



Novel therapeutic approaches for the treatment of achondroplasia

Laurence Legeai-Mallet, Ravi Savarirayan

► To cite this version:

Laurence Legeai-Mallet, Ravi Savarirayan. Novel therapeutic approaches for the treatment of achondroplasia. BONE, 2020, 141, pp.115579 -. <10.1016/j.bone.2020.115579>. <hal-03492784>

HAL Id: hal-03492784

<https://hal.science/hal-03492784v1>

Submitted on 21 Nov 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons CC BY-NC 4.0 - Attribution - Non-commercial use - International License

Novel therapeutic approaches for the treatment of Achondroplasia

Laurence Legeai-Mallet^{1*} and Ravi Savarirayan^{2*}

¹Université de Paris, *Imagine* Institute, Laboratory of Molecular and Physiopathological Bases of Osteochondrodysplasia. INSERM UMR 1163, F-75015, Paris, France.

²Victorian Clinical Genetics Services, Murdoch Children's Research Institute, and University of Melbourne, Parkville, Victoria 3052, Australia

*Corresponding authors:

Laurence.legeai-mallet@inserm.fr

ravi.savarirayan@vcgs.org.au

Abstract

Achondroplasia is the most common form of human dwarfism. The molecular basis of achondroplasia was elucidated in 1994 with the identification of the fibroblast growth factor receptor 3 (FGFR3) as the causative gene. Missense mutations causing achondroplasia result in activation of FGFR3 and its downstream signalling pathways, disturbing chondrogenesis, osteogenesis, and long bone elongation. A more accurate understanding of the clinical and molecular aspects of achondroplasia have allowed new therapeutic approaches to be developed. These are based on: clear understanding of the natural history of the disease; proof-of-concept preclinical studies in mouse models; and the current state of knowledge regarding FGFR3 and related growth plate homeostatic pathways. This review provides a brief overview of the preclinical mouse models of achondroplasia that have led to new, non-surgical therapeutic strategies being assessed and applied to children with achondroplasia through pioneering clinical trials.

Keywords

Achondroplasia; FGFR3; therapeutic approaches; preclinical studies; mouse models; clinical trials

1. Introduction

Skeletal dysplasia represents 5% of all birth defects and comprise 461 different diseases [1]. Among them, achondroplasia (ACH, OMIM# 100800) is the most common form of human dwarfism and affects over 250, 000 individuals worldwide. It is associated with a recognizable pattern of growth, medical complications over the lifespan, and related functional and psychosocial challenges [2, 3]. This condition remains the most readily recognizable phenotype consequent to abnormalities in growth plate biology.

The molecular basis of achondroplasia has been elucidated with the identification of Fibroblast Growth Factor Receptor 3 (FGFR3) as the causative gene [4,5]. Missense mutation causing ACH result in activation of FGFR3 and its downstream signaling pathways that can be enhanced in the presence FGF ligands [6]. During infancy and childhood, bone growth occurs, progressively, over 15-18 years with an active phase before puberty. The bone elongation is essentially driven by the activity of the various growth plates, and formation of the ossification centers, both affected in ACH by the FGFR3 gain-of-function mutation.

The management and the treatment of ACH are major challenges of many centers involved in rare skeletal disorders. The only current therapy (approved in Japan) that is offered to ACH patients is treatment with recombinant human growth (r-hGH). No clear long term benefit has been established with this treatment, which does not address the underlying pathogenetic mechanisms [7]. Surgical limb lengthening is also a therapy employed for achondroplasia, primarily by distraction osteogenesis. Complications are, however, common including infection, muscle contractures, increase risk of fractures, pain, and potential adverse psychological outcomes. The use of intramedullary nails might improve outcomes and is now recommended as the implant of choice of femoral lengthening [8].

This review provides a brief overview of the clinical and molecular aspects of achondroplasia, and focuses on new therapies, developed from insights gained from the ACH mouse models.

2. *FGFR3* and dwarfism

ACH is caused by a heterozygous, activating mutation localized in the transmembrane domain of Fibroblast Growth Factor Receptor 3 (*FGFR3*) gene. *FGFR3* is a member of the receptor tyrosine kinases (RTK). The protein is comprised of extracellular, ligand-binding transmembrane, and intracellular kinase domains [9]. Mutations that cause ACH (approximately 99%) arise in the same nucleotide, and result in a glycine to arginine substitution (Gly380Arg) in the transmembrane domain of the FGFR3 protein. This missense

mutation is fully penetrant and can show a modest variability in clinical features. Most cases (80%) of achondroplasia arise as new, spontaneous mutation, and the high frequency of this condition is partially due to a paternal age effect [10]. During antenatal development, the most visible impact related to dysregulation of *FGFR3* signaling is in the skeleton. Prenatal ultrasound can often detect common characteristics of achondroplasia such as shortened limbs, frontal bossing, depressed nasal bridge, short cranial base and narrow thorax, but it is important to note that these features usually develop after 26 weeks of gestation [11] and are often not present at the routine ultrasound check (performed at 20 weeks of gestation).

The spectrum of chondrodysplasia phenotypes associated with *FGFR3* missense mutations range from mild to severe. Hypochondroplasia, the mildest form of *FGFR3*-related dwarfism, is caused by mutations localized in the tyrosine kinase domain I of the protein; SADDAN syndrome (Severe Achondroplasia with Developmental Delay and Acanthosis Nigricans) by a specific mutation (K650M) localized in the tyrosine kinase domain II of *FGFR3*; and thanatophoric dysplasia, an almost uniformly lethal skeletal dysplasia, by mutations localized primarily in the extracellular domain of *FGFR3* [12].

