Silencing c-Myc Translation as a Therapeutic Strategy through Targeting PI3Kδ and CK1ε in Hematological Malignancies

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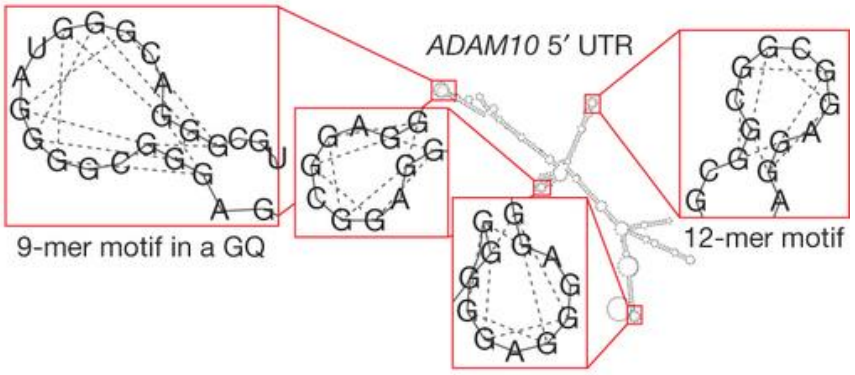
Targeting of c-Myc Translation as a Novel Therapeutic Strategy

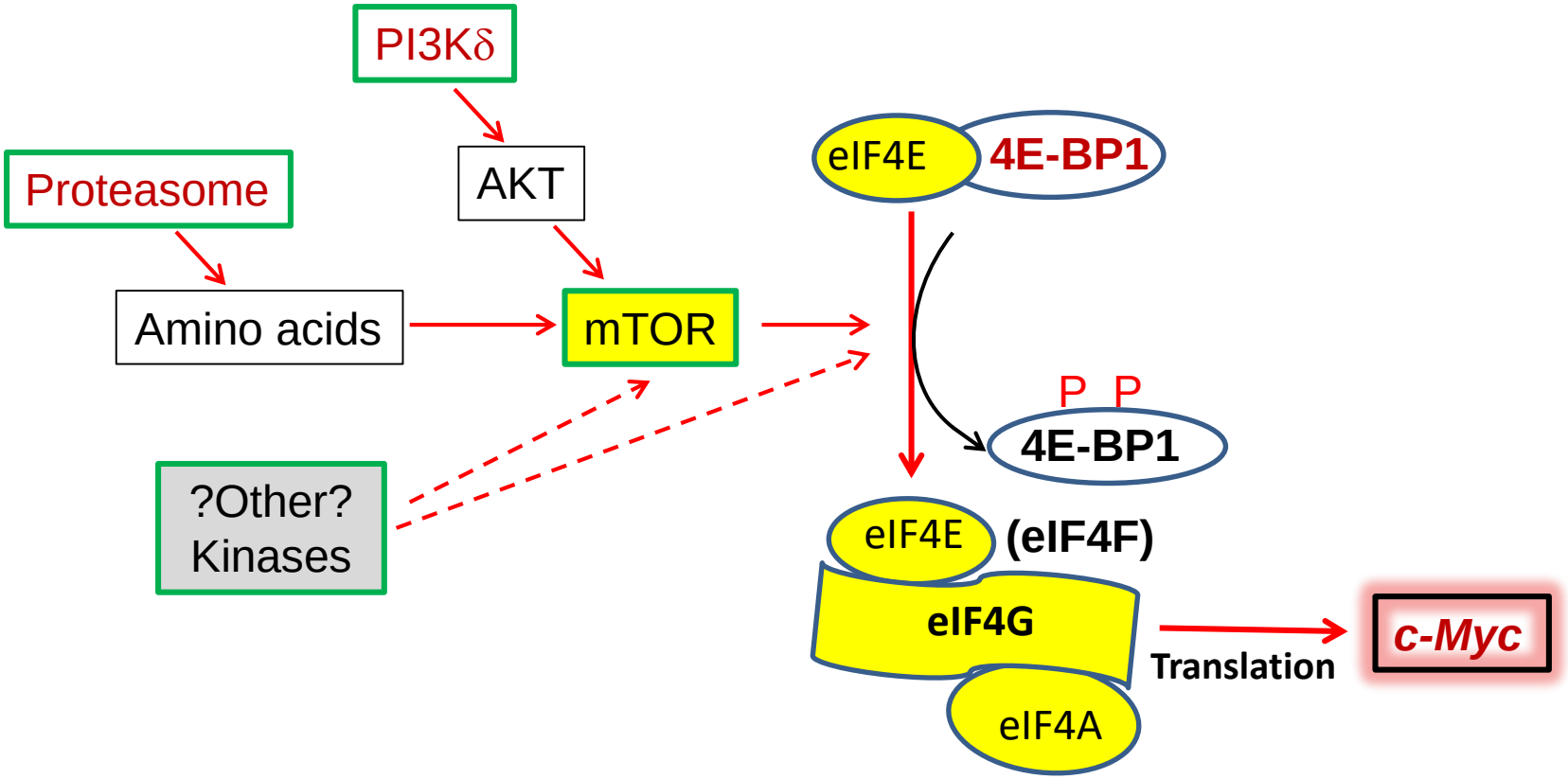
- No c-Myc targeting drugs have been approved.
- C-Myc protein has a short half life, 30 min.
- C-Myc mRNA has complex secondary structures in the 5' untranslated region (UTR), which negatively regulate cap dependent translation of c-Myc.



- Translation of c-Myc is potently inhibited by silvestrol, a selective inhibitor of the eukaryotic initiation factor 4A (eIF4A).

