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From mysteries to medicines: drug development for fibrodysplasia ossificans progressive

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Abstract

Introduction: Fibrodysplasia ossificans progressiva (FOP) is the most disabling disorder of skeletal metamorphosis in humans and leads to the formation of a second skeleton of heterotopic bone. Presently, there is no effective treatment.

Areas covered: In this review, the authors discuss heterozygous activating mutations in Activin receptor A, type I/ Activin-like kinase 2 (ACVR1/ALK2), a bone morphogenetic protein (BMP) type I receptor that are the genetic cause of FOP and reveal a promising pharmacologic target in the BMP signaling pathway. Despite these germline mutations, episodic disease activation is induced by soft tissue injury and resultant inflammatory triggers that are dependent on responding progenitor cells and a tissue microenvironment that supports heterotopic ossification.

Expert opinion: Here we review opportunities and challenges for the development of effective therapeutics for FOP. There are many potential approaches that may eventually be used to harness FOP. The long-term treatment of FOP is likely to involve not one, but several concomitant approaches that acknowledge molecular mechanisms involved in the induction and progression of the disease.

Keywords

Bone morphogenetic protein receptors; Fibrodysplasia ossificans progressiva; Heterotopic endochondral ossification; Skeletal metamorphosis

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Declaration of interests

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1. THE CLINICAL BACKGROUND OF FOP

1.1 The Natural History of FOP

Fibrodysplasia ossificans progressiva (FOP; MIM #135100) is the most catastrophic disorder of skeletal metamorphosis in humans and leads to the formation of a second skeleton of heterotopic bone [1-4]. Two invariant clinical features define classic FOP - congenital malformations of the great toes and progressive heterotopic endochondral ossification (HEO) [1-4]. Individuals with FOP appear normal at birth except for characteristic malformations of the great toes which are present in all classically affected individuals. During the first decade of life, episodic soft tissue swellings (or flare-ups) arise, usually in the neck or back. While some flare-ups regress spontaneously, most undergo pathological metamorphosis into mature heterotopic bone. Ribbons, sheets, and plates of heterotopic bone replace skeletal muscles and connective tissues through HEO and lead to an armament-like encasement of bone with permanent immobility [1-4]. Minor trauma such as intramuscular immunizations, mandibular blocks for dental work, muscle fatigue, blunt muscle trauma from bumps, bruises, falls, or influenza-like viral illnesses can trigger new flare-ups of FOP leading to progressive HEO [5-8].

Heterotopic ossification in FOP progresses in characteristic anatomic and temporal patterns, typically occurring first in the dorsal, axial, cranial, and proximal regions of the body and later in the ventral, appendicular, caudal, and distal regions [9, 10]. Several skeletal muscles including the diaphragm, tongue, and extra-ocular muscles, as well as cardiac and smooth muscle, are spared from HEO [1-4].

Bone formation in FOP is episodic, but disability is cumulative [10]. Most patients are confined to a wheelchair or are immobilized in a standing position by the third decade of life, and require lifelong assistance to perform activities of daily living [9, 10]. Severe weight loss may result following ankylosis of the jaw. Pneumonia or right-sided heart failure may complicate rigid fixation of the chest wall [11, 12]. The median survival age is 40 years; death often results from complications of thoracic insufficiency syndrome [13].

1.2 Radiographic Features of FOP

While malformation of the great toes is characteristic of FOP, other developmental anomalies are frequently observed. Characteristic anomalies of the cervical spine have been well-described [14], as well as scoliosis [15]. Other skeletal anomalies associated with FOP include malformed thumbs, short broad femoral necks, dysplasia of the hips, proximal medial tibial osteochondromas, and asymmetric ankylosis of the costo-vertebral joints [2, 16-18]. While plain radiographs are sufficient for following progression of the disease [17-19], changes have been described on magnetic resonance imaging at different stages of lesional progression [20]. Interestingly, fractures heal robustly in FOP patients [21].

1.3 The Histopathology of FOP

The histopathology of FOP lesions has been well-described [22-25]. Early FOP lesions contain intense mononuclear and perivascular infiltrates. Subsequent migration of

mononuclear inflammatory cells into affected muscle precedes widespread death of skeletal muscle [24, 26].

Following a rapid and destructive inflammatory stage, there is an intense angiogenic, fibroproliferative phase, histologically indistinguishable from aggressive juvenile fibromatosis, but replete with BMP2/4 expression in lesional cells [27]. Fibroproliferative tissue undergoes a predictable metamorphosis to bone through HEO (Figure 1). The resultant new ossicle of heterotopic bone is histologically normal and contains marrow elements [22, 24, 28].

Mast cells have been identified at every histological stage of FOP lesion formation. During the fibroproliferative stage, mast cells are found at a higher density than in any other inflammatory myopathy [29]. Recent studies suggest that neuroinflammatory signals also play an important role in the pathophysiology of the disease [30, 31].

Inflammatory cells of hematopoietic origin are associated with the induction of heterotopic ossification [28] and contribute to vasculogenesis in the late osteogenic stage [32], while multipotent Tie2⁺ stem-like cells of vascular, perivascular, or muscle interstitium origin contribute to the fibroproliferative, chondrogenic and osteogenic stages of heterotopic ossification [33-35].

The innate immune system is involved in the induction of FOP flare-ups [6, 28, 36]. Recent studies in mouse models of FOP strongly support the role of the innate immune system in inducing heterotopic ossification [37-41].

1.4 Biomarkers in FOP

Disease-specific and stage-specific biomarkers for FOP are lacking. Routine biochemical evaluations of bone mineral metabolism are normal, although the erythrocyte sedimentation rate (ESR) and serum alkaline phosphatase activity may be increased, especially during early and late disease flare-ups, respectively [42]. Urinary basic fibroblast growth factor levels may be elevated during disease flare-ups, coinciding with the angiogenic phase of early fibroproliferative lesions [43]. Circulating osteogenic cells also herald early heterotopic bone formation, but remain a research tool [32].

1.5 Diagnosis and Misdiagnosis of FOP

FOP is commonly misdiagnosed as aggressive juvenile fibromatosis, lymphedema, or soft tissue sarcoma [44]. Clinicians often fail to associate the rapidly developing soft tissue swellings that appear on the head, neck, and upper back with the malformed great toes. The misdiagnosis of FOP approaches 90 per cent of affected individuals worldwide [44]. Children often undergo unnecessary and harmful diagnostic biopsies that exacerbate the progression of the condition [44]. The definitive diagnosis of FOP can be made by simple clinical evaluation that associates rapidly appearing soft tissue lesions with malformations of the great toes [2, 3, 19]. Genetic confirmation can follow [45].

1.6 Epidemiology and Genetics of FOP

FOP is rare with a worldwide prevalence of approximately one in two million individuals. There is no ethnic, racial, gender, or geographic predisposition [46]. Most cases arise as a result of a spontaneous new mutation. A paternal age effect has been reported [47]. When observed, genetic transmission is autosomal dominant and can be inherited from either mothers or fathers [46, 48]. Maternal mosaicism may exist [49]. Fewer than ten small multigenerational families are known worldwide [46]. Phenotypic heterogeneity is observed [49].

Both genetic and environmental factors affect the phenotype of FOP. A study of three pairs of monozygotic twins with FOP found that within each pair, congenital toe malformations were identical. However, postnatal heterotopic ossification varied greatly depending on life history and environmental exposure to viral illnesses and to soft tissue trauma. Genetic determinants strongly influence disease phenotype during prenatal development while environmental factors strongly influence postnatal progression of heterotopic ossification [50].

Definitive genetic testing of FOP is available and can confirm a diagnosis of FOP prior to the appearance of heterotopic ossification. Clinical suspicion of FOP can lead to early clinical diagnosis, confirmatory diagnostic genetic testing (if appropriate), and the avoidance of harmful diagnostic and treatment procedures [45]. Clinicians should be cognizant of the early diagnostic signs of FOP which are congenital malformation of the great toes and episodic soft tissue swelling even before the appearance of heterotopic ossification [2, 3, 45]. This awareness should prompt genetic consultation and testing (if appropriate) and the institution of assiduous precautions to prevent injury and iatrogenic harm [45].

