Comparative Biochemical Kinase Activity Analysis Identifies Lirafugratinib as a Highly Selective FGFR2 Inhibitor

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Poster # 294

Presented at
AACR 2026 Annual Meeting
April 17-22, 2026

BACKGROUND

- Genomic alterations in the fibroblast growth factor receptor (*FGFR*) gene occur across tumor types.[1]
- Cholangiocarcinoma (CCA) represents about 3% of all gastrointestinal malignancies worldwide, yet the clinical burden is disproportionately high due to late-stage diagnosis and limited therapeutic options.[2]
- While chemotherapy is the cornerstone for first-line therapeutic management of advanced biliary tract cancer (BTC), the median overall survival rarely exceeds 1 year.[2,3]
- Molecular profiling has shown that BTCs are enriched with potentially actionable genomic alterations including *FGFR2* fusions and rearrangements.[4]
- Standard-of-care for second-line FGFR fusion-positive CCA is treatment with a pan-FGFR inhibitor.[5]
- Recent efforts have focused on the development of a selective and covalently binding FGFR inhibitor to improve therapeutic efficacy, safety, and tolerability.
- Lirafugratinib, a highly selective and irreversible FGFR2 inhibitor, was designed to target oncogenic *FGFR2* alterations to enhance efficacy outcomes while limiting toxicity due to off-target inhibition of FGFR isoforms, mainly, FGFR1 and FGFR4 that are associated with hyperphosphatemia and diarrhea, respectively.[6]
- The purpose of this analysis was to compare the relative selectivity of lirafugratinib to 3 FDA-approved tyrosine kinase inhibitors (TKIs) and to a TKI in development, AZD4547 (fexagratinib), in a head-to-head biochemical study.

FGFR Inhibitors Used for Kinome Scanning Analysis

	Lirafugratinib[7]	Futibatinib[8]	Pemigatinib[9]	Erdafitinib[10]	AZD4547 (Fexagratinib)[11,12]
Isoform	FGFR2	FGFR1, 2, 3, 4	FGFR1, 2, 3	FGFR1, 2, 3, 4	FGFR1, 2, 3
Bond	Covalent	Covalent	Non-covalent	Non-covalent	Non-covalent
Indication	Proposed: CCA harboring an *FGFR2* f/r who have not received FGFRi	Intrahepatic CCA harboring *FGFR2* f/r	• CCA with an *FGFR2* f/r; • Relapsed or refractory MLNs with *FGFR1* rearrangement	Urothelial carcinoma with susceptible *FGFR3* genetic alterations	Cancer harboring aberration(s) in *FGFR1-3*
FDA Status	NDA submitted Jan 2026	Accelerated approval 2022	Accelerated approval for CCA, 2020; Approved for MLNs, 2022	Approved 2019	In development

CCA, cholangiocarcinoma; *FGFR2* f/r, FGFR2 fusion or other rearrangement; FGFRi, fibroblast growth factor receptor inhibitor; MLNs, myeloid/lymphoid neoplasm; NDA, new drug application

METHODS

Kinome Scanning Analysis

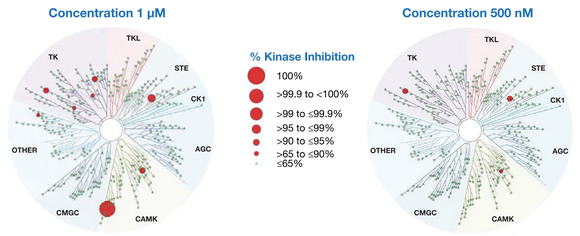
- A KINOMEscan profiling service scanMAX[13] and TREEspot[14] interaction maps were used for evaluation and visualization of inhibition of kinases.
- A panel of 468 human target kinases and disease-relevant kinase mutant variants was tested at a concentration of 500 nM for lirafugratinib and the 4 other pan-FGFR inhibitors (futibatinib, pemigatinib, erdafitinib, and fexagratinib).
- The kinome scanning analysis was also performed with 1 µM lirafugratinib using the same panel of 468 kinase targets.
- The 90% and 75% inhibition thresholds were selected based on established and published research methodologies related to historic kinome mapping.[15,16]

