# TYRA-300 Demonstrates Significant Increases in Growth and Bone Length in a Mouse Model of FGFR3-Related Skeletal Dysplasias

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## Background

Achondroplasia (ACH) is the most common human skeletal dysplasia and cause of disproportionate short stature, affecting ~1 in 25,000 births.

Infants with ACH can face serious complications related to critical foramen magnum stenosis leading to cervicomedullary compression and requiring surgical intervention[1,2].

A specific mutation in FGFR3, G380R, causes over 99% of pediatric ACH[1,3,4,5].

FGFR3 is expressed in growth plate chondrocytes where it functions to regulate endochondral bone formation[5].

The G380R mutation, as well as other mutations, results in increased FGFR3 activity, which impairs chondrogenesis in the growth plate, disturbing long bone elongation[5].

### TREATMENT

There is currently only one approved treatment option for ACH. Vosoritide, a C-naturetic peptide analogue, acting exclusively on the MAP kinase pathway, was approved in 2021 as a daily injection to increase annual growth velocity in children with open growth plates.

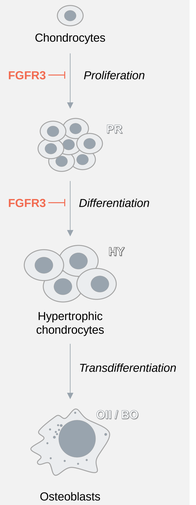
To provide an orally bioavailable therapy that acts specifically on the bone development pathway, infigratinib, a pan-FGFR1/2/3 inhibitor, was investigated in an Fgfr3$^{Y367C/+}$ mouse model[6,7] and is currently in clinical trials for ACH.

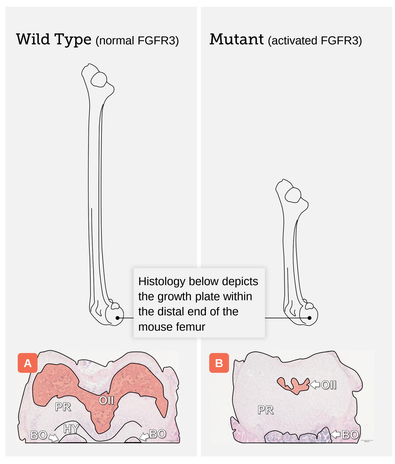
TYRA-300 is an oral, highly selective FGFR3 inhibitor currently undergoing a Phase 1/2 clinical trial, SURF301 (Study in Untreated and Resistant FGFR3+ Advanced Solid Tumors), which may provide a favorable therapeutic window with respect to anticipated toxicities compared to pan-FGFR inhibitors based on its specificity profile.

To assess the potential of TYRA-300 pre-clinically, we used a mouse model recapitulating most of the hallmarks of ACH. This Fgfr3$^{Y367C/+}$ driven mouse model is characterized by a disproportionate short stature and a growth deficit affecting both endochondral and membranous ossification[6,7,8,9,10].

### FGFR3 in ACH

FGFR3 plays an inhibitory role in the regulation of chondrocyte proliferation and differentiation.




These illustrations correspond to the histological sections at right. They illustrate the differences between zones of chondrocyte proliferation and differentiation in the Wild Type (A) and Mutant (B) in which FGFR3 is over-activated.

**PR:** Proliferating chondrocytes form clonal columns of cells that differentiate into prehypertrophic chondrocytes and then HY.

**HY:** Hypertrophic chondrocytes are master regulators of endochondral ossification. They undergo apoptosis or further differentiate.

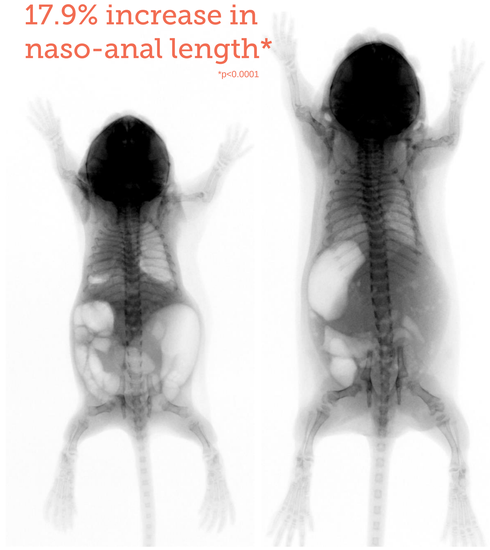
**OII / BO:** secondary ossification center / bone. Consisting of osteoblasts, the OII serves as protection for the growth plate.

