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 defining 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; Iwaki et al. 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 "unretractable" 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 oncogenic translation in 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 umbilisib (TGR-1202) and Carfilzomib (TC), known to inhibit PI3K and proteasomes, respectively, potently inhibits 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 PI3K and caspase kinase 1 (Ck1) activity. 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 the combination of TGR-1202, CCND1/3, assembly of eIF4F, and survival of MCL and DHL cells.

**Hypothesis**

If umbilisib/TGR-1202 and carfilzomib in combination effectively downregulate multiple activating signals of translation, then the TC combination may synergistically induce cell death and suppression in lymphomas driven by oncogenes such as c-MYC, BCL2-2, and CCND1.

**RESULTS**

**Figure 1**

First-in-class dual PI3K/C1 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.

**Figure 2**

TGR-1202 and Carfilzomib are highly synergistic in MCL and DHL. MCL and DHL cells were treated for 246 with indicated concentrations. Expected % inhibition was calculated using the Bliss model. EC50 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,C2-138; J:VIM-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 cell lines (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 4E-BP1/2 in MCL and DHL. MCL and DHL cells (C: Z-138; 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-dependant translation, but do not inhibit transcription of C-MYC and CCND1. (A) 4E-BP1 phosphorylation activity was measured with mTOD Sepharose agaose bead in Jeko-1 (MCL) cell line. (B) Reporter assay with capped RnaI 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 PI3K/C1 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.