Andresen et al., Nucleic Acids Res 2012
Wolfe et al., Nature 2014

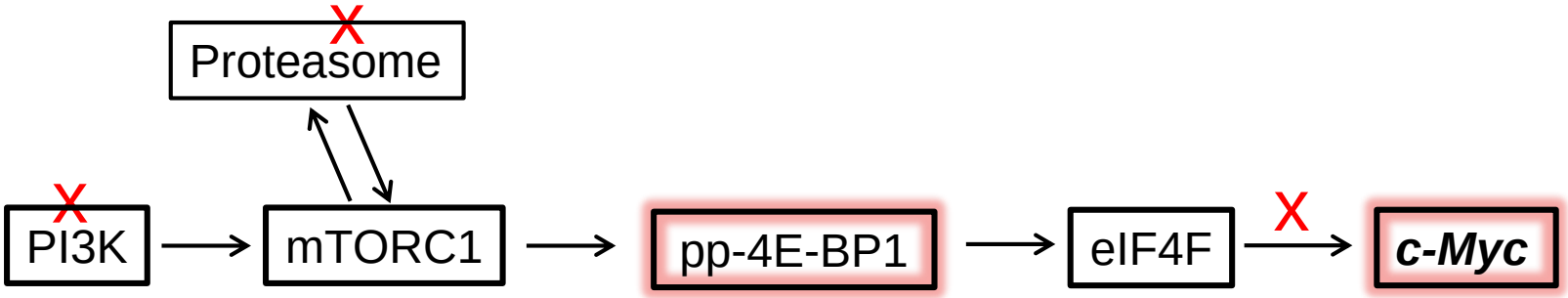
Targeting Translation of c-Myc through Inhibiting Phosphorylation of 4E-BP1



Suraweera, A., et al., Mol Cell, 2012
Quy, P.N., et al., J Biol Chem, 2013
Hutter, G., et al., Leukemia, 2012
Zhang, Y., et al., Nature, 2014

Dibble CC and Cantley LC.
Trends Cell Biol, 2015

Combining PI3K and Proteasome Inhibitors May Synergistically Inhibit Translation of c-Myc and Kill Lymphoma Cells



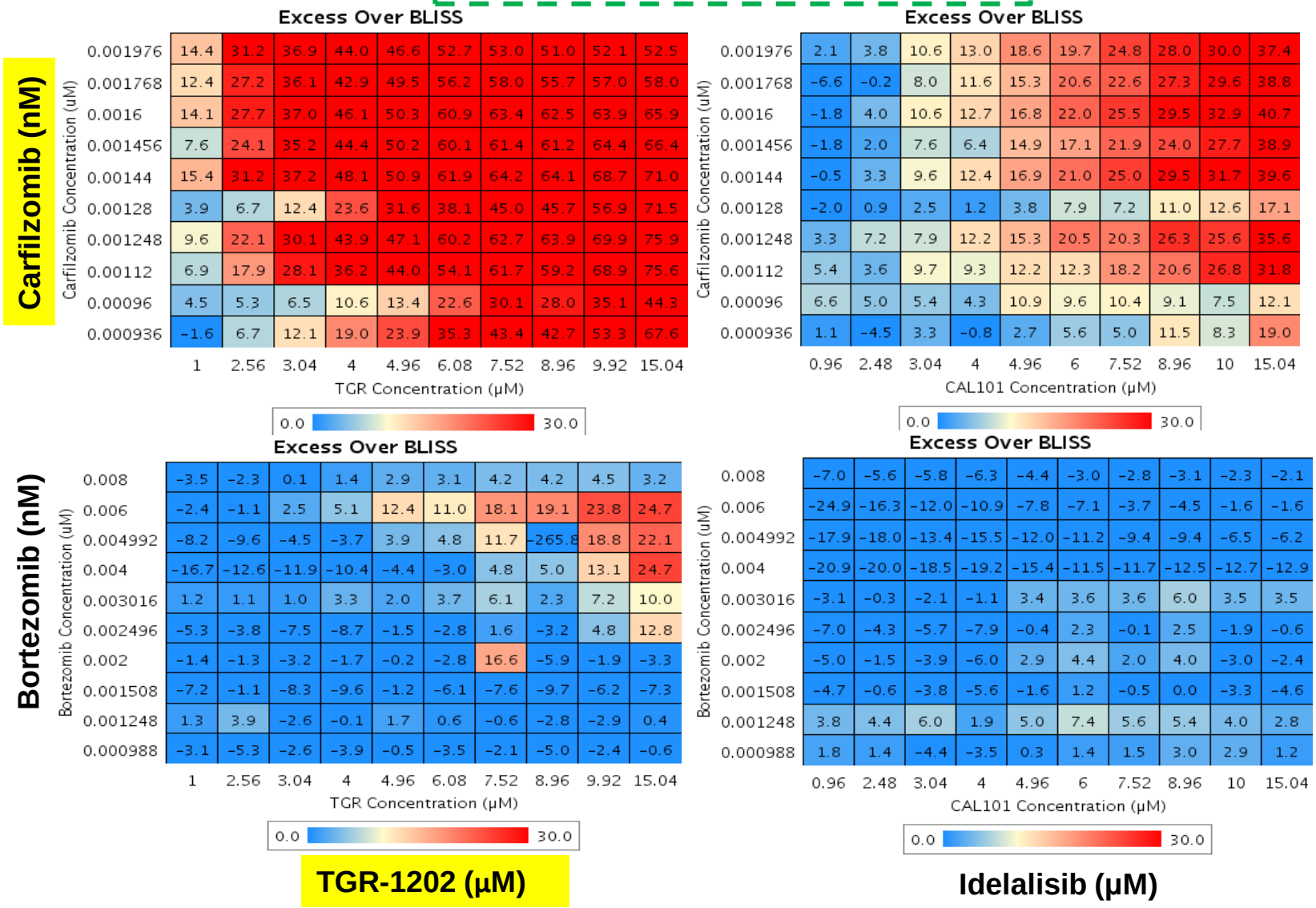
PI3Kδ Inhibitors

Proteasome Inhibitors

Drugs	TGR-1202 (TG)	Idelalisib (Ide)
Carfilzomib (Cfz)	TC	IC
Bortezomib (Bz)	TB	IB

TC Is Highly Synergistic and Superior to Other Combinations of PI3K and Proteasome Inhibitors

