Recent Patents on Achondroplasia: Latest Research Development

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Received: May 16, 2012; Accepted: June 13, 2012; Revised: June 22, 2012

Abstract: Achondroplasia (ACH) is the most common pathology of the group of disorders called chondrodysplasias. It is resulting from a mutation in the Fibroblast Growth Factor Receptor type 3 (FGFR3) gene, one of the key regulators of endochondral ossification and it is characterized by different degrees of short-limb dwarfism. The mutation of the FGFR3 receptor, leads to an over-activated receptor, producing important changes at the intracellular signaling level. The most important changes are an accelerated rate of chondrocyte maturation leading to an altered extracellular matrix and an important modification in the biochemical behavior of the cells. Subsequently, terminal hypertrophic cells undergo accelerated apoptosis, which obstructs proper bone development and leads to the observed short stature phenotype. Even though, the molecular mechanisms causing the disease are known since 1994, there is no effective solution found yet, due to difficulties in the drug delivery phase but mainly because molecular events by which FGFR3 mediates its signaling are not completely understood. In this article, we review and discuss the latest patents in this field, with their corresponding literature, regarding future possible drugs for the treatment of this group of rare diseases. Some of these patents are concerning potential medicines that entered or are about to enter clinical trials and possibly will be succeed as future drug therapies.

Keywords: Achondroplasia, chondrocytes, chondrodystrophies, FGFR, hypochondroplasia, signaling pathways, thanatophoric dysplasia, tyrosine kinase.

INTRODUCTION

Achondroplasia (ACH) is a disorder in which the gene encoding for the fibroblast growth factor receptor 3 (FGFR3) is mutated [1]. 97% of the patients who have the mutation, present a single glycine (G) to arginine (R) substitution at the position 380 (G380R) [2]. FGFR3 is a tyrosine kinase receptor which is activated by the binding of fibroblast growth factors (FGF) [3]. This activation induces receptor dimerization and a subsequent receptor autophosphorylation in the intracellular domain, triggering a downstream activation of intracellular signaling. In achondroplasia, the activated mutant FGFR3 is resistant to down-regulation and internalization, which means that instead of being degraded after its activation, the receptor returns to the plasma membrane [4, 5]. Consequently, it remains constitutively active and overexpressed in the cell membrane, causing an excessive intracellular signaling that produces critical alterations to the biochemistry of cartilage cells, the chondrocytes. In particular, chondrocyte proliferation, differentiation and the cartilage matrix production are disturbed, leading to a remarkably foreshortened epiphyseal growth plate cartilage [6]. Processes altered in achondroplasia such as chondrocyte proliferation or extracellular matrix homeostasis can be influenced by conditions such as modifications in cell volume and ionic currents. In this sense, it has been shown that the modification of normal chondrocyte volume or ionic composition can affect the turnover (synthesis and degradation) of the extracellular matrix [7]. Moreover, a relationship between ion channel activity and proliferative behavior has been suggested in normal chondrocytes [8, 9]. Despite these findings, little is known about changes in cell volume or intracellular ion concentrations in achondroplasic chondrocytes.

Bone development by endochondral ossification includes the initial formation of a cartilage template and its subsequent replacement by mineralized bone. Longitudinal bone growth is driven by regulated proliferation and differentiation of chondrocytes in the growth-plate cartilage [10]. First slow proliferating chondrocytes differentiating to flat columnar cells that form orderly columns and show a high proliferation rate. Columnar chondrocytes stop proliferating and differentiate into post-mitotic hypertrophic cells, which are subsequently replaced by bone and bone marrow [11]. Chondrocyte proliferation, differentiation and the replacement of chondrocytes by bone are tightly controlled to guarantee proper bone development [12].

Achondroplasia may be inherited as an autosomal dominant trait, which means that if a child gets the defective gene from one parent, the child will suffer the disorder. If one parent has achondroplasia, the infant has a 50% chance of inheriting the disorder. If both parents have the condition, the infant's chances of being affected increase to 75% [13]. However, most cases appear as spontaneous mutations. This means that two parents without achondroplasia may give birth to a baby with the condition. Dwarfism can affect any baby born anytime; the prevalence is from about 1 in10,000 births to 1 in 30,000 [14]. Interestingly, homozygous achondroplasia is a neonatal lethal condition [15], while the individuals affected with heterozygous achondroplasia, express

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both wild-type and mutant FGFR3 [16]. The mutated allele has always paternal origin [17].

In addition to being short, the individual may have moderate to severe orthopedic difficulties. Over time, gravity pulls on the skeleton of the achondroplasic person and progressively leads to a loss of mobility. In the literature are mentioned neurological complications such as cervicomedullary compression which can cause pain, ataxia, incontinence, apnea, and respiratory arrest. Also, nerve root compression in the neural foramina could create problems in the limbs [18]. Additionally, there were reported respiratory problems, restrictive obstructive lung disease [19] spinal stenosis, otitis media, and tibial bowing [20]. In the most severe cases, children may lose the ability to walk, and other functions are profoundly affected. In some cases, without early medical intervention, the child may pass away [21]. The orthopedic problems of achondroplasia require complex reconstructive surgery and extensive physical therapy [22]. The surgery on achondroplasic patients involves bone grafts, fusions, steel screws, pins and plates, then months of physical therapy to regain even the most basic function like walking. Unlike an accident victim, preventive treatment of the skeletal structure can lessen pain and disability that otherwise strikes achondroplasic persons [23].

PHYSIOLOGY OF THE SKELETAL DISORDERS

Amongst other signaling molecules, fibroblast growth factors (FGFs) play an important role in controlling proliferation and differentiation of chondrocytes. FGFs can activate several membrane receptors termed FGF receptors (FGFR) 1-4 and one soluble receptor termed FGFR [24]. Different mutations in FGFR1-3 have been shown to result in congenital skeletal disorders with FGFR3 being of high importance for regulating the development of the endochondral skeleton [25]. Lately, it was discovered that fibroblast growth factors 1, 2, 17, and 19 are the predominant FGF ligands expressed in human fetal growth plate cartilage [26]. The main skeletal disorders due to FGFR3 mutations are hypochondroplasia (HCH), achondroplasia (ACH) and thanatophoric dysplasia (TD). Hypochondroplasia is a skeletal dysplasia inherited in an autosomal dominant manner due, in most cases, to mutations in the FGFR3. It is similar to achondroplasia but less severe and rarely recognized before 2 years of age. It is characterized by short stature, short limbs and lumbar lordosis [27]. Thanatophoric dysplasia is characterized by severe limb shortening (micromelia), bowing of limbs, narrow thorax and protuberant abdomen. As the name thanatophoric or "death-bearing" suggests, the condition is frequently lethal in-utero or shortly after deliver. The cause of death is respiratory failure that occurs soon after birth [28].

