Prospects for Pharmaceutical Treatment of Achondroplasia

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Achondroplasia is a form of dwarfism that occurs about once in every 20,000 births. It arises from a defect in the process of bone growth in children, particularly in the "long" bones of the body such as those of the arms and legs. There is currently no known cure for achondroplasia. A surgical procedure of limb-lengthening can add up to a foot to the height of those with achondroplasia, but the procedure can take years to complete and does not address all of the medical issues with the condition. Alternatively, there are several promising drug treatments for achondroplasia being investigated. This article reviews the current status of these drugs in development. It is written in "layman's" terms; a superficial knowledge of chemistry or biology for the reader would be helpful but not necessary.[1,2]

Achondroplasia has a genetic origin. With very few exceptions, humans have 46 chromosomes, consisting of 23 pairs (one set from each parent) in most cells of the body. Each chromosome is composed of a single DNA molecule, and can be considered to be divided into many sections called genes; most chromosomes have between 300 -2000 genes. A gene generally contains the instructions (or code) to make a single protein. Proteins have a variety of critical functions in the body. Some are transport proteins such as the hemoglobin that carries oxygen; some are hormones such as insulin; and many are enzymes that facilitate the biochemical reactions necessary for metabolism.

The building blocks of proteins are amino acids; thus, a protein is a long chain (or polymer) of amino acids strung together by "peptide" bonds, and hence can be called polypeptides. Sizes of proteins are measured in units called "daltons," named after John Dalton (1766-1844), one of the fathers of chemistry. All amino acids are composed of the elements hydrogen, carbon, nitrogen, and oxygen, which are about 1, 12, 14, and 16 daltons (also called atomic mass units), respectively. There are 20 different amino acids that compose proteins, ranging in size from 75 daltons (for the amino acid glycine) to 204 (for tryptophan), or an average size of about 120 daltons. Proteins range in size from less than 100 amino acids to over 10,000 (i.e., from 10,000 daltons to over a million). For example, human insulin is composed of 51 amino acids and is 5808 daltons, while alkaline phosphatase (an enzyme frequently measured in routine blood tests) has 858 amino acids and is 86,000 daltons. A given protein has a specific amino acid sequence critical to its function; the amino acid sequence is in turn defined by the genetic code in the gene responsible for its synthesis. Changes (or mutations) in the gene will result in an incorrect amino acid sequence in the protein, which can lead to a loss of or change in its activity. Mutations result from a mistake in replication

(copying) of the DNA of the gene for that protein, and can arise from a variety of causes, such as radiation, chemical mutagens, and other factors not completely understood.

One of the most abundant proteins in humans is collagen, which is a major component of tendons, blood vessels, intestines, ligaments and other connective and structural tissues. Unlike enzymes and transport proteins (which are "globular" proteins), collagen is a fibrous (or rope-like) protein. It is also a major component of cartilage, which is a somewhat elastic tissue that is a structural component of the ears, nose, rib cage, and spine. Cartilage is important in the growth process of bones. The skeleton of a human fetus in the first trimester of pregnancy is mostly cartilage, and this cartilage is gradually replaced by bone. This process is called ossification, and continues after birth until puberty. It is primarily the addition of calcium (mostly in the form of calcium hydroxyapatite) that is important in ossification and gives bone its rigidity compared to cartilage; mature bone is about 70% calcium salts and 30% protein matrix (mostly collagen). Cells called chondrocytes are the ones involved in the ossification process; they manufacture cartilage and turn it into bone. Chondrocytes are found at the "growth plates" at the end of the long bone, which is the place where the bone is adding length. During the growth process at the growth plate, chondrocytes will proliferate (multiply), and undergo hypertrophy (grow bigger). Finally, as calcium is processed in the cell and ossification occurs, the chondrocytes die and are replaced by mature bone. When puberty is reached, the growth plates close, growth stops, and the lengthening process ceases.

