Chemical Control of Heterotopic Ossification: ALK2(R206H)-Targeted PROTACs to treat FOP

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Abstract:

Fibrodysplasia Ossificans Progressiva (FOP) is a rare and severely disabling genetic disorder in which muscles, tendons, and ligaments are gradually replaced by bone. This progressive ossification stems from a mutation in the ACVR1 gene, also known as activin-like kinase 2 (ALK2). ACVR1 encodes ALK2, a type I BMP receptor that normally guides bone growth and tissue repair. In FOP, the common R206H mutation makes ALK2 abnormally active, triggering BMP signaling without proper cues. This drives connective tissue—including endothelial cells—to become bone-forming cells, slowly creating an unwanted secondary skeleton.The mutated receptor activates pathways that are normally reserved for bone development. Because of this, bone forms in soft tissues after even minor trauma or inflammation. One drug currently being studied for FOP is palovarotene. It aims to reduce abnormal bone growth. However, it has shown limited effectiveness and can cause side effects, especially in younger patients. These challenges highlight the need for more precise and reliable therapies. One promising strategy involves PROTACs (Proteolysis-Targeting Chimeras). These are specialized molecules that guide harmful proteins—such as mutant ALK2—to the cell's natural disposal system, the ubiquitin–proteasome pathway. Unlike traditional treatments, PROTACs remove the protein entirely. This could lead to more specific and longer-lasting effects. This project explores the potential of using PROTACs to treat FOP. The focus is on the selective degradation of mutant ALK2. The study will examine how PROTACs might distinguish the mutant version from the normal one. It will also explore ways to improve delivery to bone and connective tissues. The goal is to provide well-supported suggestions on how PROTACs could become a new, targeted treatment option for FOP.

Introduction:

