CYTOTOXIC ACTIVITY OF THE CASEIN KINASE 2 INHIBITOR CX-4945 AGAINST T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA: targeting the Unfolded Protein Response Signaling

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Knowing CK2... not a lazy protein!!!

• Ubiquitously expressed and constitutively active serine/threonine kinase, considered the most pleiotropic protein kinase.

• Its central task: global promotion of cell growth and prevention of apoptosis.

• Overexpressed and up-regulated in all cancers examined, in human solid tumors but also in some blood malignancies.

• CK2 is a “cancer driver” and a “lateral player”.
CK2-dependent multisite regulation of NF-κB (A), β-catenin (B) and Akt (C) signaling. A negative effect (−) is indicated by a dot-arrow.<ce:inline-figure baseline="0.0">
CX-4945

a novel, highly specific, orally available, ATP-competitive inhibitor of CK2α
CX-4945: antiproliferative effects

PTEN +

PTEN -

% cell viability

CX-4945 [µM]

DND-41 ctrl  DND-41 24h  MOLT-4 ctrl  MOLT-4 24h

CEM-R ctrl  CEM-R 24h  CEM-S ctrl  CEM-S 24h

<table>
<thead>
<tr>
<th>Jurkat</th>
<th>CEM-R</th>
<th>CEM-S</th>
<th>MOLT-4</th>
<th>PF-382</th>
<th>ALL-SIL</th>
<th>HPB-ALL</th>
<th>DND-41</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC50 [µM]</td>
<td>4.9</td>
<td>4</td>
<td>4.6</td>
<td>8.7</td>
<td>4.5</td>
<td>5.7</td>
<td>6.1</td>
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</tbody>
</table>

Caspase 3

Cleaved caspase 3

Parp

35 kDa

19 kDa

17 kDa

116 kDa

89 kDa
CX-4945: PI3K/PTEN/Akt/mTOR pathway modulation

**PTEN +**
- HPB-ALL
- ALL-SIL
- DND-41

**PTEN -**
- Jurkat
- CEM-S
- PF-382

**CK2**
- HPB-ALL: 42 kDa
- ALL-SIL: 42 kDa
- DND-41: 42 kDa
- Jurkat: 42 kDa
- CEM-S: 42 kDa
- PF-382: 42 kDa

**p-PTEN (Ser380)**
- HPB-ALL: 54 kDa
- ALL-SIL: 54 kDa
- DND-41: 54 kDa
- Jurkat: 54 kDa
- CEM-S: 54 kDa
- PF-382: 54 kDa

**PTEN**
- HPB-ALL: 54 kDa
- ALL-SIL: 54 kDa
- DND-41: 54 kDa
- Jurkat: 54 kDa
- CEM-S: 54 kDa
- PF-382: 54 kDa

**actin**
- HPB-ALL: 45 kDa
- ALL-SIL: 45 kDa
- DND-41: 45 kDa
- Jurkat: 45 kDa
- CEM-S: 45 kDa
- PF-382: 45 kDa

**PTEN +**
- DND-41

**PTEN -**
- CEM-R

**p-Akt (Ser129)**
- DND-41: 60 kDa
- CEM-R: 60 kDa

**Akt**
- DND-41: 60 kDa
- CEM-R: 60 kDa

**actin**
- DND-41: 45 kDa
- CEM-R: 45 kDa
CX-4945: PI3K/PTEN/Akt/mTOR pathway modulation

**A**

- IP: Akt
- CEM-R
- WB: Akt
  - ctrl
  - CX-4945
  - 60 kDa
- WB: HSP-90
  - 60 kDa
  - 90 kDa

**B**

- ctrl
- GA
- p-Akt (Thr308)
  - 60 kDa
- p-Akt (Ser473)
  - 60 kDa
- p-Akt (Ser129)
  - 60 kDa
- Akt
  - 60 kDa
- HSP-90
  - 90 kDa
- actin
  - 45 kDa

**C**

- p-Akt (Thr308)
  - -
  - +
  - +
  - OA
  - CX-4945
  - 60 kDa
- Akt
  - 60 kDa
- actin
  - 45 kDa
Unfolded Protein Response
CX-4945: CK2 inactivation affects ER stress/UPR signaling

<table>
<thead>
<tr>
<th></th>
<th>ALL-SIL</th>
<th>DND-41</th>
<th>MOLT-4</th>
<th>Jurkat</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>0 h</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>1 h</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>3 h</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td><strong>6 h</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><strong>16 h</strong></td>
<td></td>
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<tr>
<td><strong>24 h</strong></td>
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<td></td>
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</tr>
<tr>
<td><strong>48 h</strong></td>
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**PTEN +**

<table>
<thead>
<tr>
<th>Protein</th>
<th>0 h</th>
<th>1 h</th>
<th>3 h</th>
<th>6 h</th>
<th>16 h</th>
<th>24 h</th>
<th>48 h</th>
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<tbody>
<tr>
<td>GRP78/BIP</td>
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<td></td>
<td></td>
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<td></td>
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<td>78 kDa</td>
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<tr>
<td>IRE1α</td>
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<td></td>
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</tr>
<tr>
<td>p-EIF2α</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>38 kDa</td>
</tr>
<tr>
<td>(Ser51)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>38 kDa</td>
</tr>
<tr>
<td>EIF2α</td>
<td></td>
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<td></td>
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<tr>
<td>CHOP</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>28 kDa</td>
</tr>
<tr>
<td>actin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>45 kDa</td>
</tr>
</tbody>
</table>

**PTEN -**
CX-4945: efficacy in primary T-ALL blasts

A

% cell viability

CX-4945 [µM]

Pt #1
Pt #2
Pt #3
Pt #4

Patient ID
24h IC50 [µM]
3
0.78
0.45
1.1

B

Propidium iodide

Pt #1

ctrl
5 µM CX-4945
10 µM CX-4945

Annexin V-FITC

Pt #4

IRE1α
GRP78/BIP
actin
CX-4945: delay of T-ALL tumor growth \textit{in vivo}
Conclusions

• **CX-4945** may be an efficient treatment for T-ALLs that have aberrant up-regulation of the **CK2/PI3K/Akt/mTOR** signaling

• **CX-4945**

• Modulation of the **ER stress/UPR signaling** through CK2 inhibition could be exploited for inducing T-ALL cell death.

Buontempo F. et al.
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