1.7 Current Symptomatic Treatment of FOP

There is no established medical treatment for FOP [3]. Medical management is currently supportive [51]. High dose glucocorticoids have a limited use in the management of the early inflammatory flare-ups [51]. The disorder's rarity, variability, and fluctuating clinical course pose substantial uncertainties when evaluating experimental therapies [52]. Bone marrow transplantation is ineffective, since even a normal immune system may trigger FOP flare-ups in a genetically susceptible chimeric host [28].

Removal of FOP lesions is often followed by recurrence. Surgical release of joint contractures has been unsuccessful and risks new, trauma-induced HEO. Spinal bracing is ineffective and surgical intervention is associated with numerous complications [15]. Dental therapy should preclude mandibular blocks and stretching of the jaw [51]. Guidelines for the administration of anesthetics are available [51]. While physical therapy to maintain joint mobility may be harmful by provoking or exacerbating lesions, occupational therapy is often helpful [51]. Intramuscular injections should be avoided. Prevention of falls, influenza, recurrent pulmonary infections, and complications of restricted chest wall disease is vital [51]. Hearing can be affected and audiology evaluations are encouraged, especially during childhood [53].

2. THE BASIC SCIENCE BACKGROUND OF FOP

The discovery of the genetic cause of FOP in 2006 immediately identified ACVR1/ALK2 as druggable target and led to investigations of initiating triggers, progenitor cells, and microenvironmental factors that are critical for disease activation and progression [54-56]. Most importantly, the identification of the FOP gene enabled the development of high-fidelity knock-in mouse models of classic FOP that are essential for pre-clinical drug testing [39, 40].

2.1 FOP & the BMP Signaling Pathway

The classic FOP phenotype of great toe malformations and progressive HEO suggested that the primary molecular pathology might involve the bone morphogenetic protein (BMP) signaling pathway [57]. A number of seminal studies provided evidence of profound dysregulation of the BMP signaling pathway in cells from FOP patients [24, 25, 27, 58-65].

2.2 The FOP Gene

Functional signaling studies and positional cloning identified the gene encoding activin receptor A, type I/activin-like kinase 2 (ACVR1/ALK2), a BMP type I receptor, as the FOP gene [54]. A recurrent heterozygous missense mutation in the glycine-serine (GS) activation domain (c.617G>A;R206H) was identified in all classically affected individuals with sporadic or familial FOP and comprises approximately 97 percent of all known FOP patients worldwide [18, 54]. Recently, additional mutations have been identified in the GS domain and kinase domain of ACVR1 in individuals with atypical forms of FOP, which are approximately three percent of all known FOP patients worldwide [18, 66-74].

2.3 Structural and Functional Consequences of the FOP Mutation

Protein homology modeling of the mutated ACVR1/ALK2 receptor predicts destabilization of the GS activation and/or Smad binding domain as the underlying cause of the ectopic chondrogenesis, osteogenesis, and joint fusions seen in FOP [54, 75-77]. Crystallographic and biochemical analysis of ACVR1/ALK2 has led to a more detailed understanding of inter- and intramolecular interactions of the mutant receptor. Evidence supports that the FOP mutation leads to allosteric destabilization of an inactive receptor conformation and the resultant loss of autoinhibition that occurs in all FOP genotypes [63, 76-81].

2.4 Animal Models of FOP

High-fidelity mouse models of FOP are necessary for pre-clinical testing. Presently, there are five mouse models of HEO associated with BMP signaling: implantation of native or recombinant BMP protein into skeletal muscle [82, 83], viral-mediated expression of BMP2 in skeletal muscle [84], transgenic over-expression of BMP4 expression under the control of a nerve specific promoter [37], a constitutively active ACVR1 mutation that does not exist in any known FOP patient but can induce heterotopic bone formation [85], and a knock-in animal model of classic FOP [39, 40]. Conventional knock-in mice [39, 40], however, cause lethality in a high percentage of chimeras, thus conditional strategies are necessary.

Clearly, the most useful mouse model for FOP would be a knock-in model of classic FOP. This is particularly important in order to answer a myriad of clinically-relevant questions for examining drug treatment strategies: Is the drug effective once a flare-up occurs? Can the drug be stopped without a rebound effect? What is the proper window for treating an FOP flare-up? What is the correct dosage and duration for treating an FOP flare-up? Is the medication effective for spontaneous as well as trauma-induced flare-ups? What are the potential short and long-term side-effects of FOP treatment in the context of the classic mutation? Until such questions are answered, effective clinical trials cannot safely be designed. Presently, with the newer animal models, these questions can and are being addressed.

3. APPROACHES AND OPPORTUNITIES FOR THE TREATMENT OF FOP

Definitive treatments and cures are not yet available for FOP, and there is a critical unmet need for such development. The discovery of the FOP gene and emerging insights into its mechanisms of action reveal at least four approaches to the treatment and/or prevention of FOP:

1. Blocking activity of the mutant receptor (ACVR1/ALK2) that causes increased BMP pathway signaling (through four possible strategies which include: monoclonal antibodies, signal transduction inhibitors (STIs), inhibitory RNA, and secreted antagonists).
2. Inhibiting triggers of FOP flare-ups.
3. Directing FOP stem cells to an alternate tissue fate other than cartilage or bone.
4. Blocking the body's response to microenvironmental signals that promote the formation of FOP lesions.

3.1 Approach 1: Blocking activity of the mutant receptor (ACVR1/ALK2) that causes increased BMP pathway signaling. (Four possible strategies include: monoclonal antibodies, signal transduction inhibitors (STIs), inhibitory RNA, and secreted antagonists.)

The discovery of the FOP gene identified ACVR1/ALK2 as a pharmacologic target for the treatment of FOP [54, 55]. Therapeutic strategies for inhibiting BMP signaling in FOP include inhibitory RNA technology to knock-down the mutant allele, blocking monoclonal antibodies directed against ACVR1/ALK2, orally administered small molecule signal transduction inhibitors (STIs) of ACVR1/ALK2, and secreted antagonists for BMP signaling.

3.1.1 Signal Transduction Inhibitor (STI strategy)—STIs are important molecular tools for studying BMP signaling in FOP, and have the potential to be developed into powerful therapeutic drugs for FOP [41, 86]. Several years ago, Dorsomorphin (Compound C) was identified in a screen for STIs that perturb BMP-regulated embryonic pattern formation in zebrafish [87]. Dorsomorphin and its derivatives (including LDN193189) inhibit all type I BMP receptors (ALK2, ALK3, and ALK6), and block downstream BMP-signaling [86]. However, a safe and effective STI for FOP must preferentially inhibit

ACVR1/ALK2 (the FOP gene) over ALK3 and ALK6, rather than completely blocking all BMP signaling, and must not affect other critical cellular receptors. Such highly-selective STIs are being developed [88, 89]. STIs designed to specifically block ACVR1/ALK2 must have specificity, efficacy, minimal or absent resistance, acceptable safety profiles, and be shown to lack toxic rebound effects before they can enter clinical trials for FOP.

Further extensive testing in animal models of classic FOP will be necessary to more completely evaluate potential efficacy, safety, dosage, duration, timing, treatment window and rebound issues.

3.1.2 Monoclonal Antibody Strategy—Although the mutated ACVR1/ALK2 receptor in FOP demonstrates leaky BMP signaling, it also exhibits ligand responsiveness and thus the opportunity for developing blocking antibodies to ACVR1/ALK2 in the prevention and treatment of FOP. The opportunities to develop monoclonal antibodies that are specific for ACVR1/ALK2 make this approach particularly appealing. Work is presently underway in this area of therapeutics.

3.1.3 Small Inhibitory RNA (a cellular RNA strategy)—A novel proof-of-principal approach using siRNA to specifically block the mutant FOP allele has been described. In two recent studies, the authors generated specific inhibitory RNA duplexes capable of suppressing the expression of the mutant copy of the gene in connective tissue progenitor cells from FOP patients and importantly show that this approach decreased the elevated BMP signaling in FOP cells to levels observed in control cells [72, 90]. The cells used in one of the studies were adult stem (or progenitor) cells obtained directly from deciduous teeth of FOP patients and thus preserve the exact stoichiometry of mutated and normal ACVR1/ALK2 receptors found in all classically affected FOP patients worldwide [90].