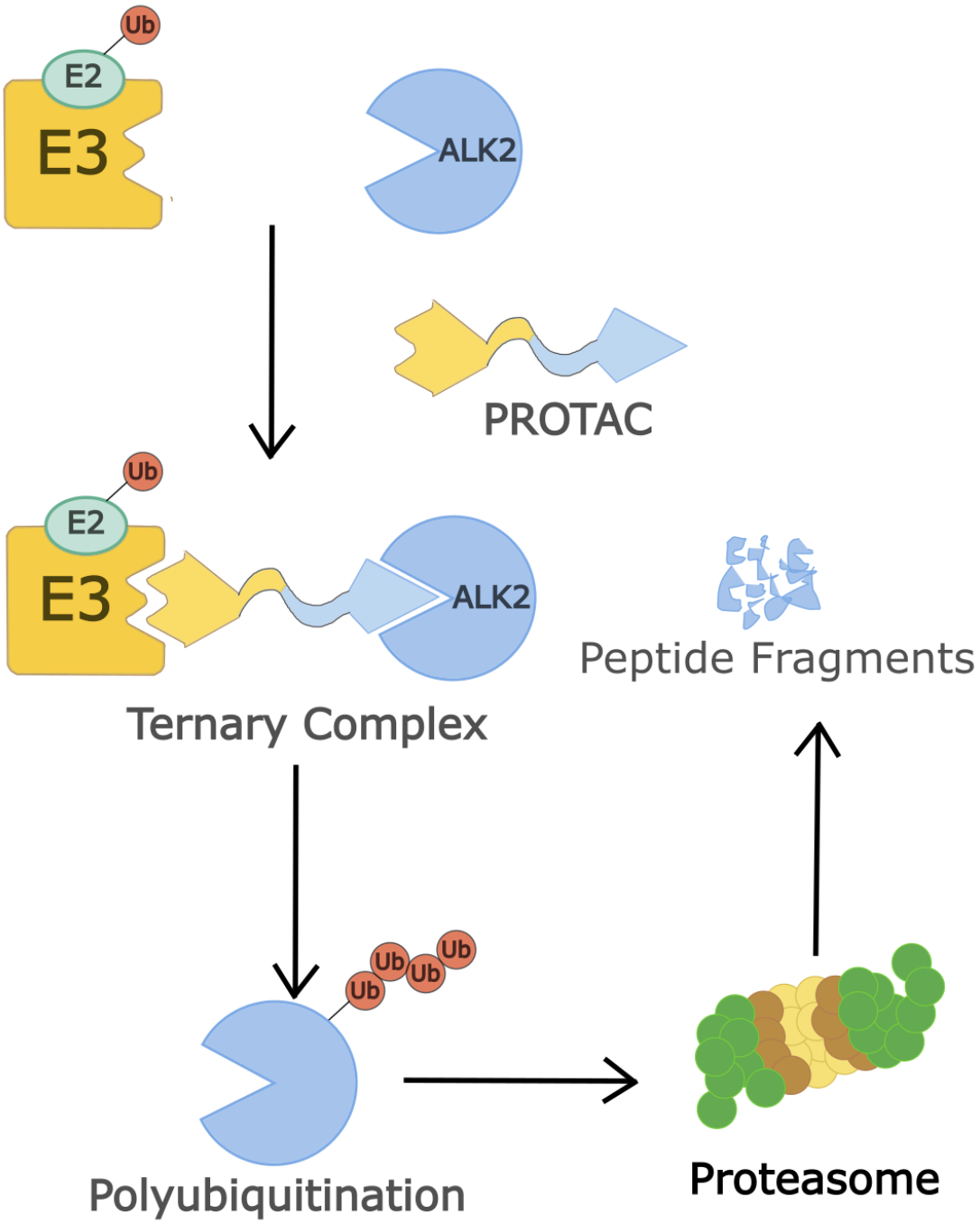

Figure 1: ALK2-targeted degrader: ternary complex and ubiquitination

Fibrodysplasia Ossificans Progressiva (FOP) is an ultra-rare, severely disabling genetic disorder in which soft connective tissues such as muscles, tendons, and ligaments are progressively replaced by bone. The abnormal process is called heterotopic ossification (HO). It results in fusion of joints, pain, and eventual extreme immobility. Even minor injuries, intramuscular injections, or viral illnesses can trigger new bone growth. Worldwide, FOP occurs

in approximately 1 in 1–2 million people (Shore, 2012). The symptoms usually become evident during early childhood. Patients characteristically have congenital malformations of the great toes. They are accompanied by intermittent flare-ups of swelling and ossification involving the soft tissues, which become progressively worse over time. As HO progresses, an unwanted "second skeleton" is formed that envelops the encased body, leaving patients wheelchair-bound and significantly reducing lifespan. The disease not only has a great physical toll but also has profound psychological and social impacts, as progressive disability limits independence and quality of life.

FOP happens because of mutations in the ACVR1 gene, which makes activin-like kinase 2 (ALK2). ALK2 is a type I receptor in the BMP signaling pathway. BMP is part of the transforming growth factor-β (TGF-β) superfamily. ACVR1 helps control bone growth, cartilage formation, and normal tissue balance. It does this through tight on-off control of ALK2 and other BMP type I receptors by BMP ligands. The most common change, R206H, swaps histidine for arginine in the juxtamembrane GS region of ALK2. This weakens binding to the inhibitory protein FKBP12. As a result, the receptor stays partly active even without a ligand. In healthy cells, Activin A acts as an antagonist to ALK2. In FOP, the mutation makes Activin A act like an agonist. This unusual response triggers receptor activation. It causes too much phosphorylation of Smad1/5/8. These proteins then activate genes involved in cartilage and bone formation. Endothelial and connective tissue cells are pushed to become chondrocytes and osteoblasts. This leads to HO, meaning bone forms in muscles, ligaments, and fascia. Small injuries, inflammation, or immune reactions can start the process. These events raise local BMP signaling, including extra BMP4. Over time, HO builds up and cannot be reversed. It results in joint fusing and restricted movement.

