

# Title: Gene therapy for Fibrodysplasia Ossificans Progressiva (FOP): feasibility and obstacles

**Short title:** Gene therapy for FOP

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#### **Contributions to the manuscript:**

This article was initiated by EMWE together with MM and TS, GP, JHS, GG, and FK, RP, and RDdR and BJS.

Contributions to all crude versions from EMWE, RDdR, BJS, TS, TJdV, CN, ECH, GP, JHS, GG and FK, RP, MM.

The subfinal version was prepared and sanctioned by this core group of authors. All remaining authors CS, NH, ZG, CLDC, MdR, PLRD, BRJD, VM, TJC, RM, CSF, MZ, who are active ICC on FOP members have had the opportunity to further improve the manuscript, these comments were incorporated by EMW, DM and by RDdR and BJS in the submitted version.

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## Consent for publication

One picture is shown with the written consent of the patient and her parents.

**Abstract:**

Fibrodysplasia ossificans progressiva (FOP) is a rare and devastating genetic disease in which soft connective tissue is converted into heterotopic bone through an endochondral ossification process. Patients succumb early as they gradually become trapped in a second skeleton of heterotopic bone.

Although the underlying genetic defect is long known, the inherent complexity of the disease has hindered the discovery of effective preventions and treatments. New developments in the gene therapy field have motivated its consideration as an attractive therapeutic option for FOP. However, the immune system's role in FOP activation and the as-yet unknown primary causative cell, are crucial issues which must be taken into account in the therapy design. While gene therapy offers a potential therapeutic solution, more knowledge about FOP is needed to enable its optimal and safe application.

**Manuscript:**

Fibrodysplasia ossificans progressiva (FOP; MIM#135100) is a rare genetic disease affecting soft connective tissues. The prevalence is reported to be 1 in 1.3 million - 2 million.<sup>1</sup> FOP is characterized by muscles, tendons and ligaments that turn into bone through an endochondral ossification process.<sup>2</sup> Bone formation typically transpires through so-called flare-ups (Figure 1), a local inflammatory response which subsequently triggers local chondrogenesis and osteogenesis.<sup>3</sup> In addition to the flare-ups, there is also a level of basal chronic heterotopic ossification (HO) present in FOP patients.<sup>4</sup> During life, FOP follows a progressive pattern first affecting the axial skeleton and later the appendicular skeleton, though it varies greatly between patients. Eventually, this highly complex disease leads to devastating contractures and severe disability and causes premature death in FOP patients due to thoracic insufficiency syndrome, trauma or sepsis.<sup>5</sup>

The underlying cause of FOP is a heterozygous, usually *de novo*, R206H gain-of-function mutation in the ubiquitously expressed bone morphogenetic protein (BMP) type I receptor activin receptor-like kinase 2 (ALK2) (Figure 2). This mutation alters the properties of the receptor by converting it to a form that is both mildly constitutively active at the basal state and hyperactive to BMP signaling in the activated state as evidenced by the phosphorylation of the downstream SMAD1/5/8 effector proteins.<sup>6,7</sup> Also, the mutation renders the ALK2 receptor aberrantly responsive to Activin A which induces phosphorylation of SMAD 1/5/8, leading to bone formation where it normally would not occur.<sup>8</sup> It is still unclear what other factors contribute to the unpredictable and episodic activity of the disease, although an important role is attributed to the immune system.<sup>9-12</sup>

The R206H (c.617G>A) mutation can be found in more than 95% of the classic form of FOP patients. Currently, at least 13 other mutations have been found in the GS or kinase domain of ALK2 that cause FOP, which appear to lead to different phenotypes than the 'classic' FOP. Nonetheless, they are all heterozygous missense mutations which enhance receptor signaling (Figure 1).<sup>13</sup>

