FGFR-selective tyrosine kinase inhibitors, such as infigratinib, show potency and selectivity for FGFR3 at pharmacologically relevant doses for the potential treatment of achondroplasia

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#5126

Background

- Germline mutations in fibroblast growth factor receptor genes 1–3 (*FGFR1–3*) can cause skeletal dysplasias and craniosynostoses.
- Mutations in *FGFR2* (e.g. *S351C, Y375C, S252W*) and *FGFR3* (e.g. *G380R, K650E, N540K, Y373C*) are known to cause skeletal dysplasias including craniosynostoses, short-stature syndromes such as achondroplasia and hypochondroplasia, and thanatophoric dysplasia.
- Over the past decade, several FGFR1–3 tyrosine kinase inhibitors (TKIs), such as infigratinib (BGJ398), AZD4547, and PD173074 have been studied in a variety of preclinical models of FGFR-driven skeletal dysplasias.
- Achondroplasia is the most common form of disproportionate short stature driven by an *FGFR* genetic alteration. It is most commonly caused by an autosomal dominant *G380R* substitution in *FGFR3*.[1]
- Achondroplasia is the most frequently studied FGFR-driven skeletal dysplaisa although, to date, no study has comprehensively examined the literature regarding the potential therapeutic usage of FGFR1–3 TKIs in achondroplasia or other FGFR-driven skeletal dysplasias.

Purpose:

- Explore the publicly available literature to evaluate the dose dependency and toxicity profiles of FGFR-selective TKIs in preclinical skeletal dysplasia models.
- Evaluate, based on the comprehensive non-clinical evidence of safety and efficacy of FGFR-selective TKIs, the potential for a therapeutic option in FGFR-driven skeletal dysplasias.

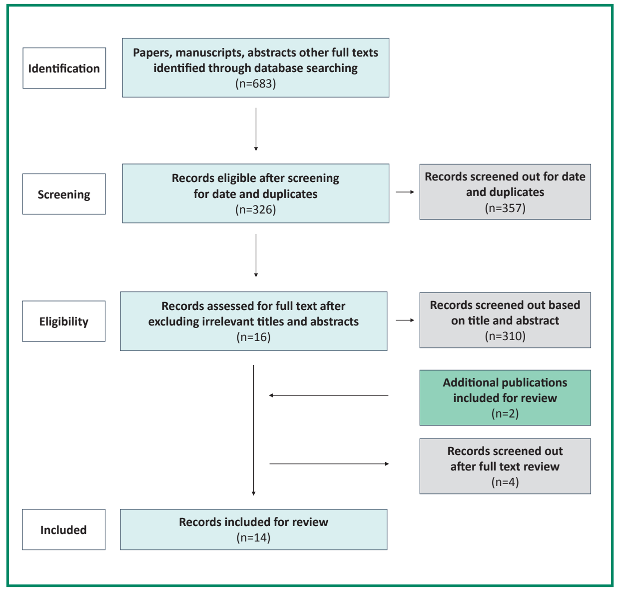
Methods

- A systematic literature review was performed to investigate non-clinical data from studies of infigratinib and other FGFR-selective TKIs relevant to FGFR-driven skeletal dysplasias.
- Two major types of sources were searched on October 22/23 2019:
  - Major databases (e.g., PubMed, Medline [NLM Catalog]) were searched for relevant articles from the past 10 years.
  - Conference archives (e.g., ENDO, ESPE, ISDS, ASHG, ASBMR) were searched for relevant abstracts from the past 5 years.
- Full text was included where possible.
- Key words used in the searches included, but were not limited to, the following:
  - Achondroplasia.
  - Skeletal dysplasia.
  - FGFR inhibition.
  - Infigratinib.
  - BGJ398.
  - AZD4547.
  - PD173074.
  - Tyrosine kinase inhibitor.
- Eligibility criteria for inclusion was determined in advance to exclude content not relevant to the purpose of this literature review.

Results

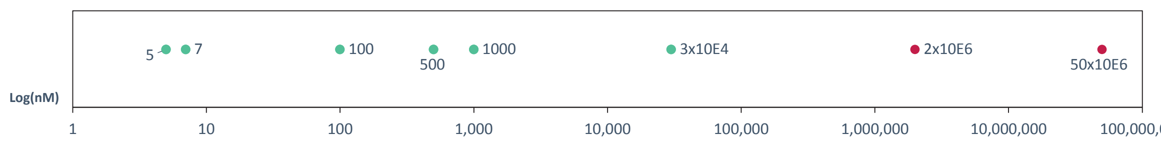
- 14 publications were included in this review (Figure 1).
  - 683 publications were identified through the initial search, with 326 remaining after screening for date and duplicates.
  - 310 publications were excluded based on title and abstract, leaving 16 remaining to assess full text.
  - Of the 16 publications reviewed as full texts, four were excluded given focus outside the scope of this literature review (e.g., focus on targets other than FGFR, discussion of therapeutic space without provision of new non-clinical data).
  - Two additional publications found through reference review of identified publications were included due to direct relevance.

