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Synergistic effects of glutaminase and proteasome inhibition in Multiple Myeloma cell lines

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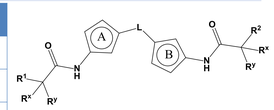
Introduction

Multiple Myeloma (MM) accounts for approximately 2.1% of all cancer deaths with a 5-year median survival rate of 49.6%. Besides chemotherapy, treatment options include monoclonal antibodies, immunomodulatory agents, and proteasome inhibitors. Carfilzomib is a selective proteasome inhibitor administered intravenously in patients with relapsed or refractory MM. With cancer cell metabolism emerging as a critical regulator of tumor progression, combining carfilzomib with an inhibitor of the metabolic machinery represents a novel therapeutic strategy to prevent MM progression. RP10107 is a 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 carfilzomib and RP10107 in MM cell lines

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/IB2015/050075

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 selectivity over GLS-2***

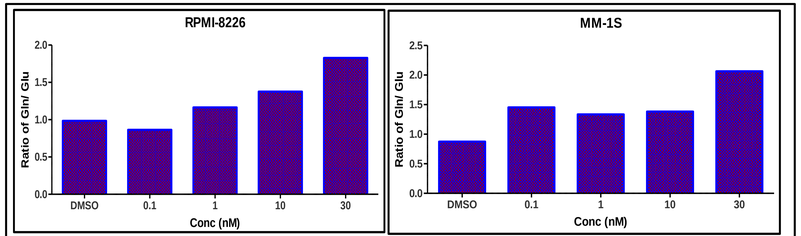


Figure 1. Glutamine to Glutamate conversion in MM cell lines. Cells were plated and incubated at 37°C for 24-hours post treatment with RP10107. Cells were collected, washed with PBS, lysed using 50% methanol in water, and incubated on ice for 30 min. Following centrifugation, supernatant was collect and glutamine and glutamate concentrations were determined on a LC-MS/MS. ***Inhibition of glutaminase by RP10107 caused accumulation of glutamine within cells with a subsequent increase in the ratio glutamine: glutamate.***

MM-1S

Fraction Inhibition	Conc	Carfilzomib (nM) 0	0.1	0.5	1	3
RP10107 (nM)	0	0	0.057	0	0	0.384
	100	0.292	0.352	0.304	0.354	0.803
	300	0.288	0.241	0.313	0.331	0.84
	500	0.446	0.517	0.568	0.548	0.963
	800	0.664	0.691	0.746	0.832	1.057
	1000	0.66	0.689	0.721	0.905	1.016

MM-1S

Excess over Bliss	Conc	Carfilzomib (nM) 0	0.1	0.5	1	3
RP10107 (nM)	0					
	100		2.05	1.17	6.27	23.93
	300		-8.74	2.55	4.31	27.89
	500		3.91	12.14	10.18	30.45
	800		0.83	8.22	16.84	26.46
	1000		1.01	6.07	24.48	22.59

RPMI-8226

Fraction Inhibition	Conc	Carfilzomib (nM) 0	0.1	0.5	1	3
RP10107 (nM)	0	0	0.167	0.172	0.248	0.475
	30	0.26	0.306	0.513	0.495	1.025
	50	0.409	0.628	0.772	0.76	1.016
	100	0.493	0.422	0.635	1.008	1.082
	300	0.468	0.484	0.675	0.996	1.059
	500	0.495	0.743	0.732	0.861	1.174

RPMI-8226

Excess over Bliss	Conc	Carfilzomib (nM) 0	0.1	0.5	1	3
RP10107 (nM)	0					
	30		-7.7	12.7	5.13	41.4
	50		12.1	26.2	20.5	32.7
	100		-16	5.53	38.9	34.8
	300		-7.3	11.6	39.7	33.9
	500		16.3	15	24.1	43.9

Table 2. Synergistic activity of RP10107 and Carfilzomib in MM cell lines. Cells were plated in media at a pre-determined density in 96-well plates Following overnight incubation, cells were treated with RP10107, Carfilzomib (CFZ), or a combination of RP10107+ CFZ. After 72 h, MTT was added. BLISS scores were calculated based on the percent inhibition data. -20 20. ***A high degree of synergism was observed in MM-1S and RPMI-8226 cell lines.***

