RLY-4008, a Novel Precision Therapy for FGFR2-Driven Cancers Designed to Potently and Selectively Inhibit FGFR2 and FGFR2 Resistance Mutations

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ABSTRACT

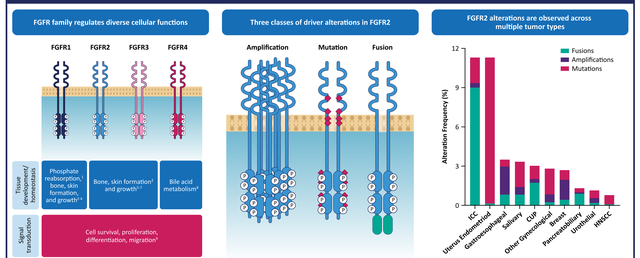
FGFR2 fusions, amplifications, and mutations are oncogenic drivers that occur across multiple tumor types. Clinical efficacy observed with pan-FGFR inhibitors has validated the driver status of FGFR2 in *FGFR2* fusion-positive intrahepatic cholangiocarcinoma (ICC); however, FGFR1-mediated toxicities (hyperphosphatemia, tissue mineralization) and the emergence of on-target FGFR2 resistance mutations limit the efficacy of pan-FGFR inhibitors.

To overcome these limitations, we designed RLY-4008, a potent and highly selective FGFR2 inhibitor. Despite significant investment in traditional structure-based drug design, selective targeting of FGFR2 has not been achieved. We leveraged differences in conformational dynamics between FGFR2 and other FGFR isoforms observed through molecular dynamics simulations to enable the design of RLY-4008. RLY-4008 inhibits FGFR2 with low nanomolar potency and demonstrates > 200-fold selectivity over FGFR1, and > 80- and > 5000-fold selectivity over FGFR3 and FGFR4, respectively, in biochemical assays. Additionally, RLY-4008 demonstrates high kinome selectivity for FGFR2 against a panel of > 400 human kinases. RLY-4008 has strong activity against primary and acquired FGFR2 resistance mutations in cellular assays, and potent antiproliferative effects on FGFR2-altered human tumor cell lines.

In vivo, RLY-4008 demonstrates dose-dependent FGFR2 inhibition and induces regression in multiple human xenograft tumor models, including *FGFR2* fusion-positive ICC, gastric, and lung cancers, *FGFR2*-amplified gastric cancer, and *FGFR2*-mutant endometrial cancer. Strikingly, RLY-4008 induces regression in an *FGFR2* fusion-positive ICC model harboring the *FGFR2*^{V565F} gatekeeper mutation and an endometrial cancer model harboring the *FGFR2*^{K659E} mutation, two mutations that drive clinical progression on current pan-FGFR inhibitors. In the *FGFR2*^{V565F} model, pan-FGFR inhibitors are ineffective, even at maximally tolerated doses. Notably, treatment of these tumors with RLY-4008 induces rapid regression and restores body weight. In rat and dog toxicology studies, RLY-4008 is well tolerated and is not associated with hyperphosphatemia or tissue mineralization at exposures significantly above those required to induce regression in all models.

In contrast to pan-FGFR inhibitors, RLY-4008 is highly selective for FGFR2 and demonstrates strong activity against FGFR2 resistance mutations, suggesting that RLY-4008 may have broader therapeutic potential via preventing and overcoming therapeutic resistance. Together, these data and the favorable pharmaceutical properties of RLY-4008 strongly support its clinical development in FGFR2-altered tumors.

Figure 1: FGFR family signaling and FGFR2 alterations in cancer.



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Adapted from Babina IS, Turner NC. *Nat Rev Cancer* 2017;17: 318-332.

Data source: FoundationInsights® database, using 8 copies as the threshold for amplification, and including only mutations with known or likely functional significance. ICC, intrahepatic cholangiocarcinoma; CUP, carcinoma unknown primary; HNSCC, head and neck squamous cell carcinoma.