The gene for the FGFR3 is located in the short arm of chromosome 4 (4p16.3). Studies carried out in different populations demonstrate the existence of a recurrent mutation (Gly380Arg) in the transmembrane domain of the receptor leading to a receptor constitutively activated [29]. This mutation has been observed in 97% of the cases. Thanatophoric dysplasia I, results from either a stop codon mutation or missense mutations in the extracellular domain of the gene. Interestingly, all missense mutations found so far created cysteine residues. In 1996, Rousseau [30] reported missense mutations (Tyr373Cys and Gly370Cys) creating cysteine residues giving further support to the view that newly created cysteine residues in the extracellular domain of the protein appear to play a key role in the severity of the disease.

The FGFR3 receptor belongs to the family of the receptor tyrosine kinases. An activation of the receptor by FGFs triggers the dimerization and autophosphorylation of Tyr residues [31]. These phosphorylated Tyr residues become biologically active binding sites for proteins and effectors that propagate FGFR3 signals in the cells [32]. How the different mutations of FGFR3 lead to the activation of the receptors has been intensively investigated and various mechanisms have been proposed. In the case of the G380R which is the most common cause of achondroplasia, the mutation seems to stabilize the dimer, necessary for the receptor activation, even in the absence of the ligand [33, 34]. On the other hand, the thanatophoric dysplasia mutations introducing Cys residues in the extracellular domain, facilitate the formation of disulphide bonds leading to the dimerization of the receptor [35, 36]. Other mutations such as K650M alter the receptor conformation producing a constitutive activation of the kinase activity [37, 38]. Interestingly, but perhaps not surprisingly, the mutations responsible for TD activate the FGFR3 receptor more strongly than the mutations causing achondroplasia. Furthermore, it has been suggested that the mutant FGFR3 uncouples ligand-mediated receptor activation by preventing lysosomal and peroxisomal degradation of the receptor, thereby inhibiting its inactivation [4, 5, 39].

The effects of FGFs are mediated by multiple intracellular signaling pathways, two of which are known to operate in chondrocytes: signal transducer and activator of transcription (STAT) and mitogen-activated protein kinase (MAPK) pathways [40, 41]. The STAT1 pathway has been shown to inhibit chondrocyte proliferation [42]. Activation of STAT1 blocks cell cycle progression through an increase in the expression of p21 and simultaneously accelerates the differentiation of chondrocytes towards the hypertrophic stage [43, 44]. Other authors reported that constitutive activation of MEK1 in chondrocytes, causes STAT1 independent achondroplasia like dwarfism and rescues the FGFR3 deficient mouse phenotype [40]. On the other hand, overexpression of C natriuretic peptide (CNP) in cartilage partially rescues achondroplasia through activation / inhibition of the ERK pathway.

In Thanatophoric dysplasia (TD) type I mutations of FGFR3 are found in different positions mostly Arg248Cys and Tyr373Cys. The resulting stronger receptor activation leads to the more severe bone phenotypes and lethality which is characteristic for TD patients[45].

TRANSGENIC MICE AND CELL LINES

Several mouse models mimicking the human achondroplasia phenotype have been created by expressing mutated forms of *Fgfr3* in the developing cartilage [46, 47]. These mice display a severe shortening of the skeletal elements due to reduced regions of proliferating and hypertrophic chondrocytes. In contrast, mice carrying a targeted deletion of *Fgfr3* are characterized by increased regions of proliferating

and hypertrophic chondrocytes [48, 49]. The transgenic mice with the mutated *Fgfr3* gene are the best models where achondroplasia can be studied [50, 51]. A mouse for *Fgfr* associated chondrodysplasia, generated by Yayon of the Hebrew University in Rehovot and its claimed with the patent US6265632 [52], presents the main characteristics of achondroplasia such as small size, facial hypoplasia, and severely reduced bone growth plates. The animal has a genetically modified fibroblast growth factor receptor gene integrated in at least one locus in its genome. The genetically modified *Fgfr* gene encodes a modified fibroblast growth factor receptor protein, which when expressed, results in an integral membrane protein having a gain of function. Both the animal and the chondrosarcoma cells (RCJ cells) stably transfected with the normal human FGFR3, and with the G380R mutation is an attractive system where the biology of the FGFR3 can be investigated [4, 53].

The group of Legeai-Mallet of the French Hospital "Necker-Enfants Malades" has generated a mouse line corresponding to the human TD Y373C mutation by introducing an Y367C point mutation into the mouse *Fgfr3* gene. This model displays a severe chondrodysplasia phenotype and its main characteristics (e.g. reduced length of long bone, narrow trunk, short ribs and macrocephaly) [54]. Additionally, the group generated immortalized human chondrocyte culture models to study the regulation of chondrocyte functions [55] and *Fgfr3* constructions (G380R, Y373C, K650 N, M, E) [56].

Another type of mouse model used is the mouse generation with strain name "B6; 129S-Fgfr3tm1Dor/J" of Dr. Ornitz [48]. It is a knockout and transgenic mouse expressing activated FGFR3 in bone growth plates. The mouse is a mutant stock with *Fgfr3* targeted mutation. Severe skeletal defects (e.g. kyphosis, scoliosis, crooked tails, curvature and overgrowth of long bones) are evident. Inner ear defects (lack of pillar cell differentiation and tunnel of Corti formation) resulting in profound deafness are also observed. The mouse was produced by targeting a vector containing neomycin resistance [57].

Recently, the group of Dr. Chen in Daping Hospital in China, generated *Fgfr3*conditional knockout mice in which *loxp* sites flank exons 9-10 in the *Fgfr3* allele. This model was created for better understanding of the roles of *Fgfr3* in different tissues at different stages of development and in pathological conditions. They also demonstrated that Cre-mediated recombination using Col2a1-Cre, a Cre line mainly expressed in chondrocyte during bone development, results in specific deletion of the gene in tissues containing cartilage. Evidently, this animal model will be useful to study several roles of FGFR3 in different tissues at different ages [58].

EXISTING THERAPIES

For the time being, there is no specific treatment for achondroplasia. Related abnormalities, including spinal stenosis and spinal cord compression, should be treated when they cause problems. The main course of therapy so far is growth hormone (GH) replacement therapy, when there is lack of growth hormone in the body. GH treatment of children with achondroplasia improves height during 4 years of therapy without adverse effect on trunk-leg disproportion [59]. The short-term effect is comparable to that reported in Turner and Noonan syndrome and in idiopathic short stature [60]. In 1997, the Company Novo Nordisk in Japan started clinical trials (Clinical Trials Identifier NCT01516229) for the drug *somatropin*, which is a Synthetic Growth Hormone (HGH). The aim of these trials is to assess the incidence rate of adverse drug reactions (ADRs) when using *somatropin* (Norditropin®) for achondroplasia's treatment, without epiphyseal line closure, under normal clinical practice conditions [61].