IC_{50} Determination

- Biochemical inhibition of FGFR1, 2, 3, and 4 was determined using Caliper Mobility-Shift Assay (PerkinElmer) with a fluorescently labelled peptide substrate.[17]
- The average IC_{50} (half-maximal inhibitory concentration) of 3 lots of lirafugratinib were assayed and reported in 3 independent experiments.
- After the kinome scanning analyses, IC_{50} values of lirafugratinib were determined using a range of 10 concentrations with a top concentration of 10 µM using both of the following:
  - SSBK-LanthaScreen Eu Kinase Binding Assay for QSK, MEK5, and MKNK2[18]
  - SSBK-Z'-LYTE Screening Assay for FGFR1, 2, and 3, MUSK, FLT3, and TNK1[19]

RESULTS

KINOMEscan Result of Lirafugratinib Visualized by TREEspot™ Interaction Maps



Kinome Analysis: >75% Inhibition of Kinases by Lirafugratinib

Concentration 1 µM

Kinase	% Inhibition
QSK (SIK3)[+]	100
MEK5 (MAP2K5)	97
FGFR2	**94**
MUSK[+]	93
MKNK2 (MNK2)	91
FLT3	85
TNK1	81
FLT3 (N841I)	80

Inhibition of 22%, 4%, and 0% for FGFR1, FGFR3, and FGFR4, respectively

Concentration 500 nM

Kinase	% Inhibition
FGFR2	**94**
MEK5 (MAP2K5)	92
MKNK2 (MNK2)	89

Inhibition of 20%, 21%, and 14% for FGFR1, FGFR3, and FGFR4, respectively; 0% inhibition for MUSK[+]; 19% inhibition for QSK[+], 52% inhibition for TNK1, and 74% inhibition for FLT3(N841I)

IC_{50} of Lirafugratinib

Kinase	IC_{50} (µM)
QSK (SIK3)[+]	>10[a]
MEK5 (MAP2K5)	0.84[a]
FGFR2	**0.15[b]**
MUSK[+]	>10[b]
MKNK2 (MNK2)	0.15[a]
FLT3	0.42[b]
TNK1	5.91[b]
FGFR1	7.55[b]
FGFR3	6.37[b]

[a]Evaluated using SSBK-LanthaScreen Eu Kinase Binding Assay;
[b]Evaluated using SSBK-Z'-LYTE Screening Assay

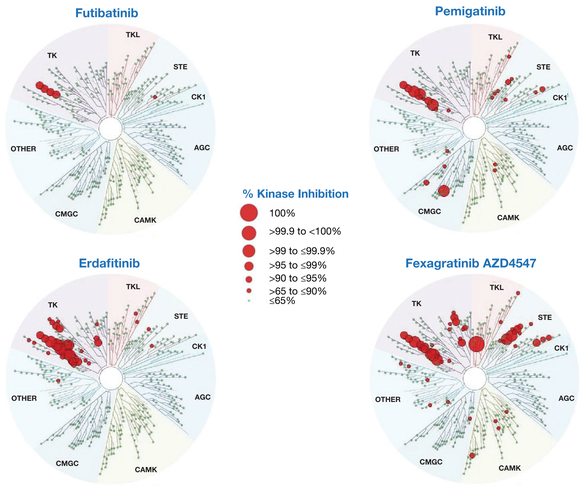
[+]High inhibition of QSK and MUSK was observed in the kinome analysis at 1µM of lirafugratinib; however, these findings were not reproduced at 500 nM or in IC_{50} determination assays. Therefore, these targets are not considered relevant for off-target potential.

