Paralog-Specific Kinase Inhibition of FGFR4: Adding to the Arsenal of Anti-FGFR Agents

Leisl M. Packer and Pamela M. Pollock

Summary: In this issue of Cancer Discovery, Hagel and colleagues report the design and the in vitro and in vivo activity of a novel, irreversible, paralog-specific kinase inhibitor of FGFR4, BLU9931. This compound binds covalently to a cysteine residue in the hinge region of FGFR4 but not in FGFR1–3. BLU9931 induces tumor shrinkage in hepatocellular carcinoma models that express a functioning ligand/receptor complex consisting of FGF19/FGFR4/KLB and adds to a growing list of anti-FGFR4 agents. Cancer Discov; 5(4); 355–7. © 2015 AACR.

See related article by Hagel et al., p. 424 (2).

Alongside the identification of cancers driven by FGFR activation, the past few years have delivered multiple second- and third-generation anti-FGFR agents. These include inhibitors with greater selectivity (e.g., AZD4547 and NVP-BGJ398), irreversible FGFR kinase inhibitors (FIN1-1, FIN1-2), mAbs specific to each family member (FGFR1–4), as well as allosteric inhibitors targeting the extracellular domain that inhibit receptor internalization (SSR128129E). With respect to the competitive kinase inhibitors, the search for greater FGFR selectivity was driven, to some extent, by the desire to reduce toxicity associated with VEGFR2 inhibition. However, the selection against VEGFR2 was accompanied by a loss of selectivity against FGFR4. As a result, almost all the competitive FGFR kinase inhibitors currently in phase I/II trials (AZD4547, NVP-BGJ398, JNJ-42756493, BAY1163877, Debio 1347, and LY2874455) show at least a 4-fold greater selectivity against FGFR1–3 over FGFR4 (1).

Hagel and colleagues (2) report the design and the activity of an FGFR4-specific inhibitor, BLU9931, in several models of hepatocellular carcinoma (HCC). This group utilized structure-based drug design to create a compound that binds covalently to Cys552 in the hinge region of FGFR4. This cysteine is unique to FGFR4, as FGFR1–3 possess a bulky tyrosine residue that clashes with BLU9931. As a result of modifying Cys552, BLU9931 is a potent and selective FGFR4 inhibitor (IC50 = 3 nmol/L) with only weak activity against FGFR1–3 (IC50 values range from ∼130 to 300 nmol/L). Furthermore, BLU9931 was unable to inhibit the six other kinases that also have a cysteine at the corresponding position within the hinge region.

The group at Blueprint Medicines shows that BLU9931 inhibits FGFR4 and blocks phosphorylation of its substrates FRS2α, MAPK, and AKT at 10 to 30 nmol/L in the MDA-MB-453 breast cancer cell line carrying an activating Y367C mutation in FGFR4, but not in DMS-11 lung cancer cells carrying amplification of FGFR1. With regard to HCC, they confirmed previous reports with pan-FGFR inhibitors showing only cells expressing a fully functional receptor complex comprising of FGF19/FGFR4/KLB and FGF19 respond to BLU9931 (3). Of the 11 HCC cell lines they tested, 4 cell lines expressed all three components, and 3 of these, all with FGF19 amplification, were sensitive to BLU9931. The authors report that twice-daily 30-mg/kg doses led to tumor shrinkage in the Hep3B xenograft model; however, twice-daily doses of 300 mg/kg were required to induce tumor shrinkage in an FGF19-overexpressing HCC patient-derived xenograft model. Which of these models represents the more common effective dose in humans is currently unknown.

