Clinicogenomic Analysis of FGFR2-Rearranged Cholangiocarcinoma Identifies Correlates of Response and Mechanisms of Resistance to Pemigatinib

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Pemigatinib, a selective FGFR1–3 inhibitor, has demonstrated antitumor activity in FIGHT-202, a phase II study in patients with cholangiocarcinoma harboring FGFR2 fusions/rearrangements, and has gained regulatory approval in the United States. Eligibility for FIGHT-202 was assessed using genomic profiling; here, these data were utilized to characterize the genomic landscape of cholangiocarcinoma and to uncover unique molecular features of patients harboring FGFR2 rearrangements. The results highlight the high percentage of patients with cholangiocarcinoma harboring potentially actionable genomic alterations and the diversity in gene partners that rearrange with FGFR2. Clinicogenomic analysis of pemigatinib-treated patients identified mechanisms of primary and acquired resistance. Genomic subsets of patients with other potentially actionable FGFR/FGFR alterations were also identified. Our study provides a framework for molecularly guided clinical trials and underscores the importance of genomic profiling to enable a deeper understanding of the molecular basis for response and nonresponse to targeted therapy.

**SIGNIFICANCE:** We utilized genomic profiling data from FIGHT-202 to gain insights into the genomic landscape of cholangiocarcinoma, to understand the molecular diversity of patients with FGFR2 fusions or rearrangements, and to interrogate the clinicogenomics of patients treated with pemigatinib. Our study highlights the utility of genomic profiling in clinical trials.
cholangiocarcinoma, including patients with FGFR2 fusions or rearrangements (22). In this study, patients were initially prescreened prior to enrollment for FGE/FGFR alterations including amplifications, mutations, fusions, or rearrangements. The primary analysis of 107 patients included only patients harboring FGFR2 fusions or rearrangements. In these patients, pemigatinib monotherapy resulted in an independent centrally confirmed objective response rate (ORR) of 35.5% and a disease control rate of 82%. With a median follow-up of 15.4 months, responses were durable, with a median duration of response of 7.5 [95% confidence interval (CI), 5.7–14.5] months. Median progression-free survival (PFS) was 6.9 (95% CI, 6.2–9.6) months.

There is significant molecular diversity of FGFR2 fusions and rearrangements in patients with intrahepatic cholangiocarcinoma including a large number of partner genes that rearrange with FGFR2 (13, 17, 18, 23). Therefore, assays that are capable of detecting known and novel FGFR2 fusions or rearrangements are necessary to ensure the comprehensive characterization of tumors, which then enhances the likelihood of identifying all patients who may respond to FGFR2-targeted therapies. It is not yet known whether the FGFR2 partner gene has an impact on response or survival associated with FGFR inhibitor treatment. In addition, the question arises of whether FGFR2 rearrangements co-occur with other GAs, and whether this may also affect response to therapy.

Using the genomic profiling and clinical results from patients prescreened and enrolled in the FIGHT-202 trial, this study explores: (i) overall genomic landscape of cholangiocarcinoma; (ii) unique genomic features characteristic of FGFR2-rearranged cholangiocarcinoma; (iii) genomic correlates of response, including correlations with FGFR2 rearrangement partner and co-occurring GAs; (iv) response to pemigatinib in patients without FGFR2 rearrangements; and (v) acquired resistance to pemigatinib.

**RESULTS**

To identify patients for enrollment in FIGHT-202, 1,206 patients with cholangiocarcinoma from any anatomic location (however, in some instances, noncholangiocarcinoma biliary tract cancers may have been sent for analysis), based in the United States (n = 138), Europe (n = 569), and Middle East and Asia (rest of the world; n = 499), were prescreened using the FoundationOne assay (Foundation Medicine Inc.) for FGE/FGFR alterations prior to eligibility criteria assessment (Supplemental Table S1). We did not capture the precise anatomic location of prescreening samples, for example, whether the primary lesion was intrahepatic or extrahepatic. Furthermore, this prescreening population does not include 85 patients with an existing FoundationOne report or an FGE/FGFR status report derived from a local assessment with retrospective central confirmation using FoundationOne. The DNA-based, targeted NGS assay detects multiple alteration classes in up to 404 genes including those encoding components of the FGFR signaling pathway: FGFR1–4, FGFR3/4/6/10/12/14/19/23, and the FGFR substrate 2 (FRS2) adapter protein (24). FoundationOne detects rearrangements in select genes, including FGFR1–3, with both known and novel partners. This is critical due to the diversity of FGFR2 rearrangement partners in cholangiocarcinoma that have been reported by multiple groups (13, 25, 26). According to FoundationOne, FGFR2 rearrangements are further defined as fusions (i) if the genomic breakpoint is within the intron 17 or exon 18 hotspot and (ii) if the fusion gene partner is either a previously described fusion partner or a novel gene partner predicted to be an in-frame fusion with FGFR2. Other reported FGFR2 rearrangements include those with genomic breakpoint within the FGFR2 intron 17 or exon 18 hotspot and with either (i) a novel partner gene predicted to be out of frame or out of strand with FGFR2, or (ii) no identifiable partner gene (designated as intron 17 rearrangement or partner N/A). Therefore, FGFR2 fusions are a subset of FGFR2 rearrangements and collectively are referred to as FGFR2 rearrangements.

