental Grants Program which is administered by The Center. The mission of the Developmental Grants Program is to foster collaborative research between The Center and other research laboratories of excellence at Penn, and at other universities in the United States and around the globe. The program has been in place for 20 years and has had vast outreach to the relevant basic and translational science community worldwide.

The Cali Developmental Grants Program is proudly one of the crown jewels of the FOP research world. Over the past 20 years, the Cali Developmental Grants Program has awarded 53 grants of $50,000 each for a total support of $2.65 million. This innovative program has expanded horizons in FOP research well beyond the physical boundaries of the FOP laboratory at Penn into a true worldwide co-laboratory.

Research partners include other laboratories within The University of Pennsylvania as well as other universities and institutions including Baylor; Brown; Harvard; Northwestern; Texas A&M; Vanderbilt; University of California, San Francisco; Children’s Hospital of Philadelphia; The Mayo Clinic; and The Max Planck Institute for Molecular Biology in Germany.

The Cali Family Fund and Developmental Grants Program has funded work that led to the discovery of new therapeutic targets for FOP and to the development of kinase inhibitors, antibodies, extracellular traps, cellular pathway inhibitors, and inhibitory RNA for critical proof-of-principle studies in FOP.

Importantly, more than 80 percent of the scientists and researchers who participated or were represented at the 2014 and 2016 IFOPA Drug Development Forums in Boston have been direct or indirect beneficiaries of a Cali Developmental Research Grant from The Center for Research in FOP & Related Disorders.
In 2016, the Cali Developmental Grants Program supported three highly innovative research projects:

1. “Molecular Basis of Pathogenic Signaling and High Throughput Testing of FOP Therapies in a Zebrafish Model System” is directed by Dr. Mary Mullins from The University of Pennsylvania.

Dr. Mullins describes the work:

“Zebrafish have been used extensively in the study of BMP signaling, and more recently have been recognized for their power as a model of human disease. Our lab is using zebrafish development as a model of the aberrant BMP signaling found in FOP, to develop high throughput screens for potential therapeutics and to elucidate the mechanism by which FOP progresses in vivo.

During zebrafish development, the head to tail axis is patterned by BMP signaling in a process conserved in human development. Altering BMP signaling causes distinct dose-dependent defects, with increased signaling leading to loss of head tissues and decreased signaling leading to loss of tail tissues in the zebrafish. Previously, we determined that injection of RNA from the human FOP mutation into zebrafish embryos causes a loss of tail structures demonstrating that the mutation activates BMP signaling. By developing a transgenic FOP zebrafish model, we can screen for compounds that mitigate this feature and may be used as potential treatments for FOP.

Using zebrafish deficient in BMP signaling components, we have begun to elucidate the receptors and ligands that are involved in mutant FOP ACVR1 signaling in vivo. Heretofore, the analysis of the FOP signaling mechanism has predominantly relied on cell culture systems lacking normal in vivo physiology. Our zebrafish system brings a relevant in vivo physiological context to the study of aberrant FOP signaling. Additional experiments will reveal which receptors cooperate with the FOP ACVR1 to signal, providing additional targets to test with potential therapeutic compounds.

Using genome editing techniques, we will generate the R206H mutation and other FOP variant mutations in zebrafish. This method will produce a model of the human FOP ACVR1 mutations, which may more closely mimic the human phenotype than the current mouse model that causes embryonic lethality. We will characterize heterotopic ossification in this line and then use it to screen for potential therapeutics specific to heterotopic ossification.”

2. “Identifying Alternative Therapeutic Targets and Genetic Interactors in FOP” is directed by Dr. Ed Hsiao of University of California, San Francisco.