3. Diagnosis, clinical features and natural history

The diagnosis of ACH is made by the combination of clinical features and radiographic findings and confirmed by molecular analysis of the *FGFR3* gene. Approximately 80% of cases arise as a new mutation and individuals with ACH experience a variety of medical (i.e. foramen magnum stenosis with cord compression, spinal stenosis, sleep apnea, increased risk of sudden death), functional (i.e. limitation with activities of daily living, self-care, and access to the environment) and psychosocial issues (i.e. issues with self-esteem and discrimination based on their physical appearance) [2,3,4, 13]

Until very recently, the treatment options for individuals with ACH have been limited to surgical or expectant (“wait and watch”) modalities, with variable outcomes. Therapeutic options for ACH that address the underlying pathology have been a longstanding and unmet need. The recent advent of clinical trials in children with ACH, has been made possible by the deeper understanding of the pathogenesis of ACH provided by studies of animal (mouse) models of ACH.

4. Preclinical models of *Fgfr3*-related dwarfism: lessons from the mouse

The understanding of the control of bone elongation was partially elucidated with the discovery of *FGFR3* as the gene, that when dysregulated causes ACH. Bone formation and

long bone elongation begin during embryonic development and mostly occurs through endochondral ossification, a process in which mesenchymal cells differentiate into chondrocytes that form the cartilage template for future bone. This template is replaced following vascular invasion by bone cells. FGFR3 is expressed in proliferating and pre-hypertrophic chondrocytes and bone during embryonic and post-natal development [14]. The generation of *Fgfr3* mouse models have significantly contributed to deciphering the impact of *Fgfr3* gain-of-function mutations on bone development. Several *Fgfr3* mouse models have been developed: a transgenic mouse (*Fgfr3*^{Ach/+}) model in which a *Col2a1* promoter drives expression of the ACH mutation in chondrocytes [15]; or where a *Fgfr3* promoter drive the expression of human mutant cDNA (*Fgfr3*^{G380R}). These were followed by the generation of numerous knock-in mouse models variably expressing the G380R mutation [16, 17] G375C mutation [18], Y367C mutation [19], K644E mutation [20], and K644M mutation [21]. The deep phenotyping of the *Fgfr3* mouse models highlighted the role of the receptor in several tissues. The *Fgfr3* gain-of-function mutation is ubiquitously expressed in mice and impairs bone formation and elongation. In the appendicular skeleton, examination of the growth plate showed that the high activation of *FGFR3* induces the disorganization of the skeletal growth plate [15,16,17,18,19,20,21]. *FGFR3* signaling inhibits chondrocyte proliferation and differentiation into pre-hypertrophic and hypertrophic chondrocytes. The size of hypertrophic chondrocytes in the *Fgfr3* mutant growth plate were extremely reduced as a consequence of this abnormal differentiation, confirming the important contribution of hypertrophic chondrocytes to bone elongation. Bone maturation delay at age 1 week followed by an accelerated secondary ossification center formation was also reported in these mice [22]. The axial skeletal is also affected, with the size of the pedicles of the lumbar vertebrae reduced, and anomalies of the intervertebral discs, specifically the nucleus pulposus and annulus fibrosus [23]. Membranous and endochondral ossification were affected in craniofacial development, and skull dysmorphology is observed in *Fgfr3* mouse models, with abnormal cartilage and premature fusion of the synchondroses at the skull base leading to abnormalities of foramen magnum shape and size, as well as premature fusion of the coronal sutures [24]. *FGFR3* gain-of-function mutations lead to structural anomalies of primary (Meckel's) and secondary (condylar) cartilages of the mandible, resulting in mandibular hypoplasia and dysmorphogenesis [25]. In addition, a deafness phenotype was also reported in the *Fgfr3*^{Y367C/+} mouse model [19].

Fgfr3 knock-out mouse models confirm the key role of *FGFR3* in controlling bone elongation. In mice that lack *Fgfr3*, the proliferative and hypertrophic zones of the growth

plate are expanded with bone elongation observed [26, 27]. This phenotype was also described in humans, and termed CATSHL syndrome (camptodactyly, tall stature and hearing loss), with patients being heterozygous for a missense *FGFR3* mutation that inactivates the receptor [28, 29].

Activation of *FGFR3* is induced by high affinity ligand binding, which results in dimerization of the tyrosine kinase domains and trans-phosphorylation of tyrosine residues, inducing tyrosine kinase activity

Interestingly, *FGFR3* gain of function mutations result in constitutive activation of *FGFR3* and its signaling pathways that can be further modulated by the presence or absence of FGF ligands. In growth plate chondrocytes, it has been well demonstrated that mutated *FGFR3* dysregulated many downstream signaling pathways that directly affect chondrocyte proliferation and differentiation in various *Fgfr3* mouse models [15,16,17,18,19,20,21]. *FGFR3* signaling can activate several MAP kinases (e.g. ERK1/2 and P38) and other protein phospholipases (i.e., Phospholipase C γ (PLC γ) and signal transducer and activator of transcription (STAT1, 3, 5) [30].

5. Current therapeutic strategies for achondroplasia: from mouse to human model

Aberrant FGF signaling is involved in both developmental disorders (e.g. dwarfism) and a wide range of cancers, including bladder, myeloma, lung, cervical, prostate, and testicular. Some potential treatments for ACH have been informed by protocols used in oncology, and adapted to target bone tissues affected by *FGFR3* gain-of-function mutations. With respect to achondroplasia, it was necessary to take into consideration that the *FGFR3* mutation is active from the early stages of prenatal skeletal development until skeletal maturity and possibly beyond.

The progress in understanding of the *FGFR3* mechanism of action and pathological bases of *Fgfr3*-related dwarfism gained from mouse models have led to potential therapeutic strategies in humans, which are now in active clinical development. Current therapeutic approaches are many and varied, and include targeting the FGFR3 receptor or the FGF ligand, inhibiting the tyrosine kinase activity of FGFR3, and antagonization of *FGFR3* downstream signaling pathways (Figure 1).

5.1. Fibroblast Growth Factor aptamer (APT-F2P/RBM 007)

An aptamer is a short, single-stranded nucleic acid molecule, raised against a range of targets and antigens. Their characteristics are their strong and specific neutralizing activities, medium size, and low antigenicity. An aptamer targeting FGF2 has been generated (APT-

F2P/RBM007; Ribomic Inc.), that blocks the binding of FGF2 to its receptors (FGFR1-4), inhibiting FGFR downstream signaling pathways.