**GAs in Cholangiocarcinoma**

In total, from 1,206 patients we identified 5,547 GAs in 335 genes, accounting for a mean of 4.6 GAs per patient. Short variants were the most frequent GAs (3,424; 2.8 alterations/patient), followed by copy-number alterations (1,676; 1.39 alterations/patient) and rearrangements (450; 0.37 alterations/patient). The most frequently altered genes were TP53 (40.0%), CDKN2A (29.0%), KRAS (22.6%), CDKN2B (19.7%), ARID1A (16.0%), SMAD4 (11.7%), IDH1 (10.2%), and BAP1 (10.2%; Fig. 1A). Potential clinically actionable alterations, defined as oncogenic driver alterations [including microsatellite instability–high (MSI-H) and high tumor mutational burden (TMB; ≥20 mutations per megabase)] with matched therapeutic agents either under investigation or approved in other tumor types, were identified in 44.5% of patients (Fig. 1B–D). These included IDH1 mutations (10.2%), ERBB2 mutations and amplifications (8.0%), FGFR2 mutations or rearrangements (7.1%), PIK3CA mutations (7.0%), and BRAF mutations or rearrangements (4.7%). Other less common oncogenic alterations (in order of decreasing frequency) were in NRAS, IDH2, EGFR, KRAS (G12C mutations only), MET, FGFR3, FGFR1, RET, JAK2, ALK, and ROS1 (Fig. 1B). MSI-H and high TMB level were identified in 0.7% and 1.2% of patients, respectively. No patient with a clinically actionable oncogenic driver alteration had co-occurring MSI-H status compared with 9 patients in the nonactionable cohort (P = 0.0042; Fisher exact test; Fig. 1C). High TMB was observed in 4 patients with actionable oncogenic driver alterations, compared with 10 patients without actionable oncogenic driver alterations (P = 0.1789; Fisher exact test; Fig. 1D). Although MSI-H and high TMB are highly correlated (27), 4 patients with actionable oncogenic driver alterations, including BRAF p.D594N, ERBB2 amplification, FGFR3–TK2 rearrangement, and PIK3CA p.E545K, had high TMB in the absence of MSI-H status. No patients with an FGFR2 fusion or rearrangement had high TMB or MSI-H status.

We also explored the co-alteration and mutual exclusivity patterns of GAs in the prescreening cohort (Fig. 1E and Supplementary Table S2). The most significant relationship between gene pairs was the co-occurrence of alterations in FGFR2 and BAP1 (OR, 9.6; q value, 1.4e-14). TP53 was significantly co-altered with ERBB2 (OR, 5.4; q value, 1.7e-10), CCNE1 (OR, 10.5; q value, 3.4e-6), and SMAD4 (OR, 2.7; q value, 6.5e-5). TP53 was mutually exclusive with BAP1 (OR,
Genomic Profiling in FGFR2-Rearranged Cholangiocarcinoma

In the prescreening cohort, we identified 74 patients (6.1%) with FGFR2 rearrangements (Supplementary Table S1). The frequency of FGFR2 rearrangements varied among regions. In the United States, 21 patients (15.2%) were positive, whereas 42 (7.4%) were positive in Europe and 11 (2.2%) were identified in the rest of the world. The frequencies of other GAs also varied among regions, with higher rates of TP53, CDKN2A, and KRAS and lower rates of BAP1 and PBRM1 observed in the rest of the world versus Europe and the United States (Supplementary Fig. S2 and Supplementary Table S4). Among patients identified as FGFR2 rearrangement–positive, 43 were enrolled on the study into the primary analysis cohort (cohort A). An additional 64 patients were enrolled into cohort A based on a preexisting FoundationOne report or other local laboratory test (report-in-hand; Fig. 2A). All patients enrolled in cohort A with local testing were confirmed by FoundationOne.

By combining the FGFR2-rearranged patients identified through prescreening with FoundationOne and patients with a preexisting FoundationOne report or those identified by local genomic testing that were confirmed by FoundationOne, we obtained genomic data on 138 patients with FGFR2 rearrangements. In total, we identified 140 FGFR2 rearrangements (2 patients each had 2 FGFR2 rearrangements), 113 of which were predicted to be fusions by FoundationOne (Supplementary Table S5; see Methods). We observed 63 unique FGFR2 rearrangement partner genes; the most frequent rearrangement found was the FGFR2–BICC1 fusion, accounting for 27.9% of FGFR2 rearrangements (Fig. 2B and Supplementary Table S5). Other recurrent FGFR2 partner genes included KIAA1217 (3.6%), TACC2 (2.9%), CCDC6 (2.9%), and AHCYL1 (2.9%). The second most frequent FGFR2 rearrangement identified was FGFR2-N/A (9.3%); N/A refers to rearrangements that occur in FGFR2 intron 17 or exon 18 fused to an intergenic region; see Methods). Strikingly, 32.9% of patients had a rearrangement partner that was unique to that patient and not shared with any other patient, whereas 15.7% of patients had a rearrangement partner that was shared with just one other patient. FGFR2 rearrangements most frequently occurred intrachromosomally on chromosome 10 (52.9%). However, interchromosomal rearrangements occurred with an additional 17 chromosomes, most commonly chromosome 1 (7.9%) and chromosome 12 (6.4%; Fig. 2C). A total of 15 patients had 17 FGFR2 mutations in the absence of FGFR2 fusions or rearrangements, most commonly p.C382R (n = 7) and p.P253R (n = 3; Supplementary Fig. S3).
Patients with FGFR2 rearrangements had fewer GAs (3.7 alterations/patient) than patients without FGFR2 rearrangements (4.7 alterations/patient). Among FGFR2-rearranged versus non–FGFR2-rearranged patients, the most frequent co-alterations were BAP1 (38.4% vs. 8.2%), CDKN2A (21.7% vs. 30.0%), CDKN2B (15.2% vs. 20.2%), and PBRM1 (9.4% vs. 10.7%; Fig. 2D). Similar to what was observed in our prescreening analysis, TP53 and KRAS alterations were observed less frequently in FGFR2-rearranged patients (5.1% vs. 10.7%), but were not significantly mutually exclusive when corrected for multiple testing (Fisher exact test; q value = 0.98; Supplementary Table S6).

Genomic Correlates of Response to Pemigatinib

We sought to interrogate the relationship between clinical response to pemigatinib and underlying molecular profiles. A total of 107 centrally confirmed FGFR2-rearranged patients were enrolled into cohort A of FIGHT-202. We first asked whether the biomarker classification of FGFR2 as a rearrangement or fusion had any impact on response to pemigatinib (Table 1). Of 107 patients with FGFR2 fusions or rearrangements, 92 were predicted to be fusions and 15 were classified as rearrangements only. No significant difference in ORR was observed for patients with FGFR2 alterations classified as rearrangements versus for patients with predicted fusions (40.0% vs.

