

Low dose, daily or intermittent administration of infigratinib (BGJ398), a selective FGFR inhibitor, as treatment for achondroplasia in a preclinical mouse model

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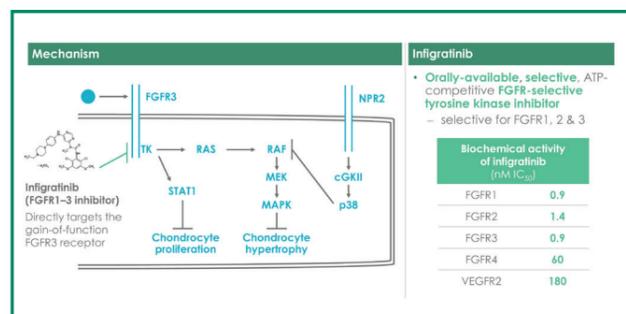


Introduction

- 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 cause 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.Gly380Arg) in *FGFR3*; this is a heterozygous mutation that demonstrates 100% penetrance and is *de novo* in 80% of cases.²
- 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 lumbar lordosis.²
- Even though they are rare, serious complications are associated with ACH, including cervical 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.³
- Current management of ACH focuses on the prevention and treatment of its complications as there is no approved 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 *Fgfr3*^{Y367C/+} mouse, which recapitulates the ACH phenotype and has been described elsewhere:^{4,5}

- 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 synchondroses, and foramen-magnum shape anomalies.⁵
- In this poster we describe studies that were designed to expand on the previously reported data,⁵ to test the effects of infigratinib at lower daily doses/intermittent doses.

Figure 1. Infigratinib directly targets the underlying cause of achondroplasia, FGFR3 overactivity^{2,6}



Objectives and methods

- We hypothesized that a dose-response relationship would be observed based on total dose with daily or intermittent dosing given to *Fgfr3*^{Y367C/+} 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 *Fgfr3*^{Y367C/+} mice using X-rays and macroscopic, histology, and immunohistology analyses.

Mouse model and drug treatment

- The ACH mouse model, *Fgfr3*^{Y367C/+} mice, has been described previously.^{4,7}
- Phosphate salt (BGJ398-AZA) 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.
- Fgfr3*^{Y367C/+} 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 Faxitron MX20 Cabinet X-ray system (Lincolnshire, IL, USA) following terminal sacrifice. 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 (VWRi819-0013, 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 $p \leq 0.05$. Statistical analyses were performed using GraphPad PRISM (v5.03).

Results

Efficacy

- We observed a statistically significant improvement vs vehicle-treated *Fgfr3*^{Y367C/+} 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 +11.9%, $p < 0.001$).
- 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 injections 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%, $p < 0.001$; ulna +5.6%, $p < 0.001$; radius +4.3%, $p < 0.001$; femur +8.7%, $p < 0.001$; 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.

Figure 2. Overall findings by dose

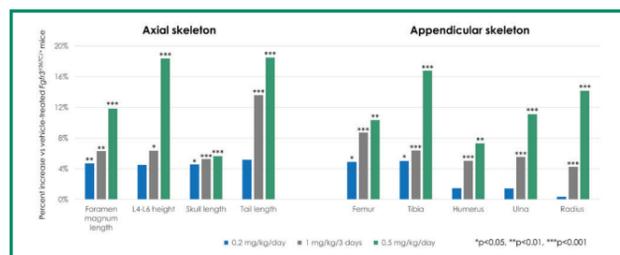
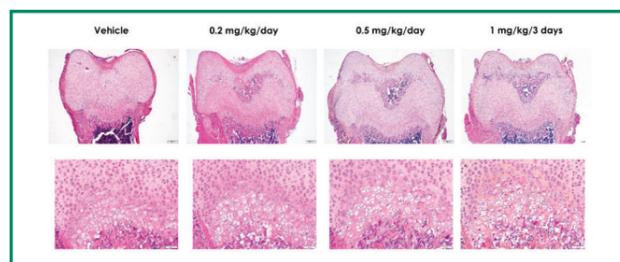


Figure 3. Infigratinib promotes the formation of the ossification center and the number of hypertrophic chondrocytes



The secondary ossification center is larger in infigratinib-treated mice vs control mice. Histological samples demonstrate efficient penetration of the growth plate by infigratinib.