Another important group of proteins are receptors, which are found on the surface (or plasma membrane) of cells. Receptors will bind some specific molecule (a "ligand" such as a hormone or enzyme) on the outside of the cell, which then causes some desired response inside the cell, analogous to a messenger arriving at the door of a manufacturing plant to deliver new instructions for the plant. An important receptor controlling bone growth is Fibroblast Growth Factor Receptor 3 (FGFR3), a protein of about 70,000 daltons in size. Like many receptors, FGFR3 initiates a cascade-like signal pathway in a series of messengers: the receptor activates a messenger that in turn activates a second messenger, which activates a third, etc., until the signal is relayed and arrives at its final destination of the pathway and the desired effect is initiated. The advantage of such a process is that the signal is amplified at every step of the relay, since each messenger in the process may activate tens or hundreds of the next messenger in the pathway. Binding of the ligand, Fibroblast Growth Factor (FGF), to its receptor, FGFR3, triggers the signal that starts the cascade; the final step in the pathway is control of chondrocyte proliferation and hypertrophy. Actually, FGFR3 normally exists as a monomer (a single unit); binding of FGF causes the FGFR3 monomers to dimerize (pair up with other monomer units to form dimers), and it is the dimers that trigger the cascade-initiating signal.

Like most physiological processes and pathways in the body, bone growth (including chondrocyte proliferation and ossification) is highly regulated. Some hormones and receptors will increase a desired effect ("up-regulate" it), while others will decrease the effect ("down-regulate" it). For example the polypeptide glucagon will stimulate release of glucose into the bloodstream, while insulin will inhibit it in response to the body's needs. Normally, the up-regulator and down-regulator work together to keep the process or pathway working at appropriate levels (analogous to a thermostat controlling temperature); both are necessary to keep the process in proper balance. In diabetes, the body does not make adequate quantities of insulin; thus the release of glucose into the blood is not down-regulated, so that blood glucose levels are too high.

There are several pathways involved in the regulation of growth. The function of FGFR3 is to down-regulate chondrocyte proliferation and hypertrophy; as mentioned above this keeps the bone growth process in proper balance. In achondroplasia, there is a mutation before conception in the gene responsible for synthesis of FGFR3. Most mutations lead to a decrease in activity or loss of function of the protein. However, in the mutation of FGFR3 in achondroplasia, there is actually a ***gain-in-function*** of the protein. Although there is still some controversy over the nature of the gain-in function in achondroplasia, it appears that the down-regulation signal will be enhanced for the mutated FGFR3. Thus, the signal to slow down the growth process in long bones will be passed down the pathway to a greater extent, thus impeding bone growth and resulting in achondroplasia. One possibility is that the mutation makes the FGFR3 dimers more stable, and would persist longer in passing the down-regulation signal to its targets.

Another substance involved in the regulation of bone growth is C-type natriuretic peptide (CNP), which up-regulates chondrocyte proliferation, and thus has the opposite effect from FGFR3; i.e., CNP is called an antagonist of FGFR3. In fact, administration of CNP has been proposed as a therapy for achondroplasia. (See Figure 1.) It has been suggested that the permanent gain-in-function of FGFR3 is analogous to a hose watering a plant that is going full blast, and the over-watering harms the plant; the action of CNP is to put a kink in the hose so that the plant gets the proper amount of water and grows healthy as a result[3]. Since FGFR3 is the down-regulator and CNP is the antagonist up-regulator, it may also be helpful to think of FGFR3 as putting a kink in the hose and CNP as removing the kink, thereby restoring the proper amount of water. One may also think of FGFR3 having the emergency brake on while driving a car, and CNP removing the brake.

CNP is a polypeptide composed of 37 amino acids. Polypeptide drugs cannot be taken orally since they are rapidly broken down in the GI tract into their constituent amino acids by digestive

enzymes, so that the amino acids can be absorbed from the small intestine into the blood stream to make proteins that the body needs. Thus, they must normally be administered by an injection, either into a vein (intravenously), into muscle (intramuscularly), or under the skin (subcutaneously).