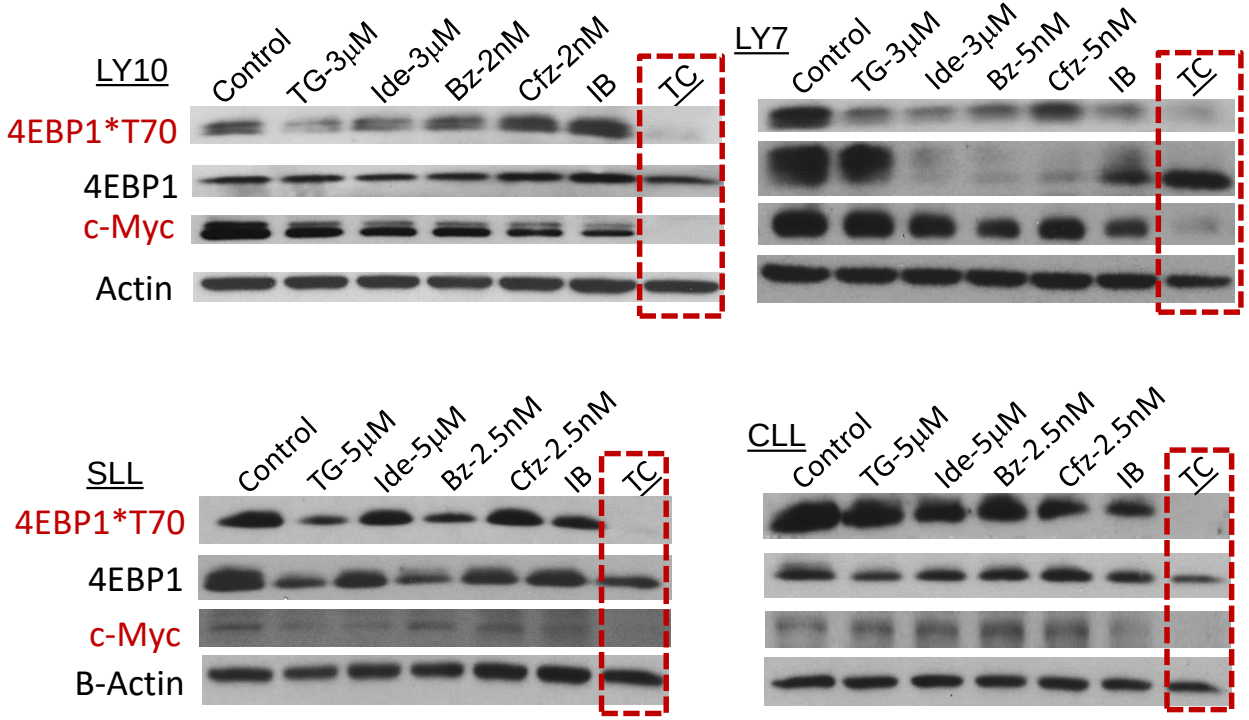
Excess over Bliss (EOB) > 0: Synergy



TC Is Highly Synergistic and Superior to Other Combinations

- TC is highly synergistic in 12 cell line models of DLBCL, MCL, MM, T-ALL, and CTCL.
- TC is highly synergistic in primary CLL, MCL, and MZL cells.
- TC synergistically induces apoptosis.

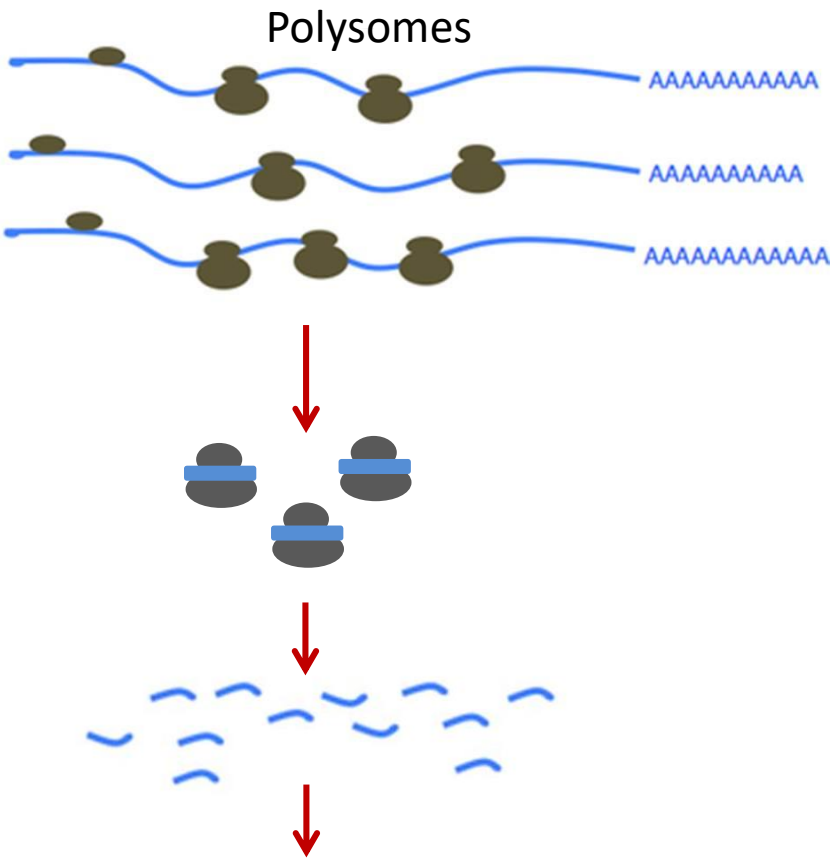
TC Uniquely and Synergistically Inhibits Translation of c-Myc and Phosphorylation of 4E-BP1



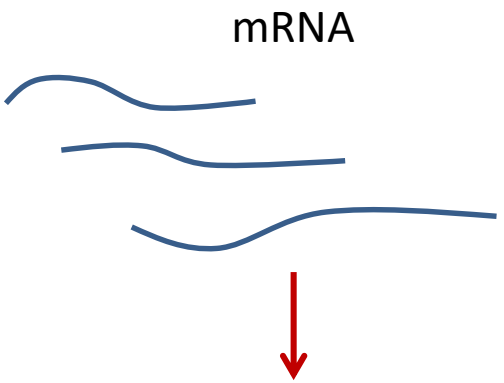
- TC does not inhibit the mRNA level of c-Myc.
- A reporter of *MYC 5'UTR* confirms TC inhibits translation of c-Myc.