- Fibroblast growth factor receptors (FGFRs) 1-4 are a family of receptor tyrosine kinases that promote cell survival, proliferation, migration and differentiation.[1] FGFRs are involved in numerous developmental and physiological processes mediating tissue homeostasis including phosphate reabsorption, bone and skin formation and growth, bile acid metabolism, and others (**Fig. 1, left panel**).
- Three classes of genetic alterations in FGFR2 result in aberrant signaling and drive oncogenesis. These include gene amplification, activating mutations, and chromosomal fusions[2] (**Fig. 1, middle panel**).
- Driver alterations in FGFR2 are observed across multiple tumor types,[3,4] suggesting a broad potential benefit of selective FGFR2 inhibition (**Fig. 1, right panel**).

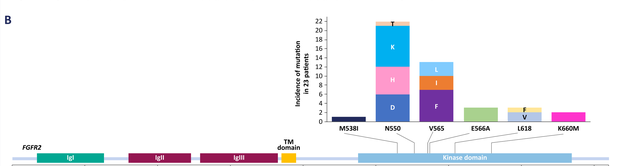
Figure 2. Pan-FGFR inhibitors suffer from limited efficacy, high toxicity, and on-target resistance.

A

Compound	Response Rate	Dosing Schedule	% of Patients with Hyperphosphatemia[a]	% of Patients with Diarrhea	% of Patients Discontinued or Dose Reduced
Pemigatinib[1]	36% (ICC)	2 weeks on, 1 week off	94%	47%	23%
Infigratinib[1]	24% (ICC)	3 weeks on, 1 week off	75%	25%	Not reported
Futibatinib[2]	37% (ICC)	Once daily dosing	88%	37%	52%
Erdafitinib[3]	32% (Urothelial Carcinoma)	Personalized dosing based on phosphate levels[b]	76%	47%	66%

[a]As defined by increased serum phosphate; except for infigratinib which is not specified.
[b]Initial dose (8 mg QD) adjusted to 9 mg QD only in absence of hyperphosphatemia.
1. Pemigatinib Prescribing Information (revised 02/2021); 2. Javle MM *et al. J Clin Oncol* 2021;39(suppl 3): 265; 3. Goyal L *et al. J Clin Oncol* 2020;38(suppl 15): 108; 4. Erdafitinib Prescribing Information (revised 04/2020).

B

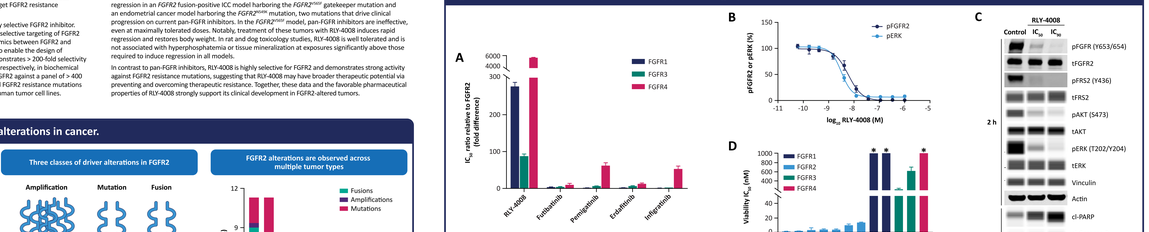


Adapted from: Goyal L *et al.* 32nd EORTC/AACR/NCI Virtual Symposium. Abstract 49 and Varghese AM *et al. JCO Precision Oncology.* 2021;5: 44-50.

- Toxicities caused by off-target inhibition of FGFR1 (hyperphosphatemia) and FGFR4 (diarrhea) lead to frequent dose interruptions or reductions, resulting in limited efficacy (**Fig. 2A**). Clinical proof-of-concept for pan-FGFR inhibitors targeting FGFR2-driven cancers has only been achieved in *FGFR2* fusion-positive intrahepatic cholangiocarcinoma (ICC).[1-3]
- Acquired resistance mutations in the FGFR2 kinase domain are commonly found in patients with *FGFR2* fusion-positive ICC treated with pan-FGFR inhibitors (**Fig. 2B**).
  - The graph indicates the number of times the indicated mutant allele was detected in tissue or ctDNA in 23 patients who developed FGFR2 kinase domain mutations at progression on pan-FGFR inhibitors. N550 is a component of the "molecular brake" and V565 is the "gatekeeper" residue (Goyal L et al, EORTC 2020, Abstract 49).