Another solution to increase patient's stature is the surgery for the lengthening of the limbs [62]. The whole procedure is controversial, but despite frequent complications, it is described that bilateral lower limb lengthening increases patients' quality of life. On the other hand, lengthening is not always possible and is a reasonable option only for selected patients. The contraindications for surgery, were the indisposition to have such a complicated surgery, the patients' medical history and the patients' susceptibility to intramedullary infection [63]. The operations are complex and prolonged, and require a special psychological approach directed to both parents and the patients. Complications are quite common and they are related to stretching of non-skeletal tissues, including nerves and blood vessels, for which patients have to be well prepared before starting the procedures. The most severe of these complications could include failure of length gain, reducible joint subluxation, transient or permanent nerve injury, angulation of >15º in femur, >10º in tibia, osteomyelitis/septic arthritis and irreversible psychological disturbances [64].

CURRENT SCIENTIFIC RESEARCH

In the last decade, a number of new approaches have emerged, due to the advance in the knowledge of the pathogenesis of achondroplasia and related disorders. The research is concentrated in suppressing FGFR3 signals [65]. Among the possible treatments are included the chemical inhibition of receptor signaling, the antibody blockade of receptor activation, and the alteration of pathways that modulate the downstream propagation of FGFR3 signals [66]. The First-generation therapies directly targeting FGFR3, such as kinase inhibitors and neutralizing antibodies are currently under heavy development [67]. The most recent and promising development is the counteracting of signal transduction pathways downstream of *FGFR3* with the discovery that administration of C-type natriuretic peptide to achondroplasic mice improves their clinical phenotype [67]. Currently, the 4 main directions for developing future therapies are the following [68]:

- Selective inhibition of the FGFR3 tyrosine kinase activity. Drugs like imatinib used in cancer chemotherapy.
- C-type natriuretic peptide (CNP)-Overexpression of CNP in mutant mice chondrocytes rescues achondroplasia through a MAPK-dependent pathway. Counteract the overactive *FGFR3* effects on endochondral bone formation.
- Blocking antibodies in order to interfere with binding of FGF ligands to *FGFR3*.These antibodies are selective

for *FGFR3* and are activating the CNP-NPR-B-cGMP pathway, which antagonizes MAPK-ERK/p38 signals downstream of *FGFR3*.

- Gene Silencing and other genetic therapies. There are used regulatory RNAs capable of interfering in the protein production, such as micro RNAs (miRNA) or small interfering RNAs (siRNA).

INHIBITING TYROSINE KINASE TRANSMEMBRANE RECEPTORS

FGFR3 is a key regulator of growth and differentiation, and its aberrant activation is not only related to achondroplasia but to a number of genetic diseases including cancer [69]. The whole effort to impede the cellular and higher level physiological disturbances that interfere with bone growth, is concentrated mainly towards the excess in tyrosine kinase activity. Jonquoy *et al.* confirmed the specific role of *FGFR3* in the cell cycle and chondrocyte differentiation and the efficacy of tyrosine kinase inhibitors for treatment of *FGFR3*. Novel FGFR3 kinase inhibitors have been developed and were successfully used in cell and organ culture experiments [70, 71]. In the patent US8076354 [72], Astex Therapeutics Ltd. is using a bicyclic heterocyclic derivative as tyrosine kinase inhibitor. Additionally in US20100234394[73], Novartis Inc. is using substituted benzimidazole to perform the role of tyrosine kinase inhibitors. The composition is effective to inhibit the activity of at least one serine/threonine kinase or a tyrosine kinase receptor. The new compounds and compositions may be used either alone or in combination with at least one additional agent for the treatment of a serine/threonine kinase or receptor tyrosine kinase mediated disorder. In the patent US20100105667 [74] of Novartis Inc., it was used quinoxaline and quinoline-carboxamide derivatives for inhibiting the tyrosine kinase activity. These compounds can inhibit the platelet-derived growth factor or p56.sup.lck. In another patent US20080312248 [75] of the same company, it was used pyrimidinyl aryl urea derivatives as tyrosine kinase inhibitors. These compounds belong to the heteroaryl aryl urea class and these are showing inhibition of a number of protein tyrosine kinases, especially of FGFR. The same company in the patent US20110045511 [76] is describing a method of monitoring the modulation of the kinase activity of FGFR. The invention relates to a method of *in vitro* diagnostics, using a compound, consisting of fibroblast growth factor 23 protein (FGF23), inorganic phosphorus (P), a product of inorganic phosphorus and total calcium (PxtCa), osteopontin (OPN) and parathyroid hormone (PTH) as biomarker. The biomarker can be used to monitor the modulation of FGFR kinase activity, in particular its inhibition, and/or the occurrence of secondary effects of its inhibition. The patent US7872016 [77] of Yale University is not using the CNP pathway, but is administering an inhibitor of the FGFR2c-FRS2 signaling. The agent inhibits signaling by antagonizing FGFR2c-FRS2 interaction. It is inhibiting the expression and/or subcellular localization of wild-type or mutant FGFR2c and/or FRS2–and the kinase activity of FGFR2c. Excessive FGFR signaling through FRS2 is responsible for achondroplasia, SADDAN (severe achondroplasia with developmental delay and acanthosis nigricans), thanatophoric dysplasia type I (TDI), thanatophoric dysplasia type II (TDII), or hypochondroplasia (HCH) among other genetic disorders [78, 79].

