#1770 Complementary Targeting of PI3K and the Proteasome Causes Potent Inhibition of mTORC1 and NF-kappaB in Models of B- and T-Cell Lymphoma

Columbia University Medical Center

Changchun Deng[1], Mark Lipstein[1], Xavier Serrano[1], Michael Mangone[1], Luigi Scotto[1], Peter Sportelli[2], Hari P. Miskin[2], and Srikant Viswanadha[3], Owen A. O'Connor[1]

[1]Center for Lymphoid Malignancies, Department of Medicine, Columbia University Medical Center, New York, NY; [2]TG Therapeutics, Inc., New York, NY; [3]Incozen Therapeutics Pvt. Ltd., Hyderabad, India

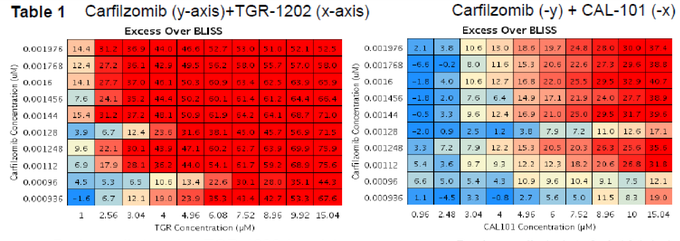
BACKGROUND

Constitutively activated PI3K/AKT/mTOR pathway plays a critical role in the proliferation and survival of cancer cells, through the expression of numerous pro-survival and proliferative genes. Specific inhibitors of the various isoforms of PI3K have shown promising activity in the treatment of indolent B-cell lymphoma. However, they have not shown similar activity in aggressive lymphoma, potentially because the expression of many PI3K/AKT/mTOR dependent pro-survival genes may be activated by alternative signals. Notable examples of signals regulated by mTOR include the pro-survival NF-kappaB (NF-κB) pathway and the eukaryotic initiation factor 4F (eIF4F). Through a feed-forward loop, eIF4F controls the expression of c-Myc, a well established oncoprotein in many cancers including highly aggressive lymphoma.

Hypothesis

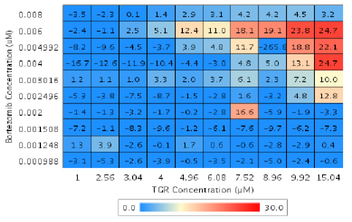
If both the proteasome and PI3K are involved in activation of mTOR, then combinations of proteasome and PI3K inhibitors will be able to potently inhibit the mTOR-eIF4F-Myc axis and kill Myc dependent cancer.

Table 1 Carfilzomib (y-axis)+TGR-1202 (x-axis)



Carfilzomib (-y) + CAL-101 (-x)

Bortezomib (y-axis)+TGR-1202 (x-axis)



Bortezomib (-y) + Cal-101 (-x)

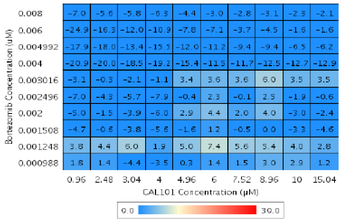


Table 1. Synergy of 4 different combinations of proteasome inhibitors and PI3K inhibitors. The LY10 cells were treated with the indicated compounds as single agent or in combinations, using a HTS platform. Synergy was indicated by values of Excess over Bliss (EOB) more than 10, with higher values of EOB indicating stronger synergy. The concentrations of the compounds were given on the X- and Y-axis, while the table included color coded EOB values.

RESULTS

Table 2

CFZ-nM	TGR-μM	Effect	CI	RRR
1	1	0.01	> 10	1.14
1.5	1	0.13	0.99	0.97
2	1	0.43	0.67	0.75
2.5	1	0.75	0.47	0.46
1	3	0.18	0.56	0.92
1.5	3	0.28	0.66	0.79
2	3	0.71	0.41	0.38
2.5	3	0.92	0.27	0.14
1	5	0.23	0.49	0.88
1.5	5	0.48	0.46	0.58
2	5	0.9	0.24	0.14
2.5	5	0.97	0.18	0.05

Bort-nM	CAL-μM	Effect	CI	RRR
1	1	0	> 10	1.35
1.5	1	0	> 10	1.22
2	1	0.09	2.33	1.28
2.5	1	0.21	1.35	1.38
1	3	0.12	1.74	1.03
1.5	3	0.15	1.5	0.99
2	3	0.27	0.987	1.02
2.5	3	0.35	0.9748	1.14
1	5	0.11	2.915	1.12
1.5	5	0.13	2.4119	1.10
2	5	0.24	1.2	1.15
2.5	5	0.27	1.278	1.38