Presently, it is not known when the disease may become active, although (minor) trauma is one of the most predictive triggering factors. Several drugs are currently being investigated in clinical trials and represent different molecular strategies.<sup>14</sup> These include blocking antibodies that stop Activin A from triggering the mutant ALK2 receptor (REGN2477),<sup>15,16</sup> ALK2 kinase inhibitors (AZD0530; IPN60130),<sup>17,18</sup> mTOR inhibitors which modulate the inflammatory response to tissue injury and aim to affect the early hypoxic stages involved in chondrogenesis (rapamycin)<sup>19</sup> and retinoic acid receptor gamma (RAR $\gamma$ ) agonists which block the chondrogenic signalling required for endochondral bone formation (palovarotene).<sup>20,21</sup> All of these experimental approaches have been shown to be effective in FOP mouse models.<sup>15,18,22,23</sup> Given the nature of this mutation and the importance of the ALK2 receptor in homeostasis and development of the skeletal system, pharmaceutical interference with the receptor can be expected to cause numerous potential side effects. Demonstrating the effect of intervention with these drugs in clinical studies has already appeared to be more difficult than initially expected in terms of acceptable risks, expected benefits and lack of comprehensive understanding of the natural history of the disease. Clinical trials are currently being conducted to further evaluate the safety and efficacy of the aforementioned drugs. However, at the time of this article, no efficacy and safety data have yet been published and, with the exception of approval of palovarotene in Canada, no drugs have been approved by regulatory authorities elsewhere.

The complexity of finding safe and effective treatments specific to the known genetic cause is why gene therapy is being explored as a new treatment option in FOP. For many monogenic diseases the gene therapy horizon is being intensively explored as it offers attractive possibilities which seem tangible in the near future; this has motivated the investigation and investment in the gene therapy approach. Considering the therapeutic benefits of commercialized gene therapy on several monogenic diseases such as lipoprotein lipase deficiency,<sup>24</sup> inherited retinal dystrophy,<sup>25</sup> and spinal muscular atrophy,<sup>26</sup> it is plausible that current gene therapy options could be beneficial for the treatment of FOP caused by a monogenic gain-of-function mutation in the ALK2 receptor.



In this perspective we summarize the different gene therapy options and their expected suitability in FOP (Table 1).

In general, gene addition aims to introduce genes encoding missing proteins or encoding corrective proteins in the event that defective proteins are produced by a genetic mutation. For FOP, where the pathological mutations cause a gain-of-function, gene therapy could conceivably employ four strategies, including gene replacement, gene silencing, combination of gene replacement and silencing, and gene editing (Table 1). First, introduction of healthy proteins via gene replacement can be used to compete against proteins with gain-of-function autosomal dominant mutations such as the classic ALK2 mutation in FOP. Second, gene silencing aims to suppress the expression of abnormal proteins at the mRNA level by using ribonucleic acid interference (RNAi). This strategy can be useful for FOP mutant allele-specific silencing of the ALK2 receptor.<sup>27,28</sup> Third, a combinatory approach of gene replacement and silencing removes abnormal proteins and expresses healthy proteins simultaneously. This strategy can be used to replace the *ACVR1* mutation in FOP with normal ALK2. Finally, gene editing aims to correct DNA mutations in the genome by using the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) system.<sup>29,30</sup> This strategy can be used to correct the ALK2 mutation in FOP at the genomic levels. However, a caveat to consider in using these therapeutic strategies in FOP is the lack of definitive identification of the HO-triggering cell types in the body. In light of this, the critical question is whether it is possible to specifically correct the ALK2 mutation in the cells involved in the various phases of the disease, which might be a solution, but at the moment still not feasible in patients. Additionally, targeting the locally affected tissue during a FOP flare-up may also pose difficulties since heterotopic ossification (HO) consists of normal bone tissue (at an ectopic site) which may be difficult to selectively target. These issues may be circumvented by improving tissue-specific tropism of the vectors that deliver therapeutic genes.

There are essentially two routes to express therapeutic genes in target cells and/or tissues (Figure 3: adapted from Dr. Gao). Genetically modified cell therapy is an *ex vivo* treatment approach that extracts target cells from the affected tissue of the patients, followed by genetic manipulation via vector-assisted transduction and reintroduction into the tissue.

By contrast, *in vivo* gene therapy aims for the direct delivery of therapeutic genes to target tissues using either a viral vector (i.e., recombinant adeno-associated virus (rAAV) or a non-viral vector (such as liposomes or nanoparticles). All FOP cells in the body with the potential to differentiate into bone need to be repaired as any untreated cell is a potential source of flare-up and HO. Therefore, *ex vivo* cell therapy, followed by reintroducing genetically manipulated cells back into the body is unlikely to substantially benefit FOP, because the presence of reintroduced cells will not affect the cells that contain the mutation. Consequently, *in vivo* gene therapy is considered the most likely treatment option in FOP as presently conceived.