FOP has no cure, so existing care involves avoidance of triggers and control of flares. Surgery is typically avoided since excision can cause sudden, "explosive" HO in incision and suture sites. Anti-inflammatory medication is used to treat flares by decreasing pain and swelling. An approved pharmacologic therapy is palovarotene, a selective RARγ agonist that inhibits chondrogenesis. It targets the cartilage phase of HO and marks meaningful progress, yet its benefits are partial. Existing bone is not removed, response is variable, and it cannot be safely used in rapidly growing children. Mechanism-directed approaches are advancing in parallel. Anti–activin A monoclonal antibodies eliminate new HO and stop growth of nascent lesions by eliminating the agonist drive to mutant ACVR1 in FOP mouse models (Davis et al., 2024). Preclinically, this is highly effective, but translating this to the clinic must protect long-term BMP/activin signaling biology. By contrast, bivalent anti-ACVR1 antibodies worsen HO in FOP mice by dimerizing and activating the mutant receptor in vivo. They still inhibit wild-type ACVR1 and can reduce trauma-induced HO in non-FOP settings. This establishes a clear "do-not-use" class for FOP and cautions against receptor-crosslinking formats. Monovalent anti-ACVR1 Fabs avoid dimerization and can attenuate HO in FOP mice, but duration of action and delivery are issues. In preclinical work, small molecules ALK2 inhibitors (e.g., LDN-193189 and analogs) inhibit Smad1/5/8 signaling and reduce ectopic ossification (Wang, 2014). However, it encounters challenges in selectivity across the BMP type I receptors, tissue exposure, and chronic tolerability. Antisense oligonucleotides against ACVR1 are in investigation but must be allele-selective in knockdown with long-term penetration to connective tissue.

PROTACs are drug-like molecules that destroy proteins instead of just blocking them. They are made of two ligands joined by a linker: one binds the target protein and the other binds an E3 ubiquitin ligase. This design pulls the E3 next to the target and tags the target with polyubiquitin, sending it to the 26S proteasome for degradation. PROTACs can in principle hit many intracellular and even transmembrane proteins, including ones long viewed as "undruggable." They act catalytically and can be recycled after a target is degraded, so effective doses may be lower than for classic inhibitors. Commonly used E3 recruiters include VHL and cereblon, among a small set of ligases that have been leveraged so far. PROTACS present mutation-centered therapy for FOP by eliminating, rather than just inhibiting, the disease driver. A PROTAC would use an ALK2-binding "warhead" linked to an E3-ligase recruiter (e.g., VHL or cereblon) to form a ubiquitinated mutant ALK2 and send it to the proteasome for degradation. Through receptor depletion, it is possible that PROTACs can inhibit premature Smad1/5/8 signaling in the presence of activin A or other activators. Perhaps providing more intense and longer duration control of HO than reversible inhibitors. Key challenges include delivery to connective tissue and bone, avoiding degradation of wild-type ALK2, optimizing E3-ligase expression in target cells, and ensuring safety with chronic use. If overcome, PROTACs could provide a precise, durable, and mechanism-based therapy for FOP.

This project proposes a PROTAC that selectively degrades mutant ACVR1/ALK2 (R206H). The goal is to shut down the abnormal BMP–Smad1/5/8 signaling that drives HO in FOP. The degrader links an ALK2-binding warhead to an E3-ligase recruiter such as VHL or cereblon. The ligase is brought to the mutant receptor. The receptor is then ubiquitinated and cleared by the proteasome. Removal, rather than inhibition, can silence the pathway even when activin A or other cues are present. It can offer deeper and more durable control than reversible kinase inhibitors. Mutant-biased design would exploit subtle structural differences between ALK2[R206H] and wild-type to preserve normal BMP biology and to avoid the receptor-crosslinking risks seen with some antibodies. If successful, the approach could reduce flare frequency and limit new bone formation. It would provide a precise, mechanism-based therapy suitable for long-term management.