ALTERATION OF SIGNALING PATHWAYS MODULATING THE DOWNSTREAM PROPAGATION OF FGFR3 SIGNALS

Yasoda *et al*. [80] using transgenic and knockout mice described that C-type natriuretic peptide (CNP) is a potent stimulator of endochondral bone growth. Natriuretic peptides (NPs) are peptide hormones that exert their biological actions by binding to three types of cell surface natriuretic peptide receptors (NPRs). Mutations in the gene NPR2 have been shown to cause acromesomelic dysplasia-type Maroteaux (AMDM), an autosomal recessive skeletal disproportionate dwarfism disorder in humans[81]. Yasoda demonstrated the efficacy and safety of the administration of CNP-22 for impaired endochondral bone growth in achondroplasic mice. These results suggest that systemic administration of CNP or CNP analogs provide a novel therapeutic strategy for human skeletal dysplasias, including achondroplasia [82]. Furthermore, Yasoda [83, 84] proposed the therapeutic use of CNP or CNP analogs activating the NPR-B signaling pathway to counter excessive FGFR3 signals. He started to translate the stimulatory effect of CNP on endochondral bone growth into a therapy and demonstrated that targeted overexpression of CNP in cartilage or systemic administration of CNP reverses the impaired skeletal growth of achondroplasic mice. Furthermore, the loss of function mutations in the gene coding for guanylylcyclase-B (GC-B), the specific receptor for CNP, has been proved to cause impaired skeletal growth in humans[85]. A variation of this approach involves therapeutically targeting NPR-C, another natriuretic peptide receptor that binds to CNP. NPR-C, which is present on hypertrophic chondrocytes in the growth plate, lacks the ability to increase intracellular cGMP and has been proposed to function as a clearance receptor to down-regulate the effects of natriuretic peptides [86]. Theoretically, blocking NPR-C would lead to an increase in CNP available to bind to natriuretic peptide B-type (NPR-B), which would be expected to antagonize FGFR3 signals in the growth plate. The full description of the guanylylcyclase-B (GC-B) activation using CNP (see Fig. (**1**)) was patented with the patent US6743425 [87] and the follow on EP1743653 [88].

Similarly, Prochon Biotech Ltd. in the patent US7276481 [89] is describing a method for the treatment of skeletal dysplasias, using as active ingredient at least one natriuretic peptide. In the description, it is shown that the natriuretic factors may be effective for bone elongation *in situ*ations of abnormal bone growth and the effects may be further enhanced by prolonging its residence time or action at the target site.

Furthermore, the company Biomarin Pharmatheutical Inc. filled the patent US20100297021 [90] about the use of more variants of C-Type natriuretic peptide (CNP) to counter the over activation of FGFR3. The method is associated to bone-related disorders, such as achondroplasia and other skeletal dysplasias. The company has developed a stabilized version of C-type natriuretic peptide called BMN-111, a positive regulator of bone growth. BMN-111 binds to its own receptor which initiates intracellular signals inhibiting the overactive FGFR3. There are used CNP variants having

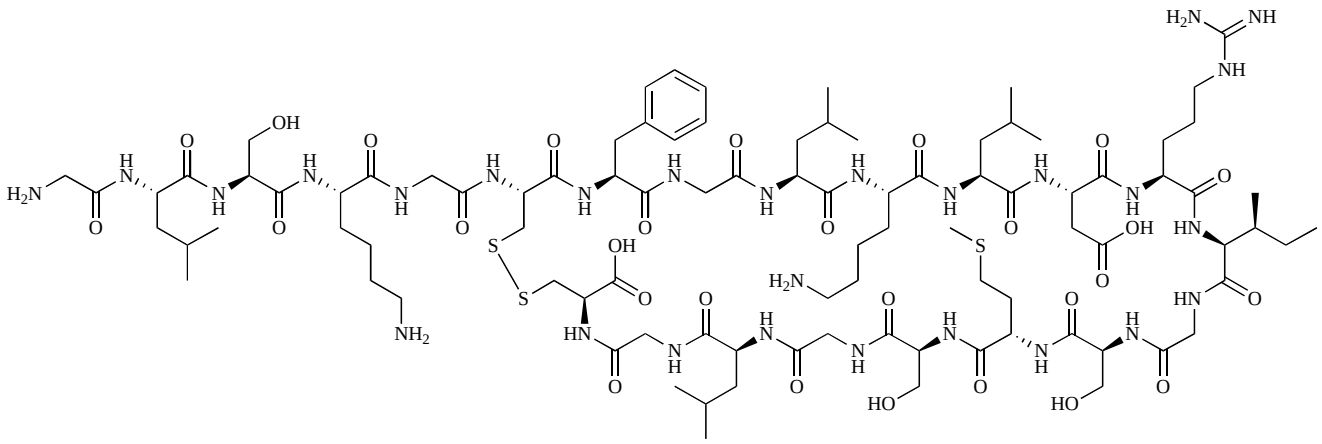

Fig. (1). Chemical structure of CNP.

increased serum half-life, e.g., as a result of reduced ability to bind to neutral end peptidase (NEP), greater resistance to proteolysis by NEP and/or reduced affinity to the clearance natriuretic peptide receptor C(NPR-C), while retaining the functionality of CNP.

To improve the delivery of the CNP variants to the cartilage, the agent has to be attached (e.g., at the N terminus and/or C terminus) to bone or cartilage targeting moieties. The bone or cartilage targeting moieties include bisphosphonates; hydroxyapatite; glucosamine; collagen (e.g., collagen type X); polyAsp; polyGlu; and amino acid sequences derived from bone-targeting domains of bone proteins such as, e.g., osteocrin, osteopontin, osteocalcin, and sialoprotein.

The BMN-111 can be administered to the patients in various ways such as by injection intravenously, intra-arterially or intraperitoneally.

Daily subcutaneous injections of BMN-111 in mouse models of the disease have demonstrated the ability of this drug to correct the dwarf phenotype. The drug was tested successfully up to the level of monkeys. It is scheduled to start Phase 1 clinical trials in 2012. The future medicine for the moment is called "BMN-111 CNP for Achondroplasia". According to the company, the BMN-111 has demonstrated benefits in moderately and severely affected animal models and has been shown to promote bone growth even in normal animals. The company expects to start the Phase 2 study in pediatric subjects in the fourth quarter of 2012 or the first quarter of 2013 [91].

Similarly, the company Alcon Research Ltd. with the patent US20110112038 [92] is describing the use of novel compounds having NPR-B agonistic action, activating the NPR-B–receptor. The used compounds are linear peptides containing 8-13 conventional or non-conventional L- or D-amino acid residues connected to one another via peptide bonds. The composition includes natriuretic peptide mimics or similar. In the patent is described, the treating of C-type natriuretic peptide (CNP)-mediated disorders, including achondroplasia using pharmaceutical compositions including the mentioned NPR-B agonist. Additionally, in the patent US2011039277 [93] is described a stimulation of bone growth using Vessel Dilator (VD). VD is a natriuretic peptide synthesized by the atrial natriuretic peptide gene whose biologic half-life is 12 times longer than CNP. VD's biologic effects on proliferating cells are mediated via inhibiting MEK 1/2 and ERK 1/2 kinases using cyclic GMP. According to the inventors, VD exhibited therapeutic effect for use in human achondroplasia, short stature and osteoporosis by stimulating osteoblast proliferation [94].