Dr. Hsiao describes the work:

“Although FOP is caused by an activating mutation in the ACVR1 gene, wide variation in clinical activity of FOP presents significant challenges for developing therapeutics that directly target ACVR1. This work seeks to identify alternate therapeutic targets through whole exome genetic analysis of FOP patients and their non-FOP family members. The work is focused on FOP patients with unusually mild or severe disease presentations. We anticipate finding modifier genetic variants that will help us understand what mitigates or worsens FOP presentations. These genes may also be useful alternative therapeutic targets if they display higher tissue specificity or can be targeted by drugs with lower toxicity. Together this study will establish a foundation for understanding the genetic contributors to the severity of FOP.”
3. “Novel Allosteric Destabilizers as Therapeutics for FOP” is directed by Dr. Jay Groppe of Texas A&M University College of Dentistry.

Dr. Groppe describes the work:

“At the 11th International BMP Conference at Harvard Medical School in October 2016, immediately following the second FOP Drug Development Forum in Boston, I presented basic and translational research supported since 2006 by the Ian Cali FOP Research Fund (ICFRF). Years of meticulous dissection of the mutant FOP receptor led to the conclusion that its damaging effects are due to too much flexibility, allowing bone-inducing signals to form in an overly-wide mouth of the receptor, in contrast to the more tight-lipped, non-mutated receptor, which keeps quiet and does not misguide cells. The paradigm-changing results revealed an entirely new therapeutic target that my colleagues and I have pursued since the spring of 2013 and that has been funded by a grant from ICFRF over the last two years.

Rather than blocking the mouth of the receptor (like Dorsomorphin or LDNs) by competing with the bone-inducing signals, novel inhibitors alter the flexibility of the receptor. With one inhibitor, flexibility can be diminished, resembling the non-mutated form. However, with other inhibitors, the flexibility of ACVR1 can be dramatically increased - so much so that the protein chain essentially comes unglued, unraveling into a structure-less, spaghetti-like strand that, in the test tube, sticks to the wall and is completely inactive.

A commonly prescribed breast cancer drug, Fulvestrant (trade name Faslodex), selectively degrades estrogen receptors in cells, by unraveling the protein. Strikingly, cells have no appetite (use) for spaghetti, sending the meaningless strands to the cellular trashcan to be chopped to bits. To determine whether this paradigm holds for degrading ACVR1, the novel inhibitors that I developed are being tested in cell-based assays - and hopefully eventually - in the FOP mouse.

By nature, the stability of the ACVR1 protein turns out to be inherently sensitive to the cellular environment (oxygen level, pH). I expect the new therapeutic approach to be highly efficacious by selectively targeting ACVR1 in inflamed soft tissues of early FOP flare-ups - thus trashing the FOP receptor when and where it is problematic. The hope is that such an approach might allow the development of a safe and effective therapeutic that could be prescribed prophylactically and lifelong.”
ASHLEY MARTUCCI FOP RESEARCH FUND

Established in 2015, the Ashley Martucci FOP Research Fund supports FOP research at The University of Pennsylvania’s Center for Research in FOP & Related Disorders – the world’s first and only comprehensive program in FOP. The Ashley Martucci FOP Research fund supports:

- **Basic Research** - This effort seeks to identify novel therapeutic targets for FOP, and investigates molecular mechanisms and immunologic triggers. The work is focused on investigating episodic bone growth – “flare-ups” – and understanding what triggers flare-ups; the molecular mechanisms in cells that amplifies flare-ups; the role of the immune system in triggering this process; and how mechanical factors (e.g. – growth, stress or mobility) and tissue hypoxia and inflammation induce and amplify flare-ups.

- **Ashley Martucci Fellowships** - Haitao Wang and Vitali Lounev, Ph.D. scientists along with Michael Convente, a Ph.D. graduate student, are investigating the immunological triggers of heterotopic bone formation in FOP.