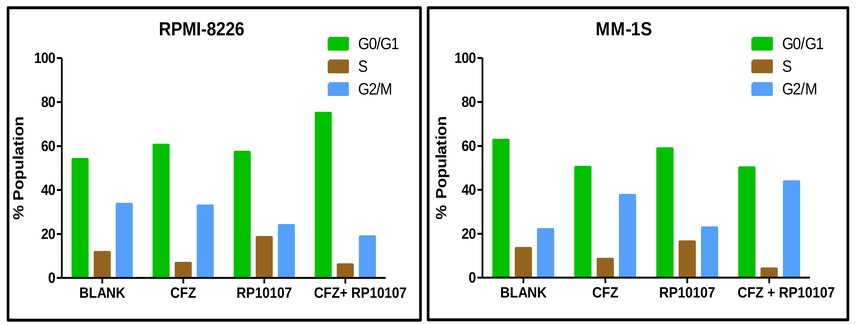


Figure 2. Effect on cell cycle in MM cells. Cells were plated in media at a pre-determined cell density in 96-well plates. Following overnight incubation, cells were treated with RP10107, Carfilzomib (CFZ), or a combination of RP10107+CFZ. After 48 h, cells were collected, washed with PBS and fixed with 70% ethanol, incubated at 4°C for 3 h, and stained with Propidium Iodide. Samples were analyzed by flow cytometry. **Concentrations**: CFZ-15 nM (RPMI-8226); RP10107- 1μM. ***Combination of CFZ and RP10107 caused cell cycle arrest in GO/G1 (RPMI-8226) and G2/M (MM-1S) phase.***

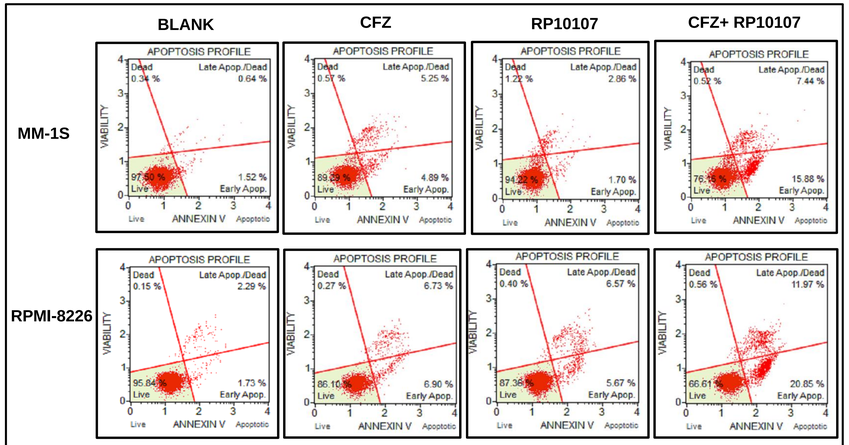


Figure 3. Induction of apoptosis in MM cell lines. Cell lines were plated in media at a pre-determined cell density in 96-well plates. Following overnight incubation, cells were treated with RP10107, Carfilzomib (CFZ), or a combination of RP10107+CFZ. After 48 h, cells were collected and stained with Annexin-V-PE and 7-AAD and flow cytometry was performed to determine % total apoptotic cells. **Concentrations**: CFZ-20 nM (MM-1S) or CFZ-15 nM (RPMI-8226); RP10107 - 1 μM. ***Combination of CFZ and RP10107 demonstrated higher apoptosis than individual agents.***

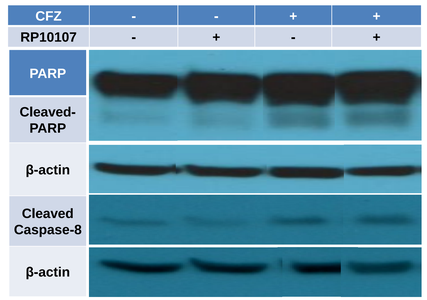


Figure 4. Addition of RP10107 accentuates carfilzomib activity on specific biomarkers in RPMI-8226 cells. Cell lines were plated in media at a pre-determined density in a 6-well plate. After overnight incubation, cells were treated with RP10107, CFZ, or a combination of RP10107+ CFZ for 48 h. Cell lysates were analyzed for markers by Western Blotting. β-actin was used as a loading control. . **Concentrations**: CFZ-15 nM; RP10107: 1 μM (PARP) or 0.5 μM (Caspase-8). ***Combination of CFZ and RP10107 caused increase in cleaved PARP and cleaved caspase-8 in RPMI-8226 cells.***

SUMMARY & CONCLUSIONS

- Glutaminase inhibition potentiated Carfilzomib activity in Multiple Myeloma cell lines
- Effect was most pronounced in the RPMI-8226 cell line.
- Anti-proliferative effect of the combination was manifested by an increase in apoptosis and cell cycle arrest
- Marked inhibition of apoptotic markers was observed
- Testing of the combination in relevant animal models is planned.

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