Preliminary data demonstrated that APT-F2P is effective for the treatment of FGF2-dependent lung cancers [31]. The efficacy of this aptamer was demonstrated in bone using mouse and rat models of arthritis and osteoporosis. APT-F2P blocked the disruption of the epiphyseal growth plate, and restored bone quality [32]. It was hypothesized that the action of APT-F2P on the growth plate could be a potential therapeutic approach for achondroplasia. Preclinical studies are currently ongoing to evaluate the effect of RBM007 on bone development and its safety in ACH mouse models.

5.2 Soluble *FGFR3* as a decoy receptor (TA-46/ Recifercept)

Soluble receptors exert their biological effects through mechanisms of action that are defined by their relationship to ligands and membrane-bound receptors. In oncology, soluble receptors have been generated in order to suppress tumor angiogenesis as a therapeutic approach in numerous forms of cancer.

As *FGFR3* activation requires ligand binding, it was hypothesized that preventing the FGF fixation to its receptor would promote the bone growth of ACH mice. A soluble form of *FGFR3* (TA-46, now called recifercept) acting as a decoy receptor was generated, and prevents FGF binding to mutant *FGFR3*. Transgenic *Fgfr3*^{Ach/+} mice were treated with TA-46 twice a week, and the defective maturation of the growth plate and bone growth were restored. These data supported the idea that the ACH mutation requires FGF-binding to be activated [33]. Currently, phase I studies employing this molecule (recifercept; Pfizer Ltd.) have been completed in healthy male volunteers without ACH. An observational study to document the baseline characteristics and growth in children with ACH aged 0-10 years (ClinicalTrials.gov number, NCT03794609; Dreambird) is now recruiting, and will likely form the basis of a phase 2 clinical drug trial commencing in the near future.

5.3 Anti-FGFR3 antibody (B-701/ Vofamatab)

Monoclonal antibody therapy is also used routinely in oncology. The mechanism of action of *FGFR3* antibodies is to bind to the extracellular domain of FGFR3, in order to block ligand binding, interfere with co-factor receptor interaction, and inhibit dimerization of the receptor.

Generation of FGFR3 antibodies were produced for the treatment of bladder cancers. Various studies have reported the efficacy of B-701 on bladder cancer lines, which are FGFR3-

dependent tumors [34, 35, 36]. In the future, *FGFR3*-specific monoclonal antibodies will potentially be an additional therapeutic option for ACH patients.

5.4 Tyrosine kinase Inhibitor (BGJ398/ Infigratinib)

Many oncologic treatments use small molecule tyrosine kinase inhibitors (TKI) to inhibit the activity of receptor tyrosine kinases. Several TKI were generated for the treatment of bladder cancers and *FGFR3*-induced myeloma, such as CHIR-258 [37], PD173074, SU5402, ARQ087, and NVP-BGJ398. NVP-BGJ398, which is more selective for *FGFR3* over other *FGFRs* [38], was used in preclinical murine models for treating rhabdoid tumors [39], hepatocellular carcinoma [40], and FGF23-mediated hypophosphatemic rickets [41]. In a mouse model mimicking ACH (*Fgfr3*^{Y367C/+}), the efficacy of NVP-BGJ398 was demonstrated showing improvement of many of the affected skeletal elements in ACH such as the long bones, vertebrae, intervertebral discs, craniofacial morphology, and the foramen magnum [23, 24,25]. Relatively low doses of NVP-BGJ398 reduced *FGFR3* phosphorylation and its downstream signaling pathways and rescued the disorganization of the growth plate and resulted in bone elongation.

Currently, this TKI (infigratinib; QED therapeutics), is in clinical development with phase 1 studies completed, and an observational natural history study recruiting (ClinicalTrials.gov number, NCT04035811; PROPEL) children with ACH aged 2.5 to 10 years to learn more about their growth and medical complications. In addition, a phase 2, multicenter, open-label, dose escalation and expansion study (ClinicalTrials.gov number, NCT04265651; PROPEL-2) to evaluate the safety, tolerability and efficacy of infigratinib has commenced in children who have completed at least 6 months of the PROPEL lead-in study. The primary endpoints of this study are to determine the incidence of treatment-emergent adverse effects and evaluate the change from baseline in annualized height velocity. Recently, (July 2020), the first child with achondroplasia was dosed orally with infigratinib, and this phase II clinical trial is now ongoing.

5.5 Drug repurposing: meclozine

Drug repurposing is also a proposed strategy for achondroplasia. Meclozine, an anti-histamine and motion sickness drug was considered as putative treatment, as it was demonstrated that meclozine downregulated the phosphorylation of ERK, a downstream *FGFR3* pathway. Preclinical studies were conducted in transgenic *Fgfr3*^{Ach/+} mice, and the preliminary data demonstrated an improvement of the skeletal phenotype of the mutant mice [42]. To date, no clinical trials have been conducted.

Statins, a class of cholesterol-lowering drugs, were also tested in transgenic *Fgfr3*^{Ach/+} mice. The preliminary data showed that statins corrected the skeletal phenotype [43]. However, others studies showed that statins retard cartilage development and reduce the expression of the key regulators of growth plate cartilage [44]. This strategy has not progressed beyond the animal model currently.