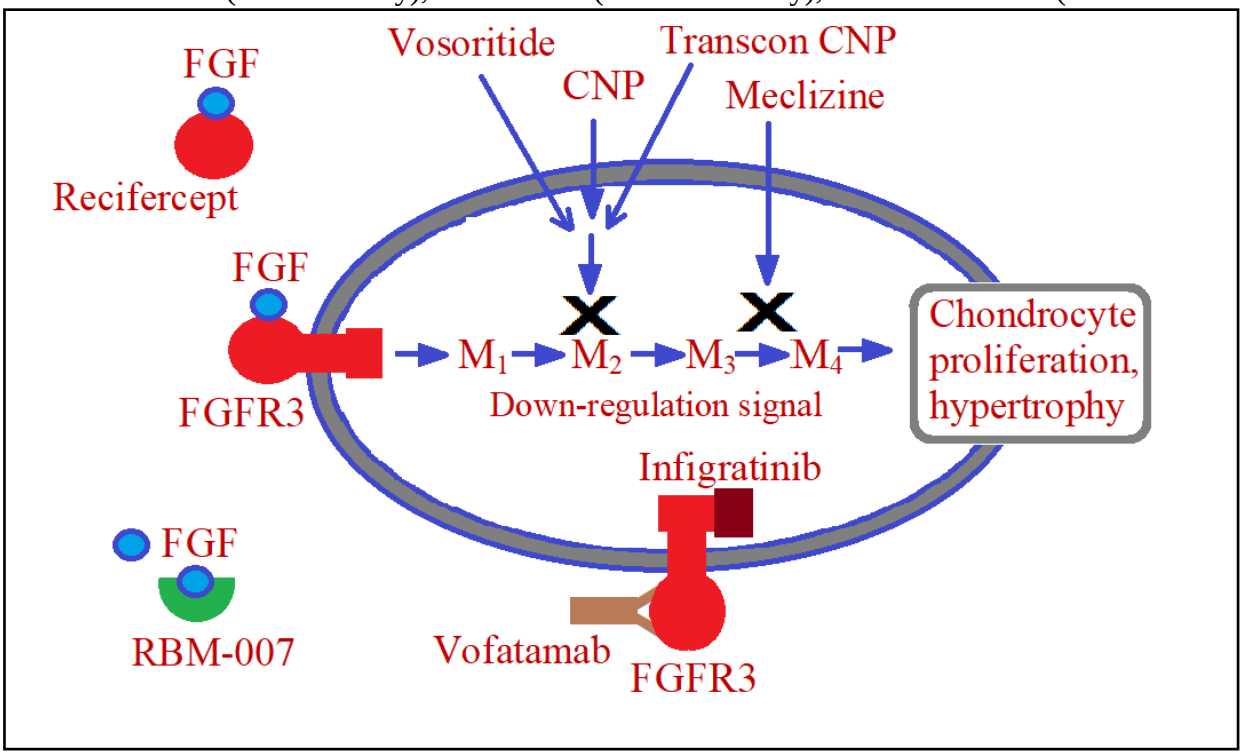

Figure 1: Role of FGFR3 in down-regulation of bone growth in the chondrocyte cell. M_1, M_2, M_3, and M_4 are the messengers that relay the down-regulation signal (their actual abbreviated names are Ras, Raf1, MEK, and ERK). CNP (as well as Vosoritide and TransCon CNP) act by blocking the signal at M_2. Meclizine acts by blocking the signal between M_3 and M_4. Recifercept is a "decoy" that prevents binding of FGF to FGFR3. RBM-007 is an aptamer oligonucleotide that binds to FGF and prevents its binding to FGFR3. Vofatamab is a monoclonal antibody against FGFR3, while Infigratinib is a tyrosine kinase inhibitor that prevents FGFR3 from initiating the down-regulation signal.

Another problem with CNP is that it is degraded rapidly in the bloodstream by enzymes called peptidases or proteases, with a half-life of about 3 minutes. (This means that after 3 minutes 50% is left, after 6 minutes, 25% remains, after 9 minutes 12.5%, etc.,) so after an hour almost all would be gone. In order to be effective, it would thus have to be continuously administered intravenously. While this has been shown to be effective in mice in which achondroplasia has been induced (by artificially altering their FGFR3), this would be unacceptable in growing children.