Since immunological triggers are known to pose a high risk for HO induction in FOP, viral vector options need to be very carefully considered. Each viral vector type has its advantages and disadvantages in terms of transduction efficiency, duration of gene expression, transgenic capacity and potential side effects (Table 2).<sup>31</sup>

Among them, rAAV has a long track record for safety and efficacy in relevant preclinical and clinical studies in non-FOP contexts and has been evaluated in over 130 clinical trials and 2,000 patients worldwide.<sup>31,32</sup> AAV, a small (26 nm) non-enveloped parvovirus with a single-stranded genome of approximately 4.7 kb in length,<sup>33</sup> has high transduction efficiency, persistent transgene expression, relatively low post-infection immunogenicity and importantly no association with any human diseases, which make it an attractive viral vector for use in gene therapy.<sup>34</sup> Additionally, a systemic disease such as FOP requires a systemic delivery via the vasculature and takes advantage of AAV's trans-vascularity and tissue-specific tropism.<sup>31</sup> However, a high dose administration of the AAV vector can potentially trigger an immunomodulatory effect in FOP complicating reliable delivery of the gene of interest to the target cell(s) and may potentially compromise the subsequent safety of this method as well as any potential therapeutic effect.<sup>35</sup> In addition, flare-ups are unpredictable and have different phases of development with involvement of other target cells and their microenvironment. During the HO developmental process, cellular hypoxia occurs and a periodic diminished blood supply is suspected, which adds an additional level of complexity in deciphering the anatomical locations and the target cells that the vector must be designed to reach.<sup>36</sup> Co-treatment with an immunosuppressor or an FOP inhibitor or the development of a new AAV vector that does not trigger FOP-associated flare-ups

may be able to address these issues. Alternatively, liposomes and nanoparticles are non-immunogenic gene therapy vectors, but they can be rapidly degraded, cleared in the circulation, have short biological half-lives, and generally exhibit non-specific uptake by cells.<sup>34</sup>

The CRISPR/Cas9 system has been developed as a genome-editing tool that can correct DNA mutations underlying human diseases. In principle, many heterozygous mutations can be individually corrected by homology-directed repair (HDR) using an exogenous DNA template.<sup>37</sup> Recently, the AAV-compatible Cas9 nuclease (SaCas9), derived from *Staphylococcus aureus*, has been engineered for *in vivo* gene editing as SaCas9 and fits within the genome packaging limits of AAV.<sup>38</sup> However, since the SaCas9 nuclease shows a low HDR-mediated gene editing efficiency and being a bacterial protein, its expression triggers immune responses in animal cells. Consequently, an alternative gene therapy technique likely needs to be considered, the so-called RNA genetic techniques.

RNA was conventionally thought to be a transient messenger (mRNA) for the passive translation of genetic information encoded by DNA into protein sequences. However, mRNA comprises only a small fraction of the RNA types and their functions in the cell. Other types of RNAs also exist which can turn genes on and off, support chemical reactions, cut and build other RNAs, and constitute the protein building machines of cells by transporting and linking amino acids. Taking this into account, RNA therapies can provide efficient silencing of target mRNA expression by inhibitory RNA (RNAi) (i.e. siRNA, shRNA, miRNA)-mediated degradation. Similar to DNA gene therapy, RNAi approaches also require a vector for delivery into cells, especially since RNA is unstable and is easily degraded in the bloodstream. For this reason, RNA therapy can be relatively short-lasting while high levels of expression can induce cytotoxicity and inflammation by perturbing the RNAi machinery or leading to significant off-target silencing. To circumvent these issues, AAV-compatible miRNA scaffolds (artificial miRNA) have been developed to increase the duration of RNAi expression, limit RNAi-related toxicity, and enable efficient gene knockdown, while reducing off-target silencing by ten-fold compared to conventional RNAi.<sup>31</sup> RNA therapy might theoretically be preferable in treating flare-ups, though the problem of not knowing which cell types to target remains.