While this approach provides proof-of-principle for the use of allele-specific inhibition of ACVR1/ALK2 in the treatment of FOP, the *in vivo* utility of this approach must be confirmed in mouse models of classic FOP. Additionally, other hurdles impair human application at the present time, most notably safe delivery of the small RNA duplexes to cells in the human body.

3.2 Approach #2: Blocking Inflammatory Triggers

Several mouse models of heterotopic ossification strongly support roles for inflammation in triggering FOP flare-ups and heterotopic ossification [28, 30, 33, 37, 38, 41]. In one study, local muscle inflammation was sufficient to induce heterotopic ossification in a transgenic mouse model in which BMP4 was over-expressed at the neuromuscular junction [37]. In a related study, the activity of circulating monocytes and tissue macrophages was inhibited pharmacologically and genetically and found to substantially abrogate heterotopic ossification [38]. In another *in vivo* study, the activation of inflammatory pathways in a constitutively active ACVR1/ALK2 mouse model led to heterotopic ossification at sites of inflammation, whereas activation of the mutant ACVR1/ALK2 gene alone did not [41]. Together, these findings strongly support that an inflammatory microenvironment enables heterotopic ossification in the setting of dysregulated BMP signaling.

In FOP and related common disorders of acquired heterotopic ossification, sensory nerves regulate the innate immune system and amplify the formation of heterotopic bone [30, 31]. Substance P (SP), an 11 amino acid neurotransmitter and potent neuroinflammatory protein, plays a key role in this metamorphosis, and provides a critical link between the sensory branch of the nervous system and the innate immune system in the induction and amplification of heterotopic ossification [30, 31].

TRPV1 (Transient Receptor Potential Vanilloid 1) cationic channel receptors are located on the sensory nerve endings in muscle and other soft connective tissue and are stimulated by soft tissue injury. Heterotopic ossification is induced when the signal reaches the dorsal root ganglia and triggers sensory neurons to release SP which is transported to nerve ending in the injured muscle where it binds to SP receptors (NK1R) on tissue mast cells. Once bound to the mast cells, SP triggers the release of inflammation-inducing and edema-causing chemicals that intensify the innate inflammatory response and amplify the heterotopic ossification [30, 31].

Experiments in mice show that blocking any major signaling hub in the sensory pathway - the TRPV1 ion channel, the dorsal root ganglion cells, the preprotachykinin (PPTA) gene that encodes SP, the neurokinin (NK1R) 1 receptor for SP, the tissue mast cells that express NK1R, or the c-kit gene (required for mast cell development) – profoundly abrogates heterotopic ossification [31].

3.3 Approach #3: Blocking Responding Connective Tissue Progenitor Cells

Retinoids, used for the treatment of acne, are known to cause skeletal birth defects because they interfere with the formation of the cartilaginous scaffold on which the embryonic skeleton is built [91]. The idea of using retinoids to treat FOP flare-ups was simple, and elegant: if retinoids caused birth defects by disrupting the formation of the cartilaginous scaffold of the normal skeleton, perhaps they might retard the formation of HEO in FOP [91].

In the mid-1980s, a clinical trial of isotretinoin (13-cis-retinoic acid; accutane) was conducted for the prevention of FOP flare-ups [91]. Although the outcome of the clinical trial was equivocal, the idea of using a retinoid to prevent or treat FOP flare-ups was far ahead of its time. Over the past 30 years, nuclear retinoid receptors have been discovered, and specific agonists that possess far greater specificity than isotretinoin have been developed.

A novel approach to inhibit heterotopic ossification, not prior to induction, but rather, after the commencement of building a second skeleton has been reported [92]. In their landmark study, Shimono et al. show that the early chondrogenic stage of the pre-osseous scaffold is exquisitely sensitive to the inhibitory effects of retinoic acid receptor gamma (RAR γ). By using compounds that specifically activate the RAR γ receptor, the authors critically targeted the pre-cartilage and cartilage cells that follow from the inflammatory start signals and that are used as the scaffold to form mature heterotopic bone. In their mouse experiments, the authors employ a comprehensive approach using implanted stem cells, BMP induction of HEO, and a conditional transgenic mouse that forms FOP-like HEO and show that RAR γ

agonists potentially inhibit HEO. Remarkably, when the RAR γ agonists are stopped, no significant rebound effect occurs, indicating that the RAR γ agonist effect may be irreversible [92].

Importantly, the authors showed that this class of compounds is effective in inhibiting HEO in animal models during a wide treatment window that includes the pre-cartilage mesenchymal stem cell phase, up to, but not including, the bone formation phase [92, 93]. These findings suggest that the successful inhibition of HEO in patients may be possible even after the clinically elusive induction phase has occurred. Most remarkably, the authors showed that this class of compounds may actually redirect cell fate decisions in mesenchymal stem cells to a non-bone lineage, an observation with wide-reaching implications for skeletal oncology, vascular biology, and tissue engineering [92, 93]. Taken together, the authors identified a potent, orally available class of compounds that can prevent HEO in animal models by inhibiting the cartilage scaffold, and by diverting stem cells to a more benign soft tissue fate while avoiding the rebound phenomena seen in other classes of experimental medications. The authors showed that the RAR γ agonists potently and irreversibly down-regulate BMP signaling by promoting the degradation of phosphorylated BMP-pathway specific Smads [92, 93].

The beauty of this approach is that it does not broadly target the BMP signaling pathway in many tissues in the body, but instead targets a specific pathological process of tissue development (cartilaginous scaffold formation) that requires the BMP signaling pathway to cause disabling disease (Figure 2). Thus, it has the desired features of targeting the molecular basis for FOP in the very cells that form HEO, hopefully with minimal collateral damage [92, 93].

3.4 Approach #4: Altering the Physiologic Response to Conductive Microenvironments that Promote Heterotopic Ossification

Generation of a hypoxic microenvironment in skeletal muscle has recently been shown to be a critical step in the formation of heterotopic bone in a mouse model [94]. To better understand the physiological implications of hypoxia in the context of the FOP mutation, we tested the hypothesis that a hypoxic microenvironment enhances signaling through the mutant ACVR1/ALK2 receptor and demonstrated that BMP signaling was both enhanced and prolonged in the presence of the canonical ACVR1/ALK2 (R206H) mutation under hypoxic conditions compared to normoxic conditions [56]. Ongoing work supports a role for hypoxia in BMP-induced heterotopic ossification. Preliminary studies indicate that blocking the response to hypoxia abrogates the resultant HEO.

4. CONCLUSION

Flare-ups of FOP are sporadic and unpredictable, and there is great individual variability in the rate of disease progression. Several large studies on the natural history of FOP have confirmed that it is impossible to predict the initiation, duration, or severity of an FOP flare-up, although characteristic anatomic patterning has been described [9, 10]. The rarity of FOP and the unpredictable nature of the condition make it extremely difficult to assess any therapeutic intervention, a fact recognized as early as 1918 by Julius Rosenstirn [52]:

“The disease was attacked with all sorts of remedies and alternatives for faulty metabolism; every one of them with more or less marked success observed solely by its original author but pronounced a complete failure by every other follower. In many cases, the symptoms of the disease disappear often spontaneously, so the therapeutic effect (of any treatment) should not be unreservedly endorsed.”

These words ring true today as they did when they were written nearly a century ago. FOP’s extreme rarity, variable severity, and fluctuating clinical course pose daunting uncertainties in developing and evaluating experimental therapies.

The FOP gene mutation in ACVR1/ALK2 creates a new form of this BMP receptor that when triggered by tissue injury unleashes a cascade of events that transforms muscle, tendon, ligament, fascia, and aponeuroses into a second skeleton of heterotopic bone through a metamorphosis that first destroys the normal tissue and then builds a second skeleton [56, 65].

There are many potential approaches that may eventually be used to harness FOP. The long-term treatment of FOP is likely to involve not one, but several concomitant approaches that acknowledge molecular mechanisms involved in the induction and progression of the disease. The challenge is controlling the renegade bone formation so that skeleton is made only where and when it is wanted and needed [95].

