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Addition of RP10107, a novel and potent glutaminase inhibitor, accentuates 5-FU activity in lung cancer cell lines *in vitro*

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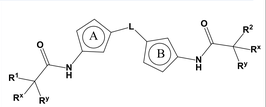
Introduction

Drug resistance remains a significant limitation to the clinical use of 5-Fluorouracil (5-FU) in lung cancer. With cancer cell metabolism emerging as a critical regulator of tumor progression, combining 5-FU with an inhibitor of the metabolic machinery represents a potential therapeutic strategy to prevent lung cancer progression. Cancer cell metabolism is reprogrammed wherein glutamine utilization is increased via elevation of glutaminase activity thereby generating the necessary substrates required for eventual ATP synthesis and energy production. RP10107 is a novel, potent, and selective glutaminase (GLS-1) inhibitor that demonstrated high potency against mouse (IC_{50}=21.2 nM), rat (IC_{50}=18.2 nM) and human (IC_{50}=26.4 nM) enzymes with selectivity over GLS-2 (>380-fold). The objective of this study was to evaluate the effect of a combination of 5-FU and RP10107 in lung cancer cells

Enzyme & Cell-based activity

Glutaminase	Species	IC_{50} (nM)
GLS-1 (Kidney type)	Human	26.4
	Mouse	21.2
	Rat	18.2
GLS-2 (Liver type)	Mouse	>10000



All variables are as defined in PCT/IIB2015/050075

Table 1. Glutaminase inhibition by RP10107. IC_{50} of RP10107 using recombinant human GLS-1 or mouse liver mitochondria derived GLS-2 was determined by measuring the conversion of glutamine to α-ketoglutarate flurometrically. Activity of RP10107 against mouse or rat GLS-1 was determined colorimetrically using brain lysates in an ammonia release assay. ***RP10107 demonstrated >380-fold selectivity over GLS-2***

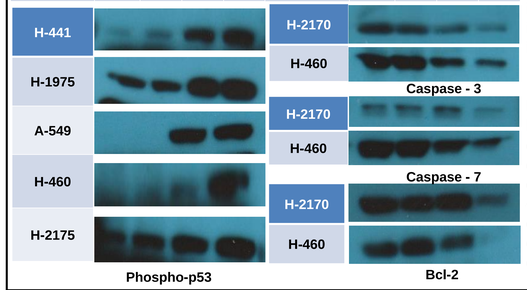


Figure 1. Addition of RP10107 accentuates 5-FU activity on specific biomarkers in Lung cancer cells. Cell lines were plated in media at a pre-determined density in a 6-well plate. After overnight incubation, cells were treated with RP10107, 5-FU or a combination of RP10107+5-FU for 72 h. Cell lysates were analyzed for markers by Western Blotting. ***Combination of 5-FU and RP10107 increased expression of phospho-p53 in all cell lines tested. Additionally, a 40-75% reduction in caspase-3, caspase-7, and Bcl-2 was observed in H-2170 and H-460 cells.***

NCI-H1975

Fraction Inhibition	Conc.	5-FU (nM) 0	500	1000	3000	5000
	0	0	0.08	0.06	0.32	0.43
RP10107 (nM)	1000	0.27	0.31	0.35	0.5	0.64
	3000	0.48	0.58	0.65	0.7	0.79
	5000	0.37	0.42	0.48	0.6	0.77
	10000	0.56	0.65	0.69	0.69	0.8

NCI-H1975

Excess over Bliss score	Conc.	5-FU (nM) 0	500	1000	3000	5000
	0					
RP10107 (nM)	1000		-1.91	3.76	0.03	6.13
	3000		6.36	13.89	5.88	8.76
	5000		-0.13	7.68	3	13.01
	10000		5.99	11	-1.28	5.51

NCI-H460

Fraction Inhibition	Conc.	5-FU (nM) 0	1000	3000	5000	10000
	0	0	0.08	0.07	0.22	0.71
RP10107 (nM)	1000	0.15	0.16	0.23	0.48	0.77
	3000	0.2	0.27	0.56	0.74	0.85
	5000	0.24	0.17	0.47	0.59	0.81
	10000	0.25	0.25	0.55	0.78	0.89

NCI-H460

Excess over Bliss score	Conc.	5-FU (nM) 0	1000	3000	5000	10000
	0					
RP10107 (nM)	1000		-5.25	1.33	14.72	1.22
	3000		0.92	29.68	36.53	8.23
	5000		-12.63	17.04	18.02	2.54
	10000		-5.92	24.44	35.9	10.22

NCI-H441

Fraction Inhibition	Conc.	5-FU (nM) 0	1000	3000	5000	10000
	0	0	0.27	0.51	0.57	1.08
RP10107 (nM)	1000	0.39	0.47	0.76	0.89	1.12
	3000	0.48	0.59	0.87	0.99	1.16
	5000	0.69	0.84	0.91	1.1	1.23
	10000	0.88	0.92	1.09	1.11	1.22

NCI-H441

Excess over Bliss score	Conc.	5-FU (nM) 0	1000	3000	5000	10000
	0					
RP10107 (nM)	1000		-8.4	6.47	14.99	7.13
	3000		-2.93	12.67	21.55	12.1
	5000		6.69	6.06	23.24	20.17
	10000		1.12	14.75	15.85	21.29