**FOP: THE SPOKEN WORD – 2016**

During 2016, major lectures on FOP were presented at:

- Advances in Mineral Metabolism; Snowmass, Colorado
- American Society for Bone and Mineral Research; Atlanta, Georgia
- Children’s Hospital of Philadelphia; Philadelphia, Pennsylvania
- FOPev Annual Meeting; Valbert, Germany
- FOP Friends and Family Conference; Manchester, United Kingdom
- FOP France; Paris, France
- FOP Italia Annual Meeting; Livorno, Italy
- Frank H. Netter School of Medicine – Quinnipiac University; North Haven, Connecticut
- IFOPA Second Drug Development Forum; Boston, Massachusetts
- IFOPA Midwest Family Meeting; St Louis, Missouri
- Institute Imagine – Necker Children’s Hospital; Paris, France
- International BMP Conference; Boston, Massachusetts
We would like to acknowledge the extraordinary medical, scientific, and patient meetings in 2016 that we were honored to attend and in which we were honored to participate in Livorno, Italy; Manchester, United Kingdom; Paris, France; St. Louis, Missouri; and Valbert, Germany. These meetings were a wonderful opportunity to meet with scientists, researchers, physicians, students, and patients from around the world.

During 2016, highlights of FOP research were presented at local, regional, national, and international FOP family meetings and gatherings in:

- Allentown, Pennsylvania
- Boston, Massachusetts
- Livorno, Italy
- Manchester, United Kingdom
- Mountainside, New Jersey
- New York City, New York
- Paris, France
- Philadelphia, Pennsylvania
- St. Louis, Missouri
- Valbert, Germany
In 2016, publications from numerous groups on FOP and FOP-related issues appeared in peer-reviewed journals including:

- Academic Radiology
- Annals of Surgery
- Bone
- Cell Signaling
- Cytokine Growth Factor Reviews
- Disease Models and Mechanisms
- Experimental Molecular Medicine
- Journal of Bone & Mineral Research
- Journal of Clinical Investigation Insight
- Journal of Clinical & Medical Research
- Journal of Medical Genetics
- Journal of Surgical Research
- Journal of Trace Elements in Medicine and Biology
- Pediatric Radiology
- PLoS One
- Proceedings of the National Academy of Sciences
- Science Translational Medicine
- Seminars in Cell and Developmental Biology
- Stem Cells
- Stem Cell Research
- Stem Cell Translational Medicine
- Trends in Biochemical Science

As of January 1, 2017, the classic paper in *Nature Genetics* (April 2006) describing the discovery of the FOP gene has been cited in 628 major scientific publications worldwide.

The articles “Cellular hypoxia promotes heterotopic ossification by amplifying BMP signaling” and, “Palovarotene inhibits heterotopic ossification and maintains limb mobility and growth in mice with human ACVR1 (R206H) fibrodysplasia ossificans progressiva (FOP) mutation” were “two (of the top five) most talked about articles” in muscle and bone research in 2016 in a special citation by *The Journal of Bone and Mineral Research*. 

Above: Whitney Weldon (Manhattan, NY) and Dr. Kaplan at FOP Ashley’s Cure Finding a Cure for FOP Charitable Event in New York City [Photo Courtesy: Miller Photo, Jack Miller]; Below: Kathy Ford (Somers Point, NJ) with Dr. Kaplan at AJ’s Avengers Driving Away FOP Golf Classic in Blackwood, NJ
CHANGING OF THE GUARD

In October, 2016, Robert J. Pignolo, M.D., Ph.D., Associate Professor of Medicine and Orthopaedic Surgery accepted a newly-created position as the Kogod Professor of Geriatrics and Chair of Geriatric Medicine and Gerontology at the Mayo Clinic, Rochester, Minnesota. Bob joined the faculty at Penn in 2003 after completing a fellowship in geriatric medicine and a research fellowship studying FOP and POH. He directed the Clinic for Osteoporosis and Related Disorders in the Division of Geriatric Medicine, and has been the principal investigator of several ongoing clinical trials in FOP at Penn. An internationally recognized expert in common and rare bone diseases, Bob was the Ian Cali Distinguished Clinician Scientist at the Center for Research in FOP & Related Disorders. He was involved in every aspect of FOP basic, translational, and clinical research and patient care while also fulfilling his primary role in geriatric medicine. Bob will be missed greatly at Penn, but his presence in the FOP community will be enduring and continuing. He will establish an FOP clinical trial site at The Mayo Clinic and will continue robust collaborations on many FOP projects at The Center for Research in FOP & Related Disorders at Penn.