Toxicity issues arising from phase I clinical trials of the FGFR1–3 inhibitors AZD4547, NVP-BGJ398, and JNJ-42756493 indicate that hyperphosphatemia and tissue mineralization represent on-target class effects, which can be clinically managed by diet modification and drugs. A specific FGFR4 kinase inhibitor is not expected to share this toxicity profile. However, one caveat to an improved toxicology profile with specific FGFR4 inhibition comes from preclinical toxicology studies with an anti-FGF19 mAb in cynomolgus monkeys (4). Although mAbs targeting FGF19 and FGFR4 have shown tumor regression in murine xenograft models, additional toxicology studies were performed in monkeys given they share similar mechanisms of cholesterol turnover and bile acid synthesis with humans. Pai and colleagues (4) reported that animals in the 10-, 30-, and 100-mg/kg cohorts but not in the 2-mg/kg cohort had to be euthanized in the subsequent weeks after their first dose due to severe diarrhea, low food consumption, and decreased body weight. Subsequent mechanistic studies revealed increases in multiple liver enzymes, suggestive of liver toxicity, as well as increased synthesis of multiple bile acids and ileal malabsorption of bile acids. Although a similar toxicity profile could be expected in monkeys due to specific FGFR4 inhibition with BLU9931, the authors propose that the combination of irreversible binding and the shorter half-life of this compound (2.3 hours), compared with the anti-FGF19 mAb (days/weeks), may allow for fine-tuning of the dosing sched-
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tation in the antiangiogenic effects associated with pan-FGFR sensitivity. Finally, another potentially significant disadvantage due to kinase switching to other FGFR family members.

It is interesting to note that, although BLU9931 had much better activity than NVP-BGJ398 in the breast cancer cell line dependent on FGFR4C567C (Fig. 2 in ref. 2), similar IC50 values were seen with both BLU9931 and BGJ398 in the three sensitive HCC cell lines (Table 1 in ref. 2). The latter data are consistent with these HCC cell lines expressing other FGFR family members that are contributing to their NVP-BGJ398 sensitivity. Finally, another potentially significant disadvantage to paralog-specific FGFR4 inhibition would be a reduction in the antiangiogenic effects associated with pan-FGFR inhibition or dual VEGFR/FGFR inhibition.

The search to identify the “best-in-class” FGFR inhibitor, in addition to finding the right balance between clinical effectiveness and patient toxicity, will involve the ability of the new agent to induce lasting tumor regressions and improve overall survival in patients. Our understanding of intrinsic and acquired mechanisms of resistance to FGFR inhibition is beginning to emerge and, although mechanisms of downstream and parallel kinase switching are likely to vary with tissue context (unpublished data, Pamela M. Pollock).

preclinical studies have already confirmed the importance of the gatekeeper residue in the FGFRs. The physical proximity between Cys552 and the V550 gatekeeper residue suggests that FGFR4 gatekeeper mutations may block BLU9931 binding. In addition, irreversible inhibitors that form a covalent bond with a cysteine residue are uniquely susceptible to mutations affecting the cysteine residue itself. Although not paralog specific, the second generation of irreversible pan-FGFR inhibitors targeting the other conserved cysteine in the hinge region has shown activity against multiple gatekeeper mutations in FGFR2–4 (6) and may offer an approach to prevent or overcome both FGFR kinase switching and the acquisition of gatekeeper mutations. Results from preclinical toxicology studies and/or comparative effectiveness studies with these two inhibitors are eagerly anticipated.

As with many kinase inhibitors, the results from early clinical trials suggest that pan-FGFR inhibition may not be as effective as first hoped. As such, combination approaches with chemotherapy, radiotherapy, or other molecularly targeted agents are likely to be required. Strategies being investigated in several FGFR-dependent malignancies include cotargeting the EGFR or PI3K/mTOR pathways. Within HCC, emerging data also implicate the potential importance of cotargeting the STAT3 pathway. A variant of FGFR19, which carries several missense mutations and a 5-codon deletion (M70), was shown to retain the ability to metabolically regulate bile acid synthesis while having no tumorigenic effect (7). M70 acted as a “biased ligand” whereby it shared similar downstream MAPK and PI3K signaling to wild-type FGFR19 but did not activate protumorigenic STAT3 signaling. The M70 molecule therefore offers a therapeutic strategy for treating both bile acid metabolism disorders and HCCs dependent on FGFR19/FGFR4/KLB signaling. Insights provided by M70 also suggest that should a dosing schedule that avoids gastrointestinal toxicity be identified for BLU9931, combination therapy with downstream STAT3 inhibition might provide more durable responses.