RESULTS

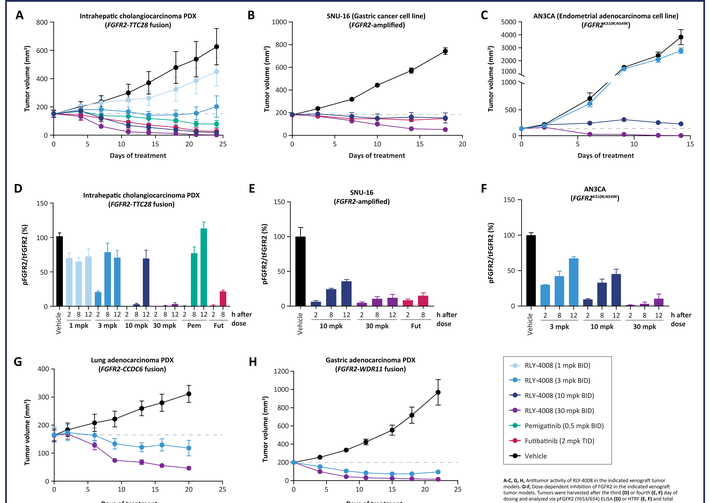
Figure 3. RLY-4008 is a novel precision therapy that is highly selective for FGFR2.



A. Fold-change in biochemical IC_{90} values of the indicated inhibitors between FGFR2 and FGFR1, FGFR3 and FGFR4. Error bars indicate SD. **B.** Inhibition of FGFR2-mediated signaling in SNU-16 cells. Cells were incubated with RLY-4008 for 2 h and analyzed via pFGFR2 (Y653/654) and pERK (T202/Y204) HTRF assays. **C.** Inhibition of FGFR2-mediated signaling in SNU-16 cells. **D.** Viability IC_{90} values for RLY-4008 in FGFR2-dependent cell lines (FGFR2-amplified gastric carcinoma (KATO III, SNU-16) and colorectal adenocarcinoma (MFE-319), FGFR2 fusion-positive ICC (JCCA-7) and FGFR2-mutant endometrial adenocarcinoma (MFE-296, FGFR2^{N549K}), ANA3CA, FGFR2$^{E566A/K660M}$) and breast carcinoma (JIMUH-2, FGFR2^{V659E}) as well as FGFR1-, FGFR3- and FGFR4-dependent cell lines. Cells were treated for 96 h and viability was assayed using CellTiter-Glo. Error bars indicate SD. *Indicates that IC_{90} > 1000 nM.

- RLY-4008 demonstrates > 200-fold selectivity over FGFR1, and > 80- and > 5000-fold selectivity over FGFR3 and FGFR4, respectively. Pan-FGFR inhibitors demonstrate little to no selectivity (**Fig. 3A**).
- The cellular potency (expressed as IC_{90}) of RLY-4008 is 6 nM (pFGFR) and 3 nM (pERK) (**Fig. 3B**).
- RLY-4008 demonstrates dose-dependent reduction of phosphorylation of FGFR2 signaling pathway nodes and induction of apoptosis (**Fig 3C**).
- RLY-4008 inhibits cellular proliferation with IC_{90} < 14 nM in *FGFR2* fusion-positive, *FGFR2*-amplified, and *FGFR2*-mutant cancer cell lines (**Fig. 3D**).
  - RLY-4008 does not have strong inhibitory activity in FGFR1, FGFR3 or FGFR4-dependent cell lines demonstrating the exquisite selectivity of RLY-4008 on FGFR2 in cellular assays (**Fig. 3D**).

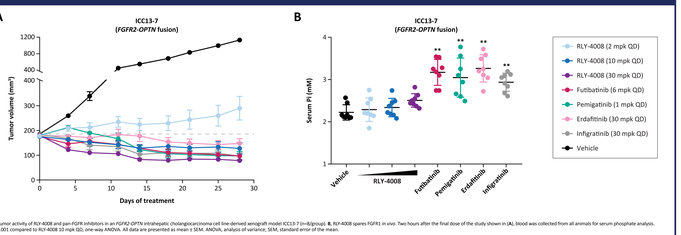
Figure 4. RLY-4008 leads to dose-dependent inhibition of FGFR2 and tumor regression in multiple FGFR2-altered tumor models.