5. EXPERT OPINION

5.1 Need for Natural History Studies in FOP

In order to advance the lessons learned from ongoing animal studies into the design of meaningful clinical trials for FOP, it is first necessary to have a comprehensive and contemporary understanding of the natural history of FOP. Detailed knowledge of how flare-ups behave and progress in the context of present symptomatic management is essential before meaningful clinical trials can be designed.

Several historical studies on the natural history of FOP confirmed the extreme difficulty in predicting the onset, duration, or severity of flare-ups, although characteristic anatomic patterns of disease progression have been described [9, 10]. However, the data are more than twenty years old, come from an era when there were no more than 45 patient-members of the International FOP Association (IFOPA) [96] and from a time when the symptomatic treatment of FOP was very different than it is now.

Today, there are nearly twenty times the numbers of known individuals with FOP worldwide. Importantly, a larger percentage of the world’s population of FOP patients routinely uses powerful steroidal and non-steroidal anti-inflammatory medications to quell the symptoms of acute flare-ups. Intervention can change outcome, and yesterday’s data may not be reliable to predict tomorrow’s therapy.

Recent reports from academia, international regulatory agencies, small biotech companies and large pharmaceutical corporations emphasize that the most common cause for failure of clinical trials in rare diseases is not a lack of appropriate molecular targets or a lack of

potentially useful drugs, but a lack of comprehensive knowledge of the natural history of the disease.

For FOP, comprehensive, contemporary knowledge of the natural history of flare-ups is of paramount importance in the design of any clinical trial. Mechanisms of drug action, safety profiles, off-target side effects, and interactions with other drugs need to be understood in the context of spontaneous and trauma-induced FOP flare-ups and in the context of real world experience across every geographic, ethnic, and cultural boundary. Such comprehensive knowledge of the natural history of FOP flare-ups becomes even more important when one contemplates the clinical complexity of FOP – the progressive developmental stages and evolving time course of each lesion, the various anatomic sites involved in the disease process, the variable clinical course of flare-ups even in the same individual, and the range of individual responses to symptomatic measures over time. Add to that the spectrum of diverse regional and cultural approaches and constraints to symptomatic management of flare-ups, the ultra-rare nature of FOP, the incredibly small number of patients worldwide, and the clinical imperative of knowing with certainty if a new pharmaceutical compound might be effective when contemporary placebo control groups might not be possible or feasible in a clinical trial. Considering the overwhelming cost and risk involved, most pharmaceutical companies simply will not embark on clinical trials unless there are adequate data on the natural history of FOP on which to base a judgment outcome.

A comprehensive, worldwide survey of FOP flare-ups was launched in December 2012 and will be vital for designing a wide range of clinical trials that are likely to emerge in the years to come.

5.2 Need for Biomarkers in FOP

Biomarkers are urgently needed for successful clinical trials in FOP:

1. To measure and monitor the variability and progression of FOP lesions in each individual and between individuals.
2. To measure and monitor the stages of disease activity during and between flare-ups.
3. 3. To measure and monitor each individual's response to the drug being studied.

Unlike in cancer, where disease biomarkers may be the same or similar throughout the course of the disease, biomarkers will likely vary in FOP, based upon the stage of the disease and the phase of the flare-up. For example, biomarkers for the earliest inflammatory phase of an FOP flare-up may be very different from those in the later phases of HEO.

It will be important to identify and monitor stage-specific biomarkers during FOP flare-ups to assess the efficacy of new medications. For example, some medications may work on one phase of a flare-up but not another. Thus entering a patient into a clinical trial in an inappropriate phase of a flare-up would be detrimental and would skew the results of any clinical trial.

Without such biomarkers, it would be difficult to know if a drug being studied might potentially be useful, but not quite reach optimal timing, dosage, or potency. Thus, disease-specific, stage-specific, and drug-specific biomarkers will be helpful in assessing the results of any clinical trial.

5.3 Clinical Trial Design in FOP

There are three scenarios for potential clinical trials in FOP each with its own implication for design and implementation. The first is the short-term treatment of acute flare-ups. The second is the long-term prevention of acute flare-ups. The third is the surgical removal of heterotopic bone and the liberation of joints presently ankylosed with heterotopic bone.

Different medications may lend themselves to different clinical trial designs. For example, drugs targeted to acute flare-ups would have to be useful once a flare-up is identified by the patient. A drug that might be used to treat acute flare-ups would have to first be shown to be effective after a spontaneous or trauma-induced flare-up has begun in a conditional knock-in mouse model of classic FOP.

Any drug targeted to prevent acute flare-ups would need to have an acceptably benign long-term safety profile. If the medication would have to be stopped for any reason, concern about rebound flare-ups would prevail. Because it is impossible to predict when a flare-up will occur, it will be necessary to study drugs over long periods of time to determine if they can alter the natural history of the disease and the survival rate of joint mobility over time.

Finally, any potential drug that is used for surgical removal of heterotopic bone would first have to be proven to be safe and effective in a prevention scenario in FOP patients, without any rebound flare-ups once the drug was discontinued. The life-threatening risk of general anesthesia will likely preclude this as a preliminary approach to testing new drugs in FOP. Nevertheless, this approach holds much appeal once a drug has been validated as a treatment or prevention of spontaneous flare-ups in a non-surgical setting.

The design of clinical trials for FOP must take into consideration not only these three scenarios, but whether or not any potential drug has pre-clinical effectiveness, dosage and safety profile that might allow it to be used in such a clinical setting.

5.4 Hurdles to Drug Development for FOP

The hurdles to drug development for FOP can be divided into five categories: Disease-related, drug-related, investigator-industry-related, regulatory-related, and support-group-related (Table 1).

Disease-related hurdles to drug development for FOP include the rarity of the condition, the variability of disease progression, the physiological similarity of heterotopic bone to normal skeletal bone, the variable stages of disease progression, the lack of target specificity (the ACVR1/ALK2 receptor and several other receptors are remarkably similar), the lack of sufficient natural history, and the paucity of disease, stage- and drug-specific biomarkers.

Drug-related hurdles to drug development for FOP include drug specificity issues, toxicity, pharmacokinetics, solubility, metabolism, and delivery issues. Each of these is specific to the various therapeutic categories under study and development.

Investigator-industry related hurdles to drug development for FOP include competing financial interests, competing academic interests, and biotechnology and pharmaceutical company proprietary issues.

Regulatory-related hurdles to drug development for FOP include meeting investigational review board criteria, and requirements from regulatory agencies including FDA, and other international regulatory bodies.

Support group-related hurdles to drug development for FOP include funding-related issues, harmonized international disease registry issues, and fragmented international patient group efforts. This latter issue is particularly important and requires that the international FOP community speak with one voice and one intention when it comes to clinical trials.

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Annotated References

- 1•. Connor JM, Evans DA. Fibrodysplasia ossificans progressiva. The clinical features and natural history of 34 patients. *J Bone Joint Surg Br.* 1982; 64:76–83. Superb general review of FOP clinical features. [PubMed: 7068725]
- 2•. Kaplan FS, Glaser DL, Shore EM, et al. The phenotype of fibrodysplasia ossificans progressiva. *Clin Rev Bone & Miner Metab.* 2005; 3:183–188. A thorough review of the clinical features of FOP.
- 3•. Kaplan FS, LeMerrer M, Glaser DL, et al. Fibrodysplasia ossificans progressiva. *Best Pract Res Clin Rheumatol.* 2008; 22:191–205. A succinct clinical review of FOP. [PubMed: 18328989]
4. Shore EM, Kaplan FS. Inherited human diseases of heterotopic bone formation. *Nat Rev Rheumatol.* 2010; 6:518–527. A seminal review of genetic disorders of heterotopic ossification. [PubMed: 20703219]
5. Glaser DL, Rocke DM, Kaplan FS. Catastrophic falls in patients who have fibrodysplasia ossificans progressiva. *Clin Orthop Rel Res.* 1998; 346:110–116.
- 6•. Lanchoney TF, Cohen RB, Rocke DM, et al. Permanent heterotopic ossification at the injection site after diphtheria-tetanus-pertussis immunizations in children who have fibrodysplasia ossificans progressiva. *J Pediatr.* 1995; 126:762–764. A classic study describing iatrogenic harm in FOP from intramuscular immunizations. [PubMed: 7752003]
- 7•. Luchetti W, Cohen RB, Hahn GV, et al. Severe restriction in jaw movement after routine injection of local anesthetic in patients who have progressive. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 1996; 81:21–25. Routine mandibular blocks cause ankylosis of the jaw in FOP. [PubMed: 8850477]
8. Scarlett RF, Rocke DM, Kantanie S, et al. Influenza-like viral illnesses and flare-ups of fibrodysplasia ossificans progressiva (FOP). *Clin Orthop Rel Res.* 2004; 423:275–279. Viral illnesses can cause FOP flare-ups.