PROTAC Design:

The goal is to obtain a degrader that removes the disease-driving ACVR1/ALK2\[R206H] receptor inside the cell, while sparing wild-type ALK2 as much as possible and keeping normal BMP/Activin biology intact. PROTACs are ideal for this because they are heterobifunctional small molecules that bring a target protein to an E3 ubiquitin ligase, catalyze ubiquitination, and hand the target to the proteasome for destruction—an event-driven pharmacology that can work at lower occupancy and on targets considered undruggable.

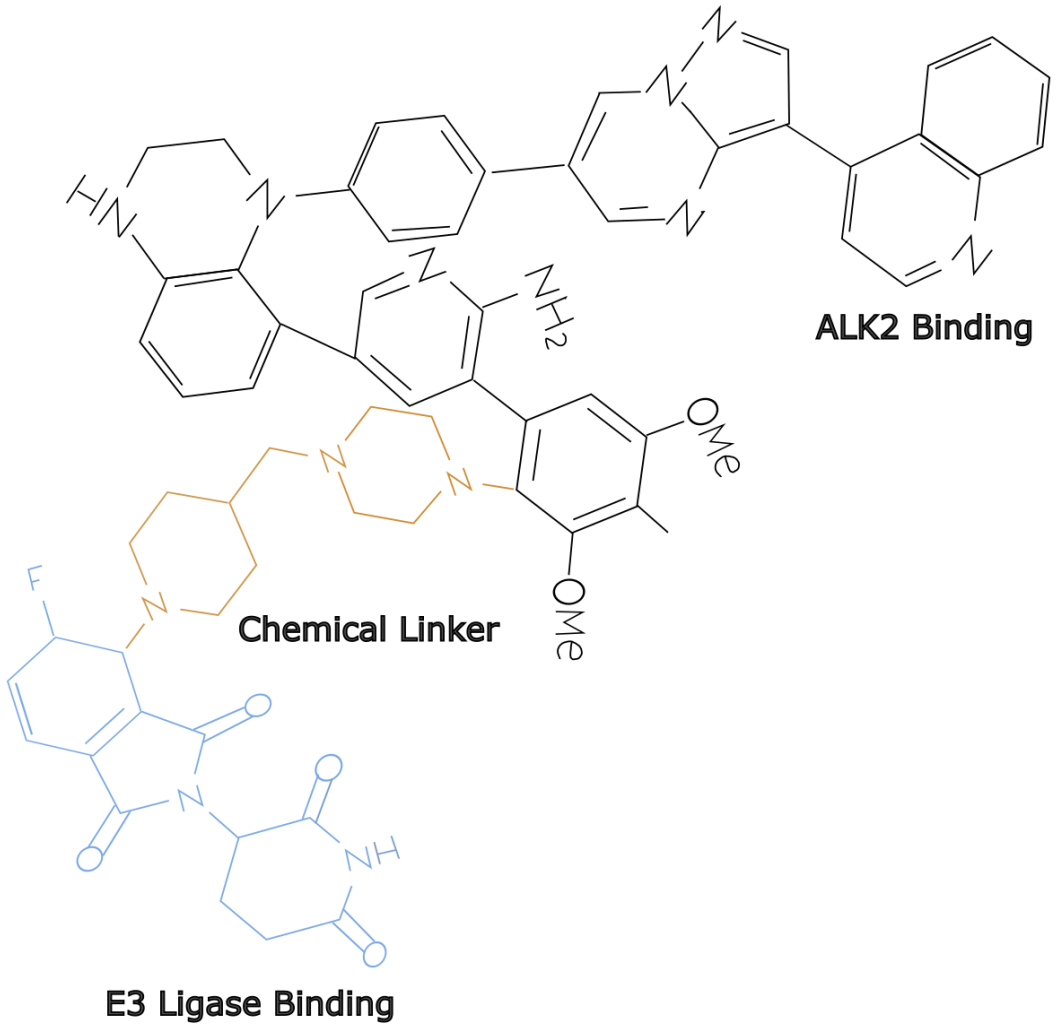

Figure 2: Degrader Design Space: ALK2 Warheads, Linkers, E3 Binders

It will be derived from ALK2-binding chemotypes that have a linker at a solvent-exposed location. Two useful starting points are the 3,5-diaryl-2-aminopyridine series (e.g., LDN-212854, LDN-193189) and the aza-indazole scaffold K02288. These series bind the ALK2 kinase domain and have been optimized for BMP type I receptor selectivity as well as potency within BMP type I receptors, so they are rational warheads for ACVR1. Some of the best of these analogs will be re-synthesized with a primary-amine “exit vector” at a location known to tolerate linker substitution, so that they may be plugged into assembly modules later. Since FOP-relevant