PF382

CFZ-nM	TGR-μM	Effect	CI	RRR
1	1	0.04	0.87	1.14
1.5	1	0.38	0.51	0.97
2	1	0.36	0.69	0.75
2.5	1	0.55	0.68	0.46
1	3	0.2	0.59	0.92
1.5	3	0.65	0.36	0.79
2	3	0.63	0.49	0.38
2.5	3	0.76	0.52	0.14
1	5	0.17	0.53	0.88
1.5	5	0.76	0.31	0.58
2	5	0.81	0.38	0.14
2.5	5	0.92	0.35	0.05

Bort-nM	CAL-μM	Effect	CI	RRR
1	1	0.07	7.32	1.11
1.5	1	0.13	1.30	1.07
2	1	0.36	1.03	1.08
2.5	1	0.67	0.86	1.06
1	3	0.11	1.97	1.06
1.5	3	0.16	1.19	1.04
2	3	0.38	1.01	1.05
2.5	3	0.65	0.89	1.11
1	5	0.15	1.00	1.05
1.5	5	0.23	0.96	0.97
2	5	0.36	1.03	1.10
2.5	5	0.72	0.80	0.91

DND41

CI/RR>1	1<CI/RR<.75	.75<CI/RR<.5	.5<CI/RR<.25	.25<CI/RR<0

Table 2. Synergy of proteasome inhibitors and PI3K inhibitors. Two T-cell lymphoma cell lines, PF382 and DND41, were cultured and treated with the indicated compounds, as single agents or in combination. The fraction of growth inhibition (Effect) was shown for the combination treatments. Synergy was indicated by combination index (CI) or relative risk ratio (RRR) values below 1. CFZ: carfilzomib, TGR: TGR-1202, bort: bortezomib, CAL: CAL-101/idelalisib.

Table 3.

Cal uM	Bort nM	Combo Effect	CI	RRR	EOB
7.5	7.5	0.1	0.91	0.93	7.3
7.5	10.0	0.16	0.87	0.91	8.5
7.5	15.0	0.21	1.00	0.82	17.1
7.5	20.0	0.34	0.99	0.94	4.5
10.0	7.5	0.19	0.78	0.84	9.0
10.0	10.0	0.18	0.93	0.88	4.6
10.0	15.0	0.25	1.01	0.78	14.1
10.0	20.0	0.4	0.97	0.85	5.6
15.0	7.5	0.28	0.80	0.77	21.4
15.0	10.0	0.3	0.87	0.79	18.8
15.0	15.0	0.37	0.96	0.68	29.3
15.0	20	0.46	1.01	0.79	14.6

TGR uM	Cfz uM	Combo Effect	CI	RRR	EOB
7.5	7.5	0.21	0.82	0.96	2.9
7.5	10.0	0.33	0.57	0.82	14.6
7.5	15.0	0.34	0.56	0.80	16.1
7.5	20.0	0.49	0.38	0.64	28.9
10.0	7.5	0.31	0.80	0.84	3.5
10.0	10.0	0.44	0.58	0.69	16.2
10.0	15.0	0.51	0.49	0.60	23.3
10.0	20.0	0.59	0.40	0.51	29.6
15.0	7.5	0.49	0.77	0.82	10.8
15.0	10.0	0.58	0.62	0.68	19.5
15.0	15.0	0.64	0.54	0.59	25.1
15.0	20.0	0.68	0.48	0.54	27.7

Table 3. Synergistic interaction of PI3K inhibitors and proteasome inhibitors, assessed by 3 methods. The pancreatic cell line Mia-Paca was incubated without any drugs, or the indicated drugs for 48 hours, as single agent and in combination. Cal: CAL-101/idelalisib, Bort: bortezomib, Combo effect: fraction of inhibition by the combination treatment, TGR: TGR-1202, Cfz: carfilzomib, CI: combination index, RRR: reletive risk ratio, EOB: excess over Bliss.

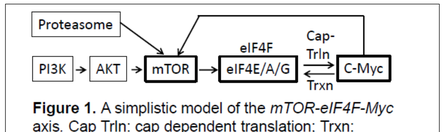


Figure 1. A simplistic model of the *mTOR-eIF4F-Myc* axis. Cap Trln: cap dependent translation; Trxn: transcription.