- 9•. Cohen RB, Hahn GV, Tabas JA, et al. The natural history of heterotopic ossification in patients who have fibrodysplasia ossificans progressiva. A study of 44 patients. *J Bone Joint Surg Am.* 1993; 75:215–219. Thorough review of the natural history of FOP.
- 10•. Rocke DM, Zasloff M, Peeper J, et al. Age and joint-specific risk of initial heterotopic ossification in patients who have fibrodysplasia ossificans progressiva. *Clin Orthop.* 1994; 301:243–248. Age-related survival curves for FOP joint involvement. [PubMed: 8156682]
11. Kaplan FS, Glaser DL. Thoracic insufficiency syndrome in patients with fibrodysplasia ossificans progressiva. *Clin Rev Bone & Miner Metab.* 2005; 3:213–216.
- 12•. Kussmaul WG, Esmail AN, Sagar Y, et al. Pulmonary and cardiac function in advanced fibrodysplasia ossificans progressiva. *Clin Orthop.* 1998; 346:104–109. The importance of baseline pulmonary function studies in FOP. [PubMed: 9577416]
- 13•. Kaplan FS, Zasloff MA, Kitterman JA, et al. Early mortality and cardiorespiratory failure in patients with fibrodysplasia ossificans progressiva. *J Bone Joint Surg Am.* 2010; 92:686–691. Comprehensive mortality data in FOP. [PubMed: 20194327]
- 14•. Schaffer AA, Kaplan FS, Tracy MR, et al. Developmental anomalies of the cervical spine in patients with fibrodysplasia ossificans progressiva are distinctly different from those in patients with Klippel-Feil syndrome. *Spine.* 2005; 30:1379–1385. A comprehensive analysis of cervical spine malformations in FOP. [PubMed: 15959366]
15. Shah PB, Zasloff MA, Drummond D, Kaplan FS. Spinal deformity in patients who have fibrodysplasia ossificans progressiva. *J Bone Joint Surg Am.* 1994; 76:1442–1450. [PubMed: 7929490]
- 16•. Deirmengian GK, Hebela NM, O'Connell M, et al. Proximal tibial osteochondromas in patients with fibrodysplasia ossificans progressiva. *J Bone Joint Surg Am.* 2008; 90:366–374. Important description of a key developmental feature of FOP. [PubMed: 18245597]
- 17•. Kaplan FS, Strear CM, Zasloff MA. Radiographic and scintigraphic features of modeling and remodeling in the heterotopic skeleton of patients who have fibrodysplasia ossificans progressiva. *Clin Orthop.* 1994; 304:238–247. The first comprehensive report of radiographic findings in FOP. [PubMed: 8020223]
- 18•. Kaplan FS, Xu M, Seemann P, et al. Classic and atypical fibrodysplasia ossificans progressiva phenotypes are caused by mutations in the bone morphogenetic protein (BMP) type I receptor ACVR1. *Hum Mutat.* 2009; 30:379–390. A seminal description of classic and variant mutations of ACVR1/ALK2 in FOP. [PubMed: 19085907]
- 19•. Mahboubi S, Glaser DL, Shore EM, Kaplan FS. Fibrodysplasia ossificans progressiva (FOP). *Pediatr Radiol.* 2001; 31:307–314. Comprehensive radiographic analysis of FOP. [PubMed: 11379597]
20. Shirkhoda A, Armin A-R, Bis KG, et al. MR imaging of myositis ossificans: Variable patterns at different stages. *J Magn Reson Imaging.* 1995; 65:287–292. [PubMed: 7633105]
21. Einhorn TA, Kaplan FS. Traumatic fractures of heterotopic bone in patients who have fibrodysplasia ossificans progressiva. *Clin Orthop.* 1994; 308:173–177. [PubMed: 7955679]
- 22•. Kaplan FS, Tabas JA, Gannon FH, et al. The histopathology of fibrodysplasia ossificans progressiva: An endochondral process. *J Bone Joint Surg (Am).* 1993; 75:220–230. The first comprehensive description of the endochondral nature of FOP lesions. [PubMed: 7678595]
23. Pignolo RJ, Suda, RK, Kaplan FS. The fibrodysplasia ossificans progressiva lesion. *Clin Rev Bone Miner Metab.* 2005; 3:195–200.
- 24•. Glaser DL, Economides AN, Wang L, et al. In vivo somatic cell gene transfer or an engineered noggin mutein prevents BMP4-induced heterotopic ossification. *J Bone Joint Surg Am.* 2003; 85:2332–2342. Proof-of-concept of Noggin gene therapy in BMP-induced heterotopic ossification and a thorough description of BMP-associated HEO. [PubMed: 14668502]
- 25•. Hegyi L, Gannon FH, Glaser DL, et al. Stromal cells of fibrodysplasia ossificans progressiva lesions express smooth muscle lineage markers and the osteogenic transcription factor Runx2/Cbfa-1: clues to a vascular origin of heterotopic ossification. *J Pathol.* 2003; 201:141–148. This paper highlights the importance of vascular cell involvement in early FOP lesions. [PubMed: 12950027]

- 26•. Gannon FH, Valentine BA, Shore EM, et al. Acute lymphocytic infiltration in an extremely early lesion of fibrodysplasia ossificans progressiva. *Clin Orthop*. 1998; 346:19–25. The first description of lymphocytic involvement in early FOP lesions. [PubMed: 9577406]
- 27•. Gannon F, Kaplan FS, Olmsted E, et al. Differential immunostaining with bone morphogenetic protein (BMP) 2/4 in early fibromatous lesions of fibrodysplasia ossificans progressiva and aggressive juvenile fibromatosis. *Hum Pathol*. 1997; 28:339–343. Early FOP lesions have active BMP signaling. [PubMed: 9042799]
- 28•. Kaplan FS, Glaser DL, Shore EM, et al. Hematopoietic stem-cell contribution to ectopic skeletogenesis. *J. Bone Joint Surg Am*. 2007; 89:347–357. Bone marrow transplantation does not cure FOP and even a normal immune system can trigger FOP flare-ups in a genetically susceptible host. [PubMed: 17272450]
- 29•. Gannon FH, Glaser D, Caron R, et al. Mast cell involvement in fibrodysplasia ossificans progressiva. *Hum Pathol*. 2001; 32:842–848. Description of an important feature of FOP lesions. [PubMed: 11521229]
30. Kan L, Lounev VY, Pignolo RJ, et al. Substance P signaling mediates BMP dependent heterotopic ossification. *J Cell Biochem*. 2011; 112:2759–2772. [PubMed: 21748788]
- 31•. Salisbury E, Rodenberg E, Sonnet C, et al. Sensory nerve induced inflammation contributes to heterotopic ossification. *J Cell Biochem*. 2011; 112:2748–2758. The importance of neuro-inflammatory triggers in FOP lesion formation. [PubMed: 21678472]
32. Suda RK, Billings PC, Egan KP, et al. Circulating osteogenic precursor cells in heterotopic bone formation. *Stem Cells*. 2009; 27:2209–2219. [PubMed: 19522009]
- 33•. Lounev V, Ramachandran R, Wosczyzna MN, et al. Identification of progenitor cells that contribute to heterotopic skeletogenesis. *J Bone Joint Surg Am*. 2009; 91:652–663. Progenitor cells of FOP lesions are endogenous to the tissue rather than hematopoietic in origin and express Tie2+ [PubMed: 19255227]
- 34•. Medici D, Shore EM, Lounev VY, et al. Conversion of vascular endothelial cells into multipotent stem-cell like cells. *Nat Med*. 2010; 12:1400–1406. Are new progenitor cells created by the FOP mutation? [PubMed: 21102460]
35. Wosczyzna MN, Biswas AA, Cogswell CA, Goldhamer DJ. Multipotent progenitors resident in skeletal muscle interstitium exhibit robust BMP-dependent osteogenic activity and mediate heterotopic ossification. *J Bone Miner Res*. 2012; 27:1004–1017. [PubMed: 22307978]
- 36•. Kaplan FS, Shore EM, Gupta R, et al. Immunological features of fibrodysplasia ossificans progressiva and the dysregulated BMP4 pathway. *Clin Rev Bone Miner Metab*. 2005; 3:189–193. The first evidence for immunological dysregulation in FOP flare-ups.
37. Kan L, Hu M, Gomes WA, Kessler JA. Transgenic mice overexpressing BMP4 develop a fibrodysplasia ossificans progressiva (FOP)-like phenotype. *Am J Pathol*. 2004; 165:1107–1115. [PubMed: 15466378]
38. Kan L, Liu Y, McGuire TL, et al. Dysregulation of local stem/progenitor cells as a common cellular mechanism for heterotopic ossification. *Stem Cells*. 2009; 27:150–156. [PubMed: 18832590]
- 39•. Chakkalakal SA, Zhang D, Culbert AL, Convente MR, et al. An *Acvr1* R206H knock-in mouse has fibrodysplasia ossificans progressiva. *J Bone Miner Res*. 2012; 27:1746–1756. First proof that the classic FOP mutation causes FOP. [PubMed: 22508565]
- 40•. Kaplan FS, Chakkalakal SA, Shore EM. Fibrodysplasia ossificans progressiva: mechanisms and models of skeletal metamorphosis. *Dis Model Mech*. 2012; 5:756–762. A thorough review of animal models for FOP. [PubMed: 23115204]
41. Yu PB, Deng, DY, Lai CS, et al. BMP type I receptor inhibition reduces heterotopic ossification. *Nat Med*. 2008; 14:1363–1369. An STI inhibits HEO in a mouse model of FOP. [PubMed: 19029982]
- 42•. Lutwak L. Myositis ossificans progressiva: mineral, metabolic, and radioactive calcium studies of the effects of hormones. *Am J Med*. 1964; 37:269–293. An attempt at finding biomarkers for FOP. [PubMed: 14206759]