Figure 1. Literature review flow chart



- Efficacy and toxicity findings were concentration- or dose-dependent (illustrative subset shown in Figure 2).
- FGFR-selectivity of TKIs included in this review varied, with FGFR3 IC_{50} ranging from 1.0 nM (infigratinib[2]) to 4.5 nM (ARQ 087[3]) for FGFR-selective compounds, and from 5 nM (PD173074[4]) to 190 nM (A31[5]) to 500 nM (NF449[a,6]) for non-selective compounds.
- Infigratinib was the most-commonly identified TKI, with eight publications on preclinical data in models of skeletal dysplasias. Key results for infigratinib show:
  - FGFR3 IC_{50} 1.0 nM, FGFR3-K650E IC_{50} 4.9 nM.[2]
  - *In-vitro* data: inhibition of FGFR1–3 activity at concentrations ranging from 5 to 500 nM, including reversal of established growth arrest in chondrocytes at 7 nM and an 'optimal concentration' of 5–10 nM (Figure 2).[7]
  - *Ex-vivo* data: overgrowth of ACH mouse femurs vs. WT at $1x10^3$ nM.[8]
  - *In-vivo* studies: dose-dependent improvements in foramen magnum and long bone length in *Fgfr3*[Y367C/+] mice at SC doses of 0.2–2 mg/kg/day (Figure 3).[8,9]
  - No preclinical studies reported a survival disadvantage and one showed a significant survival advantage for infigratinib-treated ACH mice (Figure 4).[9]
- In relation to other FGFR TKIs besides infigratinib:
  - Off-target biochemical effects on other RTKs were reported for most of these agents across studies, with the exception of ARQ 087, which was reported to only slightly inhibit KIT, FLT4, and TYRO3 at 500 nM (FGFR1–3 IC_{50} 1.8–4.5 nM).[3]

Figure 2. Representative data: concentrations of FGFR inhibitors explored preclinically in models of skeletal dysplasia

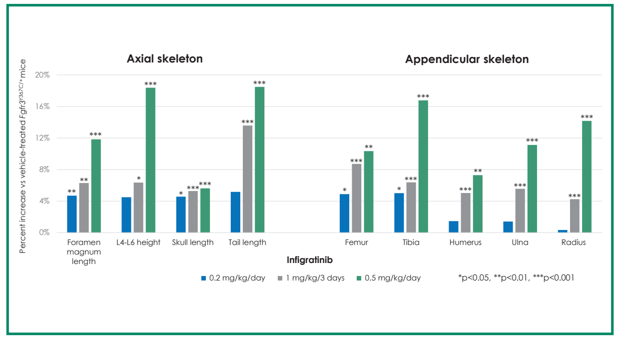


nM (drug)	5 (infigratinib)	7 (infigratinib)	100 (infigratinib)	500 (infigratinib)	1000 (infigratinib)	6000 – $3x10^4$ (NF449[a])	$1x10^6$ – $2x10^6$ (AZD4547)	$1x10^6$ – $50x10^6$ (PD173074, PD161570)
Efficacy	Rescue of FGF2-mediated inhibition of chondrocyte proliferation; optimal active concentration noted at 5-10 nM	Complete reversal of fully-established growth arrest of RCS chondrocytes	Complete rescue of bone growth defect of Fgfr3[Y367C/+] embryonic femurs *ex vivo*	Rescue of FGF2 growth-inhibited phenotype; restoration of normal growth plate architecture including Col10a1 expression in embryonic tibia cultures	Bone overgrowth vs. wt of Fgfr3[Y367C/+] embryonic femurs *ex vivo*	Significant rescue of growth arrest phenotype in RCS chondrocytes	None; significant shortening of long bones and skull bones in newborn wt mice	None; shortening of right wing or mandible in chicken embryos (HH25-29)
Toxicity	None noted	None noted	None noted	None noted	None noted	No apparent cellular toxicity throughout range	Lethality	Limb, mandible malformation
Study	*In vitro*	*In vitro*	*Ex vivo*	*Ex vivo*	*Ex vivo*	*In vitro*	*In vivo*[b]	*Ex vivo*
Ref.	Gudernova et al. 2016[7]	Gudernova et al. 2016[7]	Komla-Ebri et al. 2016[8]	Gudernova et al. 2016[7]	Komla-Ebri et al. 2016[8]	Krejci et al. 2010[4]	Gudernova et al. 2016[7]	Horakova et al. 2014[11]