Biomarin Pharmaceutical Company (California) has developed a CNP analog named **Vosoritide**, previously called BMN-111. It has 2 extra amino acids tacked onto the beginning of the CNP, and thus has 39 amino acids, and is 4100 daltons in size. This modification partially protects it from the

peptidases that degrade native CNP, and extends its lifetime to hours rather than minutes, so that it can be injected once daily. It has been tested in humans since 2016, and was the first drug for achondroplasia to enter clinical trials.[4]

Bringing a new drug to market requires a fairly long process with several phases of clinical trials. After a drug candidate has shown promise as being safe and effective in animals, it can enter a Phase I clinical trial to test safety. This involves testing in a 10 – 80 healthy adult volunteers, starting with low doses gradually increasing to higher doses and monitoring side effects. If it is shown to be safe, Phase 2 trials are initiated in 100-300 patients to test efficacy, investigate dosage, and further assess safety. If these are successful, large Phase 3 trials involving up to several thousand patients can be undertaken, in order to show that the drug is safe and effective compared to current treatments. Phase 2 and Phase 3 trials generally must include some trials using placebo (a medication-free dosage) as a control, so that the patients do not know whether or not they are actually receiving the drug. If the Phase 3 trials confirm the drug's safety and efficacy, application for approval can be made to the appropriate government agency, e.g., the FDA in the United States, or the EMA in Europe. The entire process can take up to 7 years and require up to a billion dollars before the drug's marketer can see any profit.

Fortunately, for a drug candidate for a rare disease such as achondroplasia, the situation is somewhat different, and the drug is designated an "orphan" drug. Because profits from this type of drug would be limited, pharmaceutical companies are given tax and other financial incentives from the government to develop these drugs; the development timeline is shortened and not as many volunteers and patients are required in the clinical trials.

Phase 2 trials have been completed for Vosoritide, and Phase 3 trials have been completed in children of ages 5-14; the results have been promising and "highly persuasive" according to the company. In November 2020, Biomarin announced that it had submitted an application to the FDA for approval of Vosoritide, and it is in review. If approved, Vosoritide could be available for treatment of children with achondroplasia as soon as August 2021; approval for children younger than 5 could take longer, since testing in younger children is still in progress.

Another CNP analog, currently designated **TransCon CNP**, is being developed by Ascendis Pharma (Copenhagen, Denmark). While Vosoritide must be administered once daily, TransCon CNP has a once weekly injection. It accomplishes this by chemically linking CNP to an inert polymer, polyethylene glycol (PEG). (If you have taken the laxative Miralax, you have ingested fairly large doses of PEG). The PEG protects the CNP from degradation by peptidases, and the proprietary linkages are designed to release CNP gradually over the period of a week, so that it is constantly available for absorption by the chondrocytes. The PEG linkage concept has been fairly

successful, and there have been over a dozen polypeptide drugs using this slow-release technology marketed by a number of companies since the 1990's. Phase 1 clinical trials of TransCon CNP were begun in 2018, and Phase 2 trials were initiated earlier in 2020.

Ascendis has also developed a product, TransCon hGH, for treatment of growth hormone deficiency in children, another form of dwarfism. TransCon hGH is in Phase 3 trials, and applications for approval have been submitted to the FDA and EMA. Administration of human growth hormone (hGH) was proposed as a treatment of achondroplasia as well, but it has not been shown to be effective, since it involves a different pathway for control of bone growth. Nutropin Depot, a once-weekly subcutaneous injection form of hGH was approved in 1999. This product was hGH formulated with a polymer (polylactide-glycolide, PLG), which released the drug slowly over the week. Although this product was discontinued several years ago, other depot injections of drugs with PLG have been developed and approved, and the technology may have potential for development of a once-weekly injection of a CNP analog. Another approach for delivery of polypeptides, including hGH, are microneedle systems. These are similar in appearance to transdermal patches (like the nicotine patches), but have an array of very small needles that penetrate the top layer of skin which normally forms a barrier to drug penetration. The microneedles allow the drug to enter the underlying tissues and reach the bloodstream, but are not long enough to reach the receptors that cause pain. Microneedle systems of hGH have been examined in preclinical studies but not clinical trials, and may have potential for delivery of a CNP analog.