Figure 2. Genomic analysis of FGFR2-rearranged cholangiocarcinoma. A, Prescreening and cohort diagram. B, Frequency of FGFR2 rearrangement partners. C, Distribution of FGFR2 rearrangement partners throughout the genome. D, Frequency of co-occurring alterations in FGFR2-rearranged (blue) versus non–FGFR2-rearranged (yellow) patient samples. The FGFR2 bars represent patients with FGFR2 alterations other than rearrangements. *, 31 FGFR2+ patients not meeting other eligibility criteria were excluded. †, Reflects patients with a signed informed-consent form (none were excluded); 21 patients with report-in-hand were enrolled in cohorts B/C or other. ‡, Indicates 1 patient (0.71%) each with ACLY, ARHGAP22, ATAD2, ATF2, BICD1, CCDC158, CDC42BPB, CEP128, COL16A1, CTNNA3, DBP, DNAJC12, EEA1, EIF4ENIF1, ERC1, GAB2, GTPC, INSC, KCTD1, KIAA1598, MATR3, MCU, NEDD4L1, NRPBF2, PAH, PDC1B, PXN, RABGAP1L, RASSF4, ROBO2, RPPAP3, SFI1, SOD1, SPICE1, STRN4, TBC1D1, TCTN3, TFEC, TCC28, TXLNB, U5H2A, VCL, WAC, WDHD1, ZMYM4, and ZNF521 rearrangement partners. ¶, Nonrearrangement alterations only.
Table 1. Relationship between GAs and clinical outcomes

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>ORR (%)</th>
<th>OR (95% CI), P value</th>
<th>Median PFS (95% CI), months</th>
<th>PFS P value</th>
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<tbody>
<tr>
<td>FGFR2 population (107)</td>
<td>35.5</td>
<td>—</td>
<td>6.9 (6.2–9.6)</td>
<td>—</td>
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<tr>
<td>Alteration classification</td>
<td></td>
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<tr>
<td>Fusion (92)</td>
<td>34.8</td>
<td>1.25 (0.4–3.8), 0.70</td>
<td>7.0 (6.0–10.5)</td>
<td>0.79</td>
</tr>
<tr>
<td>Rearrangement (15)</td>
<td>40.0</td>
<td>—</td>
<td>6.9 (4.7–11.7)</td>
<td></td>
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<tr>
<td>FGFR2 partner</td>
<td></td>
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<tr>
<td>Non-BICC1 (76)</td>
<td>36.8</td>
<td>0.82 (0.3–2.0), 0.65</td>
<td>9.0 (6.2–11.1)</td>
<td>0.20</td>
</tr>
<tr>
<td>BICC1 (31)</td>
<td>32.3</td>
<td>—</td>
<td>6.8 (2.6–8.9)</td>
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<tr>
<td>Tumor suppressor</td>
<td></td>
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<tr>
<td>Unaltered (43)</td>
<td>37.2</td>
<td>0.88 (0.4–2.0), 0.76</td>
<td>11.7 (9.1–17.4)</td>
<td>0.0003</td>
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<tr>
<td>Altered (64)</td>
<td>34.4</td>
<td>—</td>
<td>6.8 (4.9–6.9)</td>
<td></td>
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<tr>
<td>BAP1</td>
<td></td>
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<tr>
<td>Unaltered (68)</td>
<td>30.9</td>
<td>1.7 (0.8–3.9), 0.19</td>
<td>9.1 (6.2–11.7)</td>
<td>0.06</td>
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<tr>
<td>Altered (39)</td>
<td>43.6</td>
<td>—</td>
<td>6.9 (4.7–8.9)</td>
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<tr>
<td>CDKN2A/B</td>
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<tr>
<td>Unaltered (86)</td>
<td>38.4</td>
<td>0.50 (0.2–1.5), 0.22</td>
<td>9.0 (6.4–11.1)</td>
<td>0.03</td>
</tr>
<tr>
<td>Altered (21)</td>
<td>23.8</td>
<td>—</td>
<td>6.4 (1.7–6.9)</td>
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<td>PBRM1</td>
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<tr>
<td>Unaltered (97)</td>
<td>36.1</td>
<td>0.76 (0.2–3.1), 0.70</td>
<td>7.0 (6.8–10.5)</td>
<td>0.05</td>
</tr>
<tr>
<td>Altered (10)</td>
<td>30.0</td>
<td>—</td>
<td>4.7 (1.4–10.8)</td>
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<tr>
<td>TPS3</td>
<td></td>
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<tr>
<td>Unaltered (98)</td>
<td>38.8</td>
<td>—</td>
<td>9.0 (6.8–11.1)</td>
<td>0.0003</td>
</tr>
<tr>
<td>Altered (9)</td>
<td>0</td>
<td>—</td>
<td>2.8 (1.4–6.8)</td>
<td></td>
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<tr>
<td>PIK3CA</td>
<td></td>
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<tr>
<td>Unaltered (98)</td>
<td>35.7</td>
<td>0.90 (0.2–3.8), 0.89</td>
<td>8.8 (6.4–10.5)</td>
<td>0.10</td>
</tr>
<tr>
<td>Altered (9)</td>
<td>33.3</td>
<td>—</td>
<td>5.2 (1.5–11.1)</td>
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<tr>
<td>IDH1</td>
<td></td>
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<tr>
<td>Unaltered (102)</td>
<td>36.3</td>
<td>0.44 (0.05–4.1), 0.47</td>
<td>6.9 (6.1–9.6)</td>
<td>0.28</td>
</tr>
<tr>
<td>Altered (5)</td>
<td>20.0</td>
<td>—</td>
<td>NE (1.4–NE)</td>
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Abbreviation: NE, not estimated.

*Model did not converge.

34.8%, P = 0.70). Furthermore, median PFS was similar between these two populations (6.9 months vs. 7.0 months, P = 0.79).

Due to the large number (n = 63) of unique rearrangement partners, we chose to compare the response to pemigatinib in patients with the most frequent rearrangement partner, FGFR2–BICC1 (n = 31), with all others (n = 76; Fig. 3A). There was no significant difference in ORR (32.3% vs. 36.8%, P = 0.65) or median PFS (6.8 months vs. 9.0 months, P = 0.20) in BICC1 versus all other partners (Fig. 3B and Table 1). No other comparisons of rearrangement partners were made due to the small numbers of patients with any specific rearrangement partner other than BICC1.