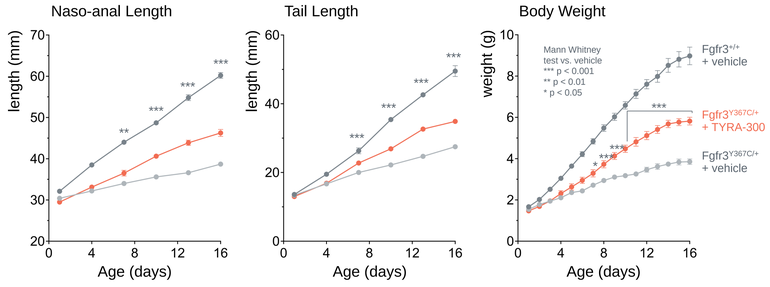
## Results

TYRA-300 increased bone growth in the Fgfr3$^{Y367C/+}$ mouse model of FGFR3-related skeletal dysplasia

17.9% increase in naso-anal length*

*p<0.0001






Data reflects separate experiments for TYRA-300 and infigratinib;

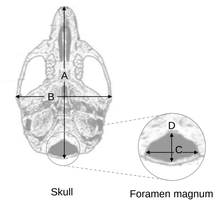
1. Daily subcutaneous injection of TYRA-300 at 1.2 mg/kg/day for 15 days starting at Day 1; 2. n=8 for TYRA-300 and n=10 for vehicle, after excluding three mice from the dataset when molecular analysis showed chimeric incorporation of mutation; 3. Data from Komra-Ebri et al 2016; 4. Demuynck, 2019; 0.167mg/kg human equivalent dose for 0.514mg/kg; 0.667mg/kg human equivalent dose for 2.058mg/kg; infigratinib human recommended phase 2 dose for ACH is 0.25mg/kg

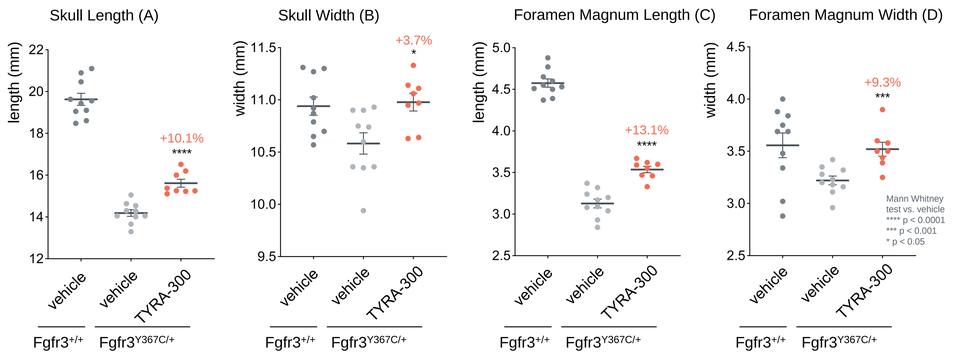
Increase in length compared to vehicle-treated Fgfr3$^{Y367C/+}$ mouse[1]

| | Dose (mg/kg/day) | Femur | Tibia | Humerus | Ulna | L4-L6 |
|---|---|---|---|---|---|---|
| TYRA-300[2] | 1.2 | 22.6%* | 33.0%* | 15.5%* | 23.5%* | 23.5%* |
| infigratinib[3] | 2.0 | 20.9% | 32.6% | 11.9% | 22.3% | 12.1% |
| infigratinib[4] | 0.5 | 10.4% | 16.8% | 7.3% | 11.1% | N/R |

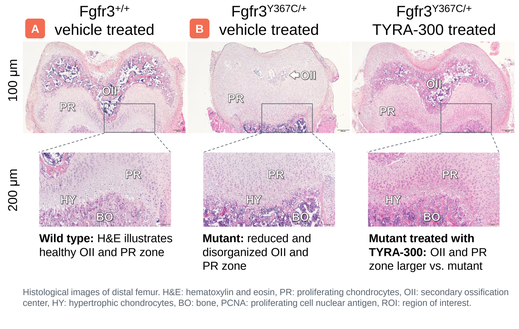
*p<0.0001

TYRA-300 improved the size and shape of the skull and foramen magnum




TYRA-300 improved proliferation and differentiation of chondrocytes within the femur growth plate