In summary, after years of setbacks,<sup>39</sup> the field of gene therapy has now achieved some success with effective applications of DNA-modulating therapy in clinical trials in previously difficult to treat hereditary diseases such as certain forms of immunodeficiency, neurological disorders, musculoskeletal disorders, blindness, hemoglobinopathies, coagulation disorders and cancer.<sup>31,40</sup> RNA-related gene therapy exists in the form of two mRNA-based therapies for hereditary transthyretin-mediated (ATTR) amyloidosis — a potentially fatal disease characterized by abnormal protein accumulation in nerves and organs including the heart,<sup>41</sup> and Nusinersen,<sup>42</sup> which targets a fatal inherited condition called spinal muscular atrophy. Regrettably, the application of Nusinersen is hampered by high costs. Eteplirsen, a treatment for Duchenne muscular dystrophy,<sup>43</sup> has been approved by the FDA. One of the biggest barriers<sup>44</sup> in the above mentioned RNA therapies has been the delivery of RNA to the correct cells. The above mentioned genetic diseases, present relatively accessible affected tissues and cells which can be distinctly targeted compared to FOP. Fundamental problems in gene therapy for FOP are the identity of the proper targets and the safety and durability of the gene targeting system. An analogy can be made with metastatic cells in cancer. Any untreated cell is still a potential source of a flare-up and HO. Since it has recently been shown that the mutant ALK2 can lead to aberrant gain of BMP signaling in different cell lines and tissue progenitor cells, with different regeneration capacities,<sup>45,46</sup> successful and comprehensive gene therapy for FOP needs may require the targeting of broader range of cell types.

Therefore, for a complex disease such as FOP, deeper insight into the underlying causative cell type(s) and the factors involved in the different phases of HO is a paramount prerequisite for efficient and safe gene therapy design. Gene therapy has the clear advantage of achieving the direct correction of the genetic cause in monogenic diseases such as FOP, which is lacking in current strategies. This justifies its pursuit as a novel therapeutic modality. While gene therapy could be a promising tool in the distant future, there are still significant obstacles to overcome until a safe therapy can be offered to the patients. Considering the complexity of FOP, it can be envisioned that its efficient treatment will involve a combination strategy of gene and pharmacological therapy. As we learn more about the nature of chronic and traumatic FOP, it will be possible to evaluate

the benefit of vector-mediated therapeutic gene and pharmacological treatment in each for optimal therapeutic outcome. Finally, the discovery of the underlying factors, the natural course of the disease, in combination with the developing variety of drug studies and new ongoing options are all very much needed to advance FOP treatment and should receive due attention in the next decade.

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**References:**

1. Baujat G, Choquet R, Bouee S, Jeanbat V, Courouve L, Ruel A, et al. Prevalence of fibrodysplasia ossificans progressiva (FOP) in France: an estimate based on a record linkage of two national databases. *Orphanet J Rare Dis.* 2017;12(1):123.
2. Kaplan FS, Tabas JA, Gannon FH, Finkel G, Hahn GV and Zasloff MA. The histopathology of fibrodysplasia ossificans progressiva. An endochondral process. *J Bone Joint Surg Am.* 1993;75:220-230.
3. Shore EM and Kaplan FS. Inherited human diseases of heterotopic bone formation. *Nat Rev Rheumatol.* 2010;6:518-527.
4. Botman E, Teunissen BP, Raijmakers P, de Graaf P, Yaqub M, Treurniet S, et al. Diagnostic Value of Magnetic Resonance Imaging in Fibrodysplasia Ossificans Progressiva. *JBMR Plus.* 2020;4:e10363..
5. Kaplan FS, Zasloff MA, Kitterman JA, Shore EM, Hong CC and Rocke DM. Early mortality and cardiorespiratory failure in patients with fibrodysplasia ossificans progressiva. *J Bone Joint Surg Am.* 2010;92:686-691.
6. van Dinther M, Visser N, de Gorter DJ, Doorn J, Goumans MJ, de Boer J, 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 osteoblast differentiation and bone formation. *J Bone Miner Res.* 2010;25, 1208-1215.
7. Shore EM, Xu M, Feldman GJ, Fenstermacher DA, Cho TJ, Choi IH, et al. A recurrent mutation in the BMP type I receptor ACVR1 causes inherited and sporadic fibrodysplasia ossificans progressiva. *Nat Genet.* 2006;38: 525-527.
8. Alessi Wolken DM, Idone V, Hatsell SJ, Yu PB and Economides AN. The obligatory role of Activin A in the formation of heterotopic bone in Fibrodysplasia Ossificans Progressiva. *Bone.* 2018;109:210-217.

