Introduction

Fibroblast growth factor receptor 3 (FGFR3) plays a crucial role in the process of endochondral ossification as shown by the FGFR3 gain-of-function mutations that result in short-stature skeletal dysplasia, such as achondroplasia (ACH).

ACH is the most common class of rhizomelic dwarfism, an autosomal dominant disorder with an incidence between 1 in 10,000 and 1 in 20,000 live births worldwide.1

In >95% of cases, ACH is caused by an arginine-to-glycine substitution at residue 380 (p.R380G in FGFR3); this is a heterozygous mutation that demonstrates 100% penetrance and is de novo in 80% of cases.1

The ACH phenotypes include rhizomelia (shortening of the limbs with proximal segments affected disproportionately), large head with frontal bossing, mid-face hypoplasia, and relatively normal trunk, with excessive lumbosacral lordosis.2

Even though they are rare, serious complications are associated with ACH, including spinal cord compression due to a narrowed foramen magnum, lumbar nerve root and/or cord compression due to spinal stenosis, and severe lower-extremity varus deformity.2

Current management of ACH focuses on the prevention and treatment of complications and involves improved therapy that targets the pathophysiology of the condition.

Various therapeutic strategies have been considered, the most advanced being an analog of C-type Natriuretic Peptide (CNP), which is given as a daily subcutaneous injection and acts by antagonizing the MAP kinase pathway.

Infigratinib (previously known as BGJ398) is an oral, selective FGFR1–3 tyrosine kinase inhibitor and was previously studied with 2 mg/kg daily dosing in the Fgfr3Y367C/+ mouse, which recapitulates the ACH phenotype and has been described elsewhere.3

– Low-dose treatment with infigratinib improved the axial and appendicular skeletons with 10 and 15 days of treatment, and reduced intervertebral disc defects of lumbar vertebrae, loss of synchondrosis, and foramen-magnus shape anomalies.2

In this poster we describe studies that were designed to expand on the process in Fgfr3Y367C/+ mice over 15 days of treatment.

The objective of the current studies was to assess the effects of two different doses and dosing regimen of infigratinib on the endochondral process in Fgfr3Y367C/+ mice using X-rays and macroscopic, histological, and immunohistological analyses.

Mouse model and drug treatment

The ACH mouse model, Fgfr3Y367C/+ mice, has been described previously.4

Salt (BGSJ38-ABA) of infigratinib was used. Infigratinib phosphate was formulated as a suspension for subcutaneous administration in DMSO (1 ml:2 mg). Infigratinib was given via subcutaneous administration due to the size of the mice, which makes oral gavage impractical.

Fgfr3Y367C/+ mice were treated from Day 1 (Day 0 = birth) to Day 15 with infigratinib subcutaneously once daily (daily dose of 0.2 mg/kg or 0.5 mg/kg) or every 3 days (intermittent dosing of 1 mg/kg every 3 days).

Animals were sacrificed on Day 16. Results were compared with those obtained from mutant mice receiving vehicle.

In-vivo observations

In-vivo observations for severity of effect were performed twice a week (hindlimb movement, posture, tail, paws) including assessment for survival. Detailed in-vivo observations were performed at the time of scoring.

X-ray assessments

Lateral X-ray images were taken of all animals by Fastscan M020 Cabinet X-ray system, Norlock AB, Sweden. Animals were placed on their right side, with the left hind leg more forward than the right, to allow both hind legs to be visible on the X-ray.

Bone length measurement

Bone length was measured using a caliper (VWR19-0013, VWR, USA) at necropsy.

Statistical analysis

Differences between experimental groups were assessed using ANOVA with Tukey’s post-hoc test or Mann-Whitney U-test. The significance threshold was set at p<0.05. Statistical analyses were performed using GraphPad PRISM (v5.03).

Results

Efficacy

We observed a statistically significant improvement vs vehicle-treated Fgfr3Y367c/+ mice in the upper (humerus +7.3%, p<0.01; ulna +11.1%, p<0.001; radius +14.2%, p<0.001) and lower (femur +10.4%, p<0.01; tibia +16.8%, p<0.001) limbs with infigratinib at a dose of 0.5 mg/kg, as well as improvement in the foramen magnum (FM length +11.0%, p<0.001).

The effect of infigratinib on bone elongation was lower at 0.2 mg/kg (Figure 2), indicating a dose-response relationship.

To test the hypothesis of whether daily treatment was needed, we performed intermittent interruptions of infigratinib (1 mg/kg, every 3 days): – The gain of growth vs vehicle-treated mice was significant for all long bones (humerus +5.0%, p<0.01; ulna +6.3%, p<0.01; radius +4.3%, p<0.01; femur +8.7%, p<0.01; tibia +6.4%, p<0.001) and the foramen magnum was increased (FM length +6.3%, p<0.01).

In addition to the gain of growth, we observed a modification of the growth plate structure, displaying a better organization of the hypertrophic zone, among other improvements.

Discussion

These data demonstrates that low, as well as intermittent, doses of infigratinib promote growth in this ACH mouse model:

– Low-dose infigratinib treatment of Fgfr3Y367C/+ mice over 15 days improved the endochondral ossification processes in an ACH mouse model.

– Skeletal changes were observed in a dose-dependent manner, based on total dose given over the 15-day treatment period.

– No apparent toxicity of infigratinib was observed; on the contrary, with infigratinib has the potential to be a valuable and relevant option for children with ACH. These findings support the continued development of infigratinib as a therapeutic option for ACH, with clinical studies planned to begin in 2020.

References