5.6 Drugs that modulate growth plate homeostasis: CNP analog (BMN111/Vosoritide) and TransCon CNP.

The C-natriuretic peptide (CNP) and its receptor, natriuretic peptide receptor B (NPR-B) are recognized as key regulators of longitudinal bone growth [45]. Loss of function mutations in NPR-B are responsible for acromesomelic dysplasia, Maroteaux type, a disproportionate form of dwarfism [46]. Mutant mice with a disruption of CNP (*Nppc*^{-/-}) also show disproportionate dwarfism with short limbs [45]. The CNP signaling pathway promotes bone growth through inhibition of MAPK signaling. Tall statures with elongated bones are observed in patients with activating *Npr3* mutations [47], and in patients that overexpress CNP [48, 49]. CNP overexpression in the cartilage or continuous delivery of CNP by intravenous infusion normalizes the dwarfism of *Fgfr3*^{Ach/+} mice [50, 51]. An analog of CNP, BMN111, with an extended plasma half -life due to its resistance to neutral endopeptidase was recently generated (vosoritide; BioMarin). Proof of principle studies were conducted in human ACH cells and a mouse model (*Fgfr3*^{Y367C/+}) and confirmed the beneficial effect of BMN111 on long bone growth and the skull [52]. In parallel, studies to assess the hemodynamic effects of BMN111, given its structural similarity to atrial natriuretic peptide, were performed in juvenile cynomolgus monkey. No clinical signs of hypotension or distress were observed in this model [53]. A multicenter, open-label, dose escalation, clinical trial using vosoritide in 35 patients with ACH aged 5 to 14 years was initiated in 2014 (Clinicaltrials.gov, number NCT02055157). The results of dose-finding and extension study (ClinicalTrials.gov number, NCT02724228) were reported in 2019 [54]. Vosoritide was given as a once daily, subcutaneous injection. A sustained increase of annualized growth velocity of 1.5cm/year above baseline was observed for up to 42 months in children receiving 15mcg/kg/day of vosoritide. Given these promising phase 2 data, a phase 3, randomized placebo-controlled, double-blind, multicenter trial (ClinicalTrials.gov number, NCT03197766), was undertaken in 121 children with achondroplasia aged 5 to less than 18 years [55]. This study confirmed a mean increase of 1.57cm/year in annual growth velocity in children receiving vosoritide as compared to placebo with a similar side effect profiles in vosoritide and placebo arms. The children recruited to this trial will be followed until final

adult height is achieved (ClinicalTrials.gov number, NCT03424018) to assess durability of drug response. It is envisaged that this randomized controlled trial (level 1) evidence will form the basis of regulatory approval of vosoritide as the first precision treatment for children with achondroplasia in this age group. In addition, a phase 2 randomized, double-blind, placebo-controlled trial is ongoing to assess the effects of vosoritide given to children aged 3 months to less than 5 years (ClinicalTrials.gov number, NCT03583697).

Another CNP derivative called, TransCon CNP, has also been engineered (Ascendis Pharma). TransCon CNP is a pro-drug consisting of CNP-38 conjugated via a cleavable linker to a polyethylene glycol carrier molecule. It is designed to provide continuous CNP exposure at the growth plate, with the goal of optimizing efficacy, with its longer half-life allowing weekly subcutaneous dosing. Preclinical data in mice and cynomolgus monkeys have shown efficacy of CNP on bone growth without cardiovascular side effects. Phase 1 studies have been completed and an observational study (ClinicalTrials.gov number, NCT03875534; ACHieve) has been recruiting children with ACH aged 0-8 years to document natural history. Recently, (June 2020), the first child with achondroplasia was recruited into a multicenter, double-blind, randomized placebo-controlled, dose-escalation clinical trial of TransCon CNP in children with ACH aged 2-10 years (ClinicalTrials.gov number, NCT04085523; ACcomplisH) to assess its safety and effect on annual growth velocity in these children.

Future perspectives

The main challenge of drug development for achondroplasia resides in characterization of the balance of harms and benefits of any proposed therapy in growing individuals based on anticipated natural history, preclinical data and clinical trials. Treating pediatric patients with achondroplasia presents challenges that relate to potential harms as, 1) some organ systems (e.g. CNS, reproductive organs, heart) are still developing, and 2) treatment is likely required to be administered for prolonged periods, often to the end of skeletal maturation/linear growth. The earliest time after birth that management and, now, treatment of ACH can be instituted is paramount to offer the best chances of ameliorating the features that cause morbidity and mortality. During infancy, the management of the foramen magnum is crucial for ACH patients as is management of sleep-disordered breathing. The combination of these two features in infants with achondroplasia is thought to contribute significantly to the increased risk of sudden death in this condition observed before age 5 years. Orthopedic concerns that need to be managed in childhood and adolescence include

spinal kyphosis, exaggerated lordosis, leg bowing, and the high prevalence of spinal stenosis leading to reduced walking distance and pain [56].

In the future, treating adult achondroplasia patients will also be an important consideration. The aim of such therapy will be to reduce the clinical impact of the *FGFR3* activating mutation on spinal stenosis, intervertebral disc disease, bone homeostasis, and other non-skeletal complications that might affect life span (i.e. cardiovascular).

Today, several major milestones for the precision treatment of achondroplasia have been achieved, best demonstrated by the results from the phase 2 and 3 clinical trials of once-daily, subcutaneous CNP therapy in children with ACH aged 5 to 18 years [54,55]. Several other therapeutic options for ACH are emerging, and we will soon have the clinical trial data to assess their safety and efficacy profiles.

It is likely that a better understanding of the multiple signalling pathways activated by the *FGFR3* gain-of function mutation in ACH, based on preclinical animal studies and the natural history of the disease will facilitate more precision therapeutic strategies to be developed. As further understanding of the benefits and harms of these drugs is gathered, these treatments can be further tailored, to provide the right therapy, at the right time and dose, to achieve maximum benefits for each individual with ACH. Combination therapy, or the sequential use of different therapies, might also become a future possibility, to first promote and control chondrogenesis and osteogenesis during early infancy and childhood and then minimize progression of the condition in adolescence and beyond skeletal maturity.

It is hoped that these new, disruptive therapies will give better, non-surgical options for individuals with ACH to optimize their health and well-being, and minimize the medical impacts of their condition.

Legend figures

Figure 1

Drug development process for achondroplasia.