9. Convente MR, Wang H, Pignolo RJ, Kaplan FS and Shore EM. The immunological contribution to heterotopic ossification disorders. *Curr Osteoporos Rep.* 2015;13:116-124.
10. Convente MR, Chakkalakal SA, Yang E, Caron RJ, Zhang D, Kambayashi T, et al. Depletion of Mast Cells and Macrophages Impairs Heterotopic Ossification in an Acvr1(R206H) Mouse Model of Fibrodysplasia Ossificans Progressiva. *J Bone Miner Res.* 2018;33:269-282.
11. Wang H, Behrens EM, Pignolo RJ and Kaplan FS. ECSIT links TLR and BMP signaling in FOP connective tissue progenitor cells. *Bone.* 2018;109:201-209.
12. Barruet E, Morales BM, Cain CJ, Ton AN, Wentworth KL, Chan TV, et al. NF-kappaB/MAPK activation underlies ACVR1-mediated inflammation in human heterotopic ossification. *JCI Insight.* 2018;3:122958.
13. Huning I and Gillessen-Kaesbach G. Fibrodysplasia ossificans progressiva: clinical course, genetic mutations and genotype-phenotype correlation. *Mol Syndromol.* 2014;5:201-211.
14. de Ruiten RD, Smilde BJ, Pals G, Bravenboer N, Knaus P, Schoenmaker T, et al. Fibrodysplasia Ossificans Progressiva: What Have We Achieved and Where Are We Now? Follow-up to the 2015 Lorentz Workshop. *Front Endocrinol (Lausanne).* 2021; 12:732728.
15. Hatsell SJ, Idone V, Wolken DM, Huang L, Kim HJ, Wang L, et al.. ACVR1R206H receptor mutation causes fibrodysplasia ossificans progressiva by imparting responsiveness to activin A. *Sci Transl Med.* 2015;7:303ra137..
16. Hino K, Ikeya M, Horigome K, Matsumoto Y, Ebise H, Nishio M, et al. Neofunction of ACVR1 in fibrodysplasia ossificans progressiva. *Proc Natl Acad Sci U S A,* 2015;112: 15438-15443.

17. Sanchez-Duffhues G, Williams E, Benderitter P, Orlova V, van Wijhe M, Garcia de Vinuesa A., et al. Development of Macrocyclic Kinase Inhibitors for ALK2 Using Fibrodysplasia Ossificans Progressiva-Derived Endothelial Cells. *JBMR Plus*. 2019;3:e10230.
18. Williams E, Bagarova J, Kerr G, Xia DD, Place ES, Dey D, et al. Saracatinib is an efficacious clinical candidate for fibrodysplasia ossificans progressiva. *JCI Insight*. 2021;6:95042.
19. Hino K, Zhao C, Horigome K, Nishio M, Okanishi Y, Nagata S, et al. An mTOR Signaling Modulator Suppressed Heterotopic Ossification of Fibrodysplasia Ossificans Progressiva. *Stem Cell Reports*. 2018;11:1106-1119.
20. Shimono K, Tung WE, Macolino C, Chi AH, Didizian JH, Mundy C, et al. Potent inhibition of heterotopic ossification by nuclear retinoic acid receptor-gamma agonists. *Nat Med*. 2011;17:454-460.
21. Pacifici M. Retinoid roles and action in skeletal development and growth provide the rationale for an ongoing heterotopic ossification prevention trial. *Bone*. 2018;109:267-275.
22. Chakkalakal SA, Uchibe K, Convente MR, Zhang D, Economides AN, Kaplan FS, et al. Palovarotene Inhibits Heterotopic Ossification and Maintains Limb Mobility and Growth in Mice With the Human ACVR1(R206H) Fibrodysplasia Ossificans Progressiva (FOP) Mutation. *J Bone Miner Res*. 2016;31:1666-1675.
23. Hino, K., Horigome, K., Nishio, M., Komura, S., Nagata, S., Zhao, C., Jin, Y., Kawakami, K., Yamada, Y., Ohta, A., Toguchida, J., et al. (2017). Activin-A enhances mTOR signaling to promote aberrant chondrogenesis in fibrodysplasia ossificans progressiva. *J Clin Invest* 127, 3339-3352. 10.1172/JCI93521.
24. Carpentier AC, Frisch F, Labbe SM, Gagnon R, de Wal J, Greentree S, et al. Effect of alipogene tiparvovec (AAV1-LPL(S447X)) on postprandial chylomicron metabolism in lipoprotein lipase-deficient patients. *J Clin Endocrinol Metab*. 2012;97:1635-1644.



