Disruption of the mTOR-eIF4F Axis by Selectively Targeting PI3Kδ and Proteasome Potently Inhibits Cap Dependent Translation of c-Myc in Aggressive Lymphomas

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Therapeutic Strategy Targeting c-Myc in Cancer Is Urgently Needed

• C-MYC rearrangement is a risk factor for poor survival in diffuse large B cell lymphoma (DLBCL)

• C-MYC expression is a risk factor for poor survival in DLBCL

• **However, no drugs specifically targeting the activity of c-Myc have been approved for any cancer.**

• C-Myc is a master transcription factor, and lacks enzymatic activity

• Structurally, c-Myc lacks globular functional domains for small molecule targeting

• The extended interaction between the c-Myc and Max offers no apparent site for positioning a small-molecule inhibitor.

• Targeting the BET bromodomains is a promising strategy for c-Myc driven cancer
  McKeown and Bradner, CSH Perspective 2014
Potential Strategies to Silence the Translation of c-Myc through Targeting the mTOR-eIF4F Axis

Proteasome

Amino acids

PI3Kδ

AKT

mTOR

eIF4E

4EBP1

P

P

4EBP1

(eIF4F)

eIF4G

eIF4A

Translation

c-Myc

Carfilzomib (Cfz)

TGR-1202 (TG)

Bortezomib (Bz)

Idelalisib (Cal)


Hutter, G., et al., Leukemia, 2012

PI3Kδ Inhibitors and Proteasome Inhibitors Synergistically Inhibit DLBCL

### Observed Inhibition in the DLBCL cell line LY10

<table>
<thead>
<tr>
<th>TGR-1202 (µM)</th>
<th>Carfilzomib (nM)</th>
<th>Bortezomib (nM)</th>
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The table shows the observed inhibition levels for different concentrations of TGR-1202, Carfilzomib, and Bortezomib in the DLBCL cell line LY10. The inhibition levels are indicated by color codes ranging from 0.0 to 100.0.
Dual Inhibition of PI3Kδ and Proteasome Is Most Synergistic with TG&Cfz Followed by Cal&Cfz > TG&Bz > Cal&Bz

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Excess Over BLISS (EOB) in the DLBCL cell line LY10

- TGR-1202 (μM)
- Cal-101 (μM)
Dual Inhibition of PI3Kδ and Proteasome Is Most Synergistic with TG&Cfz Followed by Cal&Cfz > TG&Bz > Cal&Bz
TGR-1202&Carfilzomib Is the Most Synergistic Combination in DLBCL, Mantle Cell Lymphoma, and Multiple Myeloma
TGR-1202&Carfilzomib Is the Most Synergistic Combination in T Cell Lymphoma

Observed Inhibition (%) vs. Expected Inhibition (%)

- **H9**
- **HH**
- **PF382**
- **P12**
TGR-1202 and Carfilzomib Are Synergistic in Primary CLL and MCL Cells but not Toxic to Healthy Lymphocytes

[Graphs showing cell viability for CLL #1, CLL #2, and MCL]
TGR-1202 and Carfilzomib Synergistically Induce Apoptosis in Lymphoma Cell Lines and Primary Lymphoma Cells

**PARP Cleavage**

(A: LY7) Control, TG-3uM, Cal-3uM, Bz-5nM, CFz-5nM, Cal+Bz

(B: LY10) Control, TG-3uM, CFz-2nM, Cal-3uM, Bz-2nM, Cal+Bz

(C: PF382) Control, TG-5uM, Cal-5uM, Bz-2.5nM, CFz-2.5nM, Cal+Bz

**Caspase 3/7 Activity**

(F: LY10) Cal-Bort - Cal+Bortb, TG-Cfz - TG+Cfz

Fold Change / Control

- 1uM P3K
- 3uM P3K
- 5uM P3K
- 1.5nM P1
- 2nM P1
- 2.5nM P1
- 1uM + 1.5nM
- 1uM + 2nM
- 1uM + 2.5nM
- 3uM + 1.5nM
- 3uM + 2nM
- 3uM + 2.5nM
- 5uM + 1.5nM
- 5uM + 2nM
- 5uM + 2.5nM
TGR-1202 and Carfilzomib Synergistically Inhibit Phosphorylation of 4EBP1 and Expression of c-Myc

(A: DLBCL)

- P-Akt
- P-4EBP1
- 4EBP1
- c-Myc
- HIF1α
- Actin

(B: T-ALL)

- GAPDH

(C: CLL-1)

- P-4EBP1
- 4EBP1
- c-Myc
- B-Actin

(D: CLL-2)

- TGR-1202 and Carfilzomib Synergistically Inhibit Phosphorylation of 4EBP1 and Expression of c-Myc

(E: MM)

- P-4EBP1
- 4EBP1
- C-Myc
- B-Actin
TGR-1202 and Carfilzomib in Combination Inhibit Cap Dependent Translation of c-Myc in DLBCL

(A) Western Blot of c-Myc and Actin

(B) mRNA level of MYC % control

(C) Western Blot of C-Myc and B-Actin

(D) mRNA level of MYC % control

(E) Cap dependent translation of Myc

(F) R/F Luc (% control)
TGR-1202 and Carfilzomib in Combination Inhibit the c-Myc Transcription Program in DLBCL
Overexpression of eIF4E Suppresses the Synergistic Cytotoxicity of TGR-1202 and Carfilzomib and Increases the Protein Level of c-Myc
• Optimize c-Myc-silencing therapy by targeting phosphorylation of 4EBP1

• Phase I/II clinical trial of TGR-1202 and carfilzomib in relapsed and refractory lymphoma
Thank you!

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Sulzberger Columbia Genome Center, CUMC

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