Identifying FGFR2 *fusions/rearrangements in cholangiocarcinoma patients using a novel cfDNA algorithm for treatment with RLY-4008, a highly selective irreversible FGFR2 inhibitor*

Alison M. Schram[1], Mitesh Borad[2], Vaibhav Sahai[3], Suneel Kamath[4], Richard Kim[5], Chih-Yi Andy Liao[6], Do-Youn Oh[7], Mariano Ponz-Sarvisé[8], Jeffrey Yachnin[9], Scott A. Shell[10], Philippe Cassier[11], Efrat Dotan[12], Vaia Florou[13], Victor Moreno[14], Joon Oh Park[15], David Tai[16], Oleg Schmidt-Kittler[17], Charles Ferté[17], Lipika Goyal[18], Vivek Subbiah[19]

[1]Memorial Sloan Kettering Cancer Center, New York, USA; [2]Mayo Clinic Scottsdale – PPDS, Phoenix, Arizona, USA; [3]University of Michigan, Ann Arbor, Michigan, USA; [4]The Cleveland Clinic Taussig Cancer Institute, Cleveland, Ohio, USA; [5]H Lee Moffitt Cancer Center and Research Institute, Tampa, Florida, USA; [6]University of Chicago Medical Center, Chicago, Illinois, USA; [7]Seoul National University Hospital, Seoul, Republic of Korea; [8]Clinica Universidad Navarra, Pamplona, Spain; [9]Karolinska Universitetssjukhuset Solna, Stockholm, Sweden; [10]Guardant Health, Palo Alto, California, USA; [11]Centre Léon Bérard Centre, Lyon, France; [12]Fox Chase Cancer Center, Philadelphia, Pennsylvania, USA; [13]University of Utah – Huntsman Cancer Institute – PPDS, Salt Lake City, Utah, USA; [14]START MADRID Hospital Universitario Fundación Jiménez Díaz, Madrid, Spain; [15]Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea; [16]National Cancer Centre, Singapore; [17]Relay Therapeutics, Cambridge, Massachusetts, USA; [18]Mass General Cancer Center, Boston, Massachusetts, USA; [19]University of Texas MD Anderson Cancer Center, Houston, Texas, USA

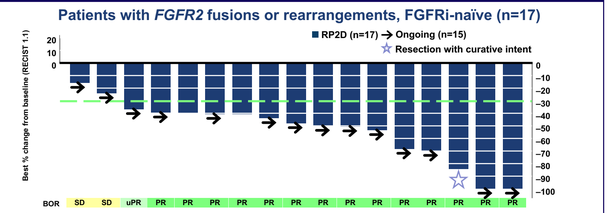
RELAY THERAPEUTICS

Poster 326

BACKGROUND

- Cholangiocarcinoma (CCA) is an aggressive malignancy with a dismal prognosis, typically treated with chemotherapy[1]
- In a subset of patients (~10–15% of intrahepatic CCA cases), CCA tumours harbour *FGFR2* fusions/rearrangements (f/r) that drive tumour growth[1]
- Identification of these patients is crucial since they are likely to benefit from FGFR2-targeted therapy
- However, genomic profiling based on tumour biopsy can pose challenges, with limited tumour tissue available, and a tendency to forego repeat biopsies in favour of initiating therapy due to the aggressive nature of CCA
- Liquid biopsies may be a non-invasive way to identify patients most likely to benefit from FGFR2-targeted therapy. Recent technical advances in cell-free DNA (cfDNA) analysis have increased the sensitivity of this method for detecting *FGFR2* f/r[2]
- We evaluated the sensitivity of liquid biopsies in detecting *FGFR2* f/r, compared to local and central tissue assessments, in patients from the ReFocus study (NCT04526106)[3]
  - ReFocus is a study of RLY-4008, a potent, selective, specific, and irreversible FGFR2 inhibitor (FGFRi),[4] in patients with advanced, *FGFR2*-driven CCA or other solid tumours. It has shown promising efficacy to date in FGFRi-naïve patients with *FGFR2* f/r CCA **(Figure 1)**[5]

Figure 1. Radiographic tumour regression per RECIST 1.1 – RP2D[5]



☆ Patient with near complete response, underwent surgical resection with curative intent.

BOR, best objective response; FGFRi, FGFR inhibitor; (u)PR, (unconfirmed) partial response; RP2D, recommended Phase 2 dose; SD, stable disease, RECIST, Response Evaluation Criteria In Solid Tumours.