- 43•. Kaplan F, Sawyer J, Connors S, et al. Urinary basic fibroblast growth factor: a biochemical marker for preosseous fibroproliferative lesions in patients with FOP. *Clin Orthop*. 1998; 346:59–65. The first report of a biomarker for early FOP flare-ups. [PubMed: 9577411]
- 44•. Kitterman JA, Kantanie S, Rocke DM, Kaplan FS. Iatrogenic harm caused by diagnostic errors in fibrodysplasia ossificans progressiva. *Pediatrics*. 2005; 116:654–661. A seminal study of catastrophic misdiagnosis of FOP.
- 45•. Kaplan FS, Xu M, Glaser DL, et al. Early diagnosis of fibrodysplasia ossificans progressiva. *Pediatrics*. 2008; 121:e1295–1300. FOP can be diagnosed before HEO appears. [PubMed: 18450872]
46. Shore EM, Feldman GJ, Xu M, Kaplan FS. The genetics of fibrodysplasia ossificans progressiva. *Clin Rev Bone & Miner Metab*. 2005; 3:201–204. An excellent review of the genetics of FOP.
- 47•. Rogers JG, Chase GA. Paternal age effect in fibrodysplasia ossificans progressiva. *J Med Genet*. 1979; 16:147–148. FOP is more common from older fathers. [PubMed: 287808]
- 48•. Kaplan FS, McCluskey W, Hahn G, et al. Genetic transmission of fibrodysplasia ossificans progressiva. Report of a family. *J Bone Joint Surg (Am)*. 1993; 75:1214–1220. The first report of a multigenerational family with classic FOP.
49. Janoff HB, Muenke M, Johnson LO, et al. Fibrodysplasia ossificans progressiva in two half-sisters. Evidence for maternal mosaicism. *Am J Med Genet*. 1996; 61:320–324.
- 50•. Hebela N, Shore EM, Kaplan FS. Three pairs of monozygotic twins with fibrodysplasia ossificans progressiva: the role of environment in the progression of heterotopic ossification. *Clin Rev Bone & Miner Metab*. 2005; 3:205–208. Importance of environmental factors in the progression of FOP lesions.
- 51•. Kaplan FS, Shore EM, Pignolo RJ. The medical management of fibrodysplasia ossificans progressiva: current treatment considerations. *Clin Proc Intl Consort FOP*. 2011; 4:1–100. A comprehensive set of guidelines to the contemporary medical management of FOP. www.ifopa.org
- 52•. Rosenstirn JA. A contribution to the study of myositis ossificans progressiva. *Ann Surg*. 1918; 68:485–520. 591–637. One of the finest and earliest reviews of FOP, patient-by-patient. [PubMed: 17864014]
- 53•. Levy CE, Lash AT, Janoff HB, Kaplan FS. Conductive hearing loss in individuals with fibrodysplasia ossificans progressiva. *Am J Audiol*. 1999; 8:29–33. An important analysis of hearing impairment in FOP. [PubMed: 10499116]
- 54•*. Shore EM, Xu M, Feldman GJ, et al. A recurrent mutation in the BMP type I receptor ACVR1 causes inherited and sporadic fibrodysplasia ossificans progressiva. *Nature Genetics*. 2006; 38:525–527. The discovery of the FOP gene. [PubMed: 16642017]
55. Kaplan FS, Glaser DL, Pignolo RJ, Shore EM. A new era of fibrodysplasia ossificans progressiva (FOP): a druggable target for the second skeleton. *Expert Opinion on Biol Ther*. 2007; 7:705–712.
- 56•. Kaplan FS, Lounev V, Wang H, et al. Fibrodysplasia ossificans progressiva: a blueprint for the metamorphosis. *Ann NY Acad Sci*. 2011; 1237:5–10. The FOP mutation is the architect, not the builder of FOP. [PubMed: 22082359]
- 57•. Kaplan F, Tabas JA, Zasloff MA. Fibrodysplasia ossificans progressiva: A clue from the fly? *Calcif Tissue Int*. 1990; 47:117–125. The seminal hypothesis of BMP dysregulation in FOP. [PubMed: 2117991]
- 58•. Shafritz AB, Shore EM, Gannon FH, et al. Dysregulation of bone morphogenetic protein 4 (BMP4) gene expression in fibrodysplasia ossificans progressiva. *N Engl J Med*. 1996; 335:555–561. The first description of dysregulation of the BMP pathway in FOP cells. [PubMed: 8678932]
59. Ahn J, Serrano de La Peña L, Shore EM, Kaplan FS. Paresis of a bone morphogenetic protein antagonist response in a genetic disorder of heterotopic skeletogenesis. *J Bone Joint Surg Am*. 2003; 85:667–674. [PubMed: 12672843]
60. Serrano de la Peña L, Billings PC, Fiori JL, et al. Fibrodysplasia ossificans progressiva (FOP), a disorder of ectopic osteogenesis, misregulates cell surface expression and trafficking of BMPRIA. *J Bone Miner Res*. 2005; 20:1168–1176. [PubMed: 15940369]

