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Drug Combination Screen Identifies Pemigatinib, an FGFR Inhibitor, as a Mechanism to Overcome KRAS G12C Inhibitor Resistance in Lung Cancer

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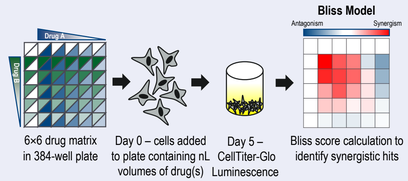
Abstract

Lung cancer is one of the most common tumors, accounting for approximately 1.8 million deaths worldwide in 2020. *KRAS* is a frequently mutated oncogene, with mutations reported in roughly 20-25% of non-small cell lung cancer cases. Specifically, *KRAS* mutations at amino acid 12, resulting in a glycine to cysteine (G12C) substitution, occur in approximately 13% and 3% of lung and colon cancers, respectively, and less frequently in other solid tumors. Recently, the development of covalent KRAS G12C inhibitors has shown meaningful anticancer activity in patients. However, many patients with *KRAS* G12C do not respond and/or develop resistance to single-agent treatment. Here we investigate combination therapies that may overcome resistance and broaden patient response to KRAS G12C inhibitors.

A curated set of 152 compounds was used in combination with screen monitoring cell-viability assays across 12 *KRAS* mutant lung and colon cancer cell lines. Compounds tested included kinase inhibitors targeting oncogenic signaling pathways, epigenetic modifiers, regulators of apoptosis, chemotherapeutics, and other anticancer agents. The activity of single-agent versus combination treatments was measured in cell viability assays, and screen hits were validated by in vitro mechanism of action studies. The screen identified synergy between covalent KRAS G12C inhibitors and the potent and selective FGFR1-3 inhibitor pemigatinib, which had a significantly high Bliss synergy score. Selectivity and siRNA knockdown experiments were performed to identify the specific FGFR isoform involved in synergistic anticancer activity. Inhibition of FGFR1 activity was shown to be essential, whereas an FGFR2-3–specific inhibitor demonstrated only modest activity in combination with KRAS G12C inhibitors. Additionally, FGFR1 knockdown combined with KRAS G12C inhibition resulted in increased drug sensitivity; in contrast, knockdown of other FGFR family members did not demonstrate similar increase in sensitivity to KRAS G12C inhibition.

Combination Screening Assay



- Large combination screen performed to assess potential synergies of a KRAS G12C inhibitor with 152 various investigational agents
- Single-agent dose-response curves for all agents were used to calculate appropriate concentrations for combination matrices
- 12 *KRAS* mutant lung and colon cancer cell lines were included in the screen
- 5-day cell viability assays were used to investigate the effect of drug combinations
- Synergy scores were calculated using the Bliss model on 6×6 drug matrices
- Hit confirmation was performed using expanded 10×10 drug matrices

Cell Lines

Cell Line	Tissue Type	Cell Morphology
SW1463	CRC	Epithelial
SW837	CRC	Epithelial
JVE-015	CRC	Epithelial
NCIH1373	Lung	Epithelial
NCIH2030	Lung	Epithelial
NCIH2122	Lung	Epithelial
NCIH358	Lung	Epithelial
NCI-H23	Lung	Epithelial
Calu1	Lung	Mesenchymal
HCC44	Lung	Mesenchymal
NCIH1792	Lung	Mesenchymal
SW1573	Lung	Mesenchymal
LU99*	Lung	Mesenchymal

*LU99 cell line was not included in the large screen. LU99 is a mesenchymal, high FGFR-expressing cell line that was used in confirmation and mechanism of action studies to further investigate pemigatinib.
CRC, colorectal cancer; FGFR, fibroblast growth factor receptor.

- Epithelial cells are specialized cells that line organs and vessels and are generally nonmotile, whereas mesenchymal cells are unspecialized cells that are capable of differentiating to multiple cell types and have increased motility
- Epithelial to mesenchymal transition (EMT) is thought to play a key role in metastasis of cancer. Studies have demonstrated that coordinated activation of multiple pathways is essential for EMT to occur[1,2]

