Low-dose infigratinib, an oral selective fibroblast growth factor receptor tyrosine kinase inhibitor, demonstrates activity in preclinical models of hypochondroplasia

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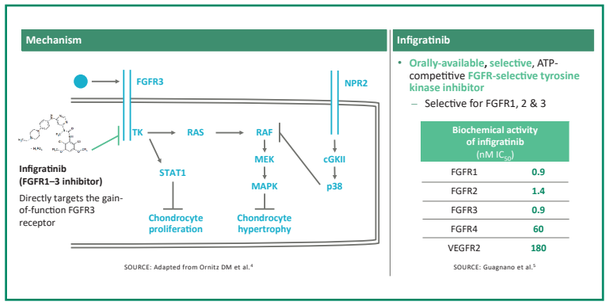
Background

- Hypochondroplasia (HCH) is a mild form of dwarfism characterized by disproportionally short stature, macrocephaly, brachydactyly, limited range of motion at the elbows, lumbar lordosis, and bowed legs.
- HCH is caused by heterozygous gain-of-function mutations in the Fibroblast Growth Factor Receptor 3 (*FGFR3*) gene.
- Approximately 70–80% of cases of HCH are due to mutations in the intracellular FGFR3 domain such as the missense mutation p.Asn540Lys.[1]
- *FGFR3* plays a crucial role in the process of endochondral ossification as shown by *FGFR3* gain-of-function mutations that result in common forms of short-stature skeletal dysplasia:
  - Achondroplasia (ACH) is the most common cause of rhizomelic dwarfism, an autosomal dominant disorder with an incidence between 1 in 15,000 and 1 in 30,000 live births worldwide.[2,3]
  - The incidence of HCH is not as well understood as ACH but is thought to be approximately the same as ACH.[1]
- There are currently no approved therapeutic options for HCH.

Aims/objectives of current study

- We generated the first mouse model of HCH (*Fgfr3*$^{Asn534Lys/+}$) expressing the *p.Asn540Lys* mutation (Loisay et al. manuscript in preparation).
- Macroscopic analysis of *Fgfr3*$^{Asn534Lys/+}$ (*Hch*) mice displayed progressive dwarfism throughout development with shortened limbs versus controls. Furthermore, chondrocyte differentiation is impaired in *Hch* mice, indicating impairment of endochondral ossification.
- We hypothesized that the oral, selective FGFR TKI infigratinib (BGJ398) could improve the phenotype and improve endochondral and membranous ossification in the *Fgfr3*$^{Asn534Lys/+}$ mouse.

Figure 1. Infigratinib directly targets the underlying cause of HCH, FGFR3 overactivity



Methods

Mouse model and drug treatment

- The *Fgfr3*$^{N534K/+}$ mouse model was generated by crossing CMV-Cre mice (C57BL/6J) to mice exhibiting the germline transmission of the *N534K* mutation corresponding to the human *N540K* (HCH) mutation.
- The *N534K* mutation was introduced into exon 12 of the mouse *Fgfr3* gene along with a NEO cassette flanked by LoxP recombination sites.
- The 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 and age of the mice, which makes oral gavage impractical.
- *Fgfr*$^{N534K/+}$ mice were treated from Day 1 (Day 0 = birth) to Day 15 with infigratinib 1 mg/kg sc every 3 days and sacrificed on Day 16 (intermittent dosing) or with infigratinib 1 mg/kg sc qd from Day 3 for 21 days (daily dosing).

In-vivo observations

- Clinical observations during treatment were performed every 3 days (hind limb movement, posture, tail, paws). Detailed 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 via caliper (VWRi819-0013, VWR, USA) at necropsy.

Histological and immunohistochemical assessment

- Samples were fixed and embedded in paraffin and the femur sections were stained and immunolabelled. For immunohistochemistry (performed for protocol 1 and protocol 2), sections were labelled with antibodies against Collagen type X.

CT scans

- µCT images of skulls were measured on a Skyscan-1172 (Bruker; 19.98 µm resolution, 100 ms exposure, 80 kV, 100 µA, 0.5° rotation step). Data were analyzed using Avizo software.

Statistical analysis

- Differences between experimental groups were assessed using Mann-Whitney U-test. The significance threshold was set at p≤0.05.
- Statistical analyses were performed using GraphPad PRISM (v5.03).

Results

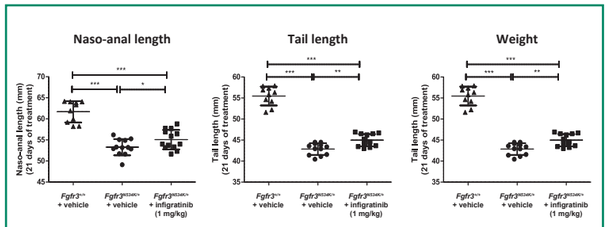
- In *Fgfr3*$^{N534K/+}$ mice, daily but not intermittent administration of infigratinib resulted in a statistically significant increase in all measured parameters vs vehicle-treated animals.

