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Vosoritide Clinical Study Data Demonstrates CXM is a Superior Biomarker of Endochondral Bone Growth

Kevin Larimore, Thom Nguyen, Yulan Qi, George Jeha, and Stephen Zoog

BioMarin Pharmaceutical Inc., Novato, CA, USA

Objectives

- Vosoritide is a C-type Natriuretic Peptide (CNP) analogue therapy for treatment of achondroplasia. Vosoritide acts on growth plate chondrocytes through the Natriuretic Peptide Receptor-B to stimulate increased endochondral bone growth, leading to increased growth velocity in treated subjects. In clinical studies, subject blood and urine samples were analyzed to monitor putative bone growth biomarkers including cross-linked C-terminal telopeptides of collagen II (CTxII), Bone-Specific Alkaline Phosphatase (BSAP), N-terminal pro-peptide of collagen I (PINP), and an N-terminal fragment of Collagen X (CXM). Changes in biomarkers over time were analyzed in relation to observed changes in growth velocity in subjects receiving vosoritide

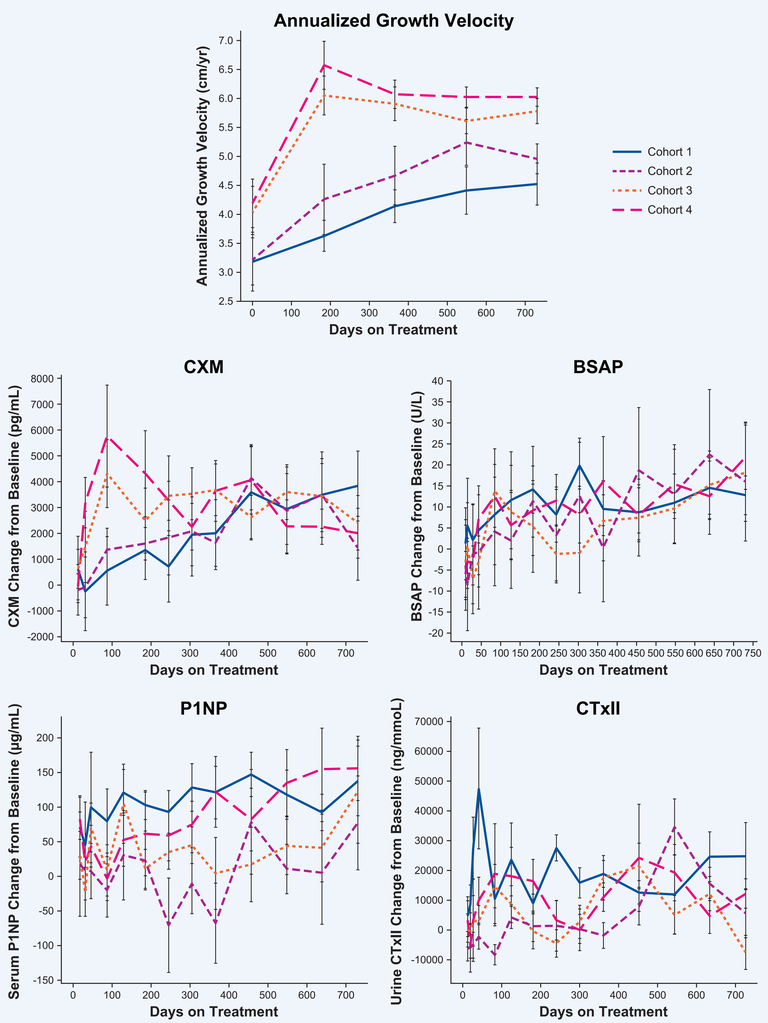
Methods

- **Collagen type X biomarker (CXM[1])** is a degradation fragment of collagen type X, released by active growth plates. A relative quantitative biomarker ECLA was developed and validated at BioMarin to measure CXM. Ninety-six-well Meso Scale Discovery (MSD) Streptavidin plats were blocked with StartingBlock PBS with Tween-20 (ThermoFisher Scientific, Waltham, MA, USA). After decanting blocking buffer, biotinylated anti-human collagen type X NC1 domain capture SOMAmer was incubated on the plate. The standard stock (recombinant human collagen type X NC1 domain in assay diluent [AD]) was serially diluted in AD, while the serum quality control samples (QC) and serum study samples were diluted 1:100 in AD. After washing the assay plate, diluted calibrators and samples were incubated on the plate. After a second wash, ruthenium-labeled mouse monoclonal anti-Collagen type X NC1 domain IgG detection antibody was incubated on the plate. The plate was then washed, MSD Read Buffer T with surfactant was added, and the plate was read on an MSD Quickplex instrument. The raw signal from each well was proportional to the collagen type X concentration in each sample. The concentration of collagen type X in each unknown sample was determined by interpolation of raw assay signal using the standard calibrator curve. The standard regression performed by Watson LIMS used a 4 Parameter Logistic (4-PL) Marquardt model with a weighting factor of 1/Y2. The assay limit of detection was 914 pg/mL CXM in human serum
- **Bone-specific alkaline phosphatase (BSAP or BAP)** is an exploratory bone growth biomarker produced by osteoblasts and osteoclasts in growth plates and mineralized bone. Changes in BSAP may reflect growth plate activity, bone growth, and / or bone remodeling activity. An enzyme immunoassay method to measure bone-specific alkaline phosphatase in human serum was validated at ICON Labs (Farmingdale, NY, USA; validation N08-024VR-1,4). The assay used monoclonal anti-BAP antibody-coated wells to capture BAP in samples. The enzyme activity of the captured BAP was detected with p-Nitrophenyl phosphate substrate. Raw assay signals were read using a SpectraMax Spectrophotometer (Molecular Devices, San Jose, CA, USA). The concentration of BAP in each sample was determined by interpolation using the standard calibrator curve with a linear curve fit. The assay limit of quantification was 2 U/L in neat urine