Targets of Investigational Agents Tested

Kinase Inhibitors		Epigenetic Modifiers	Various Others
Akt	JAK1, JAK 1/2	BET/BRD	CDC25
ALK1, 2	LRRK2	EZH2	COX2
ALK5	MEK	HDAC	FAS
ASK	mTOR	PRMT5	Glutaminase
ATM/ATR	P38	LSD1	HIF
Aurora	PAK4	DNMT	HMG CoA Reduct
AXL/MER	PDGFR	IDH	IDO
Bcr-Abl	PDK	KDM4	IKZF1
BTK	PERK		IKZF3
CDK4/6	PI3K Pan	**Antiapoptotic**	Mat2A
CDK2	PI3Kδ,γ	MCL-1	NLRP3
CDK8	PIM	Bcl-2	PARP
CDK12	PKC	Mdm2	PDE4
Chk	PDK		PDL1o
c-Met	PLK	**Chemotherapies**	Porcupine
CSF-1R	Raf	Various	PPAR
EGFR	RIPK1, 2, 3		Proteasome
ERK	RSK	**RAS Pathway (Non-kinase)**	SGLT
FAK	SphK1	SOS1	SKP2
FGFR	Syk	SHP2	SMO, Hh
FLT3	Tie-2	KRAS G12C	SQLE
GSK-3	VEGFR	KRAS G12D/V	Telomerase
HPK1	Wee1		USP7
IGF-1R	Pan-TKI		
IKK			

- Some targets are represented using multiple agents within the curated set of 152 compounds selected for combination screening

Summary of Pemigatinib Combination Synergy Scores

Synergistic Activity of KRAS G12C and Pemigatinib Combination in Lung and Colon KRAS G12C Cancer Cell Lines in Mesenchymal Cell Lines H1792 and SW1573

A

Drug Name	Nominal Targets	MAD Cutoff # of Cell Lines >2 Overall	MAD Cutoff # of Cell Lines >3 Overall	MAD Cutoff Lung # of Cell Lines >2	MAD Cutoff Colon # of Cell Lines >2	MAD Cutoff Excess Volume Average	SDapprox Cutoff # of Cell Lines >2 Overall	SDapprox Cutoff # of Cell Lines >3 Overall	SDapprox Cutoff Lung # of Cell Lines >2	SDapprox Cutoff Colon # of Cell Lines >2	SDapprox Cutoff Excess Volume Average	CI Average
Pemigatinib	FGFR	5	2	3	2	3.38	2	2	2	0	5.97	0.30

B

Synergy — Antagonism

Excess Volume

Lung								Colon			
SW1573	H1792	H358	HCC44	H1373	H23	H2030	Calu1	H2122	JVE015	SW1463	SW837	JVE371
6.86	5.09	1.08	1.04	0.97	0.60	0.47	0.42	−0.75	2.23	1.74	1.64	−4.01

C

Synergy — Antagonism

Normalized Synergy Score (MAD)

Lung									Colon			
SW1573	H1792	H358	H2122	H23	H1373	HCC44	H2030	Calu1	SW837	JVE015	SW1463	JVE371
8.85	5.39	2.60	1.80	1.16	1.12	1.07	0.65	0.27	2.72	2.28	0.98	−0.89

Normalized Synergy Score (SDapprox)

5.98	3.64	1.75	1.22	0.79	0.76	0.72	0.44	0.18	1.84	1.54	0.66	−0.60

CI, combination index; MAD, median absolute deviation; SDapprox, standard deviation approximate.

A. Pemigatinib showed drug synergism with *KRAS* G12C drug combination. CI and excess over Bliss score were used to score compounds for level of synergism. Four cutoff criteria were applied: (1) >2× MAD; (2) >2× SDapprox; (3) excess over Bliss volume average >2; and (4) median CI ≤0.5. Genedata Screener software was used for automated quality control and screen analysis

B&C. Bliss excess volume **(B)** along with MAD and SDapprox **(C)** synergy scores per cell line. Heat maps across 12 *KRAS* G12C cell lines indicate synergy or antagonisms. The most significant levels of synergy were observed in mesenchymal cell lines H1792 and SW1573

Hit Confirmation of Pemigatinib: Expanded Drug Combination Matrices in H1792 Cells

A

Pemigatinib nM	Cmpd A DMSO (0)	100	33.33	11.11	3.70	1.23	0.41	0.14	0.05	0.02
DMSO (0)	131.8	39.9	52.1	56.0	86.3	100.9	129.8	125.6	122.3	116.8
5000	19.1	−0.2	−1.1	−1.2	−0.8	2.0	16.7	16.7	19.4	21.1
1666.7	78.5	1.0	0.6	2.2	4.6	21.9	74.8	74.8	68.7	72.2
555.6	124.1	5.0	4.3	18.1	32.0	70.6	121.3	121.3	113.6	94.8
185.2	119.6	4.7	4.8	15.8	34.4	68.0	106.5	106.5	107.9	122.4
61.7	118.7	7.1	6.9	23.3	39.7	67.3	112.6	112.6	119.9	114.6
20.6	107.8	10.9	13.2	26.7	59.5	80.8	121.4	121.4	120.2	121.7
6.9	120.0	23.7	30.9	43.5	65.5	88.8	107.5	107.5	117.9	122.3
2.3	119.1	30.9	39.5	59.5	70.4	81.4	114.4	114.4	126.6	110.1
0.8	144.6	38.8	45.4	62.0	75.3	95.0	107.7	107.7	104.9	135.8