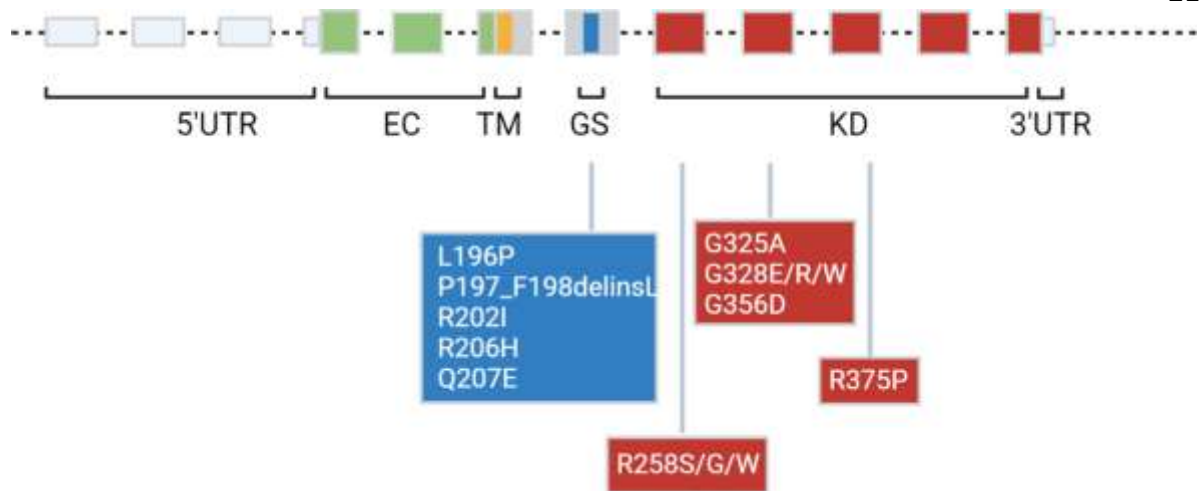
25. Chiu W, Lin TY, Chang YC, Isahwan-Ahmad Mulyadi Lai H, Lin SC, Ma C, et al. An Update on Gene Therapy for Inherited Retinal Dystrophy: Experience in Leber Congenital Amaurosis Clinical Trials. *Int J Mol Sci.* 2021;22: 10.3390/ijms22094534.
26. Mendell JR, Al-Zaidy SA, Lehman KJ, McColly M, Lowes LP, Alfano LN, et al. Five-Year Extension Results of the Phase 1 START Trial of Onasemnogene Apeparvovec in Spinal Muscular Atrophy. *JAMA Neurol.* 2021;78: 834-841.
27. Kaplan J, Kaplan FS and Shore EM. Restoration of normal BMP signaling levels and osteogenic differentiation in FOP mesenchymal progenitor cells by mutant allele-specific targeting. *Gene Ther.* 2012;19:786-790.
28. Takahashi M, Katagiri T, Furuya H and Hohjoh H. Disease-causing allele-specific silencing against the ALK2 mutants, R206H and G356D, in fibrodysplasia ossificans progressiva. *Gene Ther.* 2012;19:781-785.
29. Barrangou R, Fremaux C., Deveau H, Richards M, Boyaval P, Moineau, et al. CRISPR provides acquired resistance against viruses in prokaryotes. *Science.* 2007;315:1709-1712.
30. Makarova KS, Wolf YI, Iranzo J, Shmakov SA, Alkhnbashi OS, Brouns SJJ, et al. Evolutionary classification of CRISPR-Cas systems: a burst of class 2 and derived variants. *Nat Rev Microbiol:* 2020;18:67-83.
31. Bulcha JT, Wang Y, Ma H, Tai PWL and Gao G. Viral vector platforms within the gene therapy landscape. *Signal Transduct Target Ther.* 2021;6:53.
32. Atchison RW, Casto BC and Hammon WM. Adenovirus-Associated Defective Virus Particles. *Science.* 1965;149:754-756.
33. Gruntman AM, Bish LT, Mueller C, Sweeney HL, Flotte TR and Gao G. Gene transfer in skeletal and cardiac muscle using recombinant adeno-associated virus. *Curr Protoc Microbiol.* 2013;14(D):13.
34. Xiao Y, Muhuri M, Li S, Qin W, Xu G, Luo L, et al. Circumventing cellular immunity by miR142-mediated regulation sufficiently supports rAAV-delivered OVA expression without activating humoral immunity. *JCI Insight.* 2019;5:99052.

