#2809 Umbralisib/TGR-1202 As a Novel Dual PI3K/CK1 Inhibitor Has a Unique Therapeutic Role in Silencing Oncogenes in Aggressive Lymphomas

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BACKGROUND

Deregulation of oncogenes plays critical roles in the development and poor prognosis of aggressive lymphoma. For example, deregulation of C-MYC is prevalent in diffuse large B cell lymphoma (DLBCL), chromosome translocations involving C-MYC and BCL2 define "double hit lymphoma" (DHL), and chromosome translocation involving CCND1 is pathognomonic for mantle cell lymphoma (MCL). Translation of oncogenes such as C-MYC is highly dependent on eukaryotic translation initiation factor 4F (eIF4F), due to 'repressor' elements in the mRNA structure of these genes (Wolfe. Nature. 2014; Iwasaki. Nature 2016). Given the challenges in developing direct inhibitors of c-Myc, primarily because c-Myc lacks an enzymatic domain, disrupting eIF4F or its upstream regulators is an appealing therapeutic strategy to target the "undruggable" c-Myc oncoprotein. A number of signals have been shown to stimulate translation, including mTORC1, PI3K, AKT, and the proteasomes, by stimulating phosphorylation of 4E-BP1; however, drugs targeting these signals have not been successfully employed to silence oncogene translation as a therapeutic strategy. In diffuse large B cell lymphoma (DLBCL), where c-Myc plays a critical pathogenetic role, only limited clinical activity is observed with various mTOR inhibitors or even the combination of mTOR and proteasome inhibitors. Interestingly, we recently reported that combining umbralisib (**T**GR-1202) and **C**arfilzomib (**TC**), known to inhibit PI3Kδ and proteasomes, respectively, potently inhibited translation of C-MYC and survival of lymphoma cells (Deng. Blood. 2017). The synergy of TC is largely dependent on the activity of TGR-1202 to inhibit both PI3Kδ and casein kinase 1 epsilon (CK1ε). TC has been demonstrated to potently inhibit the phosphorylation of 4E-BP1; however, how TC silences translation remains poorly understood. In the current project, we examined the effects of TC on the translation of C-MYC, CCDND1, assembly of eIF4F, and survival of MCL and DHL cells.

Hypothesis

If umbralisib/TGR-1202 and carfilzomib in combination effectively downregulate multiple activating signals of translation, then the TC combination may synergistically induce cell death and tumor regression in lymphomas driven by oncogenes such as C-MYC, BCL-2, and CCND1.

RESULTS

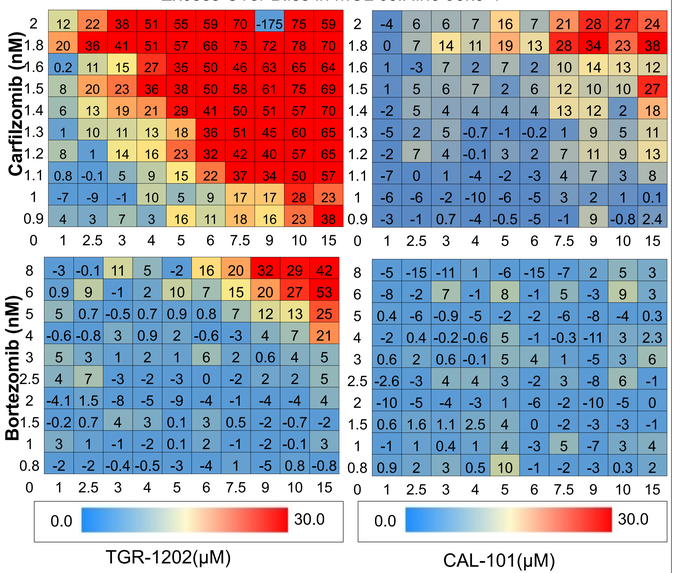

Figure 1. First-in-class dual PI3Kδ/CK1ε inhibitor TGR-1202 is uniquely synergistic with the proteasome inhibitor carfilzomib in MCL. The Mantle cell lymphoma cell line Jeko-1 was treated with single agents or two-drug combinations at the indicated concentrations for 24 hours. Growth was measured using the Cell Titer Glo assay. Percentage of inhibition was calculated relative to the untreated control.