Effects of TC on Global mRNA Translation

Ribosome footprinting & RNAseq



Gel Purify Ribosomal Footprints
& Generate Library Sequence
(Translation Rate)

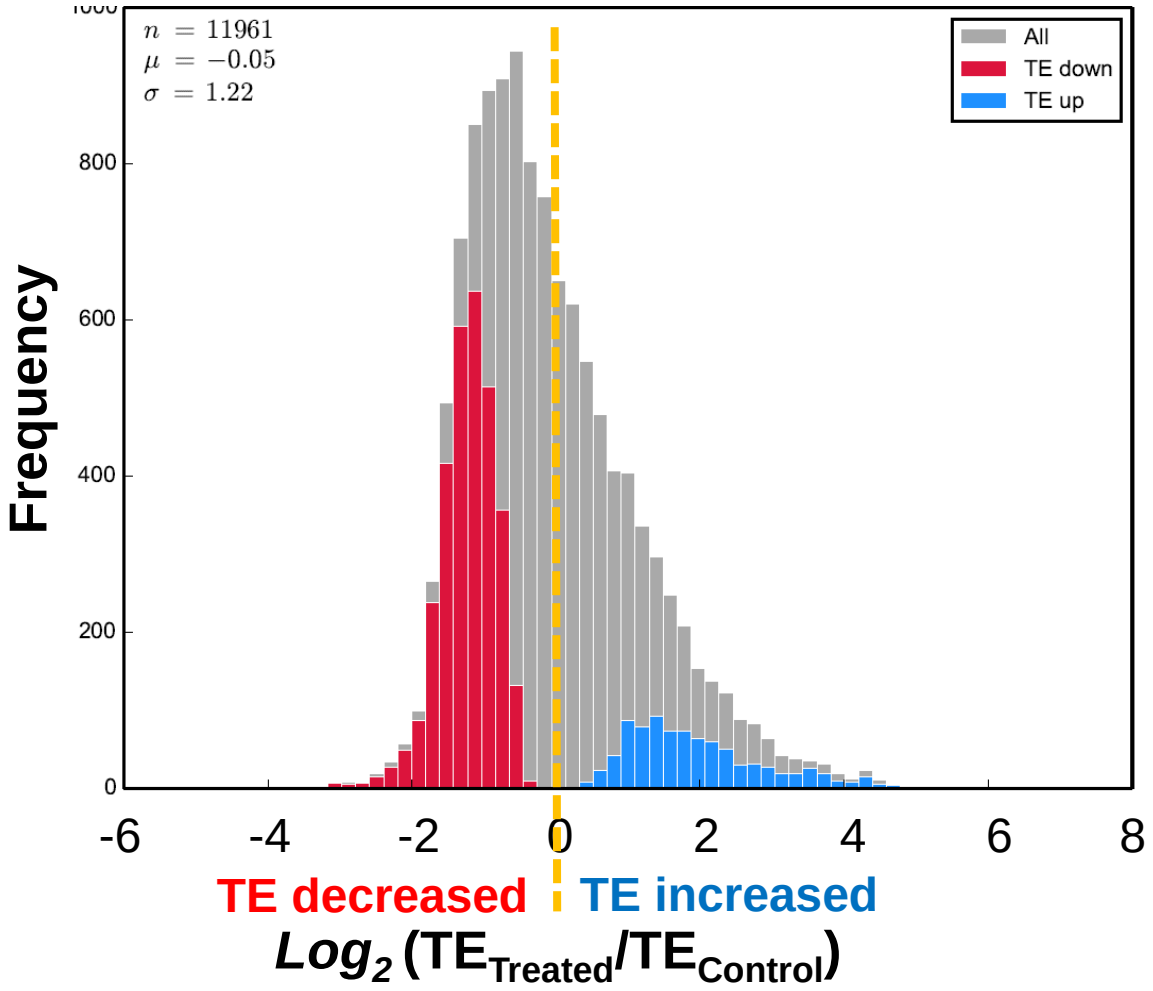


RNAseq
(mRNA expression level)