- **N-terminal pro-peptide of type I procollagen (PINP)** is an exploratory potential pharmacodynamic bone growth biomarker, released during production of type I collagen. Changes in PINP may reflect changes in growth plate activity, bone growth, and/or bone remodeling. A quantitative competitive format radioimmunoassay (RIA) method based on the UniQ PINP RIA assay kit (Orion Diagnostica, Espoo, Finland) was validated at ICON Labs (Farmingdale, NY, USA; validation N06-016VR). A known amount of 125I-labelled PINP and an unknown amount of unlabeled PINP in samples competed for a limited number of high affinity binding sites on a polyclonal rabbit anti-PINP IgG antibody. A secondary anti-rabbit IgG antibody coated on solid kaolin particles was used to separate antibody-bound PINP from matrix components. The radioactivity of the bound 125I-PINP was measured using a WIZARD automatic gamma counter (Perkin Elmer, Waltham, MA, USA). The amount of radioactivity in each tube was inversely proportional to the concentration of PINP in each sample. The concentration of PINP in each sample was determined by interpolation using a standard calibrator curve and a linear regression curve fit. The lower limit of quantitation was 5 µg/L PINP in neat human serum
- **Cross-linked C-telopeptides of type II collagen (CTXII)** is an exploratory potential pharmacodynamic bone growth biomarker released during degradation of type II collagen. Changes in CTXII may reflect changes in growth plate activity, bone growth, bone remodeling, and/or articular cartilage remodeling. A quantitative competitive format ELISA for measurement of CTXII in human urine, using the CartiLaps ELISA kit from ImmunoDiagnostic Systems (East Boldon, UK) was validated at ICON Labs to support study 111-202/205 (validation N06-114VR). The assay was based on the competitive binding of a mouse monoclonal anti-CTXII antibody to urinary fragments of type II collagen or to biotinylated, synthetic peptides bound to the surface of microtiter plates coated with streptavidin. Initially, biotinylated, synthetic peptides were bound to the surface of streptavidincoated wells of the microtiter plate. After washing, standards, controls, and urine samples containing unlabeled CTXII were pipetted into the wells followed by addition of a solution of a mouse monoclonal anti-CTXII IgG. The wells were washed, and a solution of peroxidaseconjugated rabbit anti-mouse IgG was added to the wells. Following a second washing step, tetramethylbenzidine (TMB) chromogenic substrate was added to all wells. The yellow color development was stopped with sulfuric acid and the absorbance at 450 nm was read on a SpectraMax Plus spectrophotometer (Molecular Devices, San Jose, CA, USA). The raw signal in each well was inversely related to the concentration of CTXII in each sample. The concentration of CTXII in each sample was determined by interpolation using a standard calibrator curve and a linear curve fit. The lower limit of quantitation was 0.60 ng/mL CTXII in neat urine

Results

- In study 111-202, pediatric subjects with achondroplasia age 5 to 15 received vosoritide at 2.5, 7.5, 15, or 30 µg/kg/day (cohorts 1, 2, 3, and 4, respectively) for the first 6 months. After 6 months, significant increases in annualized growth velocity (AGV) were observed in subjects receiving vosoritide at 15 or 30 ug/kg/day, but not for subjects receiving 2.5 or 7.5 ug/kg/day **(Figure 1)**. After 6 months, the dose concentration for subjects in cohorts 1 and 2 was increased to 15 ug/kg/day, resulting in increased AGV