METHODS

Samples

- Tumour tissue (archival or fresh biopsies) and pre-treatment plasma samples were obtained for central analysis from 73 patients in the ReFocus trial, until 8 September, 2022 (data cut-off)

Objective

- To evaluate the feasibility (assessed according to failure rate) and the sensitivity (assessed according to percent positive agreement) of liquid biopsies to identify *FGFR2* f/r, compared to local and central tissue assessments

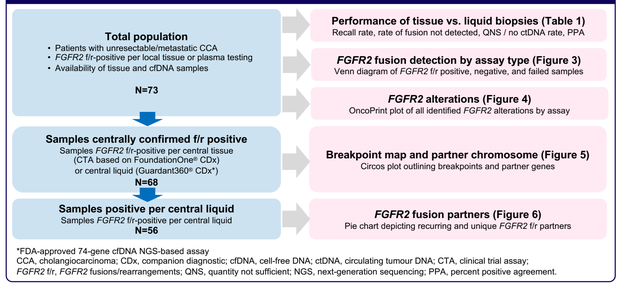
Sample analysis for *FGFR2* f/r

- Pre-treatment samples were analysed centrally, and methods applied per **Figure 2**
- Liquid cfDNA samples were then analysed *in silico* using a fusion partner agnostic algorithm that is able to detect unique *FGFR2* f/r
- Details of the Guardant360® (G360) research-only algorithm have been described previously.[2] This algorithm was applied to the G360 test to broaden the capability of detecting *FGFR2* f/r

Patient demographics

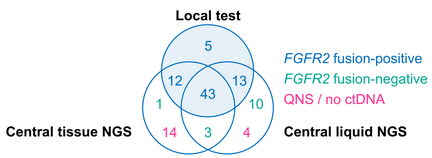
- Of the 73 patients who provided samples, 40% were male; the mean age was 56 years (standard deviation: 14 years)
- The majority of patients (93%) had Eastern Cooperative Oncology Group performance status (ECOG PS) of 0 or 1
- Almost all CCA patients (72/73) had intrahepatic disease at diagnosis
- The proportion of patients who had undergone ≥2 prior treatment regimens was 82%
- The median sum of target lesions was 79 mm (range: 13–261 mm)

Figure 2. Diagnostic analysis plan



*FDA-approved 74-gene cfDNA NGS-based assay
CCA, cholangiocarcinoma; CDx, companion diagnostic; cfDNA, cell-free DNA; ctDNA, circulating tumour DNA; CTA, clinical trial assay; *FGFR2* f/r, *FGFR2* fusions/rearrangements; QNS, quantity not sufficient; NGS, next-generation sequencing; PPA, percent positive agreement.

Figure 3. *FGFR2* f/r detection by assay type



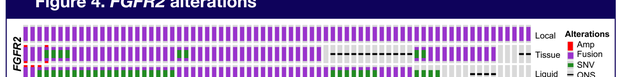
ctDNA, circulating tumour DNA; *FGFR2* f/r, *FGFR2* fusions/rearrangements; QNS, quantity not sufficient; NGS, next-generation sequencing.

RESULTS

Percentage of *FGFR2* fusions detected in tissue vs. liquid biopsies

- Of the 73 tumour tissue samples, central tissue next-generation sequencing (NGS) detected 55 *FGFR2* f/r (75.3%)
- Of the 73 plasma samples, central liquid NGS detected 56 *FGFR2* f/r (76.7%):
  - 32 *FGFR2* f/r (43.8%) were detected using the standard algorithm
  - The partner agnostic algorithm detected 24 additional *FGFR2* f/r (32.8%)
  - The majority of *FGFR2* f/r identified using the partner agnostic algorithm were unique (18/24, 75%), in contrast to 9/32 (28.1%) using the standard algorithm

Figure 4. *FGFR2* alterations



Amp, amplification; QNS, quantity not sufficient; SNV, single nucleotide variant. Credit: ComplexHeatmap r package[6]

FGFR2 alterations

- As shown in **Figure 4**, small nucleotide variants (SNV) were the second most common *FGFR2* alterations after *FGFR2* f/r
  - Liquid biopsies identified 31 patients with *FGFR2* SNVs, whereas tissue biopsy identified eight
- Analysis of liquid biopsies detected three amplifications; two amplifications were detected in tissue biopsies. All detected amplifications were low level with a copy number <4
  - Copy number determined by liquid biopsy cannot be directly compared to tissue
- Concordance of *FGFR2* SNVs and amplifications in tissue and liquid was 35/55 (63.6%), much lower than the concordance for *FGFR2* fusions, which was 46/55 (83.6%)
  - 31/33 patients with *FGFR2* SNVs had prior treatment with an FGFRi, suggesting emergence of acquired resistance as a potential cause of the observed discordance

Table 1. Performance of tissue vs. liquid biopsies

	Local tests compared to:			Central tissue compared to:	
	Central tissue	Central liquid (standard)	Central liquid (partner agnostic)	Central liquid (standard)	Central liquid (partner agnostic)
Recall rate*	55/73 (75.3%)	32/73 (43.8%)	56/73 (76.7%)	25/55 (45.5%)	43/55 (78.2%)
Fusion not detected rate†	4/73 (5.5%)	37/73 (50.7%)	13/73 (17.8%)	26/55 (47.3%)	8/55 (14.5%)
QNS / no ctDNA rate‡	14/73 (19.2%)	4/73 (5.5%)		4/55 (7.3%)	
Percent positive agreement**	55/59 (93.2%)	32/69 (46.4%)	56/69 (81.2%)	25/55 (45.5%)	46/55 (83.6%)