stromal and progenitor cells both express CRBN and VHL broadly, we'd prototype using lenalidomide/pomalidomide-based CRBN ligands and VH032-type VHL ligands that are pre-functionalized using a primary amine. Testing both often pays off because E3 selection, attachment site, and linker all govern degradation efficiency as well as selectivity in combinations.

Linker length, rigidity, and attachment points often determine cellular performance. A direct-to-biology workflow will be used to explore many variants quickly. An amide coupling will then be used to attach o-nitrobenzyl-alcohol–derived NHS "plug-in" linkers to either the warhead or the E3 fragment. PANAC photoclick chemistry will then connect the other half under light, with no metals, and under mild conditions. The reactions mainly form N-hydroxysuccinimide and water as by-products. Crude mixtures can therefore go straight into cell assays without purification. The PANAC step also installs a rigid indazolone unit in the linker. These rigid linkers will be tested head-to-head against flexible alkyl or PEG linkers to evaluate cellular performance.

The warheads will include LDN-212854-NH_2, LDN-193189-NH_2, and K02288-NH_2. The E3 ligands will include two CRBN variants (Len-NH_2 and Pom-NH_2) and one to two VHL variants (VH032-NH_2). The linkers will span short, medium, and long lengths, with rigid indazolone and flexible alkyl/PEG options, and with varied attachment sites on both ends. This focused set should yield several dozen candidates and allow clear structure–activity trends with minimal synthetic overhead. Screening will be performed directly in plates using cells that express ACVR1/ALK2 (R206H), such as fibro-adipogenic progenitors or engineered human lines. The primary endpoints are protein loss of the ALK2 by immunoblot and reduced pSMAD1/5/8 following stimulation by Activin A or BMP.Ubiquitin–proteasome dependence will be confirmed by rescue with MG132 (proteasome inhibitor) and MLN4924 (NAE inhibitor). Additional competition studies will use excess free E3 ligands (lenalidomide or pomalidomide for CRBN, or a VHL ligand) and a non-degrading ALK2 binder; where possible, tests will be repeated in E3-knockdown cells. Collectively, these controls validate target engagement, correct E3 specificity, and proteasomal clearance.

Start in cells that carry ALK2 R206H. Treat with the PROTAC and track ALK2 protein by Western blot or targeted proteomics over time. Challenge the cells with BMP6 and with activin A to stress the pathway. Measure pSMAD1/5/8 or a BRE-luciferase signal to see if signaling drops even under ligand stimulation. To prove true degradation, run rescue controls with a proteasome blocker and a neddylation blocker. Also compete with free E3 ligands and test a matched, non-degrading analog. If ALK2 levels fall and signaling stays low after washout, that is a strong sign the PROTAC works as intended.

ALK1 in BMP9-stimulated HUVECs, ALK2 in BMP6-stimulated HEK293T/ALK3-KO cells, ALK3 in BMP4-stimulated HEK293T/ALK2-KO cells, and ALK6 in GDF5-stimulated HEK293T cells, all read out by SMAD1-P. The acceptance bar is >100-fold cellular selectivity for ALK2 over ALK1/3/6. Because ALK1 inhibition is linked to serious vascular effects in the study, any design that lowers ALK1 signaling will be removed early. Activity on other FOP-linked ALK2 mutants (L196P, R258S, G328R) will also be checked to ensure the effect is not limited to R206H. In parallel, practical properties highlighted in the paper will be profiled: plasma protein

binding by equilibrium dialysis and low brain exposure (targeting a rat Kpuu,brain near ~0.028 by adding polarity). PK/PD gates will be set using their benchmark: sustain ≥70% suppression of SMAD1-P at trough (aligned with ~13.2 ng/mL plasma in their xenograft model) to predict HO control in vivo. If selectivity drifts or exposure is off, the warhead and attachment site will be refined using the same structure rules (optimize hinge-binder contacts with the gatekeeper water network, remove CLK1 off-target risk, and tune polarity to shape distribution).