Another FGFR3 inhibitor proposed is the agent NF449 and the patent filled is the US20110166223 [95]. NF449 is a suramin analog and an antagonist of FGFR3 tyrosine kinase [96]. Using a molecular library screening approach, the inventors identified NF449 based on its inhibitory activity towards FGFR3 signaling. NF449 rescued both major phenotypes of pathological FGFR3 signaling. As evidenced NF449-mediated reversal of ERK MAP kinase activation and transcript accumulation of CCL3 and CCL4 chemokines, both of which are induced by FGFR3 activation. In cell-free kinase assays utilizing STAT1 as a substrate, NF449 inhibited the tyrosine kinase activity of recombinant FGFR3 [97].

Novartis Ltd. presented in the patent US20100209418 [98], a B-Raf kinase inhibitor. B-Raf is a member of the Raf kinase family of serine/threonine-specific protein kinases. The inhibitor proposed is the protein 1-methyl-5-(2-(5-(trifluoromethyl)-1H-imidazol-2-yl)pyridin-4-yloxy)-N-(4-(trifluoromethyl)phenyl)-1H-benzo[d]imidazol-2-amine(see Fig. (**2**)). This protein plays a role in regulating the MAP kinase / ERKs signaling pathway, which affects cell division, differentiation, and secretion.

In the patent US20100218266 [99], the proposed inhibitor is the Snail1 protein. The Snail gene is involved in transducing the signaling mediated by FGFR3. Snail1 acts downstream of FGFR3 signaling in chondrocytes, regulating both STAT and MAPK pathways [100]. The de-regulated Snail activity in embryonic cartilage inducing achondroplasia and the presence of Snail in stages where it is normally repressed could be considered to be a marker of the achondroplasia phenotype of the type associated with FGFR3 [101].

Another method proposed with the invention ES2286933 [102] is the application of the pyridoxal and its analogs. The application of the pyridoxal and its derivatives is able to reduce the oxidative level of the proteins ERK1/2 that is abnormally high in achondroplasia. The pyridoxal analogs used

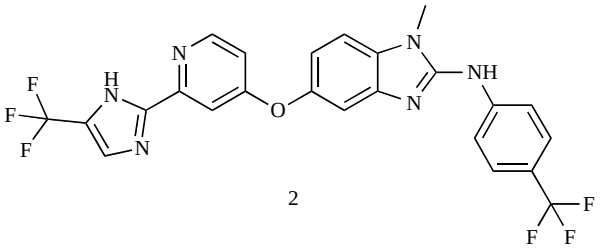

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Fig. (2). Chemical structure ofimidazol-2-amine.

are the phosphate 6 (2-naphthylazo 6- nitro-4, 8disulfonate), (PPNDS) (see Fig. (**3**)), and the pyridoxal-5′-phosphate-6-azophenyl-2′, 4′-disulfonate (PPADS) (see Fig. (**4**)). These agents are presented as able to inverse the achondroplasic chondrocytes of their pathological state. The effect of the pyridoxal and its derivatives reducing the ERK1/2 oxidative is connected to the decrease of the associated pathological effects of the disease. According to the inventors, PPADS reduced the tyrosine phosphorylation of FGF receptor type 3 triggered by fibroblast growth factor 9 (FGF9) (50% reduction), as well as the activation of extracellular signal-regulated kinases 1 and 2 (ERK1/2) pathway. As a consequence of this inhibitory effect on ERK1/2 activity the loss of extracellular matrix was also reversed by PPADS [103]. The agents were tested only *in vitro* so far.

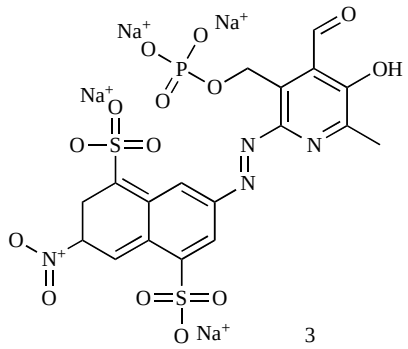

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Fig. (3). Chemical structure of PPNDS.

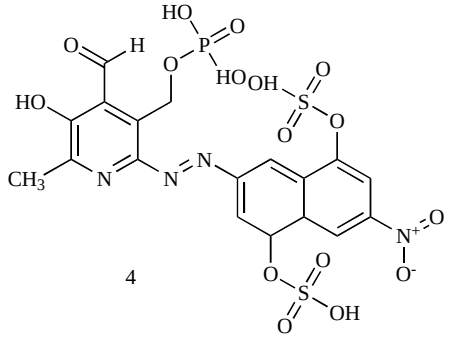

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Fig. (4). Chemical structure of PPADS.

In the patent US20100151483 [104] are proposed reagents for the detection of protein phosphorylation in signaling pathways. The method discloses phosphorylation sites identified in signal transduction proteins and pathways-using phosphorylation-site specific antibodies and heavy-isotope labeled peptides (AQUA peptides) for the selective detection and quantification of this phosphorylated sites/protein. Among the phosphorylation sites identified are sites occurring in the protein types: inhibitor proteins and Ser/Thr protein kinases [105].

Another patent US8198242 [106] is related to variants of C-type natriuretic peptide (CNP). In the method CNP variants are used having increased serum half-life exhibiting similar or improved activity to that of wild-type CNP. This is due to reduced ability to bind to neutral endopeptidase (NEP), greater resistance to proteolysis by NEP and/or reduced affinity to the clearance natriuretic peptide receptor C(NPR-C), while retaining the functionality of CNP. According to the inventors, compared to wild-type CNP22, certain CNP variants of the disclosure are much more resistant to NEP degradation *in vitro*, have a much longer plasma half-life and bioavailability in rats, stimulate a much higher level of cyclic guanosine monophosphate (cGMP) production in rats, and/or induce a significantly greater increase in long bone length and body length in achondroplasic mice. Furthermore, it has been shown that short duration dose regimen treatments with CNP22 are nearly as effective as continuous CNP22 treatment in reversing FGF2-induced arrest of chondrocyte growth *in vitro*.

ANTIBODY BLOCKADE OF RECEPTOR ACTIVATION- OTHER BINDING INHIBITORS

Another method to block FGFR3 over activation is the use of special designed antibodies. Recent research suggests that human anti-FGFR3 antibodies may have potential applications as antitumoral agents in bladder cancer [107]. In this sense, the research in achondroplasia and bladder cancer is coinciding.

In the patent US20100247531[108] an isolated anti-FGFR3 antagonist antibody named R3Mab is described, where the antibody binds FGFR3 with a Kd of 1×10^{-8} or stronger and inhibits dimerization of the FGFR3 receptor with another unit of the receptor. Biochemical analysis and 2.1-Å resolution crystallography revealed that R3Mab bound to a specific FGFR3 epitope that simultaneously blocked ligand binding, prevented receptor dimerization, and induced substantial conformational changes in the receptor [109].