As Dr. Pignolo departs, we are thrilled to welcome Mona Al Mukaddam, M.D. to The Center for Research in FOP & Related Disorders. Dr. Al Mukaddam is a renowned endocrinologist in the Division of Endocrinology, Diabetes and Metabolism at the Perelman School of Medicine at the University of Pennsylvania. She has a career interest expertise in translational medicine and clinical trials and tremendous experience in common and rare bone disorders. We are thrilled and honored to have her join our program. Dr. Al Mukaddam received her Bachelor of Science and Medical Degrees from American University in Beirut, Lebanon. She completed her internship and residency in internal medicine at the University of Iowa, a postdoctoral fellowship in the Division of Endocrinology, Diabetes and Metabolism at Penn, and received a Master of Science Degree in Translational Medicine and Therapeutics at The University of Pennsylvania. Dr. Al Mukaddam is board certified in internal medicine, endocrinology, diabetes and metabolism.

She is a member of the American Society for Bone and Mineral Research, the Endocrine Society, and the Philadelphia Endocrine Society. She is a member of the Institutional Review Board of The University of Pennsylvania and Director of The University of Pennsylvania Bone Center. Dr. Al Mukaddam has had numerous distinguished invited lectureships throughout the United States, and is currently involved in all aspects of FOP clinical research. Dr. Al Mukaddam has published articles in The Journal of Endocrinology and Metabolism, British Journal of Diabetes and Vascular Disease, and The New England Journal of Medicine. Importantly, Dr. Al Mukaddam is now the principal investigator on all ongoing clinical trials in FOP at The Center for Research in FOP & Related Disorders at Penn.

Dr. Al Mukaddam attended the IFOPA Drug Development Forum in Boston, Massachusetts in October 2016. She is a distinguished member of The International Clinical Council for FOP (ICC), and serves on the communications committee as well as the clinical trials committee. Recently, Dr. Al Mukaddam attended the IFOPA-sponsored Midwest Family Gathering and Clinics in St. Louis in November, 2016.

We extend a very warm welcome to Dr. Al Mukaddam and look forward to a brilliant career of academic and service contributions to the worldwide FOP community. We are thrilled to welcome Dr. Mona Al Mukaddam to The Center for Research in FOP and Related Disorders.
FOP: WHAT CAN WE DO TO HELP?

There is an apocryphal story that on a visit to the launch complex at Cape Canaveral in 1961, President Kennedy asked a man who was sweeping the floor what he was doing. “Mr. President,” he said, “I am helping to put a man on the moon.” What is the message of that parable? Every job is important and teamwork is essential to achieve complex goals.

Patients, families, friends, even casual visitors to The Center for Research in FOP & Related Disorders often ask: “What can we do to help?” The answer is simple. “Anything you can.”

As Kate Griffo and John Glick at The University of Pennsylvania’s Perelman School of Medicine said, “In philanthropy, as in medicine, even brief inaction can do harm. A hiatus in research funding may mean that a promising treatment or a new line of inquiry may come to an untimely and devastating end. A break in efforts could halt progress toward finding a treatment that could relieve suffering or save lives.”

Research is laborious, time consuming, often frustrating, and costly, and is filled with false starts, blind alleys, glimmers of hope and the fog of frustration, but so too is the FOP we are trying to cure. Formidable enemies require formidable opponents, and teamwork requires resources. When seminal discoveries are made and ignorance is extinguished, the fog lifts, and the summits and the paths between them become clear. When knowledge advances, it illuminates the next horizon. It is a powerful beacon that changes the world like nothing else can. The feeling of accomplishment for all who contribute to this endeavor lights a fire of personal fulfillment and brings knowledge that they have contributed something important and enduring for other human beings for generations to come.