A-C, G, H. Antitumor activity of RLY-4008 in the indicated xenograft tumor models. **D-F.** Dose-dependent inhibition of FGFR2 in the indicated xenograft tumor models. Tumors were harvested after the third **(D)** or fourth **(E, F)** day of dosing and analyzed via pFGFR2 (Y653/654) ELISA **(D)** or HTRF **(E, F)** and total FGFR2 HTRF. pFGFR2 normalized to total FGFR2 is reported (m-1)/group).

- RLY-4008, administered orally, twice daily from 1 to 30 mg/kg, exhibits dose-dependent antitumor activity and induces tumor regression in subcutaneous xenograft tumor models harboring FGFR2 alterations (**Fig. 4A-C, G, H**).
- Pharmacokinetic and pharmacodynamic analyses of plasma exposure and target engagement in tumor samples demonstrates dose-dependent inhibition of FGFR2 (**Fig. 4D-F**).

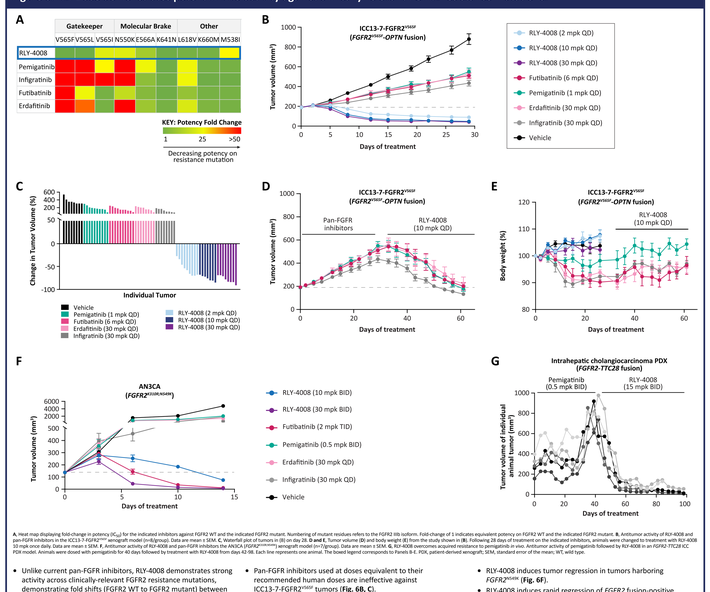
Figure 5. RLY-4008 spares FGFR1 *in vivo*.



A. Antitumor activity of RLY-4008 and pan-FGFR inhibitors in an *FGFR2*-OPTN intrahepatic cholangiocarcinoma cell line-derived xenograft model ICC13-7 (n=8/group). **B.** RLY-4008 spares FGFR1 *in vivo*. Two hours after the final dose of the study shown in (**A**), blood was collected from all animals for serum phosphate analysis. **$P < 0.001$ compared to RLY-4008 10 mpk QD, one-way ANOVA. All data are presented as mean ± SEM. ANOVA, analysis of variance; SEM, standard error of the mean.

- RLY-4008 as well as pan-FGFR inhibitors used at doses equivalent to their recommended human doses induce tumor regression in an *FGFR2* fusion-positive ICC model (**Fig. 5A**); however, pan-FGFR inhibitors cause hyperphosphatemia (32%-47% increase in serum phosphate over vehicle) due to their inhibition of FGFR1 (**Fig. 5B**).
- RLY-4008 induces tumor regression without significantly altering serum phosphate levels ($P > 0.01$, one-way ANOVA), demonstrating that RLY-4008 spares FGFR1 at efficacious exposures *in vivo* (**Fig. 5A, B**).

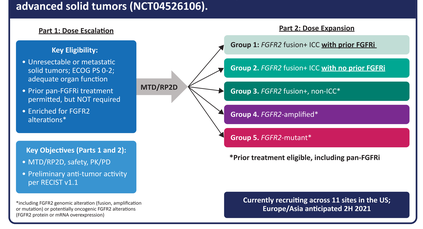
Figure 6. RLY-4008 demonstrates potent *in vivo* activity against clinically-relevant FGFR2 resistance mutations.



