ctDNA-based genomic landscape analysis to evaluate molecular brake and gatekeeper mutations in FGFR2

Abstract # 3049

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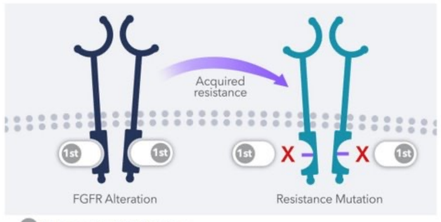
INTRODUCTION

FGFR2 and FGFR3 (FGFR2/3) in Cancer:

- Receptor tyrosine kinases (RTKs) that become aberrantly activated by gene rearrangements (including fusions), small indels, and point mutations
- Validated therapeutic targets with approved small molecule inhibitors in cholangiocarcinoma & bladder cancer
- FGFR-driven tumors can develop resistance to reversible, ATP-competitive inhibitors (1st gen) through secondary FGFR mutations that occur in the kinase domain, typically at the molecular brake and gatekeeper amino acids

Molecular Brake and Gatekeeper Mutations:

- The molecular brake is an autoinhibitory feature that consists of three amino acids (also referred to as the regulatory triad) in the FGFR hinge region. Mutations in the molecular brake amino acids relieve autoinhibition (i.e. kinase activating) and hinder the binding of most 1st gen FGFR inhibitors. Consistent with their FGFR-activating function, molecular brake mutations are known to occur in both the resistance setting and in tumors that are FGFRi-naïve
- Gatekeeper mutations cause steric clashes that can prevent binding of many FGFR inhibitors and they are typically identified in the setting of acquired resistance

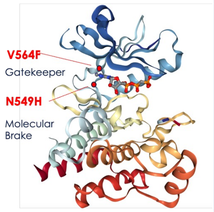


1st 1st generation FGFR inhibitors

Objective: To evaluate the occurrence and proportion of kinase domain resistance mutations in FGFR2/3 using a real-world clinical genomic database of ctDNA.

Kinase Domain (KD) Resistance Mutations

Gene	Alteration	Function
FGFR2	N549K/T/D/I/Y/H	Molecular Brake
	V562L	--
	V564F/I/L	Gatekeeper
	E565G/A	Molecular Brake
	K641N	Molecular Brake
	K659M	Activation Loop
FGFR3	V555M	Gatekeeper
	N540K/S	Molecular Brake



References: Silverman IM et al. Cancer Discov. 2021 Feb;11(2):326-339. Nakamura et al. npj Precision Oncology (2021) 5:66. Goyal L et al. Cancer Discov. 2017 Mar;7(3):252-263., Goyal L et al. Cancer Discov. 2019 Aug;9(8):1064-1079. Goyal L et al. N Engl J Med 2023;388:228-39.

METHODS

Analyses utilized GuardantINFORM™; a de-identified clinical genomic research database spanning from March 2014 to Sept. 2021 including 170,000+ patients with advanced/metastatic cancer profiled by the liquid biopsy, Guardant360® assay.

- Analyses include FGFR2 or FGFR3 alterations that are at least likely to be oncogenic or confer resistance
- Counts of patients & alterations were grouped by their sensitivity to 1st-gen FGFRi
- Pink/gray pie charts show the % of FGFR2 alterations when counting each mutation in each patient as a distinct event
- Outcomes: Real-world overall survival (rwOS, measured starting from the time of metastatic diagnosis) and real-world time to treatment discontinuation (rwTTD)
  - Included all patients regardless of FGFR detection date
  - Cohorts were compared pairwise with the log-rank test

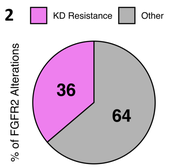
RESULTS

Pan-Cancer Oncogenic FGFR2 & FGFR3

- > 2,100 Patients with oncogenic FGFR2/3 alterations (Figure 1)
- Four tumor types with ≥ 200 FGFR2/3 positive patients: NSCLC, Breast, Bladder, Cholangiocarcinoma (CCA).

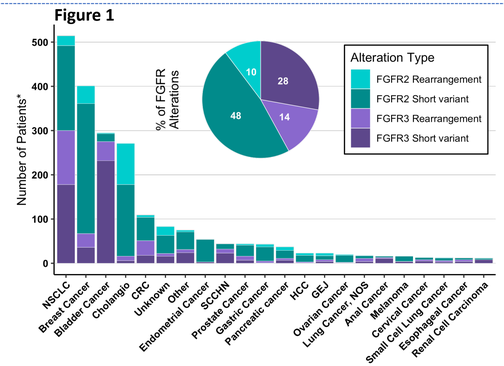
Notes: Rearrangements (including fusions) were only counted when FGFR2/3 kinase domain was predicted to be intact. Short variant category includes missense mutations and short insertion/deletions. *some patients have multiple FGFR alterations across categories (eg. cholangiocarcinoma with FGFR2-fusion & missense mutation)