61. Fiori JL, Billings PC, Serrano de la Peña L, et al. Dysregulation of the BMP-p38 MAPK signaling pathway in cells from patients with fibrodysplasia ossificans progressiva (FOP). *J Bone Miner Res.* 2006; 21:902–909. [PubMed: 16753021]
62. Billings PC, Fiori JL, Bentwood JL, et al. Dysregulated BMP signaling and enhanced osteogenic differentiation of connective tissue progenitor cells from patients with fibrodysplasia ossificans progressiva (FOP). *J Bone Miner Res.* 2008; 23:305–313. [PubMed: 17967130]
63. Shen Q, Little SC, Xu M, et al. The fibrodysplasia ossificans progressiva R206H ACVR1 mutation activates BMP-independent chondrogenesis and zebrafish embryo ventralization. *J Clin Invest.* 2009; 119:3462–3472. An important study describing the mechanism of action of the mutant BMP receptor. [PubMed: 19855136]
64. Fukuda T, Kohda M, Kanomata K, et al. Constitutively activated ALK2 and increased SMAD1/5 cooperatively induce bone morphogenetic protein signaling in fibrodysplasia ossificans progressiva. *J Biol Chem.* 2009; 284:7149–7156. [PubMed: 18684712]
65. Kaplan FS, Pignolo RJ, Shore EM. The FOP metamorphogene encodes a novel type I receptor that dysregulates BMP signaling. *Cytokine Growth Factor Reviews.* 2009; 20:399–407. A comprehensive review of the global dysregulation of BMP signaling from the FOP gene. [PubMed: 19896889]
66. Bocciardi R, Bordo D, Di Duca M, et al. Mutational analysis of the ACVR1 gene in Italian patients affected with fibrodysplasia ossificans progressiva: confirmations and advancements. *Eur J Hum Genet.* 2009; 3:311–318. [PubMed: 18830232]
67. Carvalho DR, Navarro MMM, Martins B, et al. Mutational screening of ACVR1 gene in Brazilian fibrodysplasia ossificans progressiva patients. *Clin Genet.* 2010; 77:171–176. [PubMed: 19796185]
68. Furuya H, Ikezoe K, Wang LX, et al. A unique case of fibrodysplasia ossificans progressiva with an ACVR1 mutation, G356D, other than the common mutation (R206H). *Amer J Med Genet Pt. A.* 2008; 146A(4):459–463.
69. Gregson CL, Hollingworth P, Williams M, et al. A novel ACVR1 mutation in the glycine/serine-rich domain found in the most benign case of a fibrodysplasia ossificans progressiva variant reported to date. *Bone.* 2011; 48:654–658. [PubMed: 21044902]
70. Petrie KA, Lee WH, Bullock AN, et al. Novel mutations in ACVR1 result in atypical features in two fibrodysplasia ossificans progressiva patients. *Plos One.* 2009; 4(3):e5005. [PubMed: 19330033]
71. Ratbi I, Borciardi R, Regragui A, et al. Rarely occurring mutation of ACVR1 gene in Moroccan patient with fibrodysplasia ossificans progressiva. *Clin Rheum.* 2010; 1:119–121.
72. Takahashi M, Katagiri T, Furuya H, Hohjoh H. Disease-causing allele-specific silencing against the ALK2 mutants, *R206H* and *G356D*, in fibrodysplasia ossificans progressiva. *Gene Ther.* 2012; 19:781–785. A novel proof-of-concept approach to allele-specific knockdown of the FOP mutation using RNAi. [PubMed: 22130450]
73. Whyte MP, Wenkert D, Demertzis J, et al. Fibrodysplasia ossificans progressiva: Middle-age onset of heterotopic ossification from a unique missense mutation (c.974G>C, p.G325A) in ACVR1. *J Bone Miner Res.* 2011; 27:729–737. [PubMed: 22131272]
74. Fukuda T, Kanomata K, Nojima J, et al. A unique mutation of ALK2, G356D, found in a patient with fibrodysplasia ossificans progressiva is a moderately activated BMP type I receptor. *Biochem Biophys Res Commun.* 2008; 377:905–909. [PubMed: 18952055]
75. Zhang D, Schwarz EM, Rosier RN, et al. ALK2 functions as a BMP type I receptor and induces Indian hedgehog in chondrocytes during skeletal development. *J Bone Miner Res.* 2003; 9:1593–1604. First description of the importance of ALK2 as a BMP type I receptor in skeletal development. [PubMed: 12968668]
76. Groppe JC, Shore EM, Kaplan FS. Functional modeling of the ACVR1 (R206H) mutation in FOP. *Clin Orthop Rel Res.* 2007; 462:87–92.
77. Chaikaud A, Alfano I, Kerr G, et al. Structure of the bone morphogenetic protein receptor ALK2 and implications for fibrodysplasia ossificans progressiva. *J Biol Chem.* 2012; 287:36990–36998. Crystal structure of ALK2 and implications for development of more specific signal transduction inhibitors. [PubMed: 22977237]

78. Groppe JC, Wu J, Shore EM, Kaplan FS. In vitro analysis of the dysregulated R206H ALK2 Kinase-FKBP12 interaction associated with heterotopic ossification in FOP. *Cells Tissues Organs*. 2011; 194:291–295. [PubMed: 21525719]
79. van Dinther M, Visser N, de Gorter DJ, et al. ALK2 R206H mutation linked to fibrodysplasia ossificans progressiva confers constitutive activity to the BMP type I receptor and sensitizes mesenchymal cells to BMP-induced osteoblasts differentiation and bone formation. *J Bone Miner Res*. 2010; 25:1208–1215. [PubMed: 19929436]
80. Song GA, Kim HJ, Woo KM, et al. Molecular consequences of the ACVR1 (R206H) mutation of fibrodysplasia ossificans progressiva. *J Biol Chem*. 2010; 285:22542–22553. [PubMed: 20463014]
81. Nussinov R, Tsai C-J. Allostery in disease and drug discovery. *Cell*. 2013; 153:293–305. [PubMed: 23582321]
82. Urist MR. Bone: formation by autoinduction. *Science*. 1965; 150:893–899. The classic description of bone morphogenetic proteins. [PubMed: 5319761]
83. Wozney JM, Rosen V, Celeste AJ, et al. Novel regulators of bone formation: molecular clones and activities. *Science*. 1988; 242:1528–1534. The classic description of cloning the BMP genes. [PubMed: 3201241]
84. Gugala Z, Olmsted-Davis EA, Gannon FH, et al. Osteoinduction by ex vivo adenovirus-mediated BMP2 delivery is independent of cell type. *Gene Ther*. 2003; 10:1289–1296. [PubMed: 12883525]
85. Fukuda T, Scott G, Komatsu Y, et al. Generation of a mouse with conditionally activated signaling through the BMP receptor, ALK2. *Genesis*. 2006; 44:159–167. [PubMed: 16604518]
86. Hong CC, Yu PB. Applications of small molecule BMP inhibitors in physiology and disease. *Cytokine Growth Factor Reviews*. 2009; 20:409–418. An excellent review article on STIs for BMP type I receptors. [PubMed: 19914855]
87. Yu PB, Hong CC, Sachidanandan C, et al. Dorsomorphin inhibits BMP signals required for embryogenesis and iron metabolism. *Nat Chem Biol*. 2008; 4:33–41. [PubMed: 18026094]
88. Engers DW, Frist AY, Lindsley CW, et al. Synthesis and structure relationships of a novel and selective bone morphogenetic protein receptor (BMP) inhibitor derived from the pyrazolo [1,5- α]pyrimidine scaffold of Dorsomorphin: The discovery of ML347 as an ALK2 versus ALK3 selective MLPCN probe. *Bioorganic & Medicinal Chemistry Letters*. 2013 DOI:<http://dx.doi.org/10.1016/j.bmcl.2013.03.113>. Important development of more specific ALK2 inhibitors.
89. Mohedas AH, Xing X, Armstrong KA, et al. Development of an ALK-biased BMP type I kinase inhibitor. *ACS Chem Biol*. Apr 02.2013 DOI: 10.1021/cb300655w. The development of a more specific ALK2 inhibitor.
90. Kaplan J, Kaplan FS, Shore EM. Restoration of normal BMP signaling levels and osteogenic differentiation in FOP mesenchymal progenitor cells by mutant allele-specific targeting. *Gene Ther*. 2011; 19:786–790. A novel proof-of-concept approach to allele-specific knockdown of the FOP mutation using RNAi. [PubMed: 22011642]
91. Zasloff MA, Rocke DM, Crofford LJ, et al. Treatment of patients who have fibrodysplasia ossificans progressiva with isotretinoin. *Clin Orthop*. 1998; 346:121–129. Retinoic acid derivatives are rational choices for inhibiting HEO. [PubMed: 9577419]
92. Shimono K, Tung W-e, Macolino C, et al. Potent inhibition of heterotopic ossification by nuclear retinoic acid receptor- γ agonists. *Nat Med*. 2011; 17:454–460. A seminal study describing the efficacy and mechanism of action of RAR γ agonists in the inhibition of HEO. [PubMed: 21460849]
93. Kaplan FS, Shore EM. Derailing heterotopic ossification and RARing to go. *Nat Med*. 2011; 17:420–421. A detailed rationale for the use of RAR- γ agonists in early FOP flare-ups. [PubMed: 21475232]
94. Olmsted-Davis E, Gannon FH, Ozen M, et al. Hypoxic adipocytes pattern early heterotopic bone formation. *Am J Pathol*. 2007; 170:620–32. [PubMed: 17255330]
95. Maeder T. A Few Hundred People Turned to Bone. *The Atlantic Monthly*. 1998; 281:81–89. A wonderful, human account of the FOP community and the origins of the International FOP Association (IFOPA).
96. IFOPA website. www.ifopa.org

