In-Vivo Characterization of a Selective FGFR2 Inhibitor with Potency Against Gatekeeper and Molecular Brake Mutations

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Background

- The family of fibroblast growth factor receptors (FGFRs) consists of four transmembrane receptor tyrosine kinases, FGFR1-FGFR4.[1]
- Ligand binding leads to receptor dimerization and phosphorylation to activate down stream signaling.
- FGFR signaling impacts key cellular processes including cell survival, proliferation, migration and differentiation.
- FGFR2 alterations are well-established oncogenic drivers across tumor types (Figure 1).
- These alterations are present in 2% of all cancers, 80% of which are activating mutations.[2]

Figure 1. FGFR2 Alteration Frequency by Tumor Types

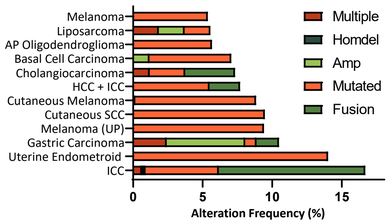


Table 1. Clinical Features and Clinical Coverage of Pan-FGFR Inhibitors

Clinical Compound	Dose Schedule	ORR	Hyper-Phosphatemia	Stomatitis	Indication	Approved FGFR2 alteration
Pemigatinib[3,4]	2 wk on/ 1 wk off	36%	94%	35%	Adv/met ICC	FGFR2 fusion
Infigratinib[5,6]	3 wk on/ 1 wk off	23%	90%	56%	Adv/met ICC	FGFR2 fusion
Erdafitinib[7]	Daily Monitor tolerability	32%	76%	56%	Adv/met UC	FGFR2 fusion
Futibatinib[8]	Daily Monitor tolerability	42%	85%	30%	Adv/met ICC	FGFR2 fusion

UC, Urothelial Carcinoma; ICC, Intrahepatic Cholangiocarcinoma

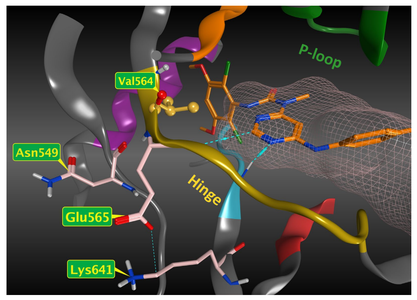
- Approved pan-FGFR inhibitors show on target toxicities. The most common DLT for these inhibitors, FGFR1 mediated hyperphosphatemia, was observed in >75% of patients regardless of the clinical compound (Table 1).
- These drugs are not approved outside of ICC/UC or for patients with FGFR2 activating and resistant point mutations.
- The gatekeeper V564X (43% of patients) and molecular brake N549K (48% of patients) mutations are the main mechanisms of resistance to existing therapies.[9-13]

Goal

Identify a selective FGFR2 inhibitor, with coverage of activating and resistance mutations, which avoids FGFR1-mediated hyperphosphatemia

Results

Figure 2. CGT3103 Maintains Mutant Activity Due to Less Steric Bulk Near the Val564 Gatekeeper Residue in the ATP Binding Pocket



- The tetra-substituted phenyl group of infigratinib (orange structure) extends into the hydrophobic pocket close to the gatekeeper amino acid Val564.
- V564 gatekeeper mutations impart a steric clash with infigratinib, and related first generation FGFR compounds, reducing activity toward these mutants.
- In-house co-crystal structure of CGT3103 FGFR2-N549K shows that CGT3103 (pink mesh) has decreased steric bulk near the gatekeeper residue and maintains activity towards these mutants as well as the molecular brake mutations.
- CGT3103 is a non-covalent reversible inhibitor.

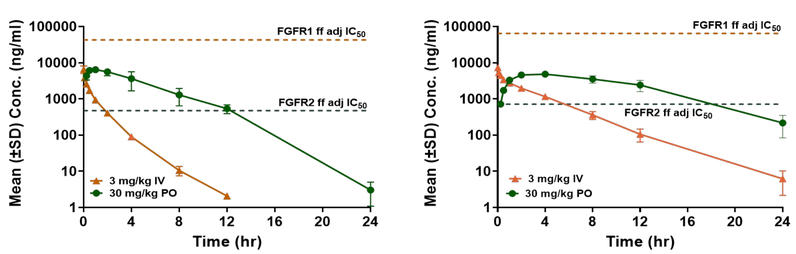
Table 2. FGFR2 Mutations are not Addressed by Approved Inhibitors

	pFGFR Inhibition IC_{50} (nM)					
Target	*Pemigatinib*	*Infigratinib*	*Futibatinib*	*RLY-4008**	*CGT1672**	*CGT3103**
FGFR2-WT	*2*	*4*	*2*	*4*	*12*	*11*
FGFR1-WT	*5x*	*3x*	*2x*	*228X*	*23x*	*94x*
FGFR2-V564F	*>500x*	*>250X*	*75x*	*0.125x*	*0.67x*	*3x*
FGFR2-V564I	*29X*	*>250X*	*1x*	*11x*	*1x*	*4x*
FGFR2-N549K	*40X*	*84x*	*1x*	*3x*	*1x*	*3x*
chemotype	*reversible*	*reversible*	*covalent*	*covalent*	*reversible*	*reversible*