The development of a specific FGFR4 inhibitor, notwithstanding the potential toxicity caveats mentioned above, might also show efficacy in a range of other cancers where preclinical data suggest FGFR4 dependency. A subset of rhabdomyosarcomas has been shown to carry activating mutations in FGFR4, and rare cases of glioblastomas, lung cancers, and breast cancers also carry activating FGFR4 mutations. In addition, a subset of cancers arising from the lung, ovary, prostate, thyroid, and colon show FGFR4 amplification or high expression, which in many cases correlates with aggressiveness and/or poor prognosis (reviewed in refs. 1 and 8). Interest in targeting FGFR4 has increased, evidenced by the increase in reported agents with anti-FGFR4 activity reported in publications, patents (9), and recent conference abstracts, or being trialed in patients (Table 1). Similar to other FGFRs, there are also some reports of FGFR4 acting as a tumor suppressor gene in some tissue types, suggesting that even with paralog-specific inhibition, some caution should be applied in the systemic application of FGFR4 inhibition (reviewed in ref. 8).

Previous studies suggest that amplification of 11q13.3 could be an effective biomarker for patients with HCC likely to

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**Table 1. Summary of anti-FGFR4 agents in preclinical and clinical development**

<table>
<thead>
<tr>
<th>Agents targeting FGF19-FGFR4 axis</th>
<th>Type of inhibitor</th>
</tr>
</thead>
<tbody>
<tr>
<td>JNJ-42756493a</td>
<td>FGFR1–4 kinase, not published</td>
</tr>
<tr>
<td>FGFR401</td>
<td>FGFR1–4 kinase, not published</td>
</tr>
<tr>
<td>LY2874455a</td>
<td>FGFR1–4 kinase, ATP competitive</td>
</tr>
<tr>
<td>SSR128129</td>
<td>FGFR1–4, extracellular domain, allosteric</td>
</tr>
<tr>
<td>BLU9931</td>
<td>FGFR4 kinase, selective irreversible</td>
</tr>
<tr>
<td>AZ709</td>
<td>FGFR4 kinase, not published</td>
</tr>
<tr>
<td>FIIN-1; FIIN-2</td>
<td>FGFR1–4 kinase, irreversible</td>
</tr>
<tr>
<td>IA6</td>
<td>mAb to FGFR1</td>
</tr>
<tr>
<td>M70a</td>
<td>FGFR19 nontumorigenic biased ligand</td>
</tr>
<tr>
<td>LD1</td>
<td>mAb to FGFR4</td>
</tr>
<tr>
<td>4D-12</td>
<td>mAb to FGFR4</td>
</tr>
</tbody>
</table>

*aCurrently being tested in clinical trials.*
respond to anti-FGFR4 therapy (10), or, alternatively, that all molecules in the FGF19–FGFR4–KLB axis need to be expressed to predict susceptibility to FGFR4 inhibition (3). The latter scenario was confirmed by Hagel and colleagues. The authors also propose that BLU9931 may be effective in treating HCC patients with high FGF19 mRNA expression in the absence of genomic amplification. If true, this assertion would result in twice the number of patients eligible for anti-FGFR4 inhibition. However, no models of HCC tested in this article represent this cohort of patients, and further preclinical evidence is required. In addition, future trials enrolling only biomarker-positive patients may allow further refinement of these biomarkers and address such questions as whether higher expression levels of KLB and FGFR4 are required to predict response in patients with increased FGF19 expression versus those with FGF19 amplification. Moreover, due to the complexity of FGF/FGFR biology in different tissues, biomarkers are likely to be different in other tumor tissue types; for example, expression of KLB is likely not required in tissues where FGFR4 is activated by mutations, amplification, and/or the expression of nonhormonal FGF ligands.

In summary, if BLU9931 can overcome the toxicity issues seen with other anti-FGF19/FGFR4 therapies, perhaps by optimization of its dosing schedule, it looks to be an effective treatment option for a subset of FGFR4-driven cancer types, such as HCC. The field looks forward with anticipation to additional preclinical and clinical data being presented for the various different agents with anti-FGFR4 activity presented in Table 1, in the hope that patients with cancer can experience durable responses to inhibiting this pathway either alone or in combination with other anticancer therapies.

**Disclosure of Potential Conflicts of Interest**

P.M. Pollock has ownership interest in PCT Application PCT/US08/58065 and has been a consultant/advisory board member for Novartis. No potential conflicts of interest were disclosed by the other author.

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