6. Article Highlights

- FOP is a rare genetic disorder of episodic and progressive heterotopic endochondral ossification for which there is presently no effective treatment or prevention.
- Heterozygous activating mutations in Activin receptor IA/Activin-like kinase-2 (ACVR/ALK2), a bone morphogenetic protein (BMP) type I receptor, are the genetic cause of FOP and reveal a promising pharmacologic target in the BMP signaling pathway.
- Possible strategies for treating FOP will be based on one or more of the following approaches:
 - a. Blocking activity of the mutant receptor (ACVR1/ALK2) that causes increased BMP pathway signaling (through four possible strategies which include: monoclonal antibodies, signal transduction inhibitors (STIs), inhibitory RNA, and secreted antagonists).
 - b. Inhibiting triggers of FOP flare-ups.
 - c. Directing FOP HEO progenitor cells to an alternate tissue fate other than cartilage or bone.
 - d. Blocking the cellular response to microenvironmental signals that promote the formation of FOP lesions.
- Pre-clinical testing in FOP should be conducted in conditional knock-in animal models of classic FOP.
- Comprehensive natural history and biomarker studies are helpful for designing meaningful clinical trials in FOP

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Metamorphosis in FOP

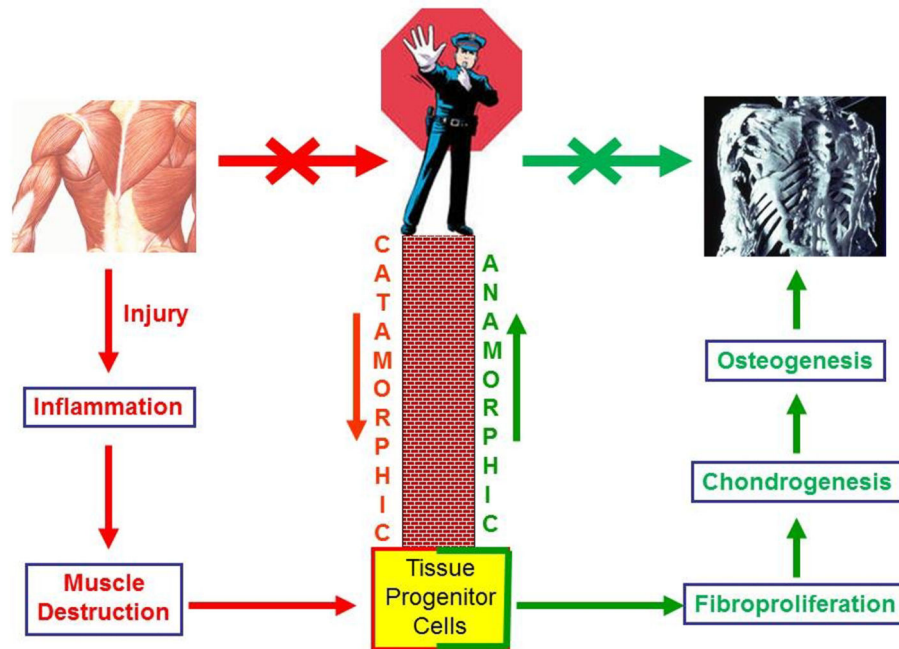
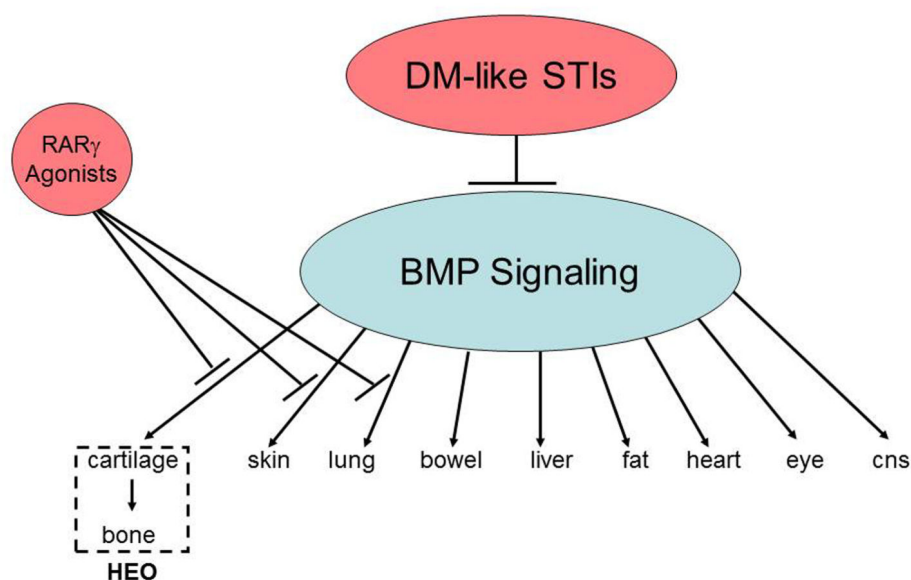


Fig. 1. Schematic Diagram of Skeletal Metamorphosis in FOP

In FOP, muscle cells do not become bone cells. Rather, skeletal muscle tissue is transformed into heterotopic bone through a process of skeletal metamorphosis [4, 56, 65]. The process of HEO in FOP involves two major phases - a catamorphic phase (tissue destruction; left column) followed by an anamorphic phase (tissue formation; right column) of transient fibroproliferative and cartilaginous scaffolds, and their replacement with mature heterotopic bone. The activation of tissue progenitor cells that contribute to the formation of heterotopic bone rather than reparative tissue is central to the process of skeletal metamorphosis [33, 34].



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Fig. 2. Action of DM-like STIs vs. RAR γ Agonists in BMP Signaling

Dorsomorphin (DM)-like STIs inhibit BMP signaling by effectively blocking the kinase domain of multiple BMP type I receptors (ALK2, ALK3, and ALK6). BMP signaling plays a critical role in the formation and maintenance of many organ systems including, but not limited to the skeleton, skin, lung, bowel, liver, fat, heart, eye, and CNS. The therapeutic efficacy of

DM-like STIs will depend largely on their ability to selectively block ALK2 signaling that drives HEO at doses that do not impair BMP signaling through ALK 3 and ALK6. RAR γ agonists inhibit BMP signaling downstream of BMP type I receptors in cartilage, skin, and both normotopic as well as ectopic chondrogenesis. The therapeutic efficacy of RAR γ agonists in FOP will depend largely on their ability to selectively block ectopic chondrogenesis at tolerable doses.

HURDLE	Disease-Related	Drug-Related	Investigator-Industry Related	Regulatory-Related	Support-Group Related
Rare	X				
Symmetry of Physiologic & Pathologic Targets	X				
Lack of Sufficient Natural History	X		X		X
Lack of Disease, Stage, & Drug-Specific Biomarkers	X	X	X		X
Lack of Relevant Animal Models	X		X		
Drug Specificity	X	X			
Drug Toxicity	X	X			
Drug Pharmacokinetics & Solubility		X			
Drug Delivery	X	X			
Competing Financial Interests			X		
Competing Academic Interests			X		
Secrecy of Labs/Biotechs/Pharma (Fragmented Effort)			X		
IRB Hurdles				X	
FDA Hurdles				X	
Market Profitability			X		
Patent Interests			X		
Lack of Funding	X		X		X
Lack of International Patient Support	X				X