**Background**

- RAS proteins occupy a critical position in the mitogen-activated protein kinase (MAPK) pathway and play a key role in its signal transduction cascade.
- Diverse mutations in RAS genes, particularly those in KRAS, have been described in up to 25% of solid tumors and have been shown to be driver oncogenes in cancers such as non-small cell lung cancer (NSCLC), endometrial cancer, and colorectal cancer.
- Monotherapy approaches targeting RAF dimers have had limited clinical success in KRAS-mutated cancers owing to an inability to adequately suppress MAPK signaling as a single agent.
- Monotherapy approaches targeting MEK have had limited clinical success in KRAS-mutated cancers owing to feedback phosphorylation of MEK and reactivation of MAPK signaling via RAF dimers.
- These limitations have been shown to be addressable in KRAS-mutated cancer models using a vertical inhibition strategy centered upon inhibiting RAF dimers in order to suppress RAF-dependent MEK reactivation while simultaneously inhibiting MEK to directly block ERK activation.
- **Lifirafenib** (BGB-283) is an oral, potent, and reversible pan-RAF inhibitor that has demonstrated monotherapy clinical activity in Braf and certain KRAS mutant solid tumors.
- **Mirdametinib** (PD-0335901) is an oral, potent, and reversible pan-MEK inhibitor that has demonstrated monotherapy clinical activity in certain tumors driven by overactivation of the MAPK pathway.

**Lifirafenib and Mirdametinib Demonstrate Synergistic Antiproliferative Effects in Cell LinesHarboring a Variety of KRAS Mutations**

- Treatment of KRAS mutated tumors with monotherapy lifirafenib inhibits RAF dimers but is unable to fully suppress signaling through ERK. Treatment of KRAS mutated tumors with monotherapy mirdametinib leads to increased RAF dimerization, increased MEK phosphorylation, and elevated ERK activity. Combination treatment disrupts RAF dimers and inhibits the MEK inhibitor-induced feedback reaction, thereby inhibiting MAPK signaling.

**Therapeutic Strategy**

1. **MAPK Pathway**: ERK
2. **MAPK Pathway**: MEK
3. **Combination**: RAF-ERK-MEK

**Combination Treatment**

- The addition of mirdametinib to lifirafenib led to potent and sustained inhibition of MAPK pathway activity, as measured by tumor pERK levels. Calu-6 cells were incubated in vitro in BRL24708 and BRL49610 (25 μM) followed by the first dose (left) and 12 hours following the fifth dose (right). pERK levels were assessed using the Sandoz Flowcytometric MAPK assay. The addition of mirdametinib to lifirafenib led to sustained and potent inhibition of pERK levels (not shown).

**Lifirafenib and Mirdametinib Drive MAPK Pathway Inhibition in Calu-6 KRASQ61K NSCLC Xenograft Model Using Clinically Relevant Doses**

- Combination of lifirafenib and mirdametinib exhibited enhanced antitumor activity in human NSCLC KRASQ61K xenograft model. NSCLC cells were inoculated subcutaneously in BRL24708 nude mice. Tumor volume was monitored and treated as indicated in vivo tumor volumes reached ~120 mm3 (n>10 per group).

**Lifirafenib and Mirdametinib Demonstrate Tumor Regressions in NCI-H358 KRASG12C NSCLC Xenograft Model Using Clinically Relevant Doses**

- Combination of lifirafenib and mirdametinib exhibited enhanced antitumor activity in human NSCLC KRASG12C xenograft model. NCI-H358 cells were inoculated subcutaneously in BRL24708 nude mice. Tumor volume was monitored and treated as indicated in vivo tumor volumes reached ~120 mm3 (n>10 per group).

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**Disclosures**

- The authors have no financial disclosures or conflicts of interest to disclose.

**Conclusions**

- We have demonstrated evidence for potent activity of lifirafenib and mirdametinib across preclinical models driven by a variety of KRAS mutations, including KRASQ61R and KRASQ61K xenografts.
- PD analysis showed strong synergistic activity in vivo against pERK using clinically relevant doses of each compound, supporting the antitumor activity of this vertical inhibition strategy.
- Lifirafenib and mirdametinib are currently being evaluated in an ongoing Phase 1b/2 clinical trial (ID: NCT03905148).

**Design of Ongoing Phase 1b/2 Clinical Trial**

- Dose Escalation
- Dose Expansion
- Evaluate antitumor activity of RFIp in patients with selected tumor types and molecular endpoints
- Additional clinical trials with promising signals (e.g., colorectal, CRC, pancreatic, biliary)

**References**

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