Figure 2



- **36% of FGFR2 alterations consist of kinase domain mutations** (Figure 2) that are potentially resistant to 1st generation FGFR inhibitors (amino acids N549, V562, V564, E565, K641, K659)

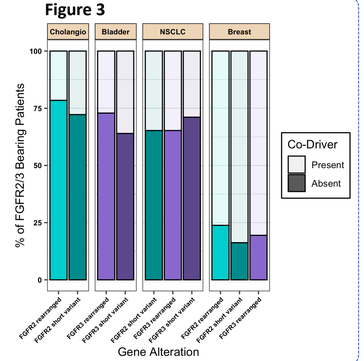
Figure 1



Mutations Co-Occurring with oncogenic FGFR2/3

- Method: Co-Mutation analysis (Figure 3) includes activated oncogenes that could circumvent/bypass the dependence on FGFR2/3 signaling (PIK3CA, BRAF, KRAS, NRAS, ERBB2, ESR1, AKT1, IDH1, EGFR, GNAS).
- In cholangiocarcinoma, bladder and NSCLC, most patients with oncogenic FGFR2/3 alterations do not harbor activating mutations in other oncogenes which **suggests that activated FGFR2/3 is the primary driver and may predict sensitivity to FGFRi monotherapy.**
- In contrast, most breast cancer patients have PIK3CA mutations in addition to FGFR2/3 alterations
- FGFR2/3 rearrangements (eg. fusions) have slightly less co-occurring driver mutations than do FGFR2/3 short variants

Figure 3

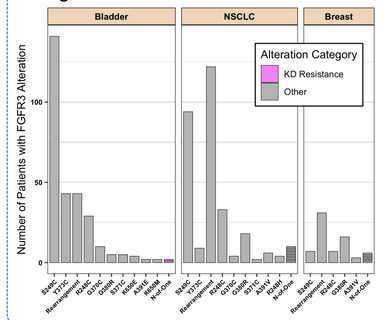


FGFR3 in Bladder, NSCLC and Breast (Figure 4)

Bladder: 286 patients with FGFR3 alteration
NSCLC: 302 patients with FGFR3 alteration
Breast: 70 patients with FGFR3 alteration

- Activating FGFR3 mutations are prevalent in NSCLC and Bladder cancers
- FGFR3 molecular brake mutations are rare in this ctDNA database. No gatekeeper mutations identified.

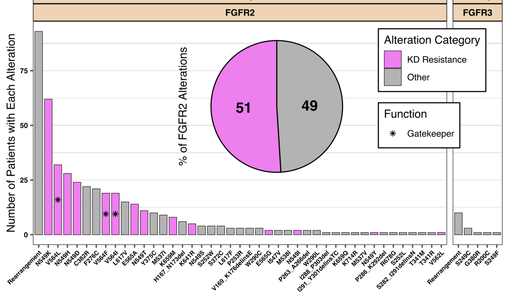
Figure 4



Cholangiocarcinoma (CCA): Half of all FGFR2 Alterations Consist of Kinase Domain Resistance Mutations

- 232 Cholangiocarcinoma patients with FGFR2 & 15 with FGFR3 alteration
- 51% of FGFR2 alterations in cholangiocarcinoma patients are potentially resistant to 1st-generation inhibitors. The most common missense mutations are N549K (molecular brake) and V564L (gatekeeper).
- 70 distinct gatekeeper(*) mutation events (V564L/F/I).

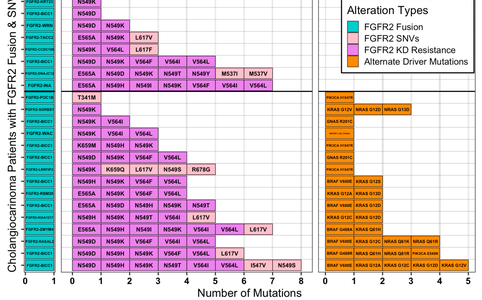
Figure 5



Out of 93 CCA patients with FGFR2 fusions (including both FGFRi-treated and naive):

- 23 have ≥ 1 FGFR2 SNV & 19 have ≥ 2 FGFR2 SNVs
  - ❖ Liquid biopsy is fit-for-purpose to detect acquired resistance
    - Non-invasive, broad survey of tumor cell populations
  - ❖ Confirms that on-target mutations are a common resistance mechanism
    - ❖ Broad coverage FGFR2 inhibition is required to combat polyclonal on-target resistance

Figure 6



Cholangiocarcinoma patients with **FGFR2 fusion** treated 2nd-line with FGFR inhibitors (pemigatinib or erdafitinib) trend toward longer survival vs other therapy (Figure 7). Although rare, **FGFR3 fusions** in cholangiocarcinoma may be associated with shorter survival than FGFR2 fusions (Figure 8) & have significantly shorter time on treatment (Figure 9). These outcomes suggest that a pan-FGFR (2/3) inhibitor may have the potential to serve a broader population of cholangiocarcinoma patients.