1. The natural history of the disease needs to be understood, with a focus on complications related to abnormality of the endochondral ossification process. 2. Relevant preclinical studies in well-characterized ACH mouse models (and cynomolgus monkeys) in order to study key disease features and proof-of-concept of drug safety and efficacy. 3. Overview of the drugs and their targets currently in clinical development (green denotes the compound in clinical trial, red denotes no clinical trial are ongoing (as of July 2020)).

References

- [1] Mortier, G. R., Cohn, D. H., Cormier-Daire, V., Hall, C., Krakow, D., Mundlos, S., Nishimura, G., Robertson, S., Sangiorgi, L., Savarirayan, R., Sillence, D., Superti-Furga, A., Unger, S., & Warman, M. L. (2019). Nosology and classification of genetic skeletal disorders: 2019 revision. *American journal of medical genetics. Part A*, 179(12), 2393–2419. <https://doi-org.proxy.insermbiblio.inist.fr/10.1002/ajmg.a.61366>
- [2] Ireland, P. J., Pacey, V., Zankl, A., Edwards, P., Johnston, L. M., & Savarirayan, R. (2014). Optimal management of complications associated with achondroplasia. *The application of clinical genetics*, 7, 117–125.
- [3] Horton, W. A., Hall, J. G., & Hecht, J. T. (2007). Achondroplasia. *Lancet (London, England)*, 370(9582), 162–172.
- [4] Rousseau F, Bonaventure J, Legeai-Mallet L, et al. Mutations in the gene encoding fibroblast growth factor receptor-3 in achondroplasia. *Nature*. 1994;371(6494):252-254.
- [5] Shiang, R., Thompson, L. M., Zhu, Y. Z., Church, D. M., Fielder, T. J., Bocian, M., Winokur, S. T., & Wasmuth, J. J. (1994). Mutations in the transmembrane domain of FGFR3 cause the most common genetic form of dwarfism, achondroplasia. *Cell*, 78(2), 335–342.
- [6] Naski, M. C., Wang, Q., Xu, J., & Ornitz, D. M. (1996). Graded activation of fibroblast growth factor receptor 3 by mutations causing achondroplasia and thanatophoric dysplasia. *Nature genetics*, 13(2), 233–237.
- [7] Miccoli, M., Bertelloni, S., & Massart, F. (2016). Height Outcome of Recombinant Human Growth Hormone Treatment in Achondroplasia Children: A Meta-Analysis. *Hormone research in paediatrics*, 86(1), 27–34.
- [8] Paley D. (2015). PRECICE intramedullary limb lengthening system. *Expert review of medical devices*, 12(3), 231–249.
- [9] Belov, A. A., & Mohammadi, M. (2013). Molecular mechanisms of fibroblast growth factor signaling in physiology and pathology. *Cold Spring Harbor perspectives in biology*, 5(6), a015958.
- [10] Wilkin, D. J., Szabo, J. K., Cameron, R., Henderson, S., Bellus, G. A., Mack, M. L., Kaitila, I., Loughlin, J., Munnich, A., Sykes, B., Bonaventure, J., & Francomano, C. A. (1998). Mutations in fibroblast growth-factor receptor 3 in sporadic cases of achondroplasia occur exclusively on the paternally derived chromosome. *American journal of human genetics*, 63(3), 711–716.
- [11] Savarirayan, R., Rossiter, J. P., Hoover-Fong, J. E., Irving, M., Bompadre, V., Goldberg, M. J., Bober, M. B., Cho, T. J., Kamps, S. E., Mackenzie, W. G., Raggio, C., Spencer, S. S., White, K. K., & Skeletal Dysplasia Management Consortium (2018). Best practice guidelines regarding prenatal evaluation and delivery of patients with skeletal dysplasia. *American journal of obstetrics and gynecology*, 219(6), 545–562.
- [12] Ornitz, D. M., & Legeai-Mallet, L. (2017). Achondroplasia: Development, pathogenesis, and therapy. *Developmental dynamics : an official publication of the American Association of Anatomists*, 246(4), 291–309.
- [13] Pauli RM. Achondroplasia: a comprehensive clinical review. *Orphanet J Rare Dis*. 2019;14(1):1. Published 2019 Jan 3. doi:10.1186/s13023-018-0972-6
- [13] Peters, K., Ornitz, D., Werner, S., & Williams, L. (1993). Unique expression pattern of the FGF receptor 3 gene during mouse organogenesis. *Developmental biology*, 155(2), 423–430
- [15] Naski, M. C., Colvin, J. S., Coffin, J. D., & Ornitz, D. M. (1998). Repression of hedgehog signaling and BMP4 expression in growth plate cartilage by fibroblast growth factor receptor 3. *Development (Cambridge, England)*, 125(24), 4977–4988.

