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Pemigatinib, an FGFR Inhibitor, Overcomes Resistance to KRAS G12C Inhibitors in Mesenchymal-Like NSCLC Tumors

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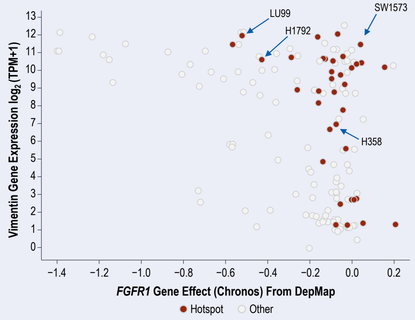
Abstract

KRAS is one of the most frequently mutated oncogenes. Clinical studies with recently developed covalent KRAS G12C inhibitors have shown promising anticancer activity in patients with *KRAS G12C* tumors (substitution of glycine to cysteine at amino acid 12). Not all KRAS G12C patients respond to single-agent treatment or, despite initial responses, develop drug resistance. Here, we investigate therapeutic options to overcome resistance to KRAS G12C inhibitor therapy in non-small cell lung cancer (NSCLC).

Analysis of a genome-wide genetic screen and corresponding mutation and expression data from DepMap identified a subset of NSCLC cells that harbor high fibroblast growth factor receptor 1 (FGFR1) expression (FGFR1hi) and *KRAS G12C* mutations. FGFR1hi cancer cells exhibited mesenchymal-like features including high levels of vimentin and low levels of E-cadherin. To assess the functional role of high FGFR1 expression in *KRAS G12C*–mutant cancer cells, pemigatinib, a potent and selective inhibitor of FGFR1-3, was tested alone or in combination with KRAS G12C inhibitors. The combination of pemigatinib and KRAS G12C inhibitors was synergistic in mesenchymal-like lung cancer cells with high FGFR1 expression, whereas no synergy was observed in cells with low FGFR1 expression. Furthermore, inhibition of FGFR1 activity was essential as an FGFR2-3–specific inhibitor demonstrated only modest activity in combination with KRAS G12C inhibitors. Notably, treatment of FGFR1hi *KRAS G12C* LU99 cells with covalent KRAS G12C inhibitors resulted in an increase in FRS2 phosphorylation, a marker of FGFR pathway activation, which was suppressed by pemigatinib. To determine whether increased FGFR1 activity may be an acquired resistance mechanism, *KRAS G12C*–mutant MiaPaCa-2 clones resistant to KRAS G12C inhibitors were generated. Subsequent protein analysis identified high levels of FGFR1 expression in a subset of resistant clones. In vivo studies with mesenchymal *KRAS G12C*–mutant xenografts confirmed increased antitumor efficacy and inhibition of pERK with the combination of KRAS G12C inhibitors and pemigatinib, compared with single-agent treatment. In contrast, in vivo combination activity was not observed in NSCLC tumors possessing an epithelial-like phenotype.

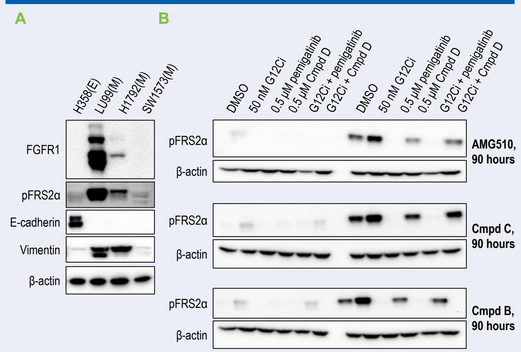
We demonstrate that NSCLC with a mesenchymal-like phenotype and harboring high FGFR1 expression and *KRAS G12C* mutations may uniquely benefit from combination treatment with current KRAS G12C–covalent inhibitors and blockade of FGFR1-mediated activity. Our results support pemigatinib as a promising agent for combination therapy with KRAS inhibitors.

FGFR1 Dependency Correlated With High Vimentin Expression



- FGFR1 dependency (Chronos score) was correlated with vimentin expression in lung cancer cells. Red circles designate cell lines with *KRAS* mutations. Source: DepMap

Treatment of Mesenchymal-Like Lung Cancer Cells With KRAS G12C Inhibitors Elevates FGFR Signaling



AMG510, Cmpd C, and Cmpd B, compound (KRAS G12C inhibitors); Cmpd D, compound (FGFR3 inhibitor); DMSO, dimethyl sulfoxide; (E), epithelial phenotype; FGFR, fibroblast growth factor receptor; (M), mesenchymal phenotype.