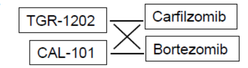


Figure 2. Two PI3K inhibitors were combined with 2 proteasome inhibitors to treat lymphoma cells. The cytotoxic and biological effects of 4 combinations were compared.

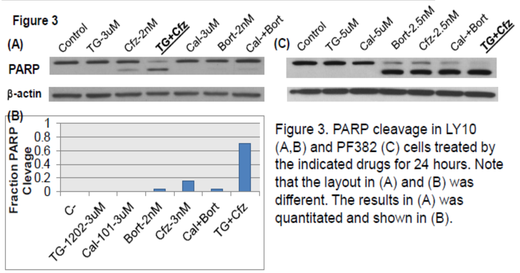


Figure 3. PARP cleavage in LY10 (A,B) and PF382 (C) cells treated by the indicated drugs for 24 hours. Note that the layout in (A) and (B) was different. The results in (A) was quantitated and shown in (B).

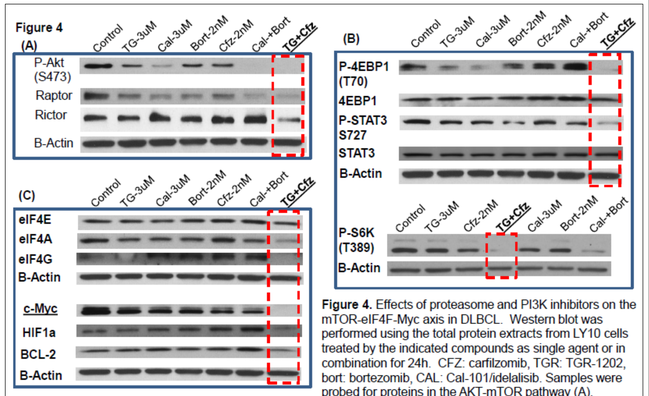


Figure 4. Effects of proteasome and PI3K inhibitors on the mTOR-eIF4F-Myc axis in DLBCL. Western blot was performed using the total protein extracts from LY10 cells treated by the indicated compounds as single agent or in combination for 24h. CFZ: carfilzomib, TGR: TGR-1202, bort: bortezomib, CAL: Cal-101/idelalisib. Samples were probed for proteins in the AKT-mTOR pathway (A), downstream signals regulated by mTOR (B), and eIF4F and its target proteins (C).

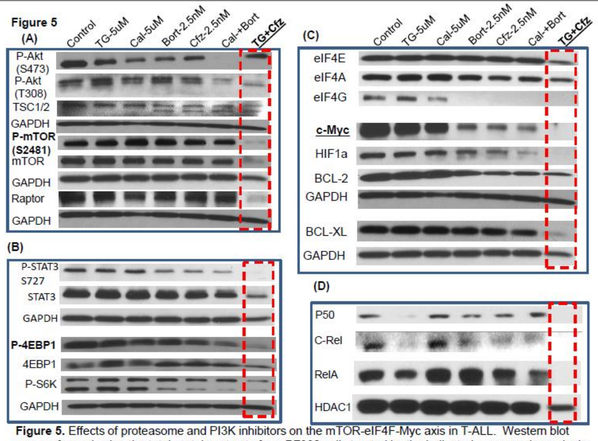


Figure 5. Effects of proteasome and PI3K inhibitors on the mTOR-eIF4F-Myc axis in T-ALL. Western blot was performed using the total protein extracts from PF382 cells treated by the indicated compounds as single agent or in combination for 24h. CFZ: carfilzomib, TGR: TGR-1202, bort: bortezomib, CAL: Cal-101/idelalisib. Samples were probed for proteins in the AKT-mTOR pathway (A), downstream signals regulated by mTOR (B), eIF4F and its target proteins (C), and the NF-kB pathway (D). Note that nuclear extract was used in (D).

Future Direction

- ***Determine whether down-regulation of c-Myc is caused by disruption of the feed-forward loop of eIF4F-Myc in lymphoma***
- ***Investigate other mechanisms of the synergy***
- ***Determine the in vivo effects of combining carfilzomib and TGR-1202***
- ***A clinical trial combining carfilzomib and TGR-1202 will be open for enrollment soon.***