* FBS Adjusted IC_{50}s

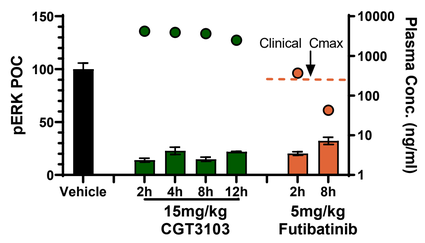
- Table 2 compares mechanistic cellular IC_{50}'s for CGT3103 to approved and clinical stage FGFR2 inhibitors.
  - (x) represents fold changes vs. FGFR2-WT cellular IC_{50}
- CGT3103 has 94x selectivity for WT FGFR2 over FGFR1 leading to reduced hyperphosphatemia risk compared to approved agents and a selectivity improvement over the previous Cogent lead CGT1672.[14]
- Cellular potency is maintained for the FGFR2 acquired resistance gatekeeper and molecular brake mutations.
- Known clinical and commercial stage FGFR2 inhibitors have FGFR1 selectivity and/or target coverage issues which prevent optimal clinical efficacy toward both gatekeeper and molecular brake mutations.
- Futibatinib and RLY-4008 are covalent inhibitors, potential resistance liability is discussed in Figure 6.

Figure 3. CGT3103 Showed FGFR2 Target Coverage in Mouse and Rat PK Studies



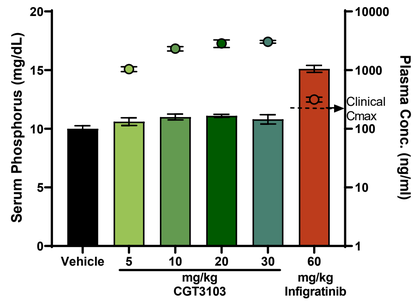
- Exploratory PK studies of CGT3103 dosed in mice and rats at 3 mg/kg IV and 30 mg/kg PO showed 12-hour coverage of the free fraction adjusted FGFR2-WT cellular IC_{50} in both species.
- CGT3103 remained below threshold of free fraction adjusted FGFR1 cellular IC_{50}, indicating a therapeutic window for efficacy without hyperphosphatemia.

Figure 4. CGT3103 Shows 12h inhibition of pERK in the Clinically Relevant AN3 CA (K310R/N549K) Mouse Model



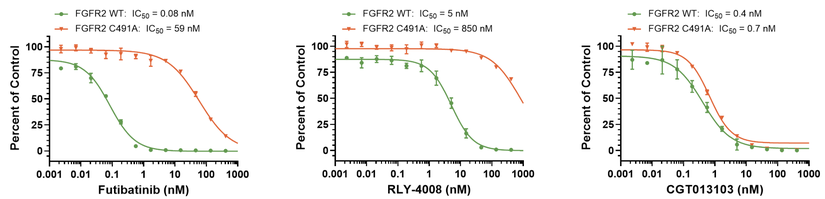
- Figure 4 shows inhibition of pERK following a single dose of CGT3103 or futibatinib in the AN3 CA PK/PD mouse model.
- Tumor samples were analyzed for pERK via western blot and normalized to GAPDH with three animals per group.
- CGT3103 shows robust 12-hour inhibition of pERK comparable to futibatinib.

Figure 5. CGT3103 Does Not Cause Hyperphosphatemia at Efficacious Plasma Concentrations



- Test compounds were dosed QD PO for three days in the rat hyperphosphatemia model. Serum phosphate and drug levels were measured 4 hours post final dose.
- CGT3103 showed no increase in serum phosphate at plasma concentrations comparable to efficacious levels observed in the PK/PD model (Figure 4).
- By comparison, treatment with infigratinib, at clinically relevant concentrations, resulted in a 50% increase in serum phosphate.
- Futibatinib also caused ≥50% increase in serum phosphate when dosed at clinically relevant concentrations in this model (data not shown).

Figure 6. CGT3103 Retains Enzymatic Activity vs. the C491A Cysteine Mutation



- Resistant mutations emerge over time in patients treated with covalent inhibitors such as ibrutinib (BTK)[15] and osimertinib (EGFR).[16] These mutations often occur at the cysteine site of covalent modification resulting in loss of inhibitor activity and resistance to therapy.
- By analogy, FGFR2 C491A protein was generated as a potential resistance mutation formed by treatment with a covalent FGFR inhibitor.
- Comparison of inhibition of WT FGFR2 and mutant C491A values gives a relative susceptibility to loss of activity against a C491 mutation.
- The covalent inhibitors futibatinib and RLY-4008 showed >100x shift of IC_{50} values between FGFR2 WT and mutant FGFR2 C491A.
- **The non-covalent inhibitor, CGT3103, maintained potency in the mutant C491A assay compared to the WT FGFR2 enzyme assay.**

Conclusions

The FGFR2 inhibitor CGT3103 exhibits:

- Less steric clash near the Val564 gatekeeper residue in the ATP binding pocket
- Inhibition of FGFR2 primary and acquired gatekeeper (V564X) and molecular brake (N549K) mutations
- Coverage of FGFR2 IC_{50} with a window for selectivity over FGFR1 dosed PO at 30 mg/kg in both mouse and rat PK studies
- Maximal PD effect in the AN3 CA (K310R/N549K) model when dosed PO at 15 mg/kg
- No increase serum phosphate levels when dosed to efficacious concentrations in rats
- No shift in activity in an FGFR2 C491A enzyme assay, a potential mutation of concern for covalent inhibitors

This series of analogs are the first publicly disclosed FGFR1 sparing, reversible FGFR2 inhibitors that address all the major activating and resistance mutations.

Work continues to identify a clinical candidate

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DISCLOSURES: All authors are employees of Cogent Biosciences

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