A. Western blots of FGFR1, pFRS2α, E-cadherin, vimentin, and β-actin in indicated cell lines

B. LU99 cells were treated with KRAS G12C inhibitors alone or in combination with pemigatinib for 90 hours. Western blots of pFRS2α and β-actin are shown. The right side of the Western blot has identical treatment; however, cells were treated with 1 ng/mL of recombinant human FGF for 15 minutes prior to harvest

Synergistic Effect Observed in LU99 Mesenchymal but not H358 Epithelial Cells With G12C and FGFR Inhibitor Combination

H358 (Epithelial)

Pemigatinib nM \ Compound B	1000	333	111	37	12	4	1	0.5
3000	0.4	0.5	1.2	1.0	3.0	7.6	11.6	3.2
1000	0.2	0.3	1.1	1.0	4.3	11.2	11.0	−3.2
333	0.0	0.0	1.3	1.0	4.9	7.8	10.4	−0.9
111	0.0	−0.4	0.8	−0.1	3.7	3.9	7.9	1.8
37	0.0	0.0	1.7	0.1	−0.2	3.0	5.3	−2.9

BLISS score average = 2.6

LU99 (Mesenchymal)

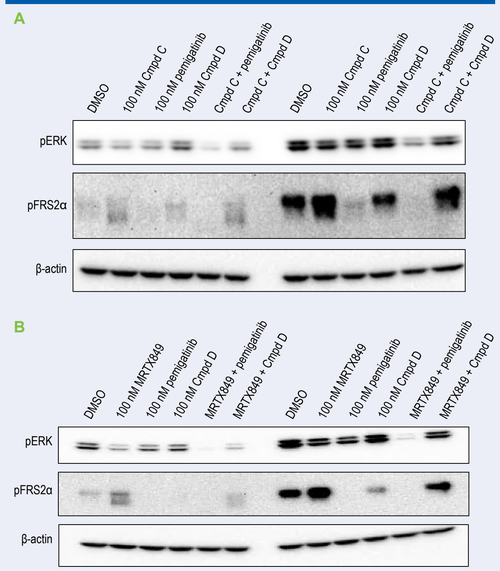
Pemigatinib nM \ Compound B	1000	333	111	37	12	4	1	0.5
1000	25	35	40	44	39	39	24	12
333	26	34	39	44	36	37	24	−1
111	31	43	48	56	47	52	37	16
37	24	32	38	44	38	41	24	10
12	14	22	28	35	26	22	14	−5

BLISS score average = 31

Compound B, KRAS G12C inhibitor.

- Cells were treated with indicated compounds alone or in combination for 5 days. Cell-Titer Glo was used to determine cell viability. Synergy score for the combination effect is calculated using a Bliss score model, where the Bliss score = Yab−(Ya+Yb−(YaYb))×100, where Ya and Yb are the monotherapies. Bliss scores >20 are strongly synergistic and a higher Bliss score indicates a higher level of synergism

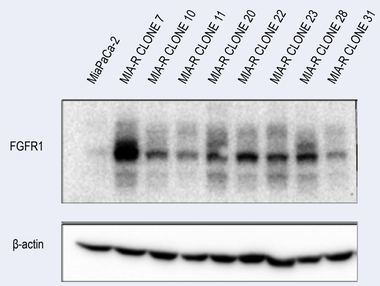
Increased Inhibition of pERK With KRAS G12C Inhibitor + Pemigatinib, but Not With G12Ci + FGFR3i



Cmpd, compound; DMSO, dimethyl sulfoxide; FGFRi, fibroblast growth factor receptor inhibitor; G12Ci, G12C inhibitor; pERK, phospho-ERK.

- LU99 cells were treated with 100 nM of compound (Cmpd) C (KRAS G12C inhibitor) (**A**) or MRTX849 (**B**) alone or in combination with pemigatinib or Cmpd D (FGFR3 inhibitor) for 24 hours. Right-side Western blot represents cells treated with 1 ng/mL of recombinant human FGF for 15 minutes prior to harvest

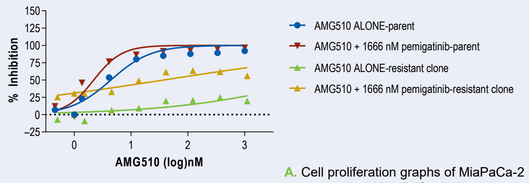
MiaPaCa-2 Cells Resistant to KRAS G12C Inhibitors Exhibited Elevated FGFR1 Expression



FGFR, fibroblast growth factor receptor.

- MiaPaCa-2 cells were cultured with increasing concentrations of AMG510 over time. Clones were generated in the presence of 1 μM AMG510. Western blot analysis of FGFR1 and β-actin in 8 MiaPaCa-2 KRAS G12C–resistant clones are shown

MiaPaCa-2 Cells Resistant to KRAS G12C Inhibitors Displayed Increased Sensitivity to G12Ci + Pemigatinib Combination



B

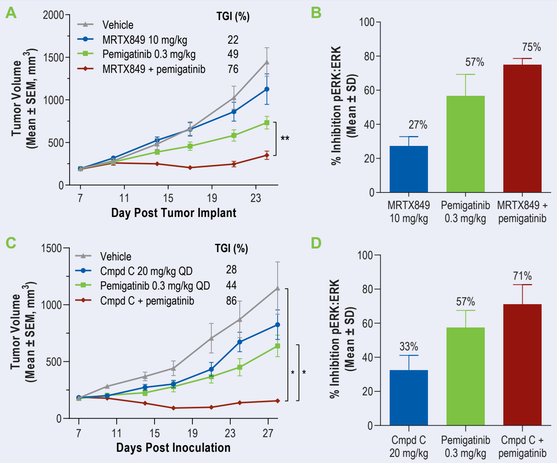
Cell Line	G12Ci + Pemigatinib Bliss Score	G12Ci + Cmpd D Bliss Score
MiaPaCa-2	6.3	2.2
MiaPaCa-2 clone 7	15.0	4.4
MiaPaCa-2 clone 11	24.9	1.8
MiaPaCa-2 clone 23	16.1	−4.4
MiaPaCa-2 clone 28	21.8	9.4

Cmpd, compound; G12Ci, G12C inhibitor.