RESULTS

Cell Line	TGR-1202 (μM)	Carfilzomib(nM) 1.25	2.5	5
Jeko-1	2.5	7.18	9.75	-0.61
	5	20.06	25.52	9.23
	10	27.44	39.46	26.02
Rec-1	2.5	-12.39	-9.56	-13.05
	5	4.44	22.99	-6.92
	10	40.56	67.6	40.31
Z-138	2.5	-2.86	-2.12	-6.93
	5	-0.62	10.66	24.96
	10	-17.88	30.62	35.98
JVM-2	2.5	7.41	4.93	5.12
	5	12.1	14.7	13.09
	10	-0.74	2.21	9.95

Cell Line	TGR-1202 (μM)	Carfilzomib(nM) 1	2.5	5
Jeko-1	2.5	0.98	0.7	5.35
	5	18.66	40.01	62.17
	10	41.65	49.94	46.71
Rec-1	2.5	-31.41	42.01	53.17
	5	8.11	37.28	33.85
	10	7.53	7.41	5.97
Z-138	2.5	-2.38	3.68	-2.37
	5	0.54	5.45	5.82
	10	-11.29	5.84	29.34

Figure 2. TGR-1202 and Carfilzomib are highly synergistic in MCL and DHL. MCL and DHL cells were treated for 24h with indicated concentrations. Expected % inhibition was calculated using the Bliss model. EOB values above zero indicate synergy, with higher positive values indicating higher levels of synergy

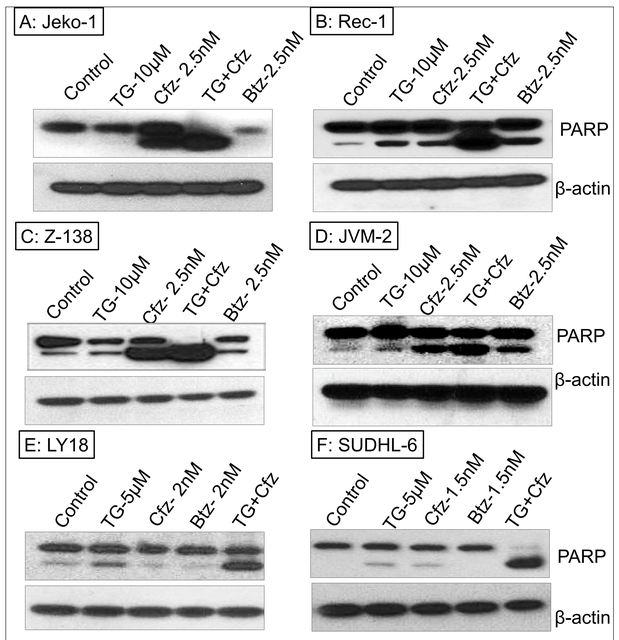

Figure 3 TGR-1202 and Carfilzomib synergistically induces apoptosis in MCL and DHL. MCL cell lines (A: Jeko-1; B: Rec-1,C:Z-138,D: JVM-2) and DHL cells (E: LY-18, F: SUDHL-6) were treated as indicated for 24 hours. Western blot was performed using the indicated antibodies.

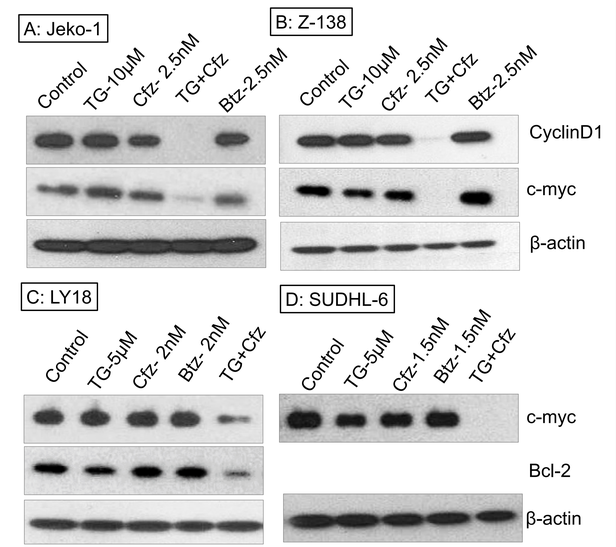

Figure 4. TGR-1202 and Carfilzomib synergistically inhibit the protein expression of C-MYC, BCL2, and CCND1 in DHL and MCL. MCL cell lines (A: Jeko-1; B: Z-138) and DHL cells (C: LY-18, D: SUDHL-6) were treated as indicated for 24 hours. Western blot was performed using the indicated antibodies.