Sanofi Aventis Inc. filled the patent US20110136856 [110]. In the patent, the use of imidazo[1,5-a]pyridine derivatives as inhibitors of FGFs is described (see Fig. (**5**)). It has been found that these compounds exhibit a powerful antagonist activity for the binding of FGFs to their receptors, demonstrating a very good activity in *in vivo* models (achondroplasic mice) and a 10mg/kg dose allowed to obtain a maximum activity of the compounds.

Prochon Biotech Ltd. presented the patent US7288406 [111], and described the use of active variants of FGF as inhibitors with enhanced receptor subtype specificity. The preferred variants retain binding to FGFR3 triggering intracellular downstream mechanisms leading to activation of a biological response.

In the patent US20090192133 [112], it is describing a method of administering an amount of a Heat Shock Protein-

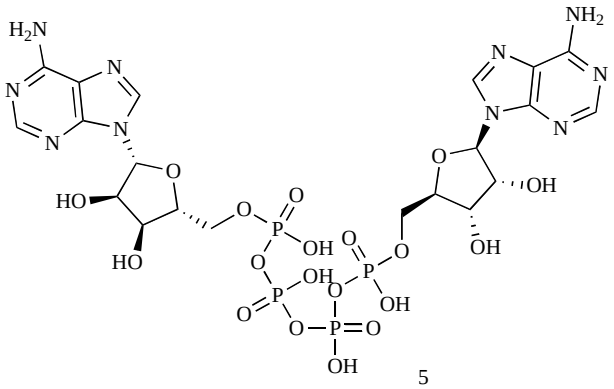

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Fig. (5). Chemical structure of imidazo-pyridine.

90 (Hsp90) inhibitor which is effective to treat the FGFR related disorder. Hsp90 is a eukaryotic chaperone that ensures proper folding of proteins. Hsp90 preferentially stabilizes mutant kinases involved in various tumors, and mutant kinases are more sensitive to the inhibition of Hsp90 than wild-type kinases. FGFR3 strongly associates with these chaperone complexes and depends on them for stability and function. Inhibition of Hsp90 function using the geldanamycin analog 17-AAG induces the ubiquitination and degradation of FGFR3 and reduces its signaling capacity. Other FGFRs weakly interact with these chaperones and are differentially influenced by Hsp90 inhibition. The Hsp90related ubiquitin ligase CHIP is able to interact and destabilize FGFR3 [69]. Finally in the patent US8187601 [113], antibody-based binding proteins that bind human FGFR3 are used. The binding proteins contain FGFR3 binding sites based on the antibody complementarity-determining regions (CDRs) of a family of antibodies that specifically bind FGFR3. The antibodies used are monoclonal 15D8, chimeric 15D8 and humanized 15D8. The binding proteins can be used as diagnostic and therapeutic agents. When used as a therapeutic agent, the binding proteins have to be humanized, to reduce or eliminate an immune response when administered to a human patient. The binding proteins prevent or inhibit the activation of human FGFR3. This is occurring by preventing FGFR3 from binding to a ligand, e.g., FGF1, thereby neutralizing FGFR3 activation. The binding proteins can be used to stimulate the proliferation of chondrocytes.

GENE SILENCING AND GENETIC THERAPIES

Lately, it is expanding the research technique of using RNA interference (RNAi) to suppress gene expression *in vitro* and *in vivo*. There are many reports to demonstrate the use of synthetic small interfering RNAs (siRNAs) in mammals, as therapeutic agents, especially in the areas of cancer and in our case for achondroplasia [65]. According to this technique in the patent US2011034354 [114] is described a method of using multi-siRNA compositions for reducing gene expression. The method is about formulations and compositions having multiple double- stranded nucleic acid complexes. Each double- stranded nucleic acid complex is comprising of an antisense strand and a continuous passenger strand or a discontinuous passenger strand. The composition decreases expression of one or more mRNAs and it is using these formulations and compositions to treat or prevent diseases or conditions associated with inappropriate gene expression [65].

Similarly, in the patent US2011034352 [115], there are used nucleic acid compounds for inhibiting FGFR3 gene expression. They are used double- stranded nucleic acid complexes having one or more hydroxymethyl substituted nucleomonomer(s) from which one strand is complementary to a FGFR3 mRNA. Nucleic complexes include short interfering RNA complexes (siRNA) capable of modulating gene expression comprising an antisense strand and a continuous or a discontinuous passenger strand ("sense strand") [65].

Furthermore, in the patent EP2327774 [116] of Precision Biosciences, there are used rationally-designed meganucleases with altered sequence specificity and DNA-binding affinity. The meganucleases are used to generate recombinant cells and organisms having a desired DNA sequence inserted into a limited number of loci within the genome. The method has been used, to identify amino acid substitutions which can alter the recognition sequence specificity and/or DNA-binding affinity of the meganucleases, and to rationally design and develop meganucleases that can recognize a desired DNA sequence that naturally-occurring meganucleases do not recognize.

Finally, the patent US20100304489 [117] of Harvard University, is describing a method for homologous recombination in human cells. It is based, in part, on the discovery of a stem cell state in human cells that resembles the morphology observed in murine-derived stem cells. Induction of such a state in human stem cells permits an increase in the efficiency of homologous recombination. Murine pluripotent stem cells can exist in two functionally distinct states, LIF-dependent embryonic stem cells (ESCs) and bFGF-dependent epiblast stem cells (EpiSCs). This method demonstrated that the hLR5 state facilitates gene targeting, and as such provides a powerful tool for the generation of recombinant human pluripotent stem cell lines [118].

OTHER ALTERNATIVE THERAPEUTIC COMPOUNDS

In this final section, the patents presented do not belong to one of the above categories. Specifically, in the patent ES2278519 [119] for the treatment of achondroplasia is proposed the use of dinucleotides. The application of the dinucleotides is able to reduce the presence of the receptor FGFR3 in the achondroplasic chondrocytes. It is demonstrated the expression of $P2Y_1$, $P2Y_2$, $P2Y_6$ and $P2Y_{11}$ receptors in achondroplasic chondrocytes, as well as the activation of these receptors after nucleotides and dinucleotides exposure. The altered calcium signaling of achondroplasic chondrocytes was confirmed, since FGF9 treatment fails to induce calcium mobilization. However, achondroplasic chondrocytes pre-treated with Ap_4A are able to respond with increases in intracellular calcium after FGF9 stimulation. These findings show the rescue effect of diadenosine-tetraphosphate (Ap_4A) (see Fig. (**6**)), acting by means of P2Y receptors, on defective calcium response triggered by achondroplasic FGFR3 [120].