A. Heat map displaying fold-change in potency (IC_{90}) for the indicated inhibitors against FGFR2 WT and the indicated FGFR2 mutant. Numbering of mutant residues refers to the FGFR2 IIIb isoform. Fold-change of 1 indicates equivalent potency on FGFR2 WT and the indicated FGFR2 mutant. **B.** Antitumor activity of RLY-4008 and pan-FGFR inhibitors in the ICC13-7-FGFR2^{V565F} xenograft model (n=8/group). Data are mean ± SEM. **C.** Waterfall plot of tumors in (**B**) on day 28. **D and E,** Tumor volume (**D**) and body weight (**E**) from the study shown in (**B**). Following 28 days of treatment on the indicated inhibitors, animals were changed to treatment with RLY-4008 10 mpk once daily. Data are mean ± SEM. **F.** Antitumor activity of RLY-4008 and pan-FGFR inhibitors the ANSCA (FGFR2$^{E566A/K660M}$) xenograft model (n=7/group). Data are mean ± SEM. **G.** RLY-4008 overcomes acquired resistance to pemigatinib *in vivo*. Antitumor activity of pemigatinib followed by RLY-4008 in an *FGFR2*-*TTC28* ICC PDX model. Animals were dosed with pemigatinib for 40 days followed by treatment with RLY-4008 from days 42-98. Each line represents one animal. The boxed legend corresponds to Panels B-E. PDX, patient-derived xenograft; SEM, standard error of the mean; WT, wild type.

- Unlike current pan-FGFR inhibitors, RLY-4008 demonstrates strong activity across clinically-relevant FGFR2 resistance mutations, demonstrating fold shifts (FGFR2 WT to FGFR2 mutant) between 0.3x (V565F) and 17x (M538I) (**Fig. 6A**).
- RLY-4008 induces rapid regression of ICC13-7-FGFR2^{V565F} tumors at a dose lower than that required to drive regression of ICC13-7 tumors (**Fig. 6B, C** and **Fig. 5A**), consistent with the increased cellular potency of RLY-4008 on FGFR2^{V565F} compared to FGFR2^{WT}.
- Pan-FGFR inhibitors used at doses equivalent to their recommended human doses are ineffective against ICC13-7-FGFR2^{V565F} tumors (**Fig. 6B, C**).
  - Treatment of ICC13-7-FGFR2^{V565F} tumors that progressed on pan-FGFR inhibitors with RLY-4008 induces rapid regression and restores body weight (**Fig. 6D, E**).
- RLY-4008 induces tumor regression in tumors harboring *FGFR2*^{K659E} (**Fig. 6F**).
- RLY-4008 induces rapid regression of *FGFR2* fusion-positive (*FGFR2-TTC28*) ICC PDX tumors that progressed on pemigatinib, demonstrating that RLY-4008 overcomes resistance to pemigatinib *in vivo* (**Fig. 6G**).

Figure 7. First-in-human study of RLY-4008 in patients with ICC and other advanced solid tumors (NCT04526106).



CONCLUSIONS

- RLY-4008 is a novel potent and highly selective FGFR2 inhibitor designed to overcome the emergence of on-target FGFR2 resistance mutations and dose-limiting toxicities associated with current pan-FGFR inhibitors.
- RLY-4008 is > 200-fold selective over FGFR1, and > 80- and > 5000-fold selective over FGFR3 and FGFR4, respectively.
- *In vivo*, RLY-4008 demonstrates dose-dependent FGFR2 inhibition and induces tumor regression in *FGFR2* fusion-positive, *FGFR2*-amplified and *FGFR2*-mutant human xenograft tumor models.
- In contrast to current pan-FGFR inhibitors, RLY-4008 spares FGFR1 *in vivo*.
- RLY-4008 demonstrates potent *in vivo* activity against clinically-relevant FGFR2 resistance mutations.
  - RLY-4008 induces regression in xenograft models expressing *FGFR2*^{V565F} and *FGFR2*^{N549K}, two common FGFR2 kinase domain mutations that drive clinical progression on current pan-FGFR inhibitors.
- A first-in-human Phase 1 trial of RLY-4008 in patients with *FGFR2* fusion-positive ICC and other advanced solid tumors harboring FGFR2 alterations is currently underway (NCT04526106).

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