35. Scarlett RF, Rocke DM, Kantanie S, Patel JB, Shore EM and Kaplan FS. Influenza-like viral illnesses and flare-ups of fibrodysplasia ossificans progressiva. *Clin Orthop Relat Res.* 2004;275-279.
36. Wang H, Lindborg C, Lounev V, Kim JH, McCarrick-Walmsley R, Xu M, et al. Cellular Hypoxia Promotes Heterotopic Ossification by Amplifying BMP Signaling. *J Bone Miner Res.* 2016;31:1652-1665.
37. Lino CA, Harper JC, Carney JP and Timlin JA. Delivering CRISPR: a review of the challenges and approaches. *Drug Deliv.* 2018;25:1234-1257.
38. Hayashi H, Kubo Y, Izumida M and Matsuyama T. Efficient viral delivery of Cas9 into human safe harbor. *Sci Rep.* 2020;10:21474.
39. Ledford H. Experimental gene therapy frees 'bubble-boy' babies from a life of isolation. *Nature.* 2019;10.1038/d41586-019-01257-9.
40. Kaiser J. Clinical research. Gene therapists celebrate a decade of progress. *Science.* 2011;334:29-30.
41. Conceicao I. Novel RNA-targeted therapies for hereditary ATTR amyloidosis and their impact on the autonomic nervous system. *Clin Auton Res.* 2019;29:11-17.
42. Darras BT, Chiriboga CA, Iannaccone ST, Swoboda KJ, Montes J, Mignon L, et al. Nusinersen in later-onset spinal muscular atrophy: Long-term results from the phase 1/2 studies. *Neurology.* 2019;92:e2492-e2506.
43. Cirak S, Arechavala-Gomez V, Guglieri M, Feng L, Torelli S, Anthony K, et al. Exon skipping and dystrophin restoration in patients with Duchenne muscular dystrophy after systemic phosphorodiamidate morpholino oligomer treatment: an open-label, phase 2, dose-escalation study. *Lancet.* 2011;378:595-605.
44. Mulligan MJ, Lyke KE, Kitchin N, Absalon J, Gurtman A, Lockhart S, et al. Phase I/II study of COVID-19 RNA vaccine BNT162b1 in adults. *Nature.* 2020;586:589-593.

