CB-839, a selective glutaminase inhibitor, synergizes with signaling pathway inhibitors to produce an anti-tumor effect in cell lines and tumor xenografts

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Calithera Biosciences
South San Francisco, California
I have the following financial relationships to disclose:
Stockholder in Calithera Biosciences
Employee of Calithera Biosciences

I will not discuss off label use and/or investigational use in my presentation.
Tumor Cells Have Increased Glutamine and Glucose Consumption

**Normal Cell**

- Lactate
- GLUCOSE
- Pyruvate
- Energy
- TCA Cycle
- α-KG
- Glutamate
- Glutaminase
- GLUTAMINE

**Tumor Cell**

- Lactate
- GLUCOSE
- Pyruvate
- Energy
- TCA Cycle
- α-KG
- Glutamate
- Glutaminase
- GLUTAMINE

Building Blocks for Cell Growth

CB-839
CB-839 Suppresses Metabolic Pathways Downstream of Glutamate

NSCLC cell lines
CB-839 (1 µM)
Timepoint: 24 h

Glutaminase

Glutamine
Glutamate
α-ketoglutarate (α-KG)
Fumarate
Malate
Citrate
Oxaloacetate
Aspartate
Glutathione

Fold Change in Metabolite

0.0625
0.125
0.25
0.5
1
2
4
8
16
Glutamate
GSH
Malate
Citrate
Aspartate

0.0625
0.125
0.25
0.5
1
2
4
8
16
Glutamine
Glutamate
GSH
Malate
Citrate
Aspartate
CB-839 Has Anti-Proliferative Activity in Multiple Cancer Types

Cell Growth (% control)

Cell Death (% plated)

Breast

<table>
<thead>
<tr>
<th>Triple-negative</th>
<th>ER+ or Her2+</th>
<th>non-RCC</th>
</tr>
</thead>
</table>

Kidney

<table>
<thead>
<tr>
<th>RCC</th>
<th>NSCLC</th>
<th>Mesothelioma</th>
<th>Myeloma</th>
<th>Lymphoma</th>
</tr>
</thead>
</table>

Cell Line

<table>
<thead>
<tr>
<th>Cell Death (% plated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-100</td>
</tr>
</tbody>
</table>

1 µM CB-839
72 h Treatment
Renal Clear Cell Carcinoma Cells are Sensitive to Glutaminase Inhibition

![Graph showing cell growth and death of various renal cell carcinoma and kidney tumor cell lines after 72 h treatment with 1 μM CB-839.](graph_image)

- **Cell Growth (% control)**
- **Cell Death (% plated)**

- **Renal Clear Cell Carcinoma**
- **Kidney Tumor (non-RCC)**

1 μM CB-839
72 h Treatment
Cross Talk Between Signal Transduction Pathways and Cancer Metabolism

Growth Factor Receptor

Ras/Raf Pathway

PI3K/mTOR Pathway

↑ Glutamine Utilization

↑ Glucose Utilization

CB-839
MTOR Signaling is Downregulated by CB-839 in Sensitive RCC Cells

<table>
<thead>
<tr>
<th></th>
<th>Sensitive Cells</th>
<th>Resistant Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TUH-10TK8</td>
<td>JMU-RTK2</td>
</tr>
<tr>
<td></td>
<td>A704</td>
<td>ACHN</td>
</tr>
<tr>
<td></td>
<td>786-O</td>
<td>RCC-ER</td>
</tr>
<tr>
<td></td>
<td>Caki-1</td>
<td>G402</td>
</tr>
<tr>
<td></td>
<td>A498</td>
<td>G401</td>
</tr>
<tr>
<td></td>
<td>786-P</td>
<td>BFTC-909</td>
</tr>
<tr>
<td>(-)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(+) CB-839</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>phospho S6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Ser240/244)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>total S6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Renal Cell Carcinoma**

**Kidney Tumor (non-RCC)**

\[ p = 0.026^* \]
MTOR Signaling is Downregulated by CB-839 in Sensitive RCC Cells

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<td>BFTC-909</td>
</tr>
</tbody>
</table>

CB-839 (1 μM; 24h)

-0.8
-0.6
-0.4
-0.2
0.0

Δ phospho 4E-BP11 (normalized A.U.)