Translation Efficiency (TE) =
Translation Rate / mRNA level

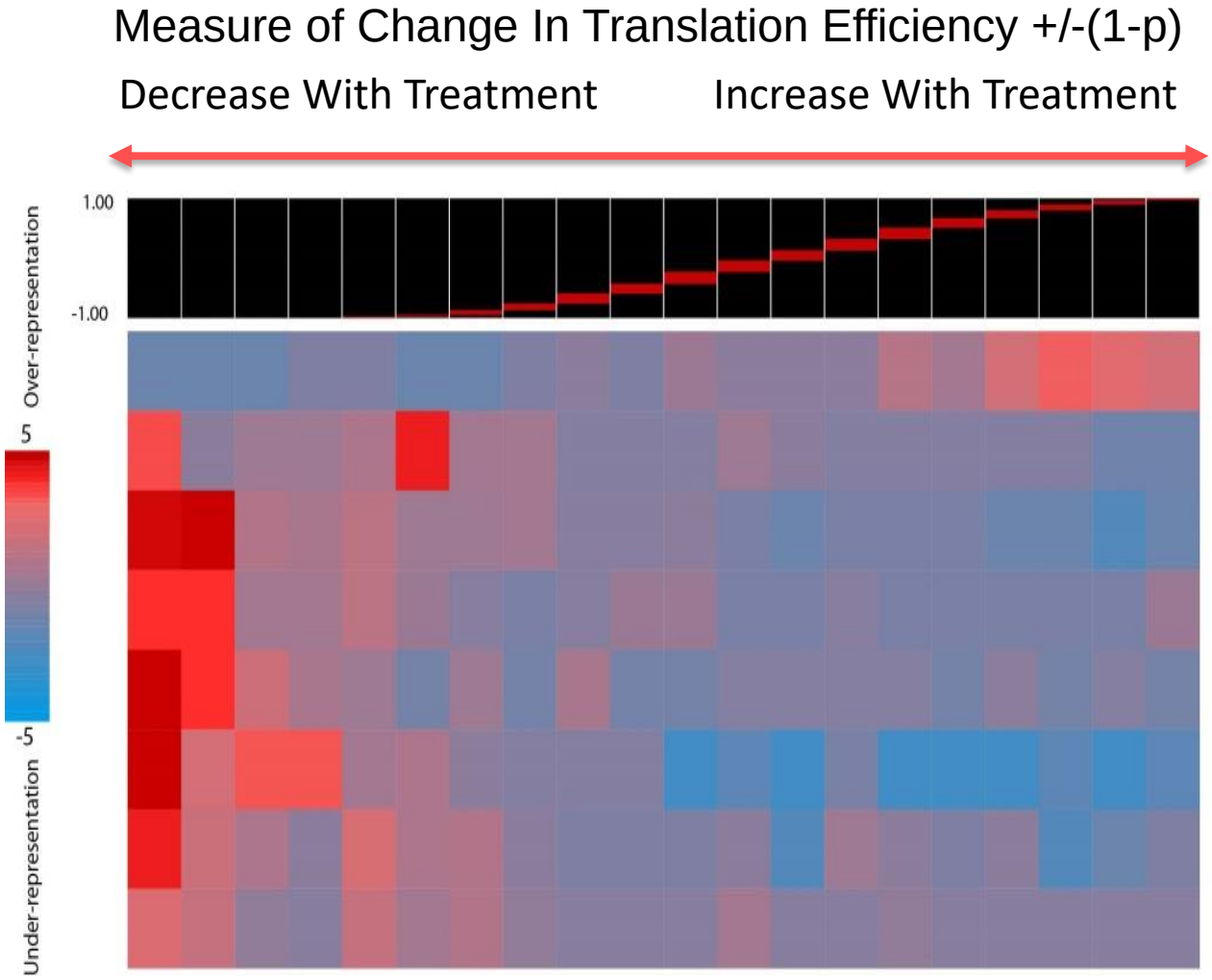
TC Inhibits Global mRNA Translation

Genome Wide Effects of TC on Translation Efficiency (TE)



TC Selectively Inhibits Translation of Genes Involved in Translation

iPAGE analysis of the ontology of translationally altered genes



Extracellular matrix

Translation factor

RNA splicing

Mitochondrial membrane

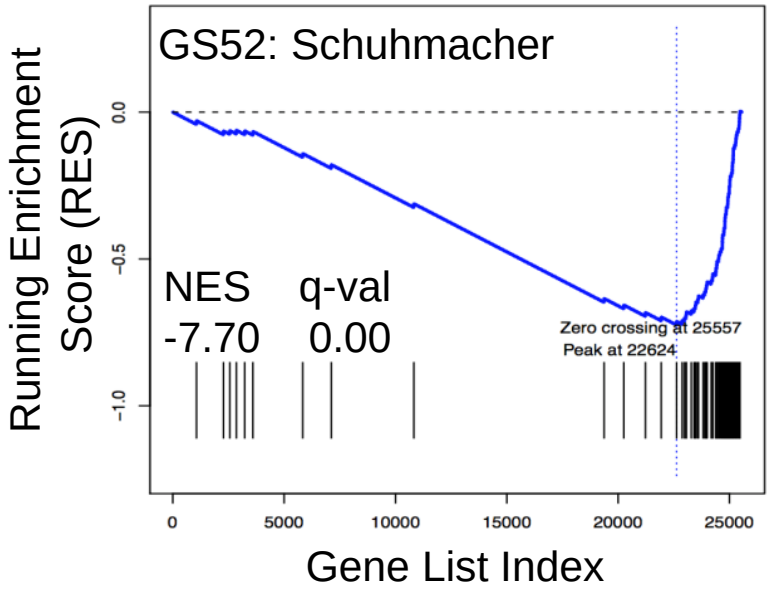
Nucleolus

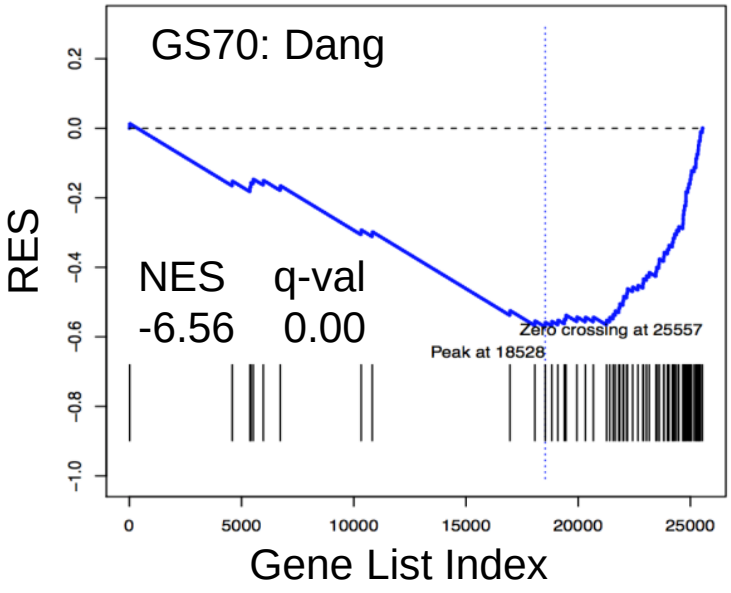
Constituents of ribosome

Mitotic cell cycle

Proteasome complex

TC Inhibits the Transcription of c-Myc Target Genes





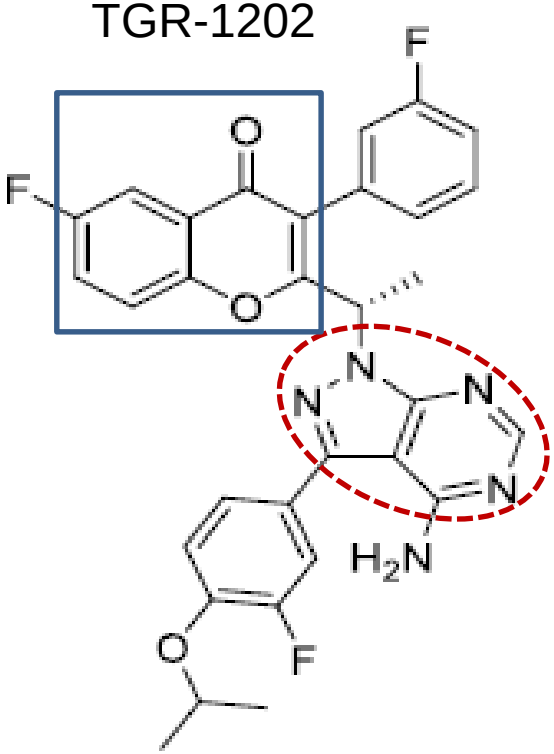
- Cytotoxicity of TC is recued by forced overexpression of c-Myc.
- Cytotoxicity of TC is recued by forced overexpression of eIF4E.