[a]Despite being referenced as an FGFR inhibitor in multiple papers, NF449 has more recently been characterized as a selective P2X1 receptor (P2RX1) antagonist.[6]
[b]In this study, AZD4547 was injected intraperitoneally.

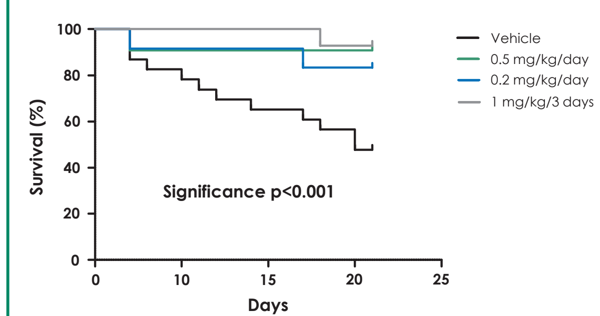
Representative data reflected.
Concentrations with reported efficacy
Concentrations with reported toxicity

Figure 3. Dose response with infigratinib in an achondroplasia mouse model[9]



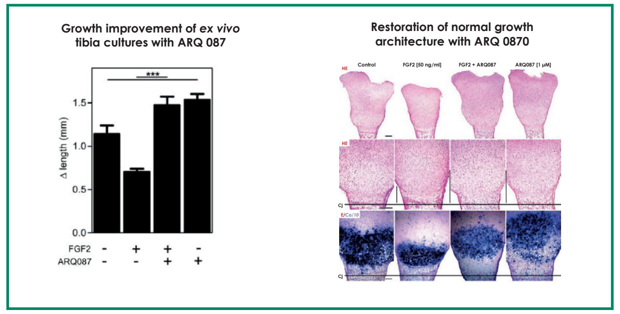
- *In-vitro* data show rescue of RCS cells from FGF2-mediated inhibition of proliferation at concentrations ranging from 10 nM for AZD4547 to 15 nM for PD173074.[7]
- One study of PD173074 (100 nM) showed significant rescue of primary cilia (PC) length in chondrocytes from FGFR3[Y367C/+] mice to 96% of the length observed in control chondrocytes, and in human fetal TD chondrocytes to 94% of that observed in control chondrocytes.[10]
- *Ex-vivo* data in one study demonstrated restoration of normal growth plate architecture and ~50% growth improvement compared with controls in mouse tibia cultures treated with $1x10^3$ nM ARQ 087, both in the presence and absence of FGF2 (Figure 5).[3]
- *In-vivo* data: one study showed that AZD4547 decreased survival in newborn wild-type mice (CD1) treated at doses of $1x10^6$ to $2x10^6$ nM.[7] Another study showed limb malformation in chicken embryos treated with PD173074 or PD161570 at doses of $1x10^6$ to $50x10^6$ nM, and increased embryo mortality above $1x10^6$ nM.[11]

Figure 4. Survival benefit with infigratinib in an achondroplasia mouse model[9]



	Number of mice at P1	Number of mice at P16
0.5 mg/kg/day	12	10
0.2 mg/kg/day	12	10
1 mg/kg/3 days	14	13
Vehicle	23	11

Figure 5. *Ex-vivo* improvement in tibia growth and Col10a[3]



Conclusions

- While two studies suggest toxicity with FGFR-selective TKIs; this was produced at doses significantly higher than pharmacologically relevant for the treatment of achondroplasia or other skeletal dysplasias.
- *In-vivo* studies in an achondroplasia mouse model treated with low doses of infigratinib showed increase in growth of long bones and foramen magnum with a good dose-response relationship. No toxic effects were observed at these low but efficacious doses.
- One study demonstrated a survival advantage in *Fgfr3*[Y367C/+] mice treated with infigratinib.

Clinical relevance:

Given the totality of evidence, low-dose infigratinib appears to be a potentially safe option for further development in children with achondroplasia.

Acknowledgements

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The data shown in this poster were accepted but not presented at ENDO 2020 as the conference was cancelled amid concerns about COVID-19