Figure 7 rwOS, Cholangio pts w/ FGFR2 Fusion w/KD, by treatment

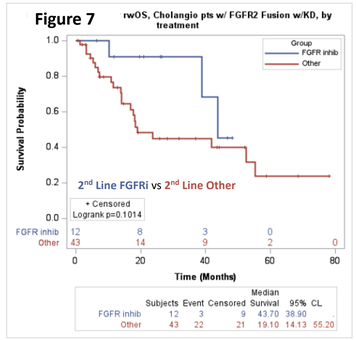


Figure 8 rwOS, Cholangio, FGFR2 Fusion w/KD vs FGFR3 Fusion w/KD

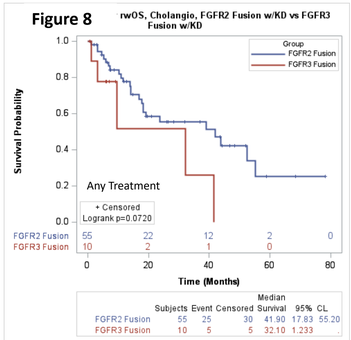
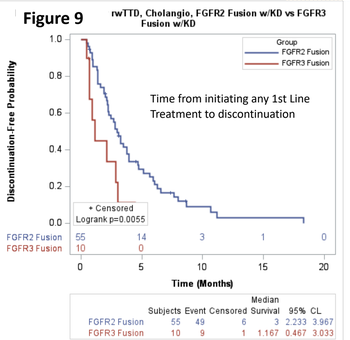


Figure 9 rwTTD, Cholangio, FGFR2 Fusion w/KD vs FGFR3 Fusion w/KD



FGFR2 in Breast, NSCLC and Bladder Cancer

Breast:
368 patients with FGFR2 alteration

- 57% of FGFR2 mutations in breast cancer patients occur in the molecular brake (N549)
- No FGFRi therapy is currently approved for breast cancer; these patients are FGFRi-naive

NSCLC:
223 patients with FGFR2 alteration

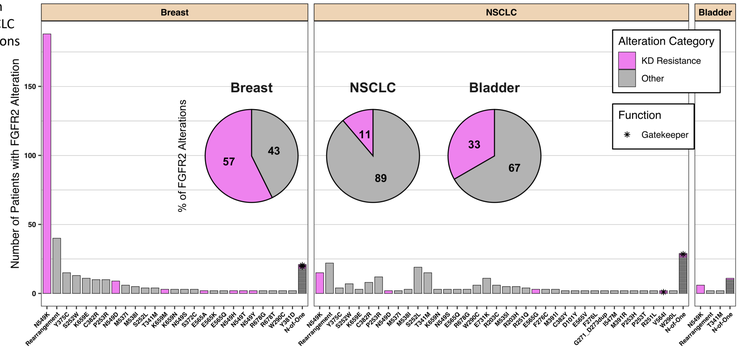
- 11% of FGFR2 alterations in NSCLC consist of kinase domain mutations

❖In Breast and NSCLC, which are predominantly FGFRi naïve, molecular brake mutations represent activating mutations which are likely to be resistant to 1st-gen FGFR inhibitors. Gatekeeper mutations are rare in the FGFRi naïve setting and are more often seen post FGFRi therapy as a mechanism of acquired resistance.

Bladder:
21 patients with FGFR2 alteration

- 33% are molecular brake mutations, typically N549K

Figure 10



CONCLUSIONS

- FGFR2/3 oncogenic alterations are found in various cancer types and are likely to be primary drivers; the highest number are found in **NSCLC, Breast, Bladder and Cholangiocarcinoma**
- 36% of the FGFR2 alterations identified across all cancer types consist of kinase domain mutations that are potentially resistant to 1st-generation FGFR inhibitors. Molecular brake mutations are most prevalent in **Cholangiocarcinoma** and **Breast Cancer**
- **This ctDNA database demonstrates that kinase domain mutations (molecular brake and gatekeeper) are common in cholangiocarcinoma patients, and they likely represent polyclonal, acquired resistance to the currently available FGFR2 inhibitors**
  - Patients with FGFR2/3 molecular brake and gatekeeper mutations may benefit from next-generation FGFR2/3-targeted therapies designed to inhibit a broad spectrum of both primary and secondary resistance mutations
  - ❖ **KIN-3248 is a next generation, potent and selective, irreversible pan-FGFR inhibitor with broad coverage of all known acquired resistance mutations. A phase1/1b clinical trial (NCT05242822) of KIN-3248 is enrolling advanced tumors with FGFR2 or FGFR3 alterations.**