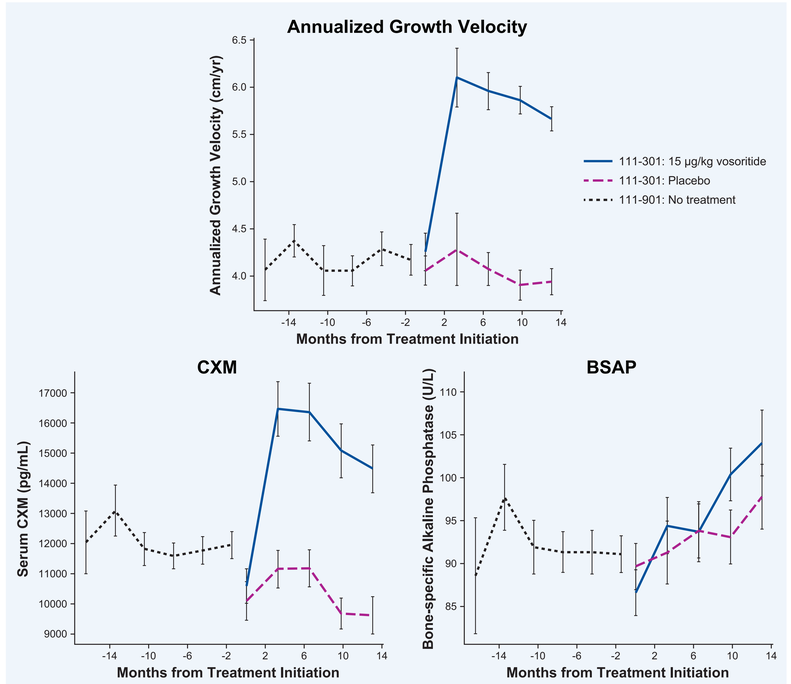
Figure 1. Phase 2 Study 111-202 growth velocity and biomarker results over time



Annualized growth velocity (AGV), serum collagen X biomarker (CXM), serum bone-specific alkaline phosphatase (BSAP), N-terminal propeptide of type I procollagen (P1NP), and Cross-linked C-telopeptides of collagen II (CTxII) were measured in subjects receiving 2.5, 7.5, 15, or 30 µg/kg/day vosoritide (cohorts 1, 2, 3, and 4). After 6 months of treatment, cohorts 1 and 2 were dose-escalated to 15 µg/kg/day. AGV or biomarker change from baseline are shown on the y-axis, days on vosoritide treatment is shown on the x axis. Lines represent the mean of each cohort, error bars represent the standard error of the mean.

- While all of the biomarkers analyzed in 111-202 were variable, CXM demonstrated dose-dependent increases, BSAP appeared to increase slightly over time on treatment for all dose levels, and there was no apparent trend or dose dependent response for CTxII or P1NP. Based on these results, BSAP and CXM were incorporated into the placebo-controlled Phase III vosoritide clinical study 111-301 and pre-treatment natural history study 111-901
- Before entering study 111-301, pediatric subjects with achondroplasia age 5 to 15 years old were monitored without treatment in study 111-901 for at least 6 and up to 15 months before treatment initiation. AGV, CXM, and BSAP were measured during natural history study 111-901 and during the placebo-controlled double-blind phase 3 study 111-301. AGV was relatively stable before treatment initiation, and in subjects receiving placebo. In contrast, AGV dramatically increased in subjects receiving vosoritide by 3 months **(Figure 2)**. Similarly, CXM levels were dramatically increased in treated subjects in study 111-301, but not in subjects receiving placebo. Serum BSAP levels increased in treated subjects, but also increased to a lesser extent in subjects receiving placebo

Figure 2. Natural History Study 111-901 and Phase 3 Study 111-301 growth velocity and biomarker results over time



Annualized growth velocity (AGV), serum collagen X biomarker (CXM), and serum bone-specific alkaline phosphatase (BSAP), were measured in untreated subjects in study 111-901 (black line), and in the same subjects receiving either 15 µg/kg/day vosoritide (blue line) or placebo (purple line) in study 111-301. AGV or serum biomarker concentrations are shown on the y-axis, months from initiation of study 111-301 (vosoritide or placebo treatment) is shown on the x axis. Lines represent the mean of each group, error bars represent the standard error of the mean.

Conclusions

Overall, the data from vosoritide clinical studies suggest that CXM is superior to CTxII, PINP, and BSAP for monitoring changes in endochondral bone growth. Data from the phase 3 study clearly demonstrated increased serum CXM levels that were associated with increased AGV in vosoritide-treated, but not placebo-treated subjects. There appeared to be some increase in BSAP in vosoritide-treated subjects over that observed in placebo-treated subjects, however this increase was relatively small compared with that observed for CXM. The study data demonstrated that serum CXM is a useful growth plate biomarker associated with changes in AGV.

Reference

1. Coghlan RF et. al., A degradation fragment of type X collagen is a real-time marker for bone growth velocity, *Sci Transl Med.* 2017 Dec 6;9(419).

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