- [16] Wang Y, Spatz MK, Kannan K, et al. A mouse model for achondroplasia produced by targeting fibroblast growth factor receptor 3. *Proc Natl Acad Sci U S A*. 1999;96(8):4455-4460.
- [17] Lee, Y. C., Song, I. W., Pai, Y. J., Chen, S. D., & Chen, Y. T. (2017). Knock-in human FGFR3 achondroplasia mutation as a mouse model for human skeletal dysplasia. *Scientific reports*, 7, 43220.
- [18] Chen, L., Adar, R., Yang, X., Monsonego, E. O., Li, C., Hauschka, P. V., Yayon, A., & Deng, C. X. (1999). Gly369Cys mutation in mouse FGFR3 causes achondroplasia by affecting both chondrogenesis and osteogenesis. *The Journal of clinical investigation*, 104(11), 1517–1525.
- [19] Pannier, S., Couloigner, V., Messaddeq, N., Elmaleh-Bergès, M., Munnich, A., Romand, R., & Legeai-Mallet, L. (2009). Activating Fgfr3 Y367C mutation causes hearing loss and inner ear defect in a mouse model of chondrodysplasia. *Biochimica et biophysica acta*, 1792(2), 140–147
- [20] Li, C., Chen, L., Iwata, T., Kitagawa, M., Fu, X. Y., & Deng, C. X. (1999). A Lys644Glu substitution in fibroblast growth factor receptor 3 (FGFR3) causes dwarfism in mice by activation of STATs and ink4 cell cycle inhibitors. *Human molecular genetics*, 8(1), 35–44.
- [21] Iwata, T., Li, C. L., Deng, C. X., & Francomano, C. A. (2001). Highly activated Fgfr3 with the K644M mutation causes prolonged survival in severe dwarf mice. *Human molecular genetics*, 10(12), 1255–1264.
- [22] Pannier, S., Mugniery, E., Jonquoy, A., Benoist-Lasselin, C., Odent, T., Jais, J. P., Munnich, A., & Legeai-Mallet, L. (2010). Delayed bone age due to a dual effect of FGFR3 mutation in Achondroplasia. *Bone*, 47(5), 905–915
- [23] Komla-Ebri, D., Dambroise, E., Kramer, I., Benoist-Lasselin, C., Kaci, N., Le Gall, C., Martin, L., Busca, P., Barbault, F., Graus-Porta, D., Munnich, A., Kneissel, M., Di Rocco, F., Biosse-Duplan, M., & Legeai-Mallet, L. (2016). Tyrosine kinase inhibitor NVP-BGJ398 functionally improves FGFR3-related dwarfism in mouse model. *The Journal of clinical investigation*, 126(5), 1871–1884.
- [24] Di Rocco, F., Biosse Duplan, M., Heuzé, Y., Kaci, N., Komla-Ebri, D., Munnich, A., Mugniery, E., Benoist-Lasselin, C., & Legeai-Mallet, L. (2014). FGFR3 mutation causes abnormal membranous ossification in achondroplasia. *Human molecular genetics*, 23(11), 2914–2925.
- [25] Biosse Duplan, M., Komla-Ebri, D., Heuzé, Y., Estibals, V., Gaudas, E., Kaci, N., Benoist-Lasselin, C., Zerah, M., Kramer, I., Kneissel, M., Porta, D. G., Di Rocco, F., & Legeai-Mallet, L. (2016). Meckel's and condylar cartilages anomalies in achondroplasia result in defective development and growth of the mandible. *Human molecular genetics*, 25(14), 2997–3010.
- [26] Colvin, J. S., Bohne, B. A., Harding, G. W., McEwen, D. G., & Ornitz, D. M. (1996). Skeletal overgrowth and deafness in mice lacking fibroblast growth factor receptor 3. *Nature genetics*, 12(4), 390–397
- [27] Deng, C., Wynshaw-Boris, A., Zhou, F., Kuo, A., & Leder, P. (1996). Fibroblast growth factor receptor 3 is a negative regulator of bone growth. *Cell*, 84(6), 911–921.
- [28] oydemir, R. M., Brassington, A. E., Bayrak-Toydemir, P., Krakowiak, P. A., Jorde, L. B., Whitby, F. G., Longo, N., Viskochil, D. H., Carey, J. C., & Bamshad, M. J. (2006). A novel mutation in FGFR3 causes camptodactyly, tall stature, and hearing loss (CATSHL) syndrome. *American journal of human genetics*, 79(5), 935–941.
- [29] Escobar, L. F., Tucker, M., & Bamshad, M. (2016). A second family with CATSHL syndrome: Confirmatory report of another unique FGFR3 syndrome. *American journal of medical genetics. Part A*, 170(7), 1908–1911.