Co-occurring alterations may be a mechanism of primary resistance to pemigatinib. We interrogated whether co-occurring GAs had any impact on response to pemigatinib (Fig. 4A and Table 1). The gene with the most frequent co-occurring GAs (BAP1) did not have a consistent impact on response criteria. ORR was higher in BAP1-altered versus BAP1-unaltered patients but did not reach statistical significance (43.6% vs. 30.9%, P = 0.19). In contrast, BAP1-altered patients trended...
 numerically toward shorter median PFS (6.9 months vs. 9.1 months, \( P = 0.06 \)). Patients with GAs in CDKN2A/B or PBRM1 alterations trended toward a lower ORR (CDKN2A/B, 23.8\% vs. 38.4\%, \( P = 0.22 \); PBRM1, 30.0\% vs. 36.1\%; \( P = 0.70 \)), although not statistically significant, and significantly shorter median PFS (CDKN2A/B, 6.4 months vs. 9.0 months, \( P = 0.03 \); PBRM1, 4.7 months vs. 7.0 months, \( P = 0.05 \)). Of note, patients with TP53 alterations (\( n = 9 \)) had no objective responses and significantly shorter median PFS (2.8 months vs. 9.0 months, \( P = 0.0003 \); Table 1 and Fig. 4B). However, 6 patients had stable disease, 3 with PFS >6 months, 2 patients experienced tumor shrinkage of 50\% and 33\%, and only 1 patient had tumor growth >20\%.

Loss of different tumor-suppressor genes may play overlapping roles in tumor development. Therefore, we asked whether patients with alterations in at least one well-known tumor suppressor, including BAP1, CDKN2A/B, TP53, PBRM1, ARID1A, or PTEN, had a different response from patients without tumor-suppressor loss. We found no significant difference in the ORR between patients with alterations in tumor-suppressor genes versus those without alterations (34.4\% vs. 37.2\%, \( P = 0.76 \)); however, we observed significantly shorter median PFS (6.8 months vs. 11.7 months, \( P = 0.0003 \)) in patients with tumor-suppressor gene loss as compared with those with no tumor-suppressor gene loss (Fig. 4C).

A small number of patients had co-occurring alterations in other known oncogenic driver genes, including PIK3CA (\( n = 9 \)) and IDH1 (\( n = 5 \)). PIK3CA, which is downstream of FGFR2, has been hypothesized to participate in signaling networks mediating primary resistance to FGFR inhibitors. However, no significant difference between PIK3CA-altered and PIK3CA-unaltered patients was observed in ORR (33.3\% vs. 38.4\%, \( P = 0.22 \)) or in median PFS (5.2 months vs. 8.8 months, \( P = 0.1 \); Table 1). A partial response was seen in 1 patient harboring an IDH1 alteration; however, the limited number of patients with co-occurring IDH1 mutations in this study makes it impossible to assess the impact of these mutations on median PFS.

Response to Pemigatinib in Patients without FGFR2 Fusions/Rearrangements

In FIGHT-202, an additional 20 patients with other FGFR or FGFR alterations were enrolled to cohort B (13 identified by prescreening and 7 with report-in-hand), and 18 patients without FGFR or FGFR alterations were enrolled to cohort C (5 identified by prescreening and 13 with report-in-hand; Supplementary Fig. S4A). One patient without FGFR or FGFR alterations was enrolled in cohort C based on a local report, but did not have sufficient sample material for confirmation by FoundationOne (data for 17 subjects are therefore presented). No independent centrally confirmed objective
responses were observed outside of cohort A, and median PFS was shorter in cohort B [2.1 (95% CI, 1.2–4.9) months] and cohort C [1.7 (95% CI, 1.3–1.8) months]; however, several patients achieved stable disease, and tumor shrinkage was noted in a subset of patients (22).

To identify additional genomic subsets of patients for whom pemigatinib may elicit antitumor activity, we examined the GAs in these patients without FGFR2 fusions or rearrangements (Supplementary Fig. S4B and S4C). Most notably, 3 of the 4 patients treated with pemigatinib who had FGFR2 p.C382R mutations achieved a best overall response of stable disease, with a PFS of 6.9, 4.0, and 9.0 months. Two of these patients had tumor shrinkage (−26.0% and −30.6%, unconfirmed) prior to disease progression. All 4 patients with the FGFR2 p.C382R mutation had co-occurring BAP1 alterations. We also observed stable disease in 3 of 6 patients with FGFR3/4/19 and CCND1 co-amplifications, with 2 patients achieving tumor shrinkage (−32.5% and −41.4%, unconfirmed). One of 8 patients with FRS2 amplifications achieved stable disease. In cohort C, tumor shrinkage was observed in 3 patients, but no unifying genomic features could be identified. Although only a small number of patients with each specific alteration type were treated in these cohorts, these data offer the potential for activity of pemigatinib in patients with other FGFR pathway alterations.

Acquired Resistance to Pemigatinib

We performed genomic profiling on 8 patients with initial tumor shrinkage followed by progressive disease using available tumor tissue (n = 6) or plasma (n = 2; Table 2); scans are provided for 5 patients (Supplementary Fig. S5). Strikingly, every patient analyzed had at least one acquired mutation in FGFR2. All mutations were located in the FGFR2 kinase domain and were previously identified as resistance mutations to pemigatinib or other FGFR inhibitors (28–30). In total, we observed six unique mutations spanning five amino acid residues. FGFR2 p.N549K/H was observed in 4 patients, whereas FGFR2 p.E565A, p.K659M, p.L617V, and p.K641R were each observed in 2 patients. Polyclonal resistance, defined by the presence of multiple acquired alterations in the same patient, was identified in 3 patients, whereas only a single mutation was observed in 5 other patients.