Summary of Lirafugratinib Selectivity and Margins Against FGFR1, FGFR2, FGFR3, and FGFR4

Kinase	IC_{50} (nM)	Selectivity	Margin[a]
FGFR1	864.27	275	144
FGFR2	3.14	---	0.5
FGFR3	274.10	87.3	46
FGFR4	17,633.30	5616	2939

FGFR, fibroblast growth factor receptor; IC_{50}, half-maximal inhibitory concentration.
[a]Margin = IC_{50}/clinical unbound C_{max} at 70 mg QD (which is 3.3 ng/mL or 0.006 µM)

KINOMEscan Results of US-approved FGFR Inhibitors and AZD4547 at 500 nM Visualized by TREEspot™ Interaction Maps



Kinome Analyses: >75% Inhibition of Kinases by Futibatinib and Pemigatinib at 500 nM

Futibatinib

Kinase	% Inhibition
FGFR1	99
FGFR3 (G697C)	98
FGFR4	98
FGFR3	97
FGFR2	97
MKK7	83

Pemigatinib

Kinase	% Inhibition	Kinase	% Inhibition
ERK5	100	EIF2AK1	90
CSF1R	100	DCAMKL3	88
FGFR3 (G697C)	99	FLT1	88
FGFR1	99	MST4	88
FGFR4	99	VEGFR2	88
FGFR3	99	PIP5K2C	83
FGFR2	97	MLK2	82
FLT4	96	PDGFRB	82
MST3	94	TAOK1	81
MAP3K2	94	CSF1R-autoinhibited	80
CDK2	91	CASK	76
		CDK7	76

Kinome Analyses: >90% Inhibition of Kinases by Erdafitinib and AZD4547 at 500 nM

Erdafitinib

Kinase	% Inhibition
ABL1(H396P)-nonP, FLT4, RET, RET(M918T), CSF1R, PDGFRB, KIT(V559D), FGFR3(G697C), ABL1(Q252H)-nonP, KIT, VEGFR2, ABL1(Y253F)-P	100
ABL1-P, ABL1(H396P)-P, **FGFR1**, **FGFR4**, ABL1(Q252H)-P, **FGFR3**, ABL1(E255K)-P	99
ABL1(M351T)-P, LCK, ABL1-nonP, BLK, **FGFR2**, EPHA1, KIT(V559D, T670I), PDGFRA	98
DDR1, KIT(L576P), FLT1	97
RET(V804M)	95
BRAF(V600E)	94
HCK, EPHB6, EPHA8, KIT(D816V)	92
DDR2	91

Plus 10 kinases with >75% inhibition

AZD4547

Kinase	% Inhibition
PIP5K2C, RIPK1, CSF1R, KIT(V559D), KIT, PDGFRB	100
FGFR1, LOK, FGFR3(G697C), HPK1, KIT(L576P), VEGFR2, **FGFR3**	99
TRKA, TRKB, RET(M918T), RET	98
FGFR2, **FGFR4**, FLT1, MAP4K5, FLT3(D835V), YSK4	97
KIT(V559D, T670I), DDR1	96
FLT3(ITD, F691L)	94
MAP3K2, FLT3(D835Y), MAP4K3	93
SNARK, MAP3K3, CSF1R-autoI, ABL1(T315I)-nonP	92
INSRR, RET(V804M), MAP4K2, KIT-autoI	91

Plus 21 kinases with >75% inhibition

autoI, autoinhibited; nonP, nonphosphorylated; P, phosphorylated

DISCUSSION

MEK5, MKNK2, and FLT3

Mitogen-activated protein (MAP) kinase kinase 5 (MAP2K5 [MEK5])

- *MEK5* encodes a dual specificity protein kinase that belongs to the MAP kinase kinase family and specifically interacts with and activates MAPK7/ERK5.
- Deregulation of the *MEK5* pathway has been implicated in metastatic prostate cancer, colon cancer, and invasive osteosarcoma, demonstrating a broad range of application across various cancer types.
- Preclinical studies show that inhibition of the *MEK5* cascade reduces intravascular invasion, circulating tumor cells, and metastatic lesion formation, underscoring its role in tumor progression and metastasis.[20]
- *MEK5* signaling is strongly linked to chemoresistance.

Mitogen-activated protein kinase (MAPK)-interacting serine/threonine kinase 2 (MKNK2 [MNK2])

- *MKNK2* encodes a member of the calcium/calmodulin-dependent protein kinase (CAMK) serine/threonine protein kinase family.
- *MKNK2* is one of the downstream kinases activated by MAPKs.
- *MKNK2* phosphorylates the eukaryotic initiation factor 4E (eIF4E), thus playing important roles in the initiation of mRNA translation, oncogenic transformation, and malignant cell proliferation.