Table 1. Effect of intermittent and daily infigratinib on weight and growth

	% change	
	Intermittent dosing	Daily dosing
Tail length	+1.20 %	+4.52 %**
Naso-anal length	–0.41 %	+5.19 %*
Weight	+5.51 %	+14.55 %*
Skull length	–0.38 %	+3.81 %*
Skull width	+0.50 %	+1.74 %
Foramen magnum length	+2.23 %*	+3.72 %***
Foramen magnum width	–1.58 %	+0.67 %
Femur	–0.81 %	+3.16 %**
Tibia	–0.35 %	+3.18 %**
Humerus	+0.14 %	+3.04 %**
Ulna	–1.52 %	+2.94 %**
Radius	–0.47 %	+3.01 %**
L4-L6	+2.65 %	+4.78 %*

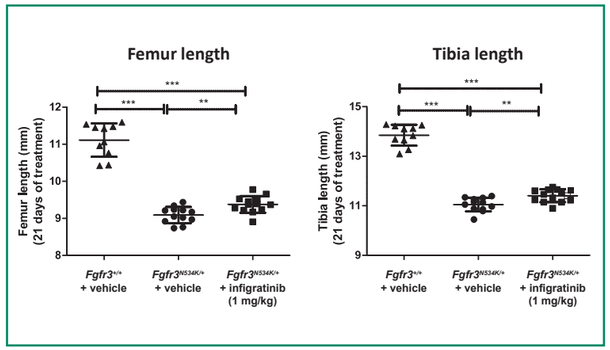
Intermittent: infigratinib 1 mg/kg q 3 days; daily: infigratinib 1 mg/kg daily in *Fgfr3*$^{N534K/+}$ mice. *p<0.05; **p<0.01; ***p<0.001 vs vehicle-treated animals.

Figure 2. Effect on axial skeleton – daily injection



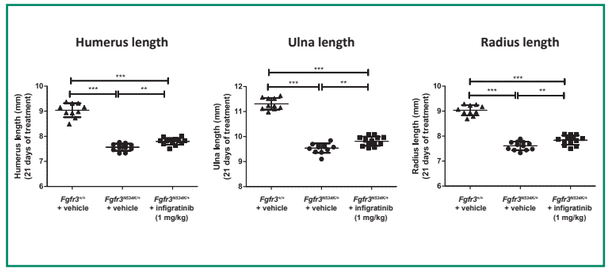
Dosing: infigratinib 1 mg/kg daily. *p<0.05; **p<0.01; ***p<0.001

Figure 3. Effect of daily infigratinib on appendicular skeleton – lower limbs



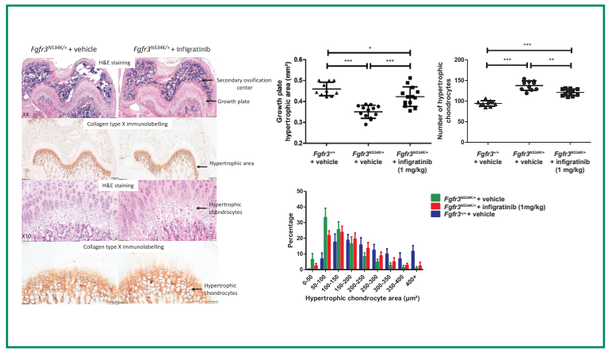
Dosing: infigratinib 1 mg/kg daily. **p<0.01; ***p<0.001

Figure 4. Effect of daily infigratinib on appendicular skeleton – upper limbs



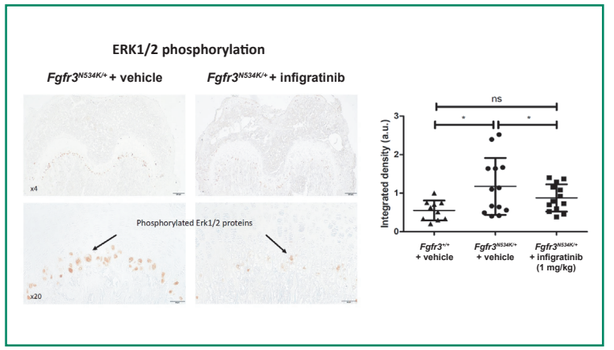
Dosing: infigratinib 1 mg/kg daily. **p<0.01; ***p<0.001

Figure 5. Effect of daily infigratinib on cartilage growth plate



- Infigratinib treatment improved the growth plate hypertrophic area (collagen type X immunolabelling). Treatment modified hypertrophic chondrocyte area and decreased the number of hypertrophic chondrocytes compared with untreated chondrocytes.

Figure 6. Effect of daily infigratinib on FGFR3 downstream signaling pathway (MAP kinase)



Dosing: infigratinib 1 mg/kg daily. *p<0.05

- ERK1/2 phosphorylation (MAP kinase pathway) in femoral growth plate was reduced by infigratinib treatment. The expression of phosphorylated ERK1/2 in treated mice is similar to that seen in the wild-type growth plate.

Discussion

- Intermittent treatment with infigratinib 1 mg/kg every 3 days did not modify endochondral and membranous ossification processes in the dwarf phenotype of the *Fgfr*$^{N543K/+}$ mouse.
- Daily infigratinib 1 mg/kg modified the whole skeleton in this mouse model of HCH:
  - Growth curves (naso-anal length, tail length, and weight) showed the efficacy of treatment after 21 days of injections.
  - Analysis of long bones after necropsy showed an impressive and significant modification of bone length. The shape and length of the skull were modified by treatment.
  - Infigratinib impacted the cartilage growth plate, significantly increasing the hypertrophic area and inhibiting the MAP kinase pathway (ERK1/2 phosphorylation) controlling the chondrocyte differentiation.
- These results demonstrate that daily infigratinib 1 mg/kg is able to counteract the constitutive activation of FGFR3 resulting from the heterozygous *N540K* mutation localized in the kinase 1 domain of FGFR3.
- **Conclusion:** These results provide a rationale for targeting FGFR3 with a TKI such as infigratinib for the treatment of children with HCH.

Acknowledgements

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