We performed in silico structural modeling to further understand the relationship between identified acquired resistance mutations in the kinase domain and pemigatinib binding (Fig. 5A and B). Similar to previous reports with other FGFR inhibitors, pemigatinib is in close contact with the FGFR2 p.V564 gatekeeper residue. FGFR2 p.N549, p.K641, and p.E565 contribute to a molecular brake that keeps the kinase in an inactive conformation (31). Mutations that disrupt hydrogen bonding between any of these amino acids lead to a conformational shift and constitutive kinase activation. The FGFR2 p.K659 residue is important for stabilizing the inactive conformation of the activation loop (32). Thus, mutation of this residue also leads to kinase activation. FGFR2 p.L617 resides in the hydrophobic spine and interacts with the phenylalanine residue of the “DFG” motif, which stabilizes a motif conformation conducive to binding of FGFR inhibitors (28). For example, mutation of FGFR2 p.L617 to a valine strengthens the hydrophobic spine of the kinase, leading to less favorable pemigatinib-binding conditions. Collectively, these data demonstrate that acquired resistance to pemigatinib mirrors that of other FGFR inhibitors in FGFR2-rearranged cholangiocarcinoma.

DISCUSSION

By using genomic profiling to prescreen patients for enrollment in FIGHT-202, we compiled a large database of mutational profiles for patients with cholangiocarcinoma. Given the rarity of cholangiocarcinoma, this information provides valuable insights into the broad mutational spectrum of patients with this disease. Mutations in known tumor suppressors were widespread and most prevalent in TP53, CDKN2A/B, ARID1A, SMAD4, and PBRM1. KRAS was the most frequently altered oncogene; however, KRAS p.G12C accounted for <5% of KRAS alterations. With the recent development of KRAS p.G12C inhibitors, this small population may benefit from targeted therapies. We estimated the frequency of actionable alterations in patients with cholangiocarcinoma to be approximately 45%, predominantly driven by alterations in IDH1/2, ERBB2, FGFR2, PIK3CA, and BRAF. However, other potentially actionable alterations were detected in <3% of patients each, including BRCA1/2, FGFR3, FGFR1, KRAS, MET, ALK, RET, and ROS1. Notably, no NTRK1–3 rearrangements were identified in this cohort, despite previous reports of identification and clinical benefit for NTRK-targeted therapeutics in patients with NTRK1 cholangiocarcinoma (33, 34). We also found a small number of patients with MSI-H tumors who may benefit from inhibition of the PD-1/PD-L1 axis. Collectively, these data suggest that a substantial proportion of patients with cholangiocarcinoma may benefit from molecularly targeted therapy in a disease with no currently approved second-line therapies.

In addition to FGFR2, clinical trials for molecular targeted agents are ongoing for other alterations in cholangiocarcinoma, including agents targeting IDH1. Patients with cholangiocarcinoma have been enrolled in basket trials for NTRK1–3 inhibitors and anti–PD-1 antibodies, which formed the basis for tumor-agnostic approvals for larotrectinib, entrectinib, and pembrolizumab. Furthermore, patients with cholangiocarcinoma were included in other basket trials, including a phase II trial of vemurafenib in patients with BRAF p.V600E mutations (35). Analysis of co-alteration patterns revealed that the strongest association between gene pairs was between FGFR2 and BAP1. BAP1 is a deubiquitinase that forms the catalytic subunit of the polycomb-repressive deubiquitinase complex involved in chromatin remodeling and is a bona fide tumor suppressor frequently mutated in mesothelioma, renal cell carcinoma, and uveal melanoma (36–38). Jain and colleagues previously reported BAP1 as the most frequently co-altered gene with FGFR in cholangiocarcinoma and observed no association with overall survival (39). Consistent with their data, we did not observe a statistically significant difference in ORR or median PFS for BAP1-altered versus BAP1-unaltered patients treated with pemigatinib. Furthermore, Jain and colleagues observed an association of patients with co-occurring TP53 or CDKN2A/B alterations with shorter
## Table 2. Identification of acquired resistance mutations in patients with FGFR2 rearrangements treated with pemigatinib

<table>
<thead>
<tr>
<th>Patient #</th>
<th>Tumor change from baseline, %</th>
<th>BOR</th>
<th>PFS, months</th>
<th>Analysis platform</th>
<th>Baseline FGFR2 rearrangement</th>
<th>Co-alteration(s)</th>
<th>Analysis platform</th>
<th>FGFR2 rearrangement</th>
<th>Acquired FGFR2 alterations (allele frequency)</th>
<th>Co-alteration(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-15.7</td>
<td>SD</td>
<td>13.1</td>
<td>MSKI</td>
<td>F1GFR2–NRBF2 fusion</td>
<td>BAP1 p.Q393*</td>
<td>F1</td>
<td>F1GFR2–NRBF2 fusion</td>
<td>p.N549K (23.19%)</td>
<td>CDKN2A/B loss, BAP1 p.Q393*</td>
</tr>
<tr>
<td>2</td>
<td>-60.5</td>
<td>PR</td>
<td>15.9 (censored)</td>
<td>F1</td>
<td>F1GFR2–KIAA1217 fusion</td>
<td>None</td>
<td>F1</td>
<td>F1GFR2–KIAA1217 fusion</td>
<td>p.K659M (6.07%)</td>
<td>None</td>
</tr>
<tr>
<td>4</td>
<td>-33.3</td>
<td>SD</td>
<td>6.8</td>
<td>F1</td>
<td>F1GFR2–AHCYL1 fusion</td>
<td>KDR p.P1243L, TP53 p.R280G, FANCG splice site</td>
<td>F1</td>
<td>F1GFR2–AHCYL1 fusion</td>
<td>p.L617V (33.9%)</td>
<td>None</td>
</tr>
<tr>
<td>6</td>
<td>-39.8</td>
<td>PR</td>
<td>6.9 (censored)</td>
<td>F1</td>
<td>F1GFR2–PAWR fusion</td>
<td>BAP1 splice site, STAG2 p.MI48fs*3</td>
<td>WESb</td>
<td>N/A</td>
<td>p.E565A (6.1%), p.K641R (23.7%)</td>
<td>BAP1 splice site, STAG2 p.MI48fs*3</td>
</tr>
<tr>
<td>7</td>
<td>-13</td>
<td>SD</td>
<td>8.8 (censored)</td>
<td>F1</td>
<td>F1GFR2–ATAD2 fusion</td>
<td>MCL1 Amp, NTRK1 Amp</td>
<td>F1L</td>
<td>F1GFR2–ATAD2 fusion</td>
<td>p.N549H (0.12%)</td>
<td>PTEN p.T319fs*1</td>
</tr>
<tr>
<td>8</td>
<td>-46</td>
<td>PR</td>
<td>9.1</td>
<td>F1</td>
<td>F1GFR2–TRIM8 rearrangement</td>
<td>EPHB6 p.R125Q</td>
<td>F1L</td>
<td>F1GFR2–TRIM8 rearrangement</td>
<td>p.E565A (0.19%), p.L617V (0.32%), p.K659M (2.71%)</td>
<td>None</td>
</tr>
</tbody>
</table>

Abbreviations: BOR, best objective response; PR, partial response; SD, stable disease; MSKI, MSK Impact; F1, FoundationOne; F1L, FoundationOne Liquid; WES, whole exome sequencing.

