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Novel therapeutic approaches for the treatment of achondroplasia

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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 signaling pathways, disturbing chondrogenesis, osteogenesis, and long bone elongation. A more accurate understanding of the clinical and molecular aspects of achondroplasia has 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.

1. Introduction

Skeletal dysplasia represents 5% of all birth defects and comprises 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. One therapy offered to ACH patients is treatment with recombinant human growth (r-hGH) (approved today only in Japan). 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 clinical therapies, developed from insights gained from the preclinical 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 kinase family (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

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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) [2–4,13].

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 has 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 [15–21]. *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 transphosphorylation 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 [15–21]. *FGFR3* signaling can activate several MAP kinases (e.g. ERK1/2 and P38) and other protein phospholipases (i.e., Phospholipase Cg (PLCg) 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 and affect mostly cartilage, an avascular tissue.

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 (Fig. 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.

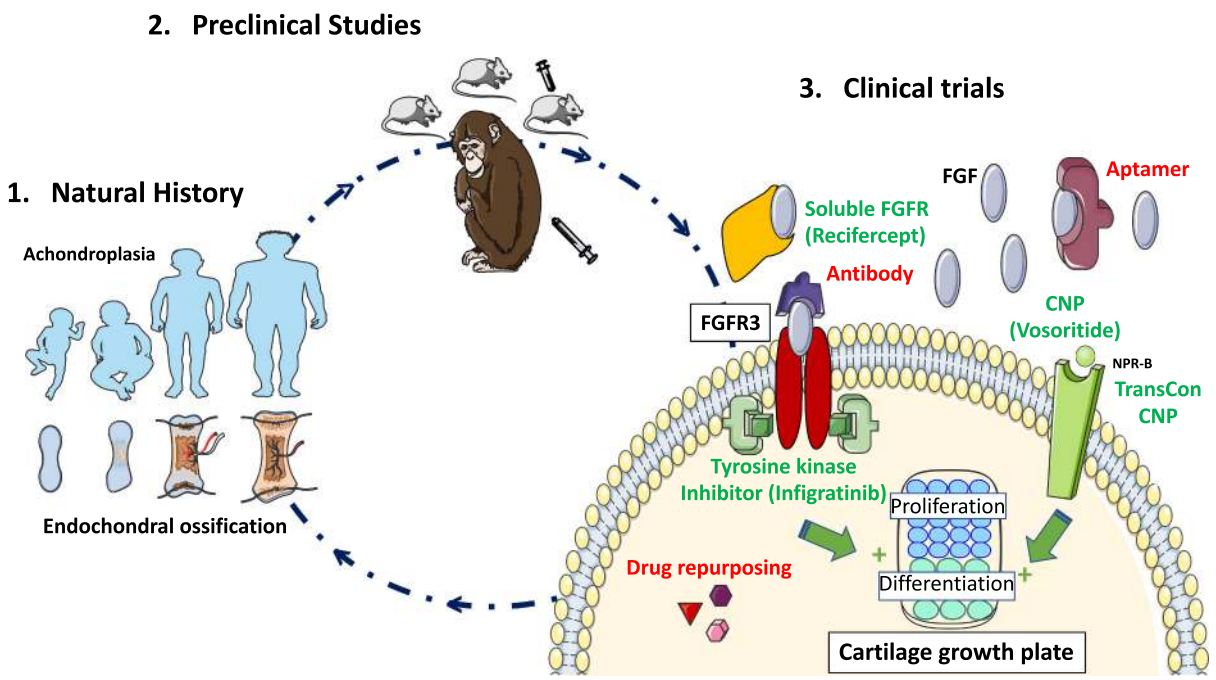

Fig. 1. Drug development process for achondroplasia.
1. The natural history of the disease needs to be understood, with a focus on complications related to abnormality of the endochondral ossification process. 2. Relevant preclinical studies in well-characterized ACH mouse models (and cynomolgus monkeys) 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). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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/vofatamab)

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 down-regulated 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 is 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 Npr2 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 1.5 cm/year above baseline was observed for up to 42 months in children receiving 15 μg/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 1.57 cm/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 asses 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 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, 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.

5.7. 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, and 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 signaling 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.

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