B

Pemigatinib nM	Cmpd A DMSO (0)	100	33.33	11.11	3.70	1.23	0.41	0.14	0.05	0.02
DMSO (0)										
5000		7.9	11.1	11.9	17.3	17.3	18.1	7.3	4.0	1.2
1666.7		30.3	40.3	41.7	63.1	57.2	49.9	23.8	27.3	19.6
555.6		44.4	60.4	51.4	75.1	54.6	75.9	34.5	38.3	50.2
185.2		43.0	57.6	51.2	68.8	52.6	71.7	43.7	38.5	17.3
61.7		40.3	55.0	43.2	62.8	52.4	57.2	36.5	25.4	24.1
20.6		32.0	43.0	33.6	33.5	27.9	34.7	14.0	11.7	4.2
6.9		24.2	31.7	23.7	38.2	32.3	55.1	43.5	29.0	18.0
2.3		16.6	22.6	7.2	32.5	38.8	61.4	35.2	19.1	29.1
0.8		18.9	29.9	19.0	49.4	50.8	90.6	73.9	72.0	33.1

Green indicates antagonism; white indicates additivity; red indicates synergy.
Cmpd, compound; DMSO, dimethyl sulfoxide.

A. Percent cell viability is shown for KRAS G12C inhibitor "Cmpd A" tested in combination with pemigatinib in an expanded matrix format

B. Heat map of Bliss synergy scores for KRAS G12C inhibitor "Cmpd A" tested in combination with pemigatinib

FGFR Selectivity

FGFR Selectivity Imperative to Combination Effect

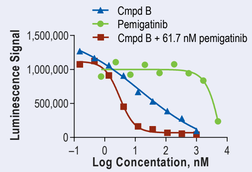
A

Cell Line	Tissue Type	Type	G12C Inhibitor	FGFR Inhibitor	Avg. Bliss Score
H358	Lung	Epithelial	Cmpd B	Pemigatinib	1.4
H358	Lung	Epithelial	Cmpd B	Cmpd D	3
H358	Lung	Epithelial	Cmpd B	TAS-120	3.3
H358	Lung	Epithelial	Cmpd B	Erdafitinib	4.4
H358	Lung	Epithelial	Cmpd B	BGJ398	−0.8
H2030	Lung	Mesenchymal	Cmpd B	Pemigatinib	4.3
H2030	Lung	Mesenchymal	Cmpd B	Cmpd D	2.4
H2030	Lung	Mesenchymal	Cmpd B	TAS-120	5.1
H2030	Lung	Mesenchymal	Cmpd B	Erdafitinib	5.8
H2030	Lung	Mesenchymal	Cmpd B	BGJ398	0.7
H1792	Lung	Mesenchymal	Cmpd B	Pemigatinib	24.2
H1792	Lung	Mesenchymal	Cmpd B	Cmpd D	1.9
H1792	Lung	Mesenchymal	Cmpd B	TAS-120	17.4
H1792	Lung	Mesenchymal	Cmpd B	Erdafitinib	12.9
H1792	Lung	Mesenchymal	Cmpd B	BGJ398	11.2
SW-1573	Lung	Mesenchymal	Cmpd B	Pemigatinib	19.3
SW-1573	Lung	Mesenchymal	Cmpd B	Cmpd D	3.1
SW-1573	Lung	Mesenchymal	Cmpd B	TAS-120	6.6
SW-1573	Lung	Mesenchymal	Cmpd B	Erdafitinib	7.9
SW-1573	Lung	Mesenchymal	Cmpd B	BGJ398	7.5
LU-99	Lung	Mesenchymal	Cmpd B	Pemigatinib	44.8
LU-99	Lung	Mesenchymal	Cmpd B	Cmpd D	13.3
LU-99	Lung	Mesenchymal	Cmpd B	TAS-120	22.0
LU-99	Lung	Mesenchymal	Cmpd B	Erdafitinib	34.7
LU-99	Lung	Mesenchymal	Cmpd B	BGJ398	31.9