*Censored at the time of the original data cutoff of March 22, 2019 (22), but progressed afterward.

*Mutation data only were used from whole-exome sequencing. FGFR2 fusions/rearrangements are not detectable in whole-exome sequencing data.

*Not present in the FoundationOne panel.
overall survival (39). Our results echo these results, finding that patients with TP53, CDKN2A/B, and PBRM1 alterations had shorter median PFS on pemigatinib than those without alterations in these genes. However, the small number of patients, particularly with TP53 or PBRM1 alterations, must be acknowledged as a limitation. In this study, we advanced these prior efforts by grouping alterations in known tumor-suppressor genes, including BAP1, CDKN2A/B, TP53, PBRM1, ARID1A, or PTEN. This group of patients, accounting for 63% of all patients, had significantly shorter median PFS, although ORR was similar between the two groups. It should be noted, however, that responses or prolonged stable disease has been observed in individual patients within this group. Collectively, these data suggest that patients with specific co-occurring alterations, especially in tumor-suppressor genes, may have worse outcomes with FGFR inhibitors. Additional strategies, such as combination therapies, may be particularly warranted in this patient population.

The co-occurrence and mutual exclusivity analysis also revealed that although IDH1 alterations were depleted in patients with FGFR2 rearrangements, they were not statistically mutually exclusive. This was surprising given the known role of both FGFR2 and IDH1 as oncogenic drivers in intrahepatic cholangiocarcinoma and in contrast to previous reports of mutual exclusivity between FGFR2 and IDH1 (13). Of 5 patients with both FGFR2 rearrangements and IDH1 alterations enrolled in FIGHT-202, 1 had a partial response, suggesting that FGFR inhibitors can have activity in the presence of both alterations; however, more data are required to determine if the IDH1 alterations affect median PFS. As both FGFR and IDH1 inhibitors are under clinical evaluation in cholangiocarcinoma, it will be important to study how this subset of patients responds to single or combination therapies.

In this study, we identified a large number of partner genes for FGFR2; in 138 patients with FGFR2 rearrangements profiled, we identified 63 unique FGFR2 partner genes. Furthermore, 27 patients were classified as having only an FGFR2 rearrangement, and 13 of these rearrangements were reported to occur without an identified partner gene. The diversity in FGFR2 rearrangement partners has been reported by previous studies (13, 15, 26, 40). For FGFR2, the mechanism of activation is unique from most other kinase fusions in that FGFR2 fusions are expressed by the endogenous FGFR2 promoter and include the majority of the protein. In general, the final exon (exon 18), corresponding to the cytoplasmic post-kinase region, is replaced with sequences from the partner gene. Although the most prevalent FGFR2 partner (BICC1) has a dimerization domain, not all FGFR2 rearrangement partners have known or predicted dimerization domains, suggesting that the pervasiveness of FGFR2 partners may be due to lack of requirement for sequence or functional specificity of partner genes. Importantly, we saw no differences in clinical response to pemigatinib in patients with BICC1 versus other partners.

DNA-based assays, such as FoundationOne, resolve the genomic breakpoints of the rearrangement and do not interrogate the fusion at the level of RNA or protein. Therefore, rearrangements not classified as fusions, including those with no identified partner gene, may express fusion proteins by utilizing posttranscriptional controls, such as alternative splicing, or be generated through more complex genomic rearrangements involving multiple breakpoints, termed “bridged fusions” (41). Alternatively, these events may represent truncation of FGFR2 in the post-kinase region. FGFR2 truncation has been shown to be sufficient to drive ligand-independent growth and may represent an alternative mechanism of FGFR2 activation, distinct from fusions (42-44). FGFR2 truncations may be missed by RNA-based approaches that are not designed to identify truncating rearrangements. Detailed analysis of these FGFR2 rearrangements, on both DNA- and RNA-based platforms, will be necessary to resolve these questions. We saw no differences in clinical outcomes for patients with FGFR2 rearrangements versus fusions and did see responses in patients with FGFR2 rearrangement with no identified partner gene. Due to the small sample size, it is not possible to suggest the functional equivalency of fusions and rearrangements.

Figure 5. In silico analysis of acquired resistance mutations in FGFR2. Full (A) and zoomed-in (B) view of the FGFR2 ATP-binding pocket interaction with pemigatinib (orange). Important kinase domain regions are highlighted; gold, hinge region; red, catalytic loop; blue, activation domain; purple, c-alpha-helix; green, P-loop; cyan, DFG motif. Relevant amino acid residues are shown as colored ball and stick models.
Although no objective responses were observed in patients treated with pemigatinib without FGFR2 rearrangements or fusions, and median PFS was shorter in these patients, post hoc genomic subset analysis identified interesting patterns that may warrant further investigation. We noted stable disease in 3 of 6 patients treated with FGFR2 p.C382R mutation (2 of whom had tumor shrinkage prior to disease progression), which is a known oncogenic mutation in the extracellular domain of FGFR2 that activates FGFR signaling (45). Interestingly, all 4 patients with FGFR2 p.C382R mutations had co-occurring BAP1 alterations. Given the strong enrichment of BAP1 alterations in FGFR2-rearranged cholangiocarcinoma, this may suggest FGFR2 p.C382R and FGFR2 rearrangements have a similar molecular etiology. FGFR inhibitors have shown clinical efficacy in other cancer types with FGFR mutations, most notably in FGFR3-mutated urothelial carcinoma (46). Although activating FGFR2 mutations are rare in cholangiocarcinoma, future tumor-agnostic studies with FGFR inhibitors, such as the FIGHT-207 study (NCT03822117) of pemigatinib, will investigate the role of FGFR inhibition in FGFR2-mutated cholangiocarcinoma.