Fms-related receptor tyrosine kinase 3 (FLT3)

- *FLT3* encodes a class III receptor tyrosine kinase that regulates hematopoiesis.
- *FLT3* regulates hematopoietic cell proliferation and differentiation. *FLT3* activates downstream signaling, including PI3K/AKT/mTOR, RAS/MAPK, and JAK/STAT.

Lirafugratinib demonstrates high selectively to FGFR2 compared with MEK5, MKNK2, and FLT3.

- The kinase inhibition of off-targets MEK5, MKNK2, and FLT3 lack translational relevancy due to:
  - Lirafugratinib covalently binds to FGFR2, unlike MEK5, MKNK2, and FLT3, which bind noncovalently.
  - The lack of a nucleophilic cysteine in the P-loop of the adenosine triphosphate pocket prevents covalent binding by MEK5, MKNK2, and FLT3[21] allowing for the potential of these kinases *in vivo* to be reversible and exposure-dependent.
  - Toxicology studies in rats and dogs showed lirafugratinib characterized by on-target FGFR2-related effects that are predictable and manageable in the clinical setting.
- MEK5, MKNK2 and FLT3 are known to be involved in tumorigenesis. Thus, inhibition of these kinases, in addition to FGFR2, may enhance the antitumor properties of lirafugratinib.

Lirafugratinib demonstrates high selectivity to FGFR2 compared with FGFR1, 3, and 4 in fundamental and clinical research.

- Lirafugratinib is 80- to >5000-fold more selective for FGFR2 over other FGFR family members; large margins to the unbound C_{max} at 70 mg QD are observed for FGFR1, 3, and 4, compared to FGFR2.
- In the repeat-dose toxicity studies in rats and dogs, hyperphosphatemia and soft tissue mineralization occurred only at non-tolerable dose levels of lirafugratinib. Lirafugratinib-related stool findings were observed only in the 28-day dog study, but not in the 91-day dog study or in the 28-day and 92-day studies in rats.
- In the clinical study with lirafugratinib, reported events of hyperphosphatemia and diarrhea were much lower in the 70 mg QD safety population (N=385) compared with those reported for pemigatinib and futibatinib.
- A side-by-side summary from clinical trials shows that lirafugratinib had lower adverse events of hyperphosphatemia and diarrhea than futibatinib or pemigatinib.

TEAEs (%)	Lirafugratinib[7]		Futibatinib[8]		Pemigatinib[9]	
	All Grades	Grade ≥3	All Grades	Grade ≥3	All Grades	Grade ≥3
Hyperphosphatemia	**21%**	0%	88%	Not reported	93%	Not reported
Diarrhea	**19%**	0.8%	39%	1%	47%	2.7%

TEAE, treatment-emergent adverse event

SUMMARY and CONCLUSIONS

- Lirafugratinib evaluated at 500 nM across 468 kinases demonstrated inhibition:
  - >90% for FGFR2 (94.1%) and MEK5 (92.4%)
  - 89% for MKNK2
  - <75% for FGFR1, FGFR3, FGFR4, and the remaining other kinases
- Lirafugratinib demonstrated the highest selectivity to inhibit FGFR2 without substantial inhibition of FGFR1, 3, 4 compared to the other FGFR inhibitors tested.
- Other FGFR inhibitors demonstrated inhibition for FGFR1-4 that is associated with hyperphosphatemia and diarrhea.
  - Futibatinib showed >90% inhibition for FGFR1, FRFR2, FRFR3, and FGFR4, 83% inhibition for MKK7, and <75% inhibition for all other kinases.
  - Pemigatinib, erdafitinib, and AZD4547 showed >90% inhibition for 11, 37, and 37 kinase targets (including FGFR1-4), respectively.
- The head-to-head kinome analysis of lirafugratinib versus four other FGFR inhibitors showed that lirafugratinib inhibited FGFR2 with high selectivity.
- In conclusion, the highly selective lirafugratinib may have fewer off-target- and off-isoform-related toxicities compared with other pan-FGFR inhibitors used in clinical practice.

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