When modern FOP research began 26 years ago in a small laboratory at The University of Pennsylvania, there was little knowledge about this terrible disease, and little hope outside an infinitesimally small circle of believers who knew in their heart that something could be done to change it. Hope prevailed - hope fueled by the faith and commitment of a dedicated and persistent few who year after year funded studies to create and sustain a team devoted to make a difference. Over the years, that team has grown and expanded and its reach now extends around the world.

Through a sustained effort at The Center for Research in FOP & Related Disorders, research is eradicating the stifling ignorance that was prevalent just two decades ago. Barrier after barrier has fallen and achievable goals are in reach. FOP research holds real promise of preventing, treating, and curing FOP. It is no longer an imaginary dream. We need your help now more than ever to make this a reality.

The often-heard comment, “Call us when you have a treatment or a cure,” is an option, but not one that will help us find a cure. Everyone has a stake in this effort. We need your help in getting there: bake sales, swimming events, Burns’ Suppers, barn dances and bingo; chicken barbecues and spaghetti dinners, garage sales and silent auctions; country fairs and benefit concerts at the Metropolitan Opera; raffles and rodeos, sales of holiday cards and embroidered quilts, 5K runs and ice fishing contests; chamber music benefits and Hard Rock concerts; horse-plowing contests and competitive swims; golf tournaments and bowling parties; wine tasting events and lemonade stands on busy street corners.

No idea or endeavor is too small or too outlandish to help. Every second counts. Please help cure FOP.
MANY THANKS TO YOU

The members of The Center for Research in FOP & Related Disorders at The University of Pennsylvania and at collaborating laboratories around the world are extremely proud to be a part of this mission, and are enormously grateful to all of those who support this vital research effort to find better treatments and a cure.

Much has been accomplished, thanks in large part to the many benefactors and partners who have supported our work. The Center for Research in FOP & Related Disorders identified the genetic cause of FOP in 2006 and used that knowledge to spearhead worldwide research efforts to develop therapies that will transform the care of individuals with FOP. In 2014, clinical trials for FOP began - a major step forward. Now, as a comprehensive center, we manage and coordinate care for FOP patients - not only at Penn, but globally - and also engage in vital clinical, basic science, and translational research that can change the course of this rare and debilitating condition. We are vitally committed to education; we want to ensure that the next generation of physicians and scientists is as passionate about FOP research as we are.

Despite the progress we have made, there are still many unanswered questions and more monumental discoveries on the horizon that will improve treatment and bring us closer to ultimately finding a cure. Our work is broad and focuses on several areas of major activity, including: clinical care and consultation worldwide; clinical research and infrastructure development; basic research to identity therapeutic targets; translational research for preclinical drug testing and biomarker discovery; clinical trial development; and education.

The generous support of our benefactors has led to new therapeutic targets for FOP, new drug discoveries, and a rich research pipeline with diverse approaches to treatment of FOP. Our lifelong goal is to propel the development of therapies and eventually a cure for children and adults with FOP. This year, new clinical trials were launched and more are anticipated in the year ahead. We envision the day when FOP patients no longer hear the words “no treatment, no cure.”

We acknowledge the generous support of:

- The International FOP Association
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- The Penn Center for Musculoskeletal Disorders (PCMD)
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- The Weldon Family Endowment for FOP Research
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- The Isaac and Rose Nassau Professorship of Orthopaedic Molecular Medicine
- The Cali-Weldon Professorship of FOP Research
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- Canadian FOP Network
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**THE LAST WORD**

“The last word belongs not to the donors and benefactors, not to the physicians, scientists, researchers, journalists, or historians - but to the patients - who struggle valiantly and who look to us for a better way. The last word always belongs to them.

“My name is Felicia Wray. My husband and I have three children. The youngest, our daughter Maria, is four years old, and she has FOP. Maria was diagnosed at 15 months, so we have spent the last 3½ years learning about and living with FOP.