We observed stable disease in 3 of 6 patients treated with pemigatinib harboring the FG3/4/19 and CCND1 amplifications (2 patients with tumor shrinkage). FG3/4/19 are signaling ligands that bind with varying specificities to the different FGF receptors to promote dimerization and transphosphorylation (47). FG3/4/19 and CCND1 are co-located on the same chromosomal region (11q13) and are often co-amplified. Of note, Jain and colleagues also described a patient with cholangiocarcinoma harboring an FG3/19 amplification who was treated with an FGFR inhibitor for 1 year and had a stable response to treatment (39). Just 1 of 8 patients with FRS2 amplifications achieved stable disease. FRS2 is an adapter protein that links FGFR signaling to the MAP kinase pathway (48). Stable disease was observed in 3 patients in cohort C, but no unifying genomic features were identified. Additional investigation into the role of pemigatinib in patients without FGFR fusions or rearrangements is warranted. However, these data suggest a requirement for strong activation of the FGFR pathway, which may be restricted to FGFR rearrangements, fusions, and possibly activating mutations.

Consistent with previous reports of resistance to other FGFR inhibitors in FGFR2-rearranged cholangiocarcinoma, we found acquired FGFR2 mutations in all 8 patients analyzed at progression (28-30). FGFR2 p.N549H was identified as a resistance mechanism to pemigatinib in a patient with cholangiocarcinoma harboring an FGFR2-CLIP1 fusion, and was shown to shift the potency of selective FGFR inhibitors, including pemigatinib, AZD4547, infigratinib, and erdafitinib, in NIH3T3 cells expressing the FGFR2-CLIP1 fusion (30). Furthermore, all 6 acquired FGFR2 mutations identified in this study were previously reported by Goyal and colleagues in their studies of patients with FGFR2-rearranged cholangiocarcinoma who progressed on either infigratinib or Debio1347 (29). These investigators demonstrated that CCLP-1 cells engineered to express the FGFR2–PHGDH fusion with a series of these resistance mutations shifted the potency of infigratinib and Debio1347 to varying levels. In a second report, Krook and colleagues identified an FGFR2–KIAA1598 fusion–positive patient treated with infigratinib who developed both the FGFR2 p.E565A and p.L617M mutations (49). These investigators performed in vitro analysis of these mutations in three different cell lines and observed potency shifts to several selective FGFR inhibitors including infigratinib, AZD4547, and erdafitinib. The only mutation identified in the current study that has not been profiled in vitro is FGFR2 p.K641R. Given the role of FGFR2 p.K641 in contributing to the molecular brake (along with p.N549 and p.E565), this mutation is expected to similarly affect pemigatinib function.

Although pemigatinib was not specifically evaluated in these later in vitro studies, the shared structural features of FGFR inhibitors combined with shared clinical resistance profiles provide compelling evidence to support the identified FGFR2 mutations as contributing to resistance.

Polyclonal resistance, involving multiple acquired FGFR2 mutations, was observed in 3 patients. This finding is reminiscent of previous evidence from other groups demonstrating polyclonal acquired resistance in patients with FGFR2-rearranged cholangiocarcinoma treated with infigratinib or Debio1347 (28, 29, 50). Collectively, these findings suggest a high level of tumor heterogeneity and a strong selective pressure by FGFR inhibitors.

Interestingly, no gatekeeper (FGFR2 p.V564) mutations were identified in this study as has been reported with other FGFR inhibitors (28, 29). Differences in mutational resistance profile are likely due to differences in drug-binding specificities. Further study into the exact resistance profile for all FGFR inhibitors is needed to ensure appropriate treatment order for patients who acquire resistance. There is a need for FGFR inhibitors whose potency is not affected by these kinase domain mutations, including the gatekeeper mutations.

Although these data offer useful insights into the overall molecular landscape of patients with cholangiocarcinoma, it is important to consider the limitations of our study. Patients prescreened for FIGHT-202 included those with both intrahepatic and extrahepatic cholangiocarcinoma subtypes, location not being captured in the clinical database. Cholangiocarcinoma subtypes are known to harbor distinct mutational profiles, and thus estimation of actionable alteration frequencies in specific cholangiocarcinoma subtypes is not possible with these data (26). For example, the frequency of FGFR2 rearrangements (6.1%) is lower than previously reported for pure intrahepatic cholangiocarcinoma populations (10%-16%), likely due to the inclusion of patients with extrahepatic cholangiocarcinoma (typically negative for FGFR2 rearrangements) in the prescreening population. Patients may have been previously prescreened for other clinical trials, and thus patients positive for clinical trial biomarkers, including FGFR2 and IDH1, may not be accurately represented in our cohort. Finally, regional differences in the availability of genomic profiling may have significantly skewed the purity of the cholangiocarcinoma population prescreened in different parts of the world. Genomic testing is regularly performed on patients with cholangiocarcinoma in the United States, but less frequently in Europe and the rest of the world. Thus, we saw large differences in the frequency of FGFR2 alterations by region (15.2% in United States, 7.4% in Europe, and 2.2% in the rest of the world), which may be due to inclusion of extrahepatic and noncholangiocarcinoma.
cancer subtypes. Consistent with the potential higher fraction of nonintrahepatic cholangiocarcinoma in Asia versus Europe and the United States, higher rates of TP53, CDKN2A, and KRAS were observed in patients from the rest of the world versus Europe and the United States and possibly reflect a difference in prevalence of extrahepatic versus intrahepatic cholangiocarcinoma rather than regional differences. Future studies should collect detailed clinical data on patients with cholangiocarcinoma around the world and analyze patients using the same genomic analysis platform.