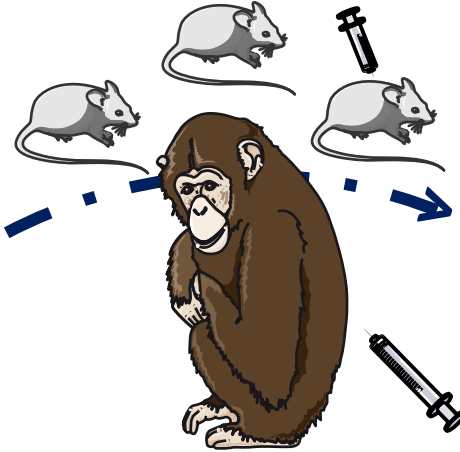
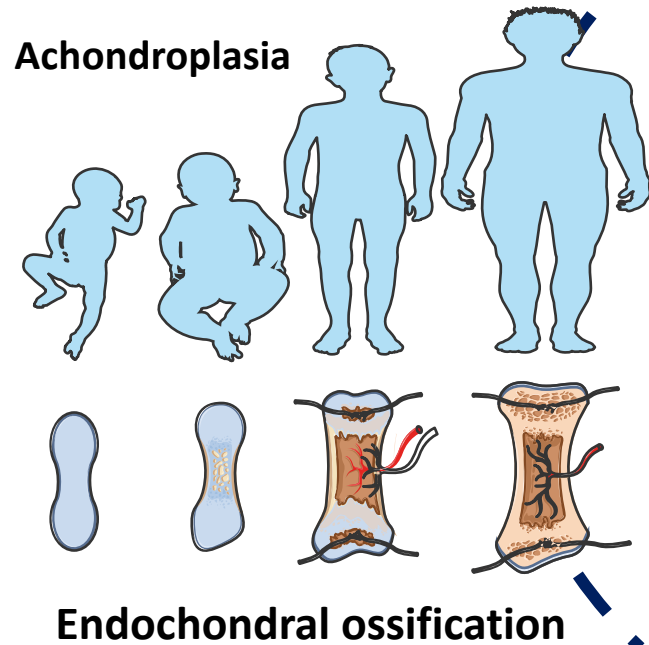
- [30] Ornitz, D. M., & Marie, P. J. (2019). Fibroblast growth factors in skeletal development. *Current topics in developmental biology*, 133, 195–234.
- [31] Hamamoto, J., Yasuda, H., Nonaka, Y., Fujiwara, M., Nakamura, Y., Soejima, K., & Betsuyaku, T. (2018). The FGF2 aptamer inhibits the growth of FGF2-FGFR pathway driven lung cancer cells. *Biochemical and biophysical research communications*, 503(3), 1330–1334.
- [32] Jin, L., Nonaka, Y., Miyakawa, S., Fujiwara, M., & Nakamura, Y. (2016). Dual Therapeutic Action of a Neutralizing Anti-FGF2 Aptamer in Bone Disease and Bone Cancer Pain. *Molecular therapy : the journal of the American Society of Gene Therapy*, 24(11), 1974–1986.
- [33] Garcia, S., Dirat, B., Tognacci, T., Rochet, N., Mouska, X., Bonnafous, S., Patouraux, S., Tran, A., Gual, P., Le Marchand-Brustel, Y., Gennero, I., & Gouze, E. (2013). Postnatal soluble FGFR3 therapy rescues achondroplasia symptoms and restores bone growth in mice. *Science translational medicine*, 5(203), 203ra124.
- [34] Rauchenberger, R., Borges, E., Thomassen-Wolf, E., Rom, E., Adar, R., Yaniv, Y., Malka, M., Chumakov, I., Kotzer, S., Resnitzky, D., Knappik, A., Reiffert, S., Prassler, J., Jury, K., Waldherr, D., Bauer, S., Kretzschmar, T., Yayon, A., & Rothe, C. (2003). Human combinatorial Fab library yielding specific and functional antibodies against the human fibroblast growth factor receptor 3. *The Journal of biological chemistry*, 278(40), 38194–38205.
- [35] Trudel, S., Stewart, A. K., Rom, E., Wei, E., Li, Z. H., Kotzer, S., Chumakov, I., Singer, Y., Chang, H., Liang, S. B., & Yayon, A. (2006). The inhibitory anti-FGFR3 antibody, PRO-001, is cytotoxic to t(4;14) multiple myeloma cells. *Blood*, 107(10), 4039–4046.
- [36] Yin, Y., Ren, X., Smith, C., Guo, Q., Malabunga, M., Guernah, I., Zhang, Y., Shen, J., Sun, H., Chehab, N., Loizos, N., Ludwig, D. L., & Ornitz, D. M. (2016). Inhibition of fibroblast growth factor receptor 3-dependent lung adenocarcinoma with a human monoclonal antibody. *Disease models & mechanisms*, 9(5), 563–571.
- [37] Trudel, S., Li, Z. H., Wei, E., Wiesmann, M., Chang, H., Chen, C., Reece, D., Heise, C., & Stewart, A. K. (2005). CHIR-258, a novel, multitargeted tyrosine kinase inhibitor for the potential treatment of t(4;14) multiple myeloma. *Blood*, 105(7), 2941–2948.
- [38] Kunova Bosakova, M., Nita, A., Gregor, T., Varecha, M., Gudernova, I., Fafilek, B., Barta, T., Basheer, N., Abraham, S. P., Balek, L., Tomanova, M., Fialova Kucerova, J., Bosak, J., Potesil, D., Zieba, J., Song, J., Konik, P., Park, S., Duran, I., Zdrahal, Z., ... Krejci, P. (2019). Fibroblast growth factor receptor influences primary cilium length through an interaction with intestinal cell kinase. *Proceedings of the National Academy of Sciences of the United States of America*, 116(10), 4316–4325.
- [39] Wöhrle, S., Weiss, A., Ito, M., Kauffmann, A., Murakami, M., Jagani, Z., Thuery, A., Bauer-Probst, B., Reimann, F., Stamm, C., Pornon, A., Romanet, V., Guagnano, V., Brümmendorf, T., Sellers, W. R., Hofmann, F., Roberts, C. W., & Graus Porta, D. (2013). Fibroblast growth factor receptors as novel therapeutic targets in SNF5-deleted malignant rhabdoid tumors. *PloS one*, 8(10), e77652.
- [40] Scheller, T., Hellerbrand, C., Moser, C., Schmidt, K., Kroemer, A., Brunner, S. M., Schlitt, H. J., Geissler, E. K., & Lang, S. A. (2015). mTOR inhibition improves fibroblast growth factor receptor targeting in hepatocellular carcinoma. *British journal of cancer*, 112(5), 841–850.
- [41] Wöhrle, S., Henninger, C., Bonny, O., Thuery, A., Beluch, N., Hynes, N. E., Guagnano, V., Sellers, W. R., Hofmann, F., Kneissel, M., & Graus Porta, D. (2013). Pharmacological inhibition of fibroblast growth factor (FGF) receptor signaling ameliorates FGF23-mediated hypophosphatemic rickets. *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research*, 28(4), 899–911.