As mentioned above, the ligand for FGFR3 is FGF; normally, binding of FGF to its receptor is what triggers the down-regulation signal. There was some evidence from that the mutated form of FGFR3 in achondroplasia has limited activity in sending the down-regulation signal even in the absence of its ligand. However, it now appears more likely that binding the ligand enhances the signal significantly even for the mutated receptor. Thus, inhibition of binding of FGF to FGFR3 will retard the down-regulation signal and restore normal chondrocyte activity and bone growth. This is the rationale behind **Recifercept** (formerly known as TA-46). This drug candidate was developed by a Swiss Company, Therachon, which was acquired by the pharmaceutical giant Pfizer. Recifercept is a genetically engineered polypeptide, probably around 10,000-20,000 daltons. It is a soluble form of FGFR3, i.e., it is the portion of the receptor that extends in the exterior of the cell rather than the part that is buried in the cell membrane or in the interior of the cell (see Figure 1). Recifercept can thus serve as a "decoy" receptor, so that FGF binds to it rather than binding to FGFR3. Hopefully it will be effective as a once-weekly injection, but may require daily injection. Phase 1 trials were completed in 2019. Phase 2 trials are underway, which will help determine the effective dosage and frequency of injection.

A similar approach is employed by **RBM-007**, an "aptamer" being developed by Ribomic, Inc. (Tokyo, Japan). An aptamer is a molecule specifically designed to bind to another target molecule. RBM-007 is a short polymer of 37 nucleotides, which are the building blocks of DNA and its smaller cousin, RNA, which is involved in protein synthesis based on the genetic code. (DNA, or DeoxyriboNucleic Acid, is a polydeoxyribonucleotide; RNA, or RiboNucleic Acid is a polyribonucleotide; and RBM-007 is an oligoribonucleotide, oligo- being Greek for "few"). RBM-007 is specific for FGF2 (one of the types of FGF), and so has been proposed to inhibit the binding of FGF to FGFR3, in a similar manner to the "decoy" action of Recifercept. RBM-007 was approved for Phase I clinical studies in June 2020 in Japan, and is also being investigated for treatment of macular degeneration.

There are several more approaches of drug therapy for achondroplasia, but which have not been tested clinically for it. For example, **Vofatamab** (B-701) is a monoclonal antibody against FGFR3 developed by Rainier Therapeutics (California). An antibody is a protein manufactured by the body in response to a potentially harmful foreign molecule (the "antigen"). The antibody will bind to the antigen and render it harmless; when you get an influenza vaccination, for example, your body manufactures antibodies against the influenza virus so that you are protected against subsequent infections. A monoclonal antibody (MAb) is a genetically engineered antibody specific for a given antigen; MAb's have a variety of diagnostic and therapeutic applications. Vofatamab was originally developed for cancer therapy. FGFR3 and other FGF receptors have been found to be altered in a number of cancers (especially bladder cancer). Hence, antibodies against FGFR3 were proposed as a therapy for bladder cancer, and Vofatamab is currently in Phase 2 clinical trials for bladder cancer. A MAb against FGFR3 would also have potential for treatment of achondroplasia, since its binding to FGFR3 would prevent binding of FGF to FGFR3. Vofatamab was being evaluated in preclinical (i.e., laboratory and animal) studies for achondroplasia, but these have apparently been terminated and not advanced to the clinic.