A. Cell proliferation graphs of MiaPaCa-2 parental and MiaPaCa-2 clone 7 treated with AMG510 alone or in combination with 1666 nM pemigatinib

B. Table of Bliss scores of MiaPaCa-2 parental cells and resistant clones when treated with either G12Ci and pemigatinib or Cmpd D (FGFR3 inhibitor). Cells were treated with indicated compounds alone or in combination for 5 days. Cell-Titer Glo was used to determine cell viability

KRAS G12C Inhibitors + Pemigatinib Produce Increased Efficacy in the Mesenchymal LU99 NSCLC Model



*P<0.05, **P<0.01; 2-way analysis of variance with Dunnett's multiple comparisons test.
Cmpd, compound; Cmpd C, KRAS G12C inhibitor; pERK, phospho-ERK; QD, once daily; SD, standard deviation; SEM, standard error of the mean; TGI, tumor growth inhibition.

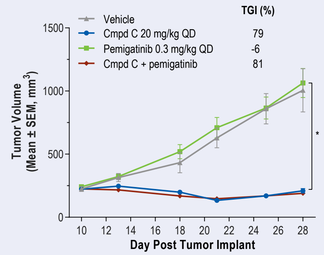
A. NCr nude mice bearing LU99 tumors were dosed orally with MRTX849 and pemigatinib as a single agent or in combination once daily starting on day 7

B. LU99 tumor–bearing nude mice (tumor volume ~390 mm^3) were given a single dose of the indicated compounds, and tumors were harvested 6 hours post-dose. The ratio of pERK/ERK was measured using a Phospho/Total ERK1/2 Whole Cell Lysate assay (Meso Scale Discovery)

C. Female NCr nude mice bearing LU99 tumors were dosed with Cmpd C or pemigatinib once daily starting on day 7

D. LU99 tumor–bearing nude mice (tumor volume ~328 mm^3) were given a single dose of the indicated compounds, and tumors were harvested 2 hours post-dose. The ratio of pERK/ERK was measured using a Phospho/Total ERK1/2 Whole Cell Lysate assay (Meso Scale Discovery)

Combination of Pemigatinib With KRAS G12C Inhibitors Is Ineffective in Epithelial-Like NSCLC Tumor Models



*P<0.05; 2-way analysis of variance with Dunnett's multiple comparisons test.
Cmpd, compound; Cmpd C, KRAS G12C inhibitor; NSCLC, non-small cell lung cancer; SEM, standard error of the mean; TGI, tumor growth inhibition; QD, once daily.

- Tumor growth was measured twice weekly in female NCr nude mice bearing H358 tumors
- Mice were dosed orally with Cmpd C and pemigatinib as a single agent or in combination once daily starting on day 10 for 18 days
- No synergistic or additive effect seen with combination dosing in epithelial-like NSCLC tumor model

Conclusions

- **Treatment of mesenchymal-like lung cancer cells with KRAS G12C inhibitors elevated FGFR signaling through increases in pFRS2**
- **Synergistic effects on cell proliferation and inhibition of pERK were observed in mesenchymal lung cancer cell lines with combination treatment of KRAS G12C and FGFR1 inhibitors, but not FGFR3 inhibitors**
- **MiaPaCa-2 cells resistant to KRAS G12C inhibitors displayed increased sensitivity to the combination of KRAS G12C inhibitors and pemigatinib**
- **Synergistic effects were observed in the LU99 mesenchymal-like lung cancer model with combination treatment of KRAS G12C inhibitors and pemigatinib**
- **No synergistic effects were observed in the epithelial-like H358 lung cancer model with combination treatment of KRAS G12C inhibitors and pemigatinib**
- **Combination of KRAS G12C inhibitors and pemigatinib may be an effective treatment option for patients with lung cancer possessing a mesenchymal phenotype warranting further investigation**

Disclosures

Favata, Weber, Abdollahi, Dostalik Roman, Farren, Gilmartin, Kim, Wang, Rios-Doria: Employment and stock ownership – *Incyte Corporation*. **Wee:** Former employment and stock ownership – *Incyte Corporation*.

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References

Manchado S, at al. *Nature*. 2016;534:647-651.
Meagan MB, et al. *Clin Cancer Res*. 2020;26:1633-1643.
Solanki HS, et al. *Clin Cancer Res*. 2021;27:2533-2548.

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