TGR-1202 and carfilzomib, but not combinations of other drugs in the same classes, synergistically inhibit c-Myc translation and c-Myc dependent gene transcription, by potently inhibiting phosphorylation of 4E-BP1.

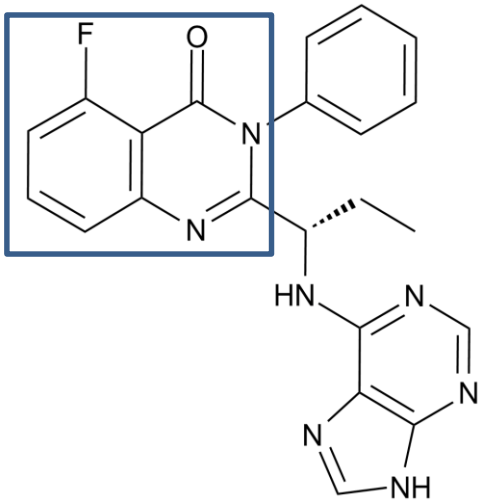
TGR-1202 and carfilzomib synergistically induce apoptosis In lymphoma cells through targeting c-Myc.

TGR-1202 Is Structurally Distinct from Idelalisib and Duvelisib

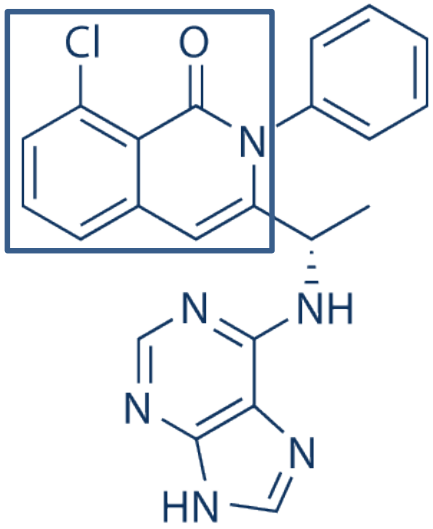
TGR-1202



Idelalisib



Duvelisib

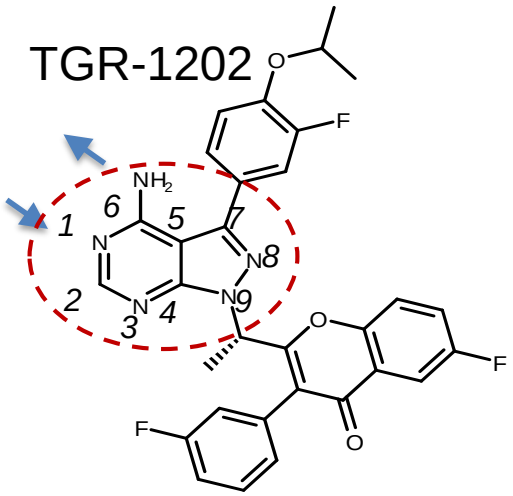


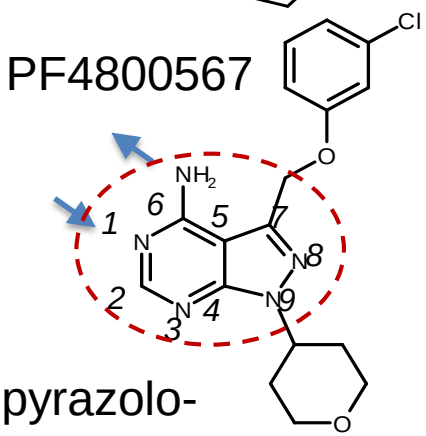
TGR-1202, but not Idelalisib or Duvelisib, Inhibits Casein Kinase 1 Epsilon (CK1ε)

Kinase activity (% of control) using the Reaction Biology Kinome Profiling platform

	TGR-1202		Idelalisib		Duvelisib	
Kinase	#1	#2	#1	#2	#1	#2
CK1a1	111	111	110	107	112	111
CK1a1L	105	103	102	101	104	99
CK1delta	105	98	96	104	100	97
CK1epsilon	*40*	*40*	*93*	*93*	*93*	*91*
CK1g1	99	98	105	105	102	98
CK1g2	104	104	102	100	99	99
CK1g3	96	95	94	93	93	93
CK2a	83	78	97	96	95	84
CK2a2	86	86	94	92	102	100

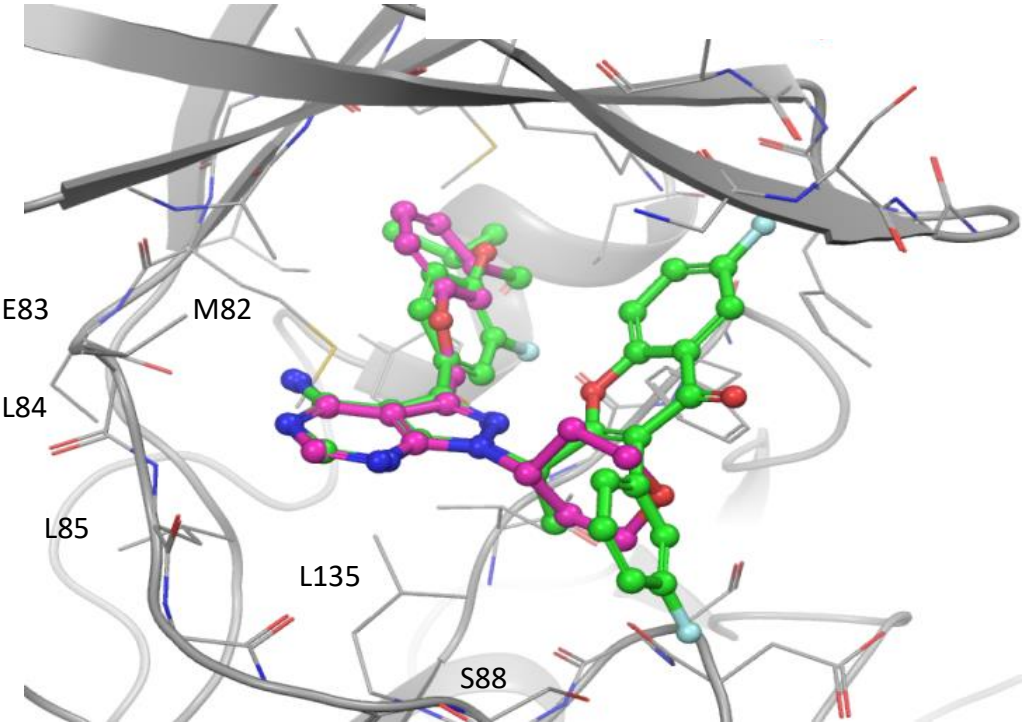
TGR-1202 and the CK1ε Inhibitor PF4800567 Share an Identical Structural Moiety