Sensitive Resistant

p = 0.04*

Renal Cell Carcinoma
Kidney Tumor (non-RCC)
Cross Talk Between Signal Transduction Pathways and Cancer Metabolism

Growth Factor Receptor

Ras/Raf Pathway

PI3K/mTOR Pathway

↓ Glutamine Utilization

↑ Glucose Utilization

Everolimus (mTOR inhibitor)

CB-839
Synergistic Anti-Proliferative Activity of CB-839 and Everolimus in Renal Clear Cell Carcinoma Cells

**Cell Survival (relative to DMSO)**

- **ACHN**

<table>
<thead>
<tr>
<th>CB-839 (nM)</th>
<th>Everolimus (nM)</th>
<th>Mixture (Comb. Index)</th>
</tr>
</thead>
<tbody>
<tr>
<td>300</td>
<td>100</td>
<td>0.38</td>
</tr>
<tr>
<td>150</td>
<td>50</td>
<td>0.33</td>
</tr>
<tr>
<td>75</td>
<td>25</td>
<td>0.20</td>
</tr>
<tr>
<td>37.5</td>
<td>3.1</td>
<td>0.36</td>
</tr>
<tr>
<td>18.8</td>
<td>1.6</td>
<td>0.19</td>
</tr>
</tbody>
</table>

**Plating Density**

- **4h Treatment**

**Glucose Consumption**

<table>
<thead>
<tr>
<th>24 h Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nutrient Consumption (relative to DMSO)</td>
</tr>
<tr>
<td>0.0</td>
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</table>

**Glutamine Consumption**

<table>
<thead>
<tr>
<th>24 h Treatment</th>
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<td>Nutrient Consumption (relative to DMSO)</td>
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**Extracellular Acidification Rate**

<table>
<thead>
<tr>
<th>24 h Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline ECAR (relative to DMSO)</td>
</tr>
<tr>
<td>0.0</td>
</tr>
</tbody>
</table>

**Oxygen Consumption Rate**

<table>
<thead>
<tr>
<th>24 h Treatment</th>
</tr>
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<tbody>
<tr>
<td>Baseline OCR (relative to DMSO)</td>
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<tr>
<td>0.0</td>
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</tbody>
</table>

**phospho 4E-BP1 (Ser65)**

- **4h Treatment**
Anti-Proliferative Activity of CB-839 in NSCLC

Cell Growth (% control)

Cell Death (% plated)

Sensitive

Resistant

1 μM CB-839
72 h Treatment
KRAS and EGFR Mutated Tumor Cells are More Sensitive to Glutaminase Inhibition with CB-839

**Correlation Between CB-839 Sensitivity & KRAS/EGFR Status**

<table>
<thead>
<tr>
<th>Test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chi-square</td>
<td>0.005**</td>
</tr>
<tr>
<td>t test</td>
<td>0.026*</td>
</tr>
</tbody>
</table>

1 µM CB-839
72 h Treatment
Cross Talk Between Signal Transduction Pathways and Cancer Metabolism

Growth Factor Receptor

Ras/Raf Pathway

PI3K/mTOR Pathway

↑ Glutamine Utilization

↑ Glucose Utilization

Erlotinib (EGFR inhibitor)

Selumetinib (Mek inhibitor)