In conclusion, our study highlights the utility of genomic profiling in the context of molecularly guided clinical trials. We utilized prescreening data from the FIGHT-202 clinical trial to gain additional insights into the broad molecular profile of patients with cholangiocarcinoma. We further investigated the unique molecular features of patients with FGFR2-rearranged cholangiocarcinoma and interrogated the relationship between genomic profile and response to pemigatinib. Finally, utilizing additional NGS approaches, we identified mechanisms of acquired resistance to pemigatinib. Collectively, these data advance our understanding of patients with cholangiocarcinoma, FGFR2 rearrangements, and mechanisms of primary and acquired resistance to pemigatinib. These data should serve as a foundation for the advancement of treatment for patients with FGFR2-rearranged cholangiocarcinoma.

METHODS

Study Design

Details on the study design, eligibility criteria, and efficacy and safety findings of FIGHT-202 have been published previously (22). FIGHT-202 (NCT02924376), a phase II, open-label, multicenter, global study of pemigatinib in patients with previously treated advanced or metastatic cholangiocarcinoma, with or without FGF/FGFR alterations, was conducted at 146 sites in the United States, Europe, the Middle East, and Asia. Key eligibility criteria for enrollment in FIGHT-202 were as follows: age ≥18 years; confirmed diagnosis of locally advanced or metastatic cholangiocarcinoma based on histology/cytology; disease progression following ≥1 prior systemic therapy (prior receipt of selective FGFR inhibitors was not allowed); Eastern Cooperative Oncology Group performance status ≤2; radiologically measurable disease per RECIST v1.1. Before eligibility screening, patients were initially prescreened centrally for FGF/FGFR status using NGS (FoundationOne). Patients were also permitted to proceed to eligibility screening if they possessed an existing FoundationOne report or an FGF/FGFR status report derived from a local assessment. Local documentation of FGF/FGFR status required retrospective central confirmation using FoundationOne. Based on central results, patients were assigned to cohorts A (FGFR2 fusions or rearrangements), B (other FGF/FGFR GAs), or C (no FGF/FGFR GAs). Regardless of cohort assignment, patients orally self-administered pemigatinib at a starting dose of 13.5 mg once daily on a 21-day (2-weeks-on, 1-week-off) cycle, until radiologic disease progression, unacceptable toxicity, withdrawal of consent, or decision to discontinue treatment by the patient or physician.

The primary endpoint of FIGHT-202 was ORR in cohort A per independent central review; secondary endpoints included were ORR in cohorts B, A + B (all patients with FGF/FGFR alterations), and C; duration of response, disease control rate, PFS, overall survival, and safety in all cohorts. Tumor response was assessed by independent review according to RECIST v1.1; disease status was assessed until disease progression or discontinuation due to any reason except disease progression. The present analysis investigated the relationship between genomic features and clinical outcomes in patients with cholangiocarcinoma harboring FGFR2 alterations receiving pemigatinib in FIGHT-202.

Genomic Analysis

Archival, formalin-fixed, paraffin-embedded tumor samples from all prescreened or enrolled patients were analyzed for GAs using the FoundationOne-targeted next-generation DNA-sequencing assay (Foundation Medicine Inc.), which uses hybrid capture–based DNA target enrichment to identify somatic GAs in the coding regions of 315 cancer-related genes and introns from 28 genes often rearranged in cancer (12). The assay detects multiple classes of GAs including base substitutions, insertions and deletions, copy-number alterations, and rearrangements. Patients entering the study with an existing FoundationOne report may have used a different version of the panel containing up to 404 genes. The FGFR2 content is the same, independent of which assay version was used. Amino acid numbering for the FGFR2 gene was based on the RefSeq transcript NM_000141.

Molecular Modeling

In silico modeling was performed using pemigatinib docked to FGFR2 (PDB structure 1EOC.pdb) Molecular operating environment (Chemical Computing Group Inc.).

Statistical Methods

Statistical analyses of genomic correlates of response, including FGFR2 rearrangement partner and co-occurring GAs, were performed using a log-likelihood ratio test. PFS distributions were estimated using the Kaplan–Meier method; statistical differences in PFS distributions in the presence or absence of FGFR2 alterations were calculated using the nonparametric log-rank test. Logistic regressions were employed to compare ORR between groups. Statistical analyses were performed using SAS (Enterprise Guide 7.1) and R version 3.5.2 [R-3.5.2 for Windows (32/64 bit); https://cran.r-project.org/bin/windows/base/old/3.5.2/].

Data Reporting

Researchers may request anonymized datasets from any interventional study (except phase I studies) for which the product and indication have been approved on or after January 1, 2020, in at least one major market (e.g., United States, Europe, and Japan). Information on Incyte’s clinical trial data sharing policy and instructions for submitting clinical trial data requests are available at: https://www.incyte.com/Portals/0/Assets/Compliance%20and%20Transparency/clinical-trial-data-sharing.pdf?vver=2020-05-21-132838-960

Authors’ Disclosures

J.M. Silverman reports other from Incyte Corporation outside the submitted work. A. Hollebecque reports grants from Incyte during the conduct of the study, personal fees and other from AstraZeneca, personal fees from Amgen, personal fees from Eisiol, personal fees from Servier, personal fees from QED Therapeutics, grants and other from AstraZeneca, grants, personal fees, and other from Incyte, other from Lilly, personal fees from Spectrum Pharmaceuticals, and other from Roche outside the submitted work. L. Friboulet reports grants from Incyte during the conduct of the study and grants from Debiopharm outside the submitted work. S. Owens reports other from Incyte Corporation outside the submitted work. R.C. Newton reports being an employee of the Incyte Research Institute. L. Félix reports other from Incyte during the conduct of the study; other from Incyte outside the submitted work; and is an employee from Incyte and owns stocks. T.C. Burn reports other from Incyte Corporation outside the submitted work. No disclosures were reported by the other authors.
Authors’ Contributions

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A. Hollebecque: Resources, investigation, writing-review and editing.
L. Friboulet: Resources, investigation, writing-review and editing.
S. Owens: Data curation, formal analysis, visualization, writing-review and editing.
R.C. Newton: Supervision, writing-review and editing.
H. Zhen: Data curation, supervision, methodology, writing-review and editing.
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D. Melisi: Resources, investigation, writing-review and editing.
T.C. Burn: Conceptualization, supervision, methodology, writing-review and editing.

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