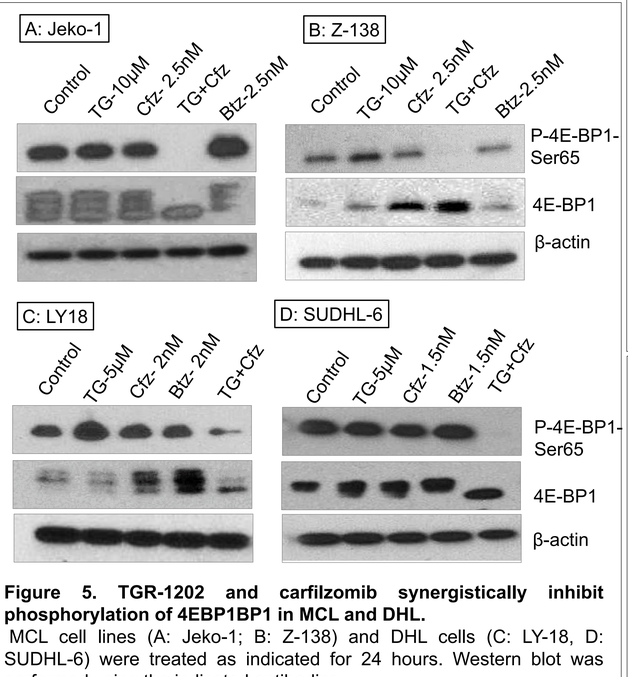

Figure 5. TGR-1202 and carfilzomib synergistically inhibit phosphorylation of 4EBP1BP1 in MCL and DHL. MCL cell lines (A: Jeko-1; B: Z-138) and DHL cells (C: LY-18, D: SUDHL-6) were treated as indicated for 24 hours. Western blot was performed using the indicated antibodies.

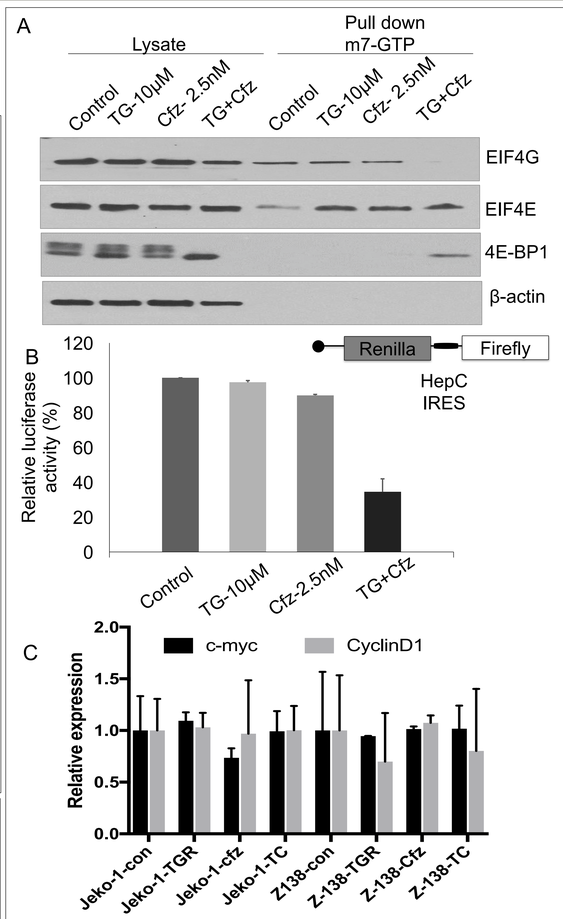

Figure 6. TGR-1202 and carfilzomib synergistically inhibits the assembly of eIF4F and repress cap-dependent translation, but do not inhibit transcription of C-MYC and CCND1

(A) 4E-BP1 cap binding activity was measured with m^{7}GTP Sepharose agarose bead in Jeko-1 (MCL) cell line. (B) Reporter assay with capped Renilla luciferase and Firefly luciferase under the HCV IRES was performed in Jeko-1 cell line . (A) MCL cell lines Jeko-1 and Z-138 were treated for 24h at the indicated conditions and qPCR was carried out to investigate the effect of TGR and Carfilzomib on transcriptional level of C-MYC and CCND1.

Summary

- Umbralisib/TGR-1202 as a first-in-class dual PI3Kδ/CK1ε inhibitor is highly synergistic with the proteasome inhibitor Carfilzomib in cell line models of double hit lymphoma and mantle cell lymphoma.
- The mechanism underlying the synergy of TGR-1202 and Carfilzomib is potent inhibition of 4E-BP1 phosphorylation, leading to disruption of eIF4F assembly, silencing of translationally susceptible oncogenes such as C-MYC and CCND1, and ultimately apoptosis of DHL and MCL cells.
- Umbralisib and Carfilzomib in combination represent a promising regimen for aggressive lymphoma, including DLBCL, DHL, and MCL. A phase I clinical study of this regimen (NCT02867618) is currently enrolling patients.