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Development of targeted FGFR2 therapies in cholangiocarcinoma

Overcoming adverse effects and acquired drug resistance

Cholangiocarcinoma (CCA) is the second most common primary hepatic malignancy after hepatocellular carcinoma, representing ~2% of all cancer-related deaths worldwide yearly[1]. Surgery is a potential curative option for CCA. However, frequent post-surgical relapse appears, and furthermore, many tumors are unresectable since most patients (~70%) are diagnosed at late stages due to lack of specific symptoms[1]. Given this background, targeted drug therapies are expected to play a significant role in the treatment of CCA.

FGFR2 fusion-positive cholangiocarcinoma

The past few years have witnessed a rapid advancement of genomics-informed therapies in biliary tract cancer. A particularly exciting case is represented by the pharmacological targeting of oncogenic FGFR2 fusions in intrahepatic cholangiocarcinoma (iCCA). FGFR2 fusion occurs with a high frequency of 10-15% of patients in iCCA[2][3] (Fig.1). Pan-FGFR inhibitors (pemigatinib, infigratinib and futibatinib) were approved by the FDA in CCA cases harboring the FGFR2 fusion based on evidence from Phase 2 studies[2]. However, negative aspects such as FGFR1- and FGFR4-mediated toxicities and the emergence of on-target FGFR2 resistance mutations have limited the efficacy of existing pan-FGFR inhibitors[4][5].

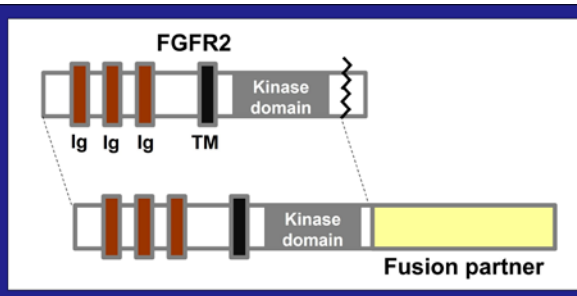

Fig.1. Schematic representation of the FGFR2 gene fusion.
Ig, Ig-like domain; TM, transmembrane domain

Adverse effects and on-target FGFR2 resistance mutations

The most common adverse effects of pan-FGFR inhibitors are hyperphosphatemia caused by the inhibition of FGFR1, which is required for phosphate reabsorption in the kidney, and diarrhea caused by FGFR4 inhibition. Elevated serum phosphate levels are reported in 55% to 85% of patients with CCA in Phase 2 clinical trials[6][7][8] and require active management such as dietary modification, phosphate binders, and dose reductions, or treatment interruptions[4][5].

Acquired secondary FGFR2 resistance mutations were frequently (60%) observed in FGFR2-altered iCCA patients with disease progression following FGFR inhibitor therapy, representing V564 gatekeeper and N549, E565, K641 molecular brake mutations as the most common[9]. Amino acid substitutions at this gatekeeper position adjacent to the hinge region alter the mode of drug-FGFR2 interactions. The triad of residues N549 (in the loop between the αC helix and the β4 strand), E565 (in the kinase hinge), and K641 (in the β8 strand) interact with each other through a network of hydrogen bonds in the unphosphorylated FGFR2 structure, keeping the kinase in an autoinhibited state (Fig.2) [10]. Therefore, mutations in this region lead to constitutive kinase activation[11][12].

To address these challenges, ongoing development of inhibitors is expected to overcome adverse effects and on-target FGFR2 drug resistance mutations.

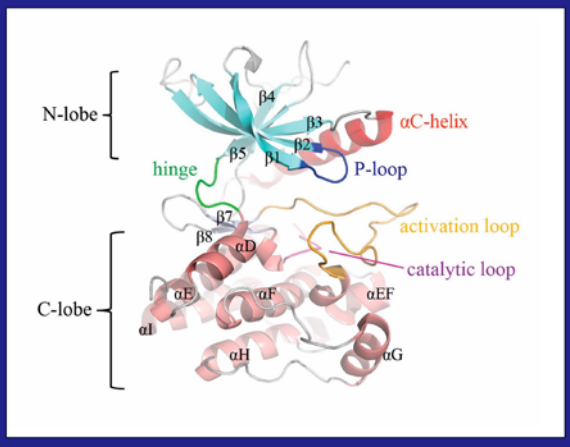

Fig.2. Structure of the FGFR kinase domain[10]