Figure 4. Infigratinib leads to improvement of chondrocyte differentiation in proximal and distal femurs

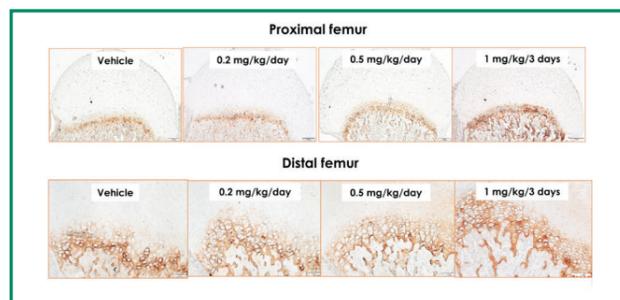
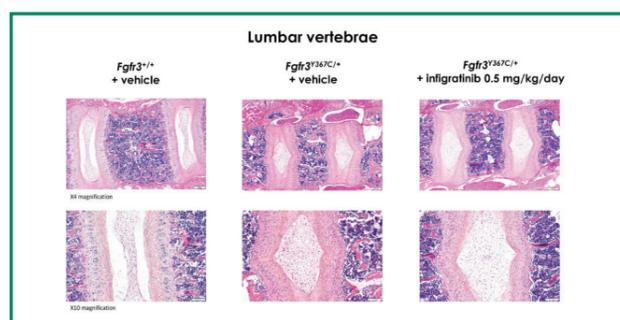


Figure 5. Effects of infigratinib treatment on the intervertebral disc

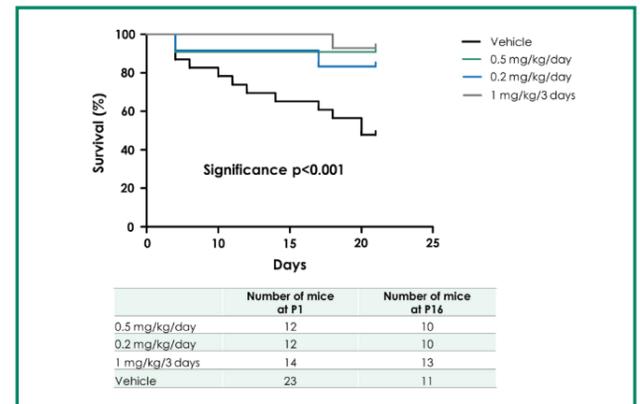


Treatment with infigratinib modified the morphology of the nucleus pulposus and annulus fibrosus.

Survival

- Survival of *Fgfr3*^{Y367C/+} mice treated with infigratinib was improved after 15 days vs vehicle-treated mice ($p < 0.001$ at all doses compared to vehicle).

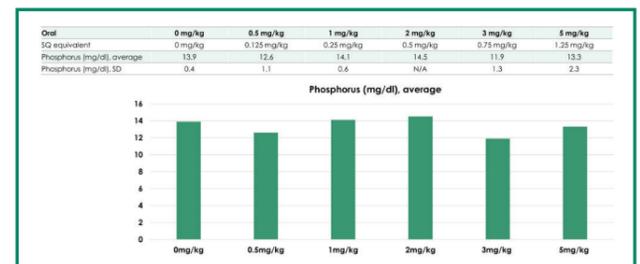
Figure 6. Survival data



Safety from separate preclinical and clinical studies

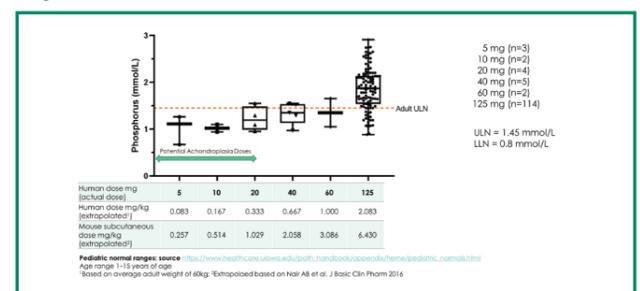
- In adult humans treated at much higher doses of infigratinib (10–20x the potential dose in achondroplasia), hyperphosphatemia is the most commonly observed side effect.^{8,9}
- In adult mice (male and female) treated with oral infigratinib at low doses, no differences in phosphorus 1 hour after dosing were observed vs vehicle-treated mice (Figure 7).

Figure 7. Mouse phosphorus data



- In adult humans treated with infigratinib in previously conducted clinical studies, hyperphosphatemia was not seen at the doses expected in ACH (Figure 8). Based on doses studied in adults to date, the median phosphorus levels above the upper limit of normal for adults are reached at doses of 125 mg daily.

Figure 8. Phosphorus concentration vs. infigratinib dose on Day 15 in adults



Discussion

- These data demonstrate that low, as well as intermittent, doses of infigratinib promote growth in this ACH mouse model:
 - Low-dose infigratinib treatment of *Fgfr3*^{Y367C/+} 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, treatment of *Fgfr3*^{Y367C/+} mice with infigratinib improved survival compared with untreated *Fgfr3*^{Y367C/+} mice.
- Conclusion:** these results suggest that, at low doses, TKI therapy 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

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