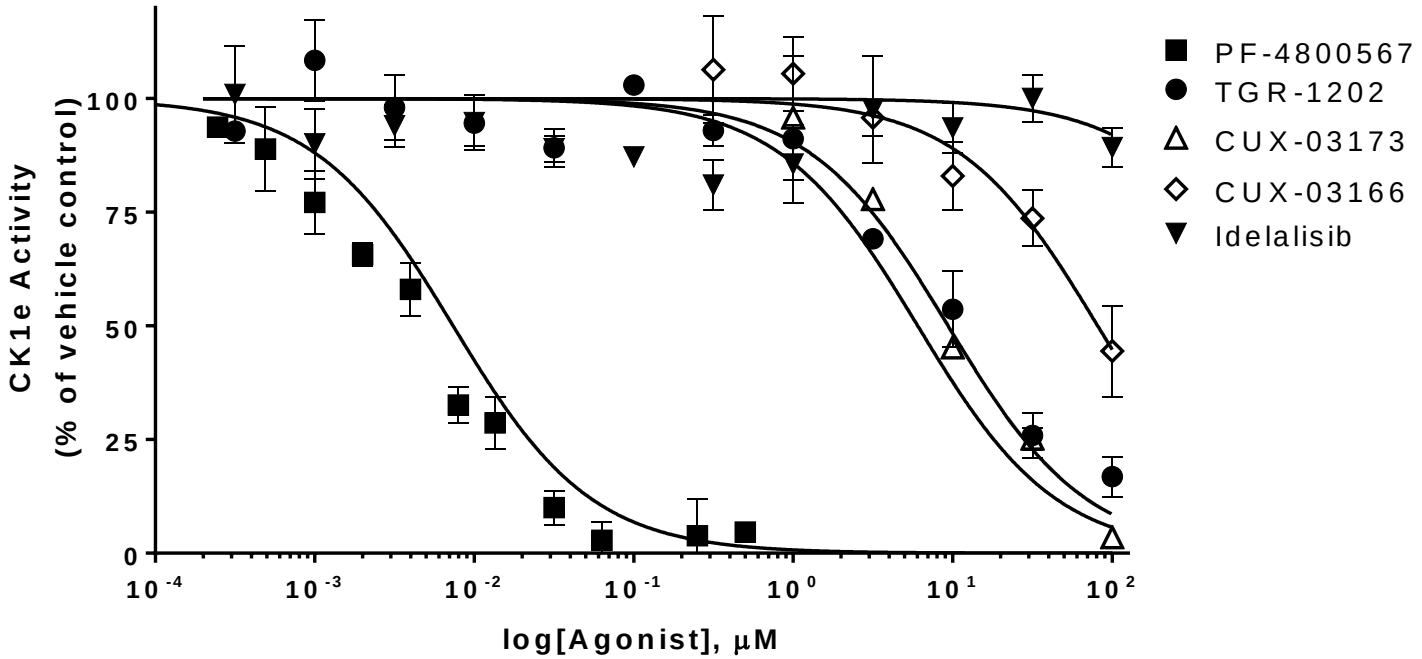
Central pyrazolo-pyrimidine moiety

PF4800567 (X-Ray)
TGR-1202 (*In silico* docking)



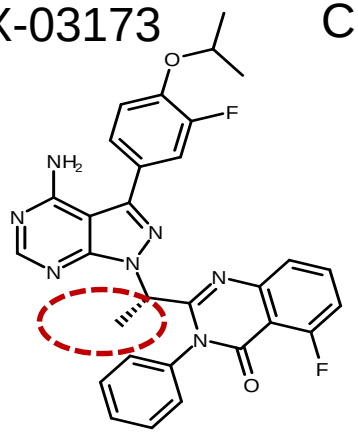
TGR-1202 and Its Analogs Inhibit CK1ε

Kinase activity (% of control) using recombinant CK1ε

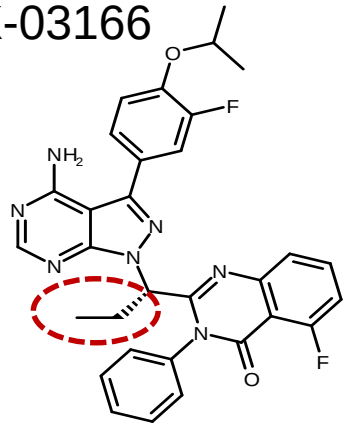


- Two analogs of TGR-1202, CUX-03173 and CUX-03166, demonstrate markedly different potency targeting CK1ε, despite they differ by only one methyl group.
- Idelalisib does not inhibit CK1ε.