In that time, Maria has been pretty significantly affected by her FOP. She can’t move her head more than a tiny bit in any direction and her back has a lot of bumps and lumps. More significantly, her shoulders are completely locked – her upper arms are fixed at her sides, and she cannot move her arms away from her body at all, whether to break a fall or just to put on a jacket. Her left elbow is locked at a 90 degree angle and can’t move at all, either to bend or to straighten. Her right elbow is also bent at a right angle and cannot straighten; however, she can move that joint just enough to get her right hand up as high as her nose.

Three and a half years can seem like such a short time. But these years have been long ones for us. It was hard to watch our baby learn to walk, with us fearing every step. It was terrifying to watch her one-year-old body swell up: whether it was her forehead swelling her eye shut, or the back of her neck, her shoulders, her arms, her back. Each time was shocking. We tried different medications, but how do you explain an intravenous treatment to a child who isn’t even two? Those years didn’t feel short.

Hope. My definition of hope has changed over time. In the last 3½ years, I have hoped that Maria’s 18 months straight of flare-ups would end, I have hoped that we would get access to a new medication, I have hoped that medication would make a difference, I have hoped that she would feel better the next day, I have hoped that steroids would work, I have hoped that a pain she described wasn’t a flare-up. But above all else, every day, what I hope for, first and last, is a cure. And when I think about how close that day could be, it fills up my heart. I can only imagine how it feels for all of the families who have come before us.

Last year, I had the opportunity to meet a few FOP parents, all with children who are now young adults. They were kind and they remembered what it was like when their children were little, and the diagnosis was new. I imagined myself, maybe 20 years in the future, one day eating lunch with other parents of young adults with FOP. I hope that, when it is our turn, we will talk about how it felt to be on the verge of something huge and amazing. I hope we will use only the past tense to talk about flare-ups. I hope that Maria’s journey will be different – that there will be something to stop the FOP progression. She is only four.

The pace of research right now is inspiring. When we first met Dr. Kaplan, he told us there could be a drug trial starting up within the year, but it would only be for adults, and it would likely be many..."
years before children could be involved. Yet, amazingly, less than four years later, there are now two
drugs in clinical trials, and one of those trials was opened to kids as young as six! And there is so
much other work happening!

One day, in those early months, Dr. Kaplan told me, “While we are sleeping, people are working
on FOP.” Sometimes I remind myself of this. Right now, as I write this at 11:30 p.m., somewhere,
someone is working on ultra-rare FOP. All of those researchers around the world, along with Maria’s
team of doctors, whose kind and attentive care is so much more than clinical, are “the good guys”
in our story.

Maria is resilient and happy and strongly opinionated. I want to keep her that way. What else do
I want? A cure for FOP. First, something that would stop bone from forming during a flare-up.
Or something to stop flare-ups as soon as they start. But really, something that will stop flare-ups
before they ever start. Something that can be taken preventatively, every day, and turn off FOP forever.
Something so that if my daughter does fall down, she only has to worry about “regular” getting hurt,
and not worry about further permanent loss of mobility. And, not to be greedy, but if we could turn off
that gene, what about surgery to remove some of the extra bone? Could I be able to lift her arms away
from her body to put on her coat? To wash her armpits? Could she do it herself?

The point is, FOP research is not just an academic issue. It’s Maria’s life. It’s the life of every
individual with FOP, and it affects the lives of everyone who loves them.

What will we want in the future? I don’t know – I’m sure that as Maria ages, and as her FOP
progresses, there will be things she wants or needs that I can’t even imagine now. But one thing
that I don’t want to have to just imagine is a cure for FOP.”
The Center for Research in FOP & Related Disorders at The University of Pennsylvania. (Seated): Drs. Fred Kaplan and Eileen Shore. (Standing left to right): Dr. John Fong, Patsy Hooker, Dr. Haitao Wang, Dr. Deyu Zhang, Meiqi Xu, Renee Jurek, Kamlesh (Kay) Rai, Will Towler, Katherine Toder, Alexandra Stanley, Michael Convente, Dr. Sun Peck, Niambi Brewer, Dr. Vitali Lounev, Ruth McCarrick-Walmsley, Dr. Salin Chakkalakal and Bob Caron