CB-839
CB-839 is Synergistic with Selumetinib in KRAS^{mut} Cells

**Glucose Consumption**
- CB-839
- Selumetinib
- Combo

**Glutamine Consumption**
- DMSO
- CB-839
- Selumetinib
- Combo

**Malate**
- DMSO
- CB-839
- Selumetinib
- Combo

**GSH**
- DMSO
- CB-839
- Selumetinib
- Combo

**Cell Survival** (relative to DMSO)
- 100
- 50
- 25
- 12.5
- 6.25
- CB-839 (nM)
- 1000
- 500
- 250
- 125
- 62.5
- Selumetinib (nM)
- 0.30
- 0.25
- 0.40
- 0.56
- 0.41
- Mixture (Comb. Index)

**H2122 Xenograft**
- Vehicle
- CB-839 TGI=46%
- Selumetinib TGI=49%
- Combo TGI=78%

*** vs. CB-839
**** vs. Selumetinib
** vs. CB-839
*** vs. Selumetinib

**Tumor Volume (mm^3)**
- 0
- 500
- 1000
- 1500

**Days Post-Implant**
- 5
- 10
- 15
- 20
- 25

**Plating Density**
- 72 h Treatment

**CB-839 is Synergistic with Selumetinib in KRAS^{mut} Cells**
CB-839 is Synergistic with Erlotinib in EGFR^{mut} Cells

CB-839 is Synergistic with Erlotinib in EGFR^{mut} Cells

**Cell Survival**
(relative to DMSO)

<table>
<thead>
<tr>
<th>HCC827 Plating Density</th>
<th>CB-839 (nM)</th>
<th>Erlotinib (nM)</th>
<th>Mixture (Comb. Index)</th>
</tr>
</thead>
<tbody>
<tr>
<td>500 500 300 100 100</td>
<td>25 12.5</td>
<td>12.5 6.25 3.125</td>
<td>0.67 0.45 0.43 0.44 0.81</td>
</tr>
</tbody>
</table>

**Glucose Consumption**
(relative to DMSO)

<table>
<thead>
<tr>
<th>24 h Treatment</th>
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<tbody>
<tr>
<td>Nutrient Consumption</td>
</tr>
<tr>
<td>DMSO</td>
</tr>
<tr>
<td>1.00</td>
</tr>
</tbody>
</table>

**Glutamine Consumption**
(relative to DMSO)

<table>
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**Malate**
(relative to DMSO)

<table>
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<tr>
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<td>1.00</td>
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**GSH**
(relative to DMSO)

<table>
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<tr>
<td>DMSO</td>
</tr>
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<td>1.00</td>
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</table>
CB-839 Enhances the Anti-Tumor Effect of Erlotinib in an Erlotinib Resistant Model

**H1650 Xenograft**

- **Tumor Volume (mm³)**
  - **Vehicle**
  - **CB-839**
  - **Erlotinib**
  - **Combo**

- **Dosing Start**
- **TGI = 26% n.s.**
- **TGI = 66%***
- **TR = 11%****
- **P < 0.001***

**Cell Survival** (relative to DMSO)

- **H1650**
- **72 h Treatment**

- **Plating Density**
- **62.5** 62.5 62.5 62.5 62.5 CB-839 (nM)
- **5,000** 2,500 1000 500 250 Erlotinib (nM)
- **0.23** 0.27 0.43 0.39 0.46 Mixture (Comb. Index)

**Cellular Metabolite (relative to DMSO)**

- **Malate**
- **24 h Treatment**

- **GSH**

**Tumor Volume (mm³)**

- **Vehicle**
- **CB-839**
- **Erlotinib**
- **Combo**

**Dosing Start**

**TGI = 26% n.s.**

**TGI = 66%***

**TR = 11%****

**P < 0.001***
Conclusions

• CB-839 is anti-proliferative in multiple tumor types and suppresses mTOR pathway signaling

• Clear cell RCC lines are sensitive to CB-839

• KRAS and EGFR mutant NSCLC lines show enhanced sensitivity to CB-839

• CB-839 in combination with signal transduction inhibition offers a novel therapeutic strategy for the treatment of cancer:
  – Everolimus in RCC
  – Mek inhibitor in KRAS mutant NSCLC
  – EGFR inhibitor in EGFR mutant NSCLC
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