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

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Disclosure

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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

Proteasome → PI3Kδ → AKT → mTOR → eIF4E → 4E-BP1 → PI3Kδ → mTOR

Amino acids → mTOR

?Other? Kinases

?Other? Kinases

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

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

PI3K → mTORC1 → pp-4E-BP1 → eIF4F → c-Myc

Proteasome

PI3Kδ Inhibitors

<table>
<thead>
<tr>
<th>Drugs</th>
<th>TGR-1202 (TG)</th>
<th>Idelalisib (Ide)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carfilzomib (Cfz)</td>
<td>TC</td>
<td>IC</td>
</tr>
<tr>
<td>Bortezomib (Bz)</td>
<td>TB</td>
<td>IB</td>
</tr>
</tbody>
</table>
TC Is Highly Synergistic and Superior to Other Combinations of PI3K and Proteasome Inhibitors

Excess over Bliss (EOB) > 0: Synergy

TCR-1202 (μM)

Idelalisib (μM)
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

Polysomes

RNAseq (mRNA expression level)

Translation Efficiency (TE) = Translation Rate / mRNA level

Gel Purify Ribosomal Footprints & Generate Library Sequence (Translation Rate)
TC Inhibits Global mRNA Translation

Genome Wide Effects of TC on Translation Efficiency (TE)

\[ \log_2 \left( \frac{\text{TE}_{\text{Treated}}}{\text{TE}_{\text{Control}}} \right) \]

- 6
- 4
- 2
  0
  2
  4
  6
  8

Frequency

\[ n = 11961 \]
\[ \mu = -0.05 \]
\[ \sigma = 1.22 \]
TC Selectively Inhibits Translation of Genes Involved in Translation

iPAGE analysis of the ontology of translationally altered genes

Measure of Change In Translation Efficiency +/- (1-p)
Decrease With Treatment   Increase With Treatment

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

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

<table>
<thead>
<tr>
<th>Kinase</th>
<th>TGR-1202 #1</th>
<th>TGR-1202 #2</th>
<th>Idelalisib #1</th>
<th>Idelalisib #2</th>
<th>Duvelisib #1</th>
<th>Duvelisib #2</th>
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</thead>
<tbody>
<tr>
<td>CK1a1</td>
<td>111</td>
<td>111</td>
<td>110</td>
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<td>97</td>
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<td>84</td>
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<tr>
<td>CK2a2</td>
<td>86</td>
<td>86</td>
<td>94</td>
<td>92</td>
<td>102</td>
<td>100</td>
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</tbody>
</table>
TGR-1202 and the CK1ε Inhibitor PF4800567 Share an Identical Structural Moiety

Central pyrazolo-pyrimidine moiety
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.
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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