- [42] (Matshushita et al 2017) Matsushita, M., Hasegawa, S., Kitoh, H., Mori, K., Ohkawara, B., Yasoda, A., Masuda, A., Ishiguro, N., & Ohno, K. (2015). Meclozine promotes longitudinal skeletal growth in transgenic mice with achondroplasia carrying a gain-of-function mutation in the FGFR3 gene. *Endocrinology*, 156(2), 548–554.
- [43] Yamashita, A., Morioka, M., Kishi, H., Kimura, T., Yahara, Y., Okada, M., Fujita, K., Sawai, H., Ikegawa, S., & Tsumaki, N. (2014). Statin treatment rescues FGFR3 skeletal dysplasia phenotypes. *Nature*, 513(7519), 507–511.
- [44] Bush, J. R., Bérubé, N. G., & Beier, F. (2015). A new prescription for growth? Statins, cholesterol and cartilage homeostasis. *Osteoarthritis and cartilage*, 23(4), 503–506.
- [45] Chusho, H., Tamura, N., Ogawa, Y., Yasoda, A., Suda, M., Miyazawa, T., Nakamura, K., Nakao, K., Kurihara, T., Komatsu, Y., Itoh, H., Tanaka, K., Saito, Y., Katsuki, M., & Nakao, K. (2001). Dwarfism and early death in mice lacking C-type natriuretic peptide. *Proceedings of the National Academy of Sciences of the United States of America*, 98(7), 4016–4021.
- [46] Bartels, C. F., Bükülmez, H., Padayatti, P., Rhee, D. K., van Ravenswaaij-Arts, C., Pauli, R. M., Mundlos, S., Chitayat, D., Shih, L. Y., Al-Gazali, L. I., Kant, S., Cole, T., Morton, J., Cormier-Daire, V., Faivre, L., Lees, M., Kirk, J., Mortier, G. R., Leroy, J., Zabel, B., ... Warman, M. L. (2004). Mutations in the transmembrane natriuretic peptide receptor NPR-B impair skeletal growth and cause acromesomelic dysplasia, type Maroteaux. *American journal of human genetics*, 75(1), 27–34.
- [47] Hannema, S. E., van Duyvenvoorde, H. A., Premisler, T., Yang, R. B., Mueller, T. D., Gassner, B., Oberwinkler, H., Roelfsema, F., Santen, G. W., Prickett, T., Kant, S. G., Verkerk, A. J., Uitterlinden, A. G., Espiner, E., Ruivenkamp, C. A., Oostdijk, W., Pereira, A. M., Losekoot, M., Kuhn, M., & Wit, J. M. (2013). An activating mutation in the kinase homology domain of the natriuretic peptide receptor-2 causes extremely tall stature without skeletal deformities. *The Journal of clinical endocrinology and metabolism*, 98(12), E1988–E1998.
- [48] Bocciardi, R., Giorda, R., Buttgerit, J., Gimelli, S., Divizia, M. T., Beri, S., Garofalo, S., Tavella, S., Lerone, M., Zuffardi, O., Bader, M., Ravazzolo, R., & Gimelli, G. (2007). Overexpression of the C-type natriuretic peptide (CNP) is associated with overgrowth and bone anomalies in an individual with balanced t(2;7) translocation. *Human mutation*, 28(7), 724–731.
- [49] Moncla, A., Missirian, C., Cacciagli, P., Balzamo, E., Legeai-Mallet, L., Jouve, J. L., Chabrol, B., Le Merrer, M., Plessis, G., Villard, L., & Philip, N. (2007). A cluster of translocation breakpoints in 2q37 is associated with overexpression of NPPC in patients with a similar overgrowth phenotype. *Human mutation*, 28(12), 1183–1188.
- [50] Yasoda, A., Komatsu, Y., Chusho, H., Miyazawa, T., Ozasa, A., Miura, M., Kurihara, T., Rogi, T., Tanaka, S., Suda, M., Tamura, N., Ogawa, Y., & Nakao, K. (2004). Overexpression of CNP in chondrocytes rescues achondroplasia through a MAPK-dependent pathway. *Nature medicine*, 10(1), 80–86.
- [51] Yasoda, A., Kitamura, H., Fujii, T., Kondo, E., Murao, N., Miura, M., Kanamoto, N., Komatsu, Y., Arai, H., & Nakao, K. (2009). Systemic administration of C-type natriuretic peptide as a novel therapeutic strategy for skeletal dysplasias. *Endocrinology*, 150(7), 3138–3144. <https://doi-org.proxy.insermbiblio.inist.fr/10.1210/en.2008-1676>
- Format:
- [52] Lorget, F., Kaci, N., Peng, J., Benoist-Lassel, C., Mugniery, E., Oppeneer, T., Wendt, D. J., Bell, S. M., Bullens, S., Bunting, S., Tsuruda, L. S., O'Neill, C. A., Di Rocco, F., Munnich, A., & Legeai-Mallet, L. (2012). Evaluation of the therapeutic potential of a CNP analog in a Fgfr3 mouse model recapitulating achondroplasia. *American journal of human genetics*, 91(6), 1108–1114.

- [53] Wendt, D. J., Dvorak-Ewell, M., Bullens, S., Lorget, F., Bell, S. M., Peng, J., Castillo, S., Aoyagi-Scharber, M., O'Neill, C. A., Krejci, P., Wilcox, W. R., Rimoin, D. L., & Bunting, S. (2015). Neutral endopeptidase-resistant C-type natriuretic peptide variant represents a new therapeutic approach for treatment of fibroblast growth factor receptor 3-related dwarfism. *The Journal of pharmacology and experimental therapeutics*, 353(1), 132–149
- [54] Savarirayan, R., Irving, M., Bacino, C. A., Bostwick, B., Charrow, J., Cormier-Daire, V., Le Quan Sang, K. H., Dickson, P., Harmatz, P., Phillips, J., Owen, N., Cherukuri, A., Jayaram, K., Jeha, G. S., Larimore, K., Chan, M. L., Huntsman Labeled, A., Day, J., & Hoover-Fong, J. (2019). C-Type Natriuretic Peptide Analogue Therapy in Children with Achondroplasia. *The New England journal of medicine*, 381(1), 25–35.
- [55] Savarirayan R, Tofts, L, Irving MI, Wilcox W, Bacino CA, Hoover-Fond J, Font RU, Harmatz P, Rutsch F, Bober MB, Polgreen LE, Ginebreda I, Mohnike K, Charrow J, Hoernschmeyer D, Ozono K, Alanay Y, Arundel P, Kagami S, Yasui N, White KK, Saal HM, Leiva-Gea A, Luna-Gonzalez F, Mochizuki H, Basel D, Porco DM, Jayaram K, Fischeleva E, Huntsman-Labeled A, Day J. Once-daily, subcutaneous vosoritide therapy in children with achondroplasia: a randomised, double-blind, phase 3, placebo-controlled, multicentre trial. In the press (Lancet); accepted 11June 2020.
- [56] White, K. K., Bober, M. B., Cho, T. J., Goldberg, M. J., Hoover-Fong, J., Irving, M., Kamps, S. E., Mackenzie, W. G., Raggio, C., Spencer, S. A., Bompadre, V., Savarirayan, R., & Skeletal Dysplasia Management Consortium (2020). Best practice guidelines for management of spinal disorders in skeletal dysplasia. *Orphanet journal of rare diseases*, 15(1), 161.

2. Preclinical Studies

1. Natural History



3. Clinical trials