Development of FGFR inhibitors

RLY-4008 (Lirafugratinib) is an FGFR2-selective inhibitor that covalently binds to C491 locating at the tip of the P-loop. It was designed by leveraging differences in conformational dynamics between FGFR2 and other FGFRs, observed through long timescale molecular dynamics simulations. In FGFR1, the P-loop has a wide range of motion and displays rapid dynamics, whereas the FGFR2 P-loop is less dynamic. The reversible binding of RLY-4008 exploits these differences to promote a rigid and extended P-loop in FGFR1 that disfavors covalent bond

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formation while minimally affecting the conformation of the P-loop in FGFR2, enabling efficient covalent bond formation and leading to FGFR2 selectivity[4)5)].

KIN-3248 was designed to be active against many acquired resistance mutations including the V564F gatekeeper mutation. The gatekeeper is positioned on a β-strand that is adjacent to a hinge region that connects the N- and C-terminal lobes of the kinase domain. Upon mutation to a larger phenylalanine residue, the back pocket region of the protein becomes more occluded and molecules that occupy this space will no longer be accommodated. To address this challenge, a strong anchoring interaction with the backbone NH of the DFG-Asp residue of the activation loop and the reduction of steric bulk near the gatekeeper residue became the focus of drug design efforts. KIN-3248 has an acrylamide warhead which covalently binds to C491 in the P-loop, resulting in irreversible inhibition of FGFR2[13)14)].

RLY-4008 and KIN-3248 both demonstrated the potential to inhibit the activity of gatekeeper and molecular brake mutations of FGFR2 in vitro, as well as tumor suppression in multiple Xenograft models including models with these FGFR2 resistance mutations. Notably, RLY-4008 leads to tumor regression without clinically meaningful serum phosphate elevation in patients with iCCA[4)13)14)]. These recent developments of FGFR inhibitors such as RLY-4008 and KIN-3248 have positioned FGFR-directed therapies at the front line of precision oncology.

FGFR products & services from Carna to support inhibitor development.

• Kinase Proteins

Carna offers a wide range of FGFR2 mutant protein products and other FGFR protein products.

(Carna's FGFR2 mutant protein products) (Table.1)

- Gatekeeper mutants: **V564F, V564I, V564L**
- Molecular brake mutants: **N549D, N549H, N549K, E565A**
- Mutant at covalent bond site: **C491A**
- Double mutants (covalent bond site and gatekeeper): **C491A/V564I, C491A/V564L**
- Mutants identified frequently in patients with iCCA who acquired resistance to FGFR inhibitors after treatment with a selective FGFR inhibitor: **M537I, M538L, L617V, K659M, M537I/M538L** (Currently in development)

(Carna's other FGFR protein products)

- **FGFR1, FGFR3, FGFR4**

All Carna's FGFR kinase proteins shown above are manufactured in-house, and available with a GST tag, as well as with a single biotin-tag at the protein's N-terminus, non-activated biotin-tag protein products also available. These proteins are useful in various biochemical assays, including kinase activity and inhibitor binding assays.

Table 1. FGFR2 variant's sensitivity to FGFR inhibitors (S, Sensitive; R, Resistance)[4)9)13)14)15)16)]

FGFR2 Variant	Pemigatinib	Infigratinib	Futibatinib	Lirafugratinib /RLY-4008	KIN-3248
V564F	R	R	R	S	S
V564I	S, R	R	S	S	S
V564L	R	R	S, R	S	S
N549D	R	R	S	S	N.D.
N549H	S	S	S	S	S
N549K	R	R	S	S	S
E565A	S, R	S, R	S	S	S
C491A	N.D.	S	N.D.	N.D.	N.D.
M537I	S	S	S	S	S
L617V	S	R	S	S	S
K659M	S, R	S, R	S	S	S
M537I/M538L	S	S	S	N.D.	S

(N.D.; No data)

Also Available are:

• Kinase Assay Kits (MSA)

• Activity-based (cell-free) Biochemical Screening/Profiling Assay Services

• Cell-based NanoBRET™ TE Intracellular Kinase Assay Services

If you are interested in our products or services, please feel welcome to contact us at info@carnabio.com.

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