Table 2. Synergistic effect of RP10107 and 5-FU on lung cancer cell growth. Cells were plated in media at a pre-determined density in 96-well plates Following overnight incubation, cells were treated with either 5-FU, RP10107, or a combination of the two agemts. After 72 h, MTT was added. BLISS scores were calculated based on the percent inhibition data. -20 20 ***Additivity or synergism was observed in all cell lines with effect being more pronounced in NCI-H-441 and NCI-H460 cells.***

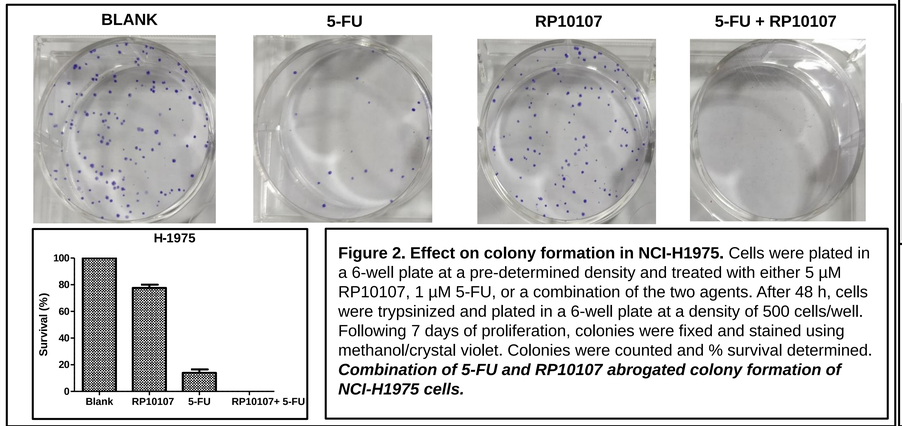


Figure 2. Effect on colony formation in NCI-H1975. Cells were plated in a 6-well plate at a pre-determined density and treated with either 5 μM RP10107, 1 μM 5-FU, or a combination of the two agents. After 48 h, cells were trypsinized and plated in a 6-well plate at a density of 500 cells/well. Following 7 days of proliferation, colonies were fixed and stained using methanol/crystal violet. Colonies were counted and % survival determined. ***Combination of 5-FU and RP10107 abrogated colony formation of NCI-H1975 cells.***

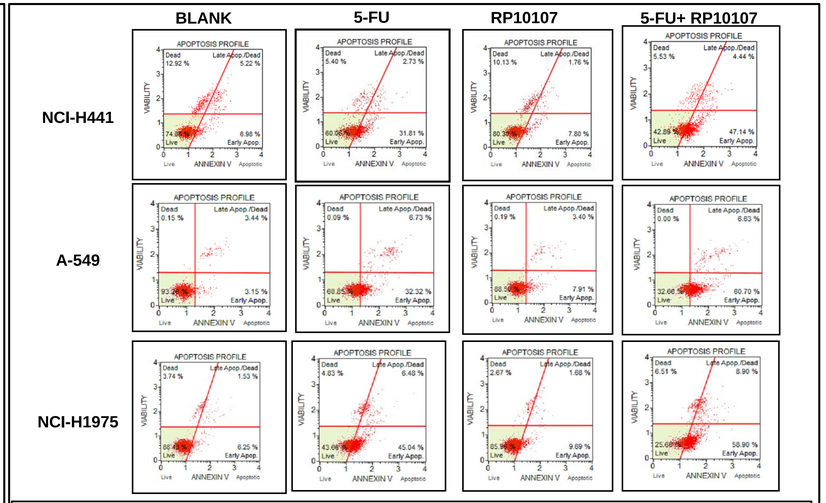


Figure 3. Induction of apoptosis in lung cancer cell lines. Cell lines were plated in media at a pre-determined cell density in 6-well plates Following overnight incubation, cells were treated with 3 μM RP10107 5 μM 5-FU, or a combination of the two agents. After 72 h, cells were collected and stained with Annexin-V-PE and 7-AAD and flow cytometry was performed to determine % total apoptotic cells. ***Combination of 5-FU and RP10107 demonstrated higher apoptosis than individual agents. Apoptosis was higher in A-549 and NCI-H1975 compared to NCI-H-2170.***

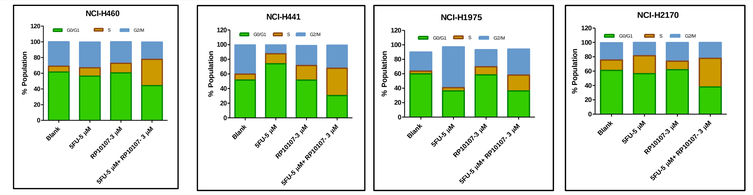


Figure 4. Effect on cell cycle in lung cancer cells. Cells were plated in media at a pre-determined density in 6-well plates Following overnight incubation, cells were treated with RP10107, 5-FU, or a combination of the two agents. After 72 h, cells were collected, washed with PBS and fixed with 70% ethanol, incubated at 4°C for 3 h, and stained with Propidium Iodide. Samples were analyzed by flow cytometry. ***Combination of 5-FU and RP10107 arrested cells in the S phase for NCI-H460, NCI-H441, NCI-H1975 and NCI-H2170 while the arrest was observed in G2/M phase for A-549 cells.***

SUMMARY & CONCLUSIONS

- Glutaminase inhibition potentiated 5-FU activity in lung cancer cell lines
- Anti-proliferative effect of the combination was manifested by an increase in apoptosis and cell cycle arrest and inhibition of caspase markers.
- Survival of NCI-H1975 colonies was reduced upon treatment with the combination
- Testing of the combination in animal models of lung cancer is planned.

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