Compound	Selectivity
Pemigatinib	Pan-R1/2/3
Cmpd D	R3 selective
TAS-129	Pan-R1/2/3/4
Erdafitinib	Pan-R1/2/3/4
BGJ398	Pan-R1/2/3

B

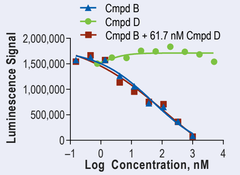
Single-Agent vs Combination Cmpd B and Pemigatinib



	Cmpd B	Pemigatinib	Cmpd B + 61.7 nM Pemigatinib
HillSlope	−0.3673	−1.618	−1.772
IC_{50}	14.98	~9310	3.071

C

Single-Agent vs Combination Cmpd B and Cmpd D



	Cmpd B	Cmpd D	Cmpd B + 61.7 nM Cmpd D
HillSlope	−0.4764	1.295	−0.2973
IC_{50}	48.67	>5000	142.7

Cmpd, compound; Cmpd B, KRAS G12C inhibitor; Cmpd D, FGFR3 inhibitor; FGFR, fibroblast growth factor receptor; IC_{50}, half maximal inhibitory concentration.

A. Bliss synergy scores for various FGFR inhibitors in combination with KRAS G12C inhibitor "Cmpd B." Bliss scores are an average across the entire drug combination. Pemigatinib combinations demonstrate the highest level of synergy over other FGFR inhibitors across multiple epithelial and mesenchymal cell lines. Bliss scores in mesenchymal cell lines were more significant compared with epithelial cell lines

B&C. KRAS G12C inhibitor "Cmpd B" and FGFR single agents vs combination. A significant shift in potency is observed with combination including pemigatinib **(B)** versus combination with "Cmpd D" (FGFR3 selective) **(C)**, indicating that the inhibition of FGFR1 and FGFR2 are critical for combination effect

Validation of FGFR siRNA Knockdown

siRNA Knockdown of FGFR1-4 in LU99 Mesenchymal Cell Line

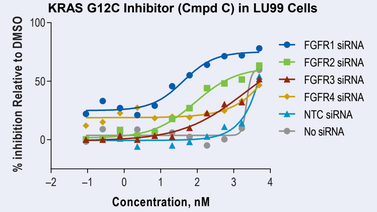
FGFR, fibroblast growth factor receptor.

- Western blot indicates successful knockdown of FGFR1-4 in LU99 mesenchymal cells using siRNA. Vimentin was used as a marker to indicate mesenchymal origin
  - LU99 cells were treated with individual siRNA for 48 hours prior to lysis

FGFR Knockdown in Combination With KRAS G12C Inhibition

Inhibition of FGFR1 Is Essential for Synergistic Anticancer Activity

Effect of siRNA in Combination With KRAS G12C Inhibitor (Cmpd C) in LU99 Cells



	FGFR1 siRNA	FGFR2 siRNA	FGFR3 siRNA	FGFR4 siRNA	NTC siRNA	No siRNA
HillSlope	1.016	0.7580	0.5057	0.7267	1.001	−7.063
EC_{50}	41.45	85.72	1902	42,115	~240,087	~2251

Cmpd, compound; DMSO, dimethyl sulfoxide; EC_{50}, half maximal effective concentration; FGFR, fibroblast growth factor receptor; NTC, non-silencing control.

- LU99 cells treated with respective FGFR siRNA for 48 hours followed by KRAS G12C inhibitor (Cmpd C) treatment for 120 hours
- FGFR1 knockdown combined with KRAS G12C inhibition demonstrates increased drug sensitivity

Conclusions

- **High-throughput drug combination screen identified synergy between an internal covalent KRAS G12C inhibitor and inhibitors of several other targets**
- **Analysis of Bliss scores from the screen across 12 *KRAS* G12C cancer cell lines identified strong synergy between an internal KRAS G12C covalent inhibitor and the FGFR1-3 inhibitor pemigatinib**
- **The synergistic effect of pemigatinib and KRAS G12C inhibition was notably strongest in mesenchymal lung cancer cell lines**
- **In vitro selectivity studies confirmed pemigatinib synergy scores were higher when compared with other FGFR inhibitors**
- **siRNA knockdown of individual FGFR isoforms indicate that inhibition of FGFR1 is essential to the synergistic effect and increased drug sensitivity**
- **Pemigatinib is a promising agent for combination therapy with KRAS G12C inhibitors in lung cancer**

Disclosures

Abdollahi, Favata, DiMatteo, Schuette, Boarder, Rupar, Macarron, Gilmartin, Wang, Amador-Arjona: Employment and stock ownership – *Incyte Corporation*.

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