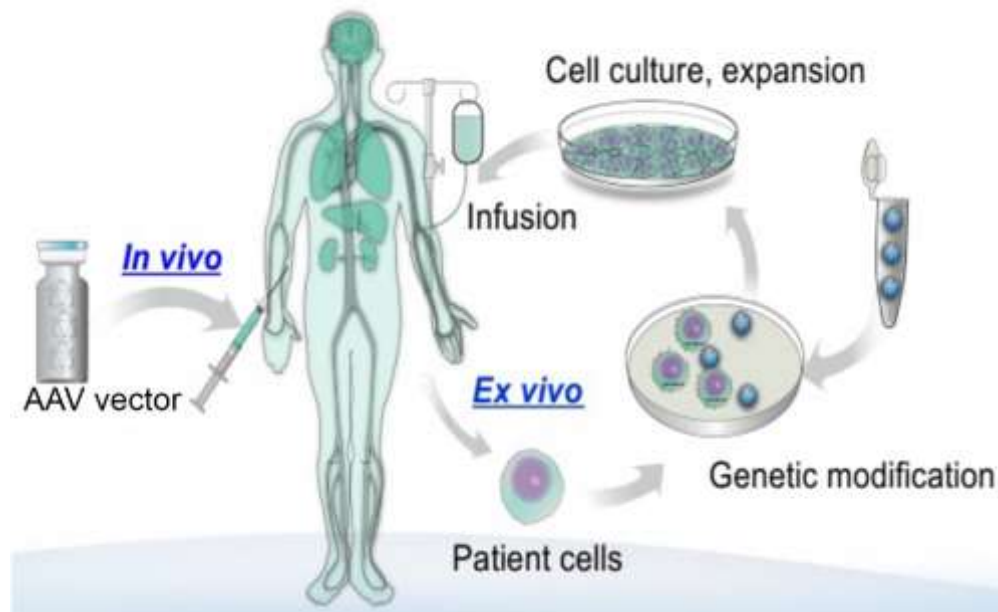
45. Dey D, Bagarova J, Hatsell SJ, Armstrong KA, Huang L, Ermann J, et al. Two tissue-resident progenitor lineages drive distinct phenotypes of heterotopic ossification. *Sci Transl Med.* 2016;8:366ra163.
46. Stanley A, Tichy ED, Kocan J, Roberts DW, Shore EM and Mourkioti F. Dynamics of skeletal muscle-resident stem cells during myogenesis in fibrodysplasia ossificans progressiva. *NPJ Regen Med.* 2022;7:5.



**Figure 1.** A flare-up with swelling of the back of a young girl diagnosed with FOP. The picture is shown with the consent of the patient and her parents.



**Figure 2.** Overview of FOP mutations in the exons of the different domains of the ACVR1 gene. UTR: untranslated region, EC: extracellular, TM: transmembrane, GS: glycine-serine rich, KD: kinase domain.



**Figure 3.** Two ways to express therapeutic genes in target cells and/or tissues. 1) Ex vivo gene therapy: Genetic modification is executed on isolated patient cells using a viral vector, and after cell expansion in the culture, treated cells are introduced to patients via infusion. 2) In vivo gene therapy: AAV vector carrying a therapeutic gene is directly introduced to patient via systemic or local administration.

Table 1. Gene therapy options in FOP for *in vivo* treatment.

	<b>Approach</b>	<b>Target</b>	<b>Effect</b>
<b>Gene replacement</b>	Expression of wild type ALK2	mRNA	Normal ALK2 competes against mutant ALK2 receptor
<b>Gene silencing</b>	Mutant ALK2 specific RNAi	mRNA	Suppression of mutant ALK2 receptor expression
<b>Gene replacement and silencing</b>	Combination of the 2 above	mRNA	Combined effect of the 2 above
<b>Gene editing</b>	CRISPR/CAS-mediated correction of ALK2 mutation	DNA	Only normal ALK2 receptor is produced

**Table 2:** Comparison of different viral vectors in transduction efficacy, duration of expression, transgenic capacity, and potential side effects.<sup>31</sup>

	<b>AAV</b>	<b>Retrovirus</b>	<b>Lentivirus</b>	<b>Adenovirus</b>
<b>Broad host range (Infects many cell types)</b>	Yes (tissue-specific tropism)	Yes (dividing cells only)	Yes	Yes
<b>Infects both dividing and non-dividing cells</b>	Yes	No (dividing cells only)	Yes	Yes
<b>Genome integration (Genotoxicity)</b>	No	Yes	Yes (integrase-deficient versions available)	No
<b>Very high level of protein expression</b>	No	No	No	Yes
<b>Insert size capacity</b>	2.5 kb	2.5-5.0 kb	2.5-5.0 kb	3.0-8.0 kb
<b>Typical titer</b>	$10^{12} - 10^{13}$ GCs/ml	$10^6$ IFU/ml	$10^7$ - $10^8$ IFU/ml	$10^9$ IFU/ml