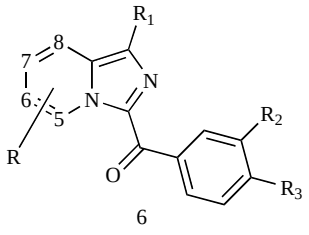

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Fig. (6). Chemical structure ofAP_4A.

In another patent US20110189306 [121] the use of hydrazide compounds is proposed (see Fig. (**7**)).V-ATPases are complex molecular motors engaged in diverse specialized roles contributing to development, tissue function and pH homeostasis within complex organisms. Mutations and misappropriation of V-ATPase function has been linked to bone-loss disorders. The proposed strategy is based on targeting a3-B2 subunit interactions within the V-ATPase complex [122].

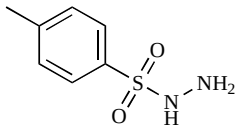

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Fig. (7). Chemical structure of P-toluenesulfonylhydrazide.

In the patent US20100239631 [123] the use of thiopyrimidine-based compounds is proposed. The thiopyrimidine-based compounds (see Fig. (**8**)) are inhibitors of protein kinases including JAK kinases. In particular, the compounds are selective for JAK1, JAK2 or JAK3 kinases and combinations such as JAK1 and JAK2. The kinase inhibitors can be used in the treatment of kinase associated diseases [124].

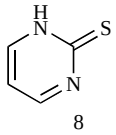

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Fig. (8). Chemical structure of thiopyrimidine.

In the patent US7078059 [125] a method for the treatment of bone diseases enhancing bone formation, inhibiting osteoclastic differentiation and/or activating osteoblastic differentiation is described. The therapeutic or prophylactic effect is obtained using an amount of a lanthanum compound. Lanthanum (III) compounds enhance bone formation and bone density and have beneficial effects on the activity and differentiation of bone cells [126]. In the patent US20100047251 [127], there are proposed antibodies with a binding affinity to fibroblast growth factor receptor 2 (FGFR2). These antibodies have binding affinity to other FGF receptors, blocking both ligand-dependent and constitutive ligand independent receptor activation. It looks like they display high affinity to more than one FGF receptor subtype and fragments. These antibodies comprise at least one, two, three, four, five or/and six hypervariable region (HVR) sequences of various positions. HVR is a location within nuclear DNA or the D-loop of mitochondrial DNA in which base pairs of nucleotides repeat (in the case of nuclear DNA) or have substitutions (in the case of mitochondrial DNA). Changes or repeats in the hypervariable region are highly polymorphic [128]. Another antibody used is comprised from a light chain variable domain of humanized 4D5antibody (huMAb4D5-8) HERCEPTIN®. Herceptin is approved for the treatment of early-stage breast cancer [129]. Astex Therapeutics Ltd. of UK, presented the patent US8131527 [130] about a method using FGFR pharmacophores, which are compounds capable of binding to FGFR with greater affinity than their binding to vascular endothelial growth factor receptors (VEGFR) and how to identifying such compounds using the pharmacophore. A pharmacophore is an abstract description of molecular features which are necessary for molecular recognition of a ligand by a biological macromolecule [131]. The invention relates to the identification of modulators of FGFR, capable of binding to FGFR and inhibiting its activity. These modulator compounds exhibit selectivity for FGFR tyrosine kinase over other tyrosine kinases in particular VEGFR2, and may be capable of binding to FGFR and inhibiting its activity.

Finally, the patent WO2011143307 [132] described a method using high concentration antibody formulations comprising an anti-scerostin immunoglobulin and an acetate salt and/or an acetate buffer. The method is based on the discovery that the addition of calcium acetate at low concentrations, e.g., 5-10mM, reduced the effective viscosity in formulations comprising a high concentration of a selected anti-scerostin antibody.

In the invention US8143271 [133], there are presented compounds active on protein kinases in general, including FGFR3 and its mutations. In particular, the invention concerns compounds of the type2-[2,4-difluoro-3-(5-pyridin-3-yl-1 H -pyrrolo[2,3-b]pyridin-3-ylmethyl)-phenoxy]-N-ethyl-acetamide(P-1475)(see Fig. (**9**)). These compounds are dedicated to therapeutic methods involving modulation of protein kinases. The used inhibitors are useful in treating both achondroplasia and thanatophoric dysplasia. A key factor for the invented inhibitor is that FGFR3 is activated by translocation in approximately 15% of multiple myeloma [134].

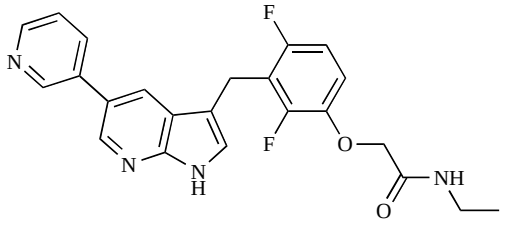

Fig. (9). Chemical structure of2-[2,4-difluoro-3-(5-pyridin-3-yl-1 H -pyrrolo[2,3-b]pyridin-3-ylmethyl)-phenoxy]-N-ethyl-acetamide.

TARGETING THE THERAPEUTIC AGENTS TO THE CARTILAGINOUS GROWTH PLATE: DELIVERY OF THE AGENTS

The delivery of the target agents to the cartilage requires the development of special procedures and technics. Cartilage is avascular and the extracellular matrix surrounding the

Table 1. Patents Reviewed in This Review Article.