All of the drugs discussed so far are biological polymers that cannot be taken orally and must be administered by injection. There are a few non-peptide "small" molecules (i.e., less than 1000 daltons) that have been considered for treatment of achondroplasia. One approach is **Infigratinib** (BGJ398), being developed by QED Therapeutics (California), a subsidiary of BridgeBio Pharma. FGFR3, like many other receptors, has an enzyme as part of its structure, and binding of the receptor's ligand (FGF in this case) will trigger the enzyme reaction which then triggers the signal for the pathway. FGFR3 is a tyrosine kinase enzyme; a kinase always transfers a phosphate group (which consists of one phosphorus atom and 4 oxygens) from one molecule to another, while tyrosine is an amino acid that is part of the receptor structure that receives the phosphate group. If

the receptor's tyrosine kinase activity can be inhibited, then the down-regulation signal from FGFR3 can be reduced. Infigratinib is a tyrosine kinase inhibitor that blocks FGFR3; it is 560 daltons and orally available. It is currently being examined in Phase 2 and Phase 3 clinical trials for bladder, bile duct, and urinary tract cancer. Phase 2 trials for achondroplasia have been initiated in 2020.

It may be possible to "re-purpose" some "small" molecule drugs currently marketed for other indications for treatment of achondroplasia. An example is **Meclizine** (brand names Bonine and Antivert), an antihistamine used for motion sickness. It is 391 daltons in size and is orally available. Animal studies have shown that it can reduce the down-regulation signal from FGFR3 by inhibiting the step between the third and fourth messengers (See Figure 1). It has been reported that Meclizine was entering Phase 1 and Phase 2 clinical trials for achondroplasia in Japan starting in 2018.

Table 1 summarizes the drugs currently being investigated for achondroplasia. A number of these drugs are quite promising for treatment of achondroplasia, and there may be even more that will be developed or re-purposed later. Thus, the prospects for widely available effective and safe drugs for treatment of achondroplasia in the very near future appear excellent.

Table 1: Summary of drugs investigated for acondroplasia

Name	Company	Description	Status
Vosoritide (BMN-111)	Biomarin (California)	Polypeptide, CNP analog, 4100 daltons; daily injection	Phase 3 trials; approval possible by August 2021
TransCon CNP	Ascendis (Denmark)	CNP analog conjugated to PEG; weekly injection	Phase 2 trials.
Recifercept (TA-46)	Therachon / Pfizer (Switzerland/US)	Polypeptide, 10,000-20,000 daltons; "decoy" soluble FGFR3; weekly injection	Phase 2 trials
RBM-007	Ribomic (Japan)	Oligoribonucleotide aptamer binding to FGF; ca. 12,000 daltons; injection	Phase I trials; also for macular degeneration
Vofatamab (B-701)	Rainier Therapeutics (California)	Monoclonal antibody for FGFR3; ca. 145,000 daltons; injection	Preclinical studies for achondroplasia; clinical trials for bladder cancer
Infigratinib (BGJ398)	QED Therapeutics (California)	Tyrosine kinase inhibitor, 560 daltons; oral administration	Phase 1 trials for achondroplasia; Phase 3 clinical trials for cancer
Meclizine	-	Marketed for motion sickness; 391 daltons; oral administration	Phase 2 clinical trials for achondroplasia in Japan

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1. At the risk of over-simplification, some details have been omitted. For more information, the reader can refer to Wikipedia articles suggested below, as well as the references to technical journal articles listed above:

achondroplasia
Fibroblast growth factor receptor 3
protein cartilage
clinical trial
epiphyseal plate
vosoritide
Receptor (biochemistry)

2. The author is a retired pharmaceutical chemist with a career in the pharmaceutical industry as well as in teaching chemistry in several universities. You can learn more about me in the following link (now outdated as I am now retired and living in Wisconsin):
https://www.routledge.com/authors/i333-john-cannon?utm_source=cjaffiliates&utm_medium=referral&cjevent=8664883a279211eb809501e50a240612

3. This analogy was referred to by Dr. Lynda Polgreen (UCLA) in a webinar sponsored by Biomarin and the Magic Foundation on Vosoritide, June 4, 2020.

4. The reader is encouraged to consult the websites of the various companies mentioned:
www.biomarin.com
https://ascendispharma.com
https://www.ribomic.com/eng/
https://www.qedtx.com/

5. The references listed are articles in technical journals; all are open access, and the links to the full text are given.