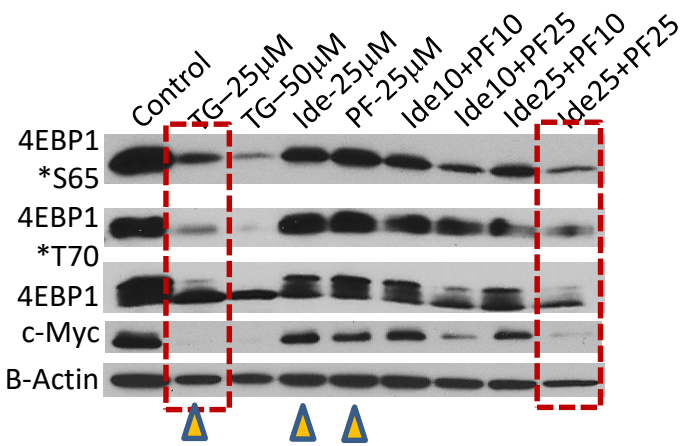
CUX-03173

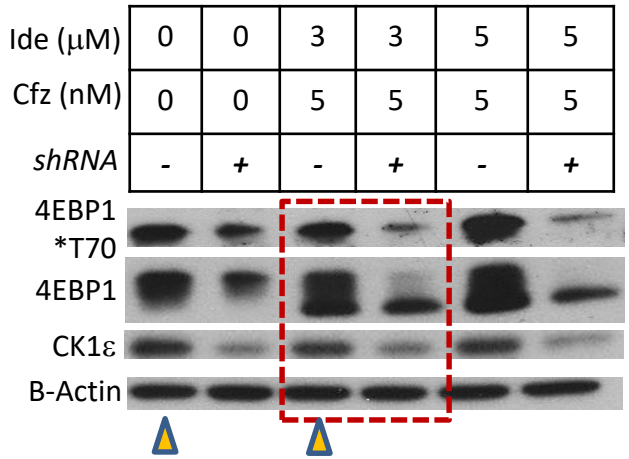


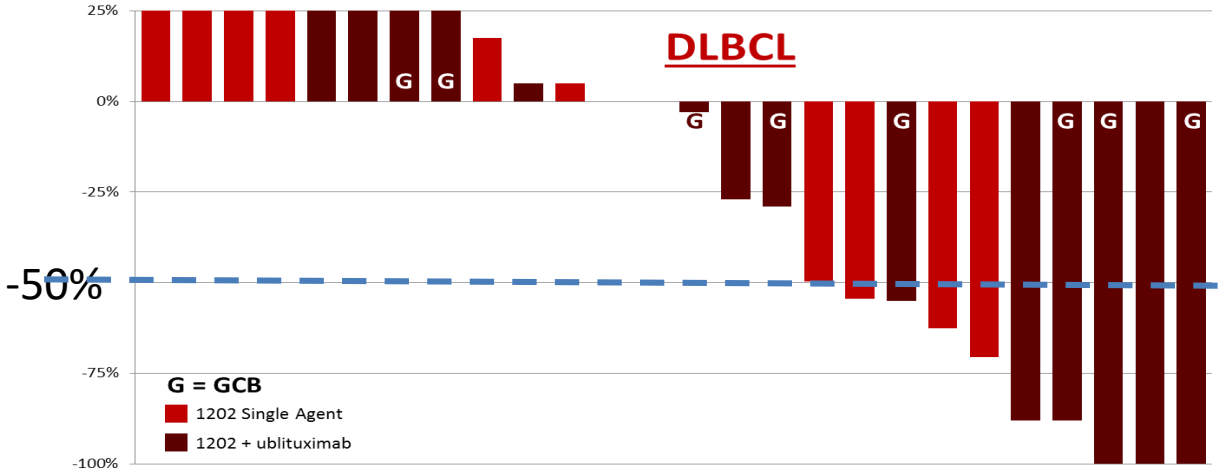
CUX-03166



Dual Targeting of PI3Kδ and CK1ε Underscores the Unique Activity of TGR-1202 in DLBCL

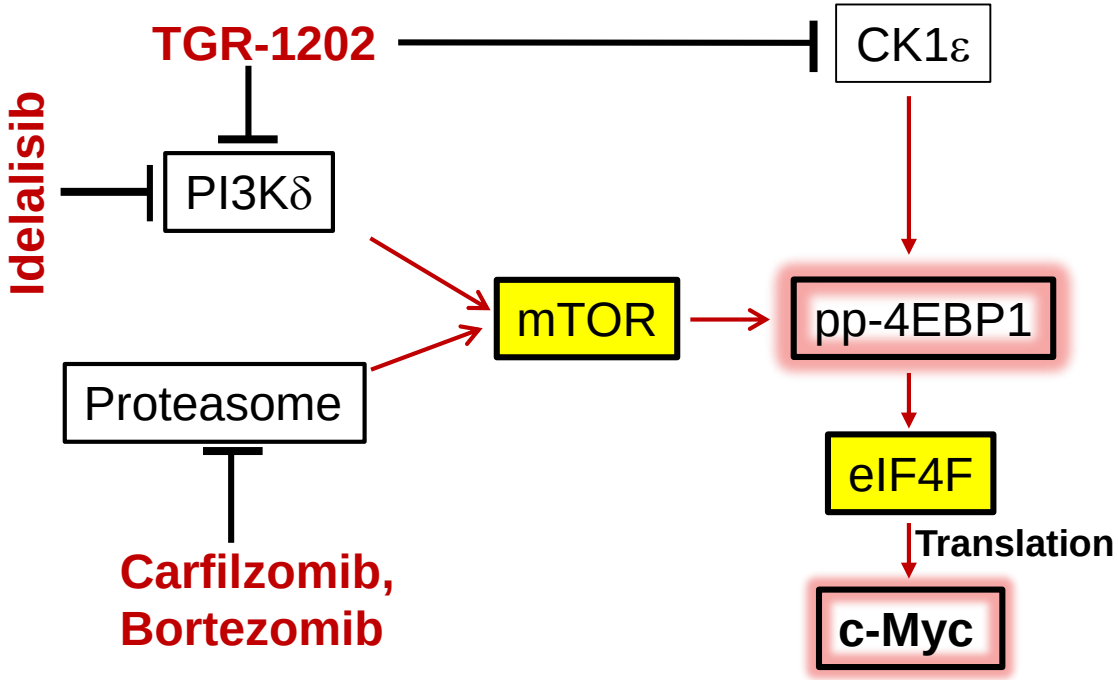






- 38% (6/16) Combo Responders.
- 30% (3/10) single responders.
- CR only in combo responders.

TGR-1202 as the First CK1ε Inhibitor Available for Patients May Have a Unique Therapeutic Role in c-Myc Driven Lymphoma



NCT02867618: actively enrolling patients

Phase I/II Study of TGR-1202 and Carfilzomib in the Treatment of Patients with Relapsed or Refractory Lymphoma

Thank You!!

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