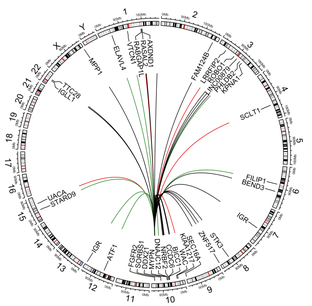
*Recall rate = *FGFR2* f/r-positive cases by assay / total population; †Fusion not detected = successfully reported samples without *FGFR2* f/r call / total population; ‡QNS / no ctDNA rate = samples failed QC or no ctDNA / total population; **Percent positive agreement = matched results / number of successfully reported samples.

ctDNA, circulating tumour DNA; *FGFR2* f/r, *FGFR2* fusions/rearrangements; QC, quality control; QNS, tumour quantity not sufficient.

FGFR2 fusion detection by biopsy and assay type

- In 68 patients, tissue or liquid biopsies identified *FGFR2* fusion events **(Figure 3; Table 1)**
  - In 43 patients, the local and central NGS tests detected identical FGFR2 f/r events
- Five *FGFR2* fusions identified in local tests were not confirmed by the central tests
  - Three patients were *FGFR2* fusion-negative according to results of both tumour tissue and liquid biopsy analyses
  - Two patients were negative by central liquid biopsy and tumour 'quantity not sufficient' (QNS) in central tissue NGS
- The frequency of central NGS *FGFR2* f/r negativity was similar for both biopsy types but differed in reason:
  - Analysis of tissue biopsy resulted in a higher rate of pathology QNS (14/73)
  - Analysis of liquid biopsy resulted in a higher rate of *FGFR2* fusion-negative outputs (10/73)

Figure 5. Breakpoint map and partner chromosomes (tissue and liquid biopsies)



Black lines: Translocations identified in both tissue and liquid biopsies

Red lines: Fusions detected in tissue only

Green lines: Translocations detected in liquid biopsies only

N=68

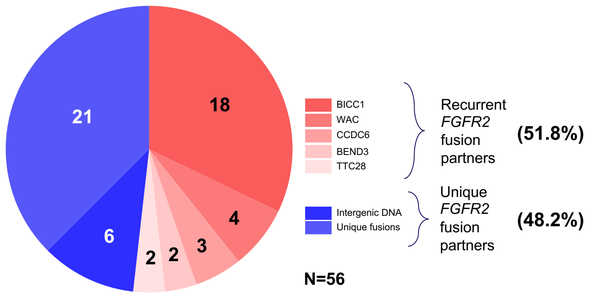
Credit: Circlize r package[7]

Location of *FGFR2* fusions and partner chromosomes

Analysis of tumour tissue and liquid biopsies

- As shown in **Figure 5**, *FGFR2* f/r partners were predominantly located in chromosome 10 (38/68; 55.9%) and chromosome 1 (6/68; 8.8%)
- Nearly half of the *FGFR2* fusion partners identified in this study were unique and/or intergenic DNA (30/68; 44.1%)
- *FGFR2* f/r included 21 *FGFR2-BICC1* fusions and 47 non-*FGFR2-BICC1* fusions

Figure 6. *FGFR2* fusion partners (liquid biopsies)



Analysis of liquid biopsies

- As shown in **Figure 6**, the predominant *FGFR2* fusion partner gene was *BICC1* (18/56; 31.2%)
- The next most common fusion event seen in liquid biopsies was fusion with intergenic DNA (6/56; 10.7%)
- About half of the *FGFR2* fusion partners were unique (27/56; 48.2%)

CONCLUSIONS

- The fusion partner agnostic *FGFR2* f/r calling algorithm is superior to the standard algorithm in cfDNA, identifying approximately a third of fusions that would otherwise have been missed
- The majority of *FGFR2* f/r detected by the partner agnostic algorithm were unique
- Liquid biopsy NGS identified a high proportion of *FGFR2* f/r, with a rate comparable to tissue NGS
- cfDNA testing offers a rapid and non-invasive mechanism for the detection of resistance mutations
- These encouraging results suggest a role for liquid biopsy in *FGFR2* f/r profiling, providing a non-invasive option to identify *FGFR2* f/r-positive CCA patients
- The role of cfDNA in prospective *FGFR2* f/r profiling will be validated in the ReFocus clinical study, which includes patients with CCA and other solid tumours

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Acknowledgments

The authors would like to thank the patients and their families, all study investigators, sub-investigators, and research staff at participating institutions. The authors also gratefully acknowledge Kai Yu Jen, PhD, Relay Therapeutics, for her contribution to this research, and Guardant Health for use of Guardant360® to enable this work.
Medical writing support was provided by Christine Elsner of BOLDSCIENCE Inc. funded by Relay Therapeutics.

Presented at the 34th EORTC-NCI-AACR Symposium, 26–28 October 2022, Barcelona, Spain. For more information, please contact: ClinicalTrials@relaytx.com.