Inventors	Patent Number	Title
Yayon, A. and Nevo Z.	WO9641620	FGFR 3 as marker for mesenchymalskeltal progenitor cells
Yayon A. and Orit S.	US6265632	Animal model for FGFR associated chondrodysplasia.
Nakao, K.	US6743425	Therapeutic agents for achondroplasia.
Atherton N. D., Totten J. W. *et al.*	US7078059	Treatment of bone diseases
Yayon, A.	WO2006061824	Chondrocyte-based implant for the delivery of therapeutic agents.
Golembo M. and Yayon A.	US7276481	Method and composition for treatment of skeletal dysplasias.
Nakao, K., Yasoda A., *et al*.	EP1743653	Composition for increasing body height.
Oren Bogin, Rivka Adar, *et al.*	US7288406	Active variants of FGF with improved specificity.
Bold G., Furet P., *et al.*	US20080312248	Pyrimidinyl Aryl Urea Derivatives Being Fgfr Inhibitors
Pintor, J., Peral A., *et al*.	ES2278519	Treatment of achondroplasia by administrating dinucleotides.
Horton W.A. and L. M. B.	US20090192133	Treatment for achondroplasia.
Pintor, J., Guzman Aranguez A. I., *et al*	ES2286933	Treatment of achondroplasia with the administration of pyridoxal and its derivates.
Ashkenazi Avi , Qing Jing , *et al*	US20100247531	Anti-FGFR3 antibodies and methods using same.
Bourke D.G., Bu Xianyong , *et al.*	US20100239631	Thiopyrimidine-based compounds and uses thereof.
Hashash Ahmad , Kangwen L. Lin, *et al.*	US20100209418	Solid Forms of a Raf Kinase Inhibitor.
Mina E. Aikawa, Payman Amiri, *et al*.	US20100234394	Substituted benzimidazoles and methods of their use.
Geijsen N. and Buecker C.	US20100304489	Methods and compositions for homologous recombination in human cells.
Nieto Toledano M. A., Alvarez De Frutos C., *et al.*	US20100218266	Methods of identifying and using SNAIL1 inhibitory compounds in chondrodysplasia treatment and preparation of pharmaceutical compositions.
Furet P., Graus D. P., *et al.*	US20100105667	Quinoxaline- and Quinoline-Carboxamide Derivatives.
Hornbeck P., Goss V., *et al.*	US20100151483	Reagents for the detection of protein phosphorylation in signaling pathways.
Wendt D. J. , Long Shinong, *et al.*	US20100297021	Variants of C-Type Natriuretic Peptide.
Yayon A. and Eran R.	US20100047251 US20128101721	Antibodies blocking fibroblast growth factor receptor activation and methods of use thereof.
Alcouffe Chantal , Badorc Alain , *et al*	US20110136856	Novel imidazo[1,5-a]pyridine derivatives, method for preparing same and pharmaceutical compositions containing same.
Hellberg M. R. and Pang I.-H.	US20110112038 WO2011038066 US20110077381 WO2011038061	Novel NPR-B agonists.
Eswarakumar V.P. Schlessinger J. *et al.*	US7872016	Method for treating skeletal disorders resulting from FGFR malfunction.
Kartner N. and Manolson M. F.	US20110189306	Compounds, compositions and treatments for V-ATPase related diseases.
Porta Diana Graus Guagnano Vito, *et al*.	US20110045511	Methods of monitoring the modulation of the kinase activity of fibroblast growth factor receptor and uses of said method.
Saxty G., Berdini V. *et al.*	US20118076354 US20118071614	Bicyclic heterocyclic compounds as protein tyrosine kinase inhibitors
Vesely David, Lynn	WO2011153531	Method of treating skeletal dysplasias using vessel dilator
Osslund, Timothy, D	WO2011143307	High concentration antibody formulations

(Table 1) Contd.…

Inventors	Patent Number	Title
Seth, S., Fosnaugh, K.L. *et al.*	WO2011139843 US2011034354	Multi-siRNA compositions for reducing gene expression
Vaish, Narendra, K. Seth, Shaguna *et al.*	WO201139842 US2011034352	Nucleic acid compounds for inhibiting FGFR3 gene expression and uses thereof
Krejci, Pavel Wilcox, William	US20110166223	Methods of inhibiting fgfr3 signaling
Weng, Z. Winston Jr., W.M. *et al.*	US8187601	Fibroblast growth factor receptor 3 (FGFR3) binding proteins.
Wendt, D.J. Long, S. *et al.*	US8198242	Variants of C-type natriuretic peptide.
Saxty, G. Berdini, V. *et al.*	US8131527	FGFR pharmacophore compounds.
Ibrahim, P.N. Artis, D.R. *et al.*	US8143271	Compounds and methods for kinase modulation, and indications therefor.

chondrocytes is a tough obstacle to surpass. In this sense, the invention WO2006061824 [135] is describing the delivery of bioactive molecules including peptides, proteins and RNAi molecules to a mammalian subject, using a genetically modified chondrocyte-based implant. The genetically modified chondrocyte-based implant is provided as a chondrocyte pellet. From the same inventor, the patent WO9641620 [136] describes FGFR3 as marker for mesenchymal skeletal progenitor cells. Using this marker is possible both to identify and locate mesenchymal skeletal progenitor cells in a tissue, as well as to obtain a substantially pure culture of such cells. The pure culture of the mesenchymal skeletal progenitor cells may be used, optionally after various manipulations *ex vivo*, as an active ingredient in pharmaceutical compositions or implants for the purpose of bone and/or cartilage repair. FGFR3 may also be used as a marker for the identification and the localization of cartilage and bone derived tumors. Agents capable of binding to FGFR3 may also be used for targeting cytotoxic agents to cartilage and bone derived tumors [137].

CURRENT & FUTURE DEVELOPMENTS

In this paper, we reviewed the most important patents of the research laboratories dealing with achondroplasia (see Table **1**). The most significant development in the pursue of a remedy to this disease, is the most expected drug BMN-111 from BioMarin Pharmaceutical Inc. The drug is an analog of C-type Natriuretic Peptide (CNP) and the results *in vitro* and *in vivo* are promising. The company expects to initiate the Phase 1 trial in the first quarter of 2012 in humans. Questions remain on the delivery vehicle. Most of other efforts failed in this point. Cartilage is avascular. There are emerging some solutions, like the targeted nanoparticle opening of cartilage for drug delivery [138], but BioMarin chose to use the technique of agent attachment to bone or cartilage targeting moieties. The field of targeted delivery for musculoskeletal diseases is still in its early development with many significant challenges. Transportation to areas with limited blood supply can be puzzling. Stability of the drug during formulation and delivery is another factor to be considered [139].

Another issue is the age of the patients. The treatment should start the sooner possible in order to maximize the effect of the growth of the skeleton and is due to be prolonged for a very long time perhaps for all the growth period of the child. This prerequisite is adding challenges concerning the possible adverse effects, which are intensified from such a lengthy treatment. BioMarin did not publish yet the adverse effect report so the information on this very sensitive issue is limited.

In any case, more drugs are expected to start clinical trials in the future. An interesting fact is that the same mutation causing achondroplasia is responsible for causing cancer under different circumstances [140]. So, the research for achondroplasia is interrelated to the cancer research and of course to other diseases like skeletal dysplasias and osteoarthritis. This common effort is producing faster results and is quite possible some of the tested agents to be converted to drugs in the near future.

ACKNOWLEDGEMENTS

We would like to thank the Foundations, Ramon Areces (Proyecto Acondroplasia) and MAGAR and Universidad Complutense de Madrid (Project GR35/10-A-920777) for their funding contribution to this publication.

CONFLICT OF INTEREST

It is certified that there is no actual or potential conflict of interest in relation to this article and the authors have no financial relationship with the presented pharmatheutical companies.

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