For in-vivo context, published FOP models that express ALK2 R206H will be mirrored. One model uses a calf-muscle “pinch” to trigger soft-tissue HO; another uses a fibular osteotomy to model combined bone and muscle injury. Similar readouts will be used: MRI to track edema after injury, micro-CT to measure new bone volume, and histology to assess cartilage and bone at the site. Because this is a degrader, tissues will also be tested for ALK2 loss, pSMAD1/5/8 reduction, and evidence of ubiquitination. Groups will include no drug, a known ALK2 inhibitor control, and the PROTAC. Dosing will begin before injury and at defined delays after injury to determine how late treatment remains effective.

Clear progress will be characterized by less edema on MRI, much lower HO on micro-CT, and normal muscle repair on histology. In the fracture model, bone should still heal with a normal callus and remodeling. “Durability” will be tested by stopping dosing after healing and monitoring for rebound HO. Safety checks will include body weight, behavior, basic blood tests, and evaluation for off-target bone effects in wild-type mice. If the PROTAC lowers ALK2 in tissue, keeps pSMAD1/5/8 quiet, prevents HO in both models, and does not block normal bone healing, then it meets the bar set by strong inhibitors while offering a removal-based mechanism—supporting further study as a targeted option for FOP.

Future Direction:

Going forward, focus will shift on chemistry and PK to achieve once-daily exposure with strong mutant-selective ALK2(R206H) degradation.The warhead exit vector and stereochemistry will be optimized and rigid vs flexible linker comparisons made. These include small length modifications to control the ternary-complex geometry and exclude hook effects. If the selectivity shifts, CRBN and VHL must be reversed. Property goals will be reasonable: modest lipophilicity, control of polar surface area and low hydrogen-bond donor count, able to be solubilized by salts or by ionizable handles when needed. Plasma protein binding will be minimized to elevate the free drug and clearance decreased by targeting predicted metabolic soft spots. It also monitors efflux risk to increase permeability. Distribution goals will stress optimal muscle and connective tissue concentrations without assuming a need for the Central Nervous System. Finally, simplified PBPK-style predictions will convert the unbound trough level through the cellular degradation window and only those designs that meet both the selectivity and the exposure goals move on.

PROTACs technology will likely move toward degraders that are smaller, more “drug-like,” and easier to deliver, with better ternary-complex modeling, smarter linker designs, and a wider set of E3 ligases to improve selectivity and tissue targeting; conditional control (turning on only in certain environments) could further boost safety and precision. These ideas could also be adapted to Alexander disease by tuning chemistry for brain entry (lower polarity

and efflux) and to post-traumatic heterotopic ossification by aiming for steady exposure in injured muscle and fascia with low CNS penetration. This forward-looking outlook is different from what this paper proposes, which focuses narrowly on mutant-selective ALK2(R206H) for FOP, and serves only as examples that would need their own validation and safety testing.

Conclusion:

This study offers a practical path to seal the HO-driving signal in FOP by clearing ALK2(R206H) with a selective PROTAC rather than merely inhibiting it. Cell assays confirm bona fide degradation (decrease in ALK2 protein, decrease in pSMAD1/5/8 upon BMP6 or activin A challenge, rescue by proteasome/neddylation inhibitors) and define stringent selectivity criteria to leave ALK1/3/6 and wild-type ALK2 intact. Together, these establish a clean decision process: only molecules showing mutant-biased degradation, stable pathway silencing after washout, and acceptable drug-like properties advance.

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