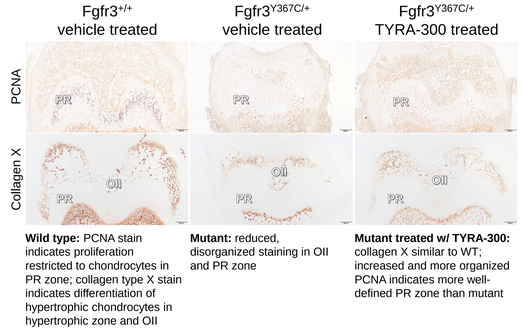



**Wild type:** H&E illustrates healthy OII and PR zone

**Mutant:** reduced and disorganized OII and PR zone

**Mutant treated with TYRA-300:** OII and PR zone larger vs. mutant

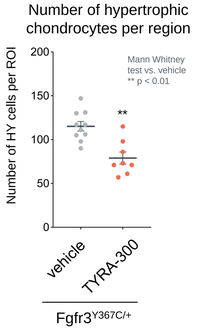
Histological images of distal femur. H&E: hematoxylin and eosin, PR: proliferating chondrocytes, OII: secondary ossification center, HY: hypertrophic chondrocytes, BO: bone, PCNA: proliferating cell nuclear antigen, ROI: region of interest.




**Wild type:** PCNA stain indicates proliferation restricted to chondrocytes in PR zone; collagen type X stain indicates differentiation of hypertrophic chondrocytes in hypertrophic zone and OII

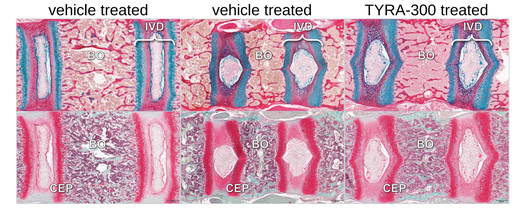
**Mutant:** reduced, disorganized staining in OII and PR zone

**Mutant treated w/ TYRA-300:** collagen X similar to WT; increased and more organized PCNA indicates more well-defined PR zone than mutant




## Results

TYRA-300 improved the architecture of the lumbar vertebrae




Histological images of lumbar vertebrae. IVD, intervertebral disc, BO: bone, CEP: cartilage endplate

**Wild type:** Stains indicate healthy lumbar vertebrae; Alcian Blue (blue = glycosaminoglycan), Sirius Red (red = collagen in bone), Safranin O (red = cartilage)

**Mutant:** modified CEP and IVD shape; disorganized architecture of nucleus pulposus and annulus fibrosus and reduced size of bone trabeculae vs. WT

**Mutant treated w/ TYRA-300:** modified shape of IVD and architecture of nucleus pulposus and annulus fibrosus compared to mutant, bone trabeculae mimics WT

TYRA-300 showed significant selectivity for FGFR3 and G380R

TYRA-300 vs. Ba/F3 Cellular $IC_{50}$ (nM)

| | infigratinib | TYRA-300 |
|---|---|---|
| FGFR1 | 15.3 | 113 |
| FGFR2 | 5.8 | 34.9 |
| FGFR3 | 6.9 | 1.8 |
| FGFR4 | 459 | 98.4 |

NanoBRET™ binding assay $IC_{50}$ (nM)

| | infigratinib | TYRA-300 |
|---|---|---|
| FGFR3 WT | 24 | 21 |
| FGFR3 G380R | 22 | 21 |

Fold Selectivity for FGFR3

| | | |
|---|---|---|
| FGFR1 | 2.2x | 63x |
| FGFR2 | 0.8x | 19x |
| FGFR4 | 67x | 55x |

TYRA-300 shows significant selectivity for FGFR3 over other FGFR isoforms

## Conclusions

TYRA-300 increased bone length of the appendicular and axial skeleton in the Fgfr3$^{Y367C/+}$ mouse model.

Improvements in the foramen magnum diameter were observed with TYRA-300.

Histological staining indicated that TYRA-300 restored the architecture of the growth plate by improving proliferation and differentiation of chondrocytes.

TYRA-300 was equally active against the FGFR3 G380R mutant and wild-type FGFR3 in a NanoBRET™ binding assay.

The FDA granted TYRA-300 Orphan Drug Designation for the treatment of ACH.

Using the data from SURF-301 and additional preclinical data, TYRA expects to submit an IND to initiate a Phase 2 clinical study in pediatric achondroplasia in 2024.

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