Targeting the PI3K-Akt-mTOR pathway with GDC-0068, a novel selective ATP competitive Akt inhibitor

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Akt pathway is frequently activated in cancer

- Compelling evidence for targeting Akt in human malignancies
GDC-0068: Novel, specific, ATP competitive Akt inhibitor

Enzymatic potency and selectivity of GDC-0068

<table>
<thead>
<tr>
<th>Kinase</th>
<th>IC50 (nM)</th>
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<tbody>
<tr>
<td>Akt1/2/3</td>
<td>5/18/8</td>
</tr>
<tr>
<td>PKG1a/b</td>
<td>98/69</td>
</tr>
<tr>
<td>p70S6K</td>
<td>860</td>
</tr>
<tr>
<td>PKA</td>
<td>3100</td>
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<tr>
<td>SGK</td>
<td>&gt;1000*</td>
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<tr>
<td>PDK1</td>
<td>&gt;1000*</td>
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<tr>
<td>AMPK</td>
<td>&gt;1000*</td>
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*No inhibition when screened at 1000 nM in a protein kinase panel

Cellular potency of GDC-0068

<table>
<thead>
<tr>
<th>Cell line</th>
<th>pPRAS40 IC50 (nM)</th>
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<tbody>
<tr>
<td>LNCaP</td>
<td>157</td>
</tr>
<tr>
<td>BT474M1</td>
<td>208</td>
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</tbody>
</table>
GDC-0068: Effectively blocks Akt signaling and induces cell cycle arrest in human cancer cell lines in vitro

PC-3 (PTEN-)

<table>
<thead>
<tr>
<th>GDC-0068 (μM)</th>
<th>DMSO</th>
<th>0.0038</th>
<th>0.011</th>
<th>0.034</th>
<th>0.10</th>
<th>0.31</th>
<th>0.93</th>
<th>2.8</th>
<th>8.3</th>
<th>25</th>
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<tbody>
<tr>
<td>pAkt^{S473}</td>
<td></td>
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<td>pPRAS40^{T246}</td>
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<td>pGSK3b^{S9}</td>
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<td>pS6^{S235/6}</td>
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<tr>
<td>β-actin</td>
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<td>GAPDH</td>
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PC-3 48 hours

DMSO Control

- G0/G1 = 42.0%
- S = 36.7%
- G2/M = 21.3%

5 µM GDC-0068

- G0/G1 = 72.0%
- S = 18.9%
- G2/M = 9.1%
High Akt activity predicts sensitivity to GDC-0068

Breast cancer cell lines

Sensitivity in breast cancer cell line panel:

- Strongest in HER2+ and Luminal subtypes.
- Driven by PI3K kinase domain mutations, PTEN loss and HER2 amplification.
- Negative association with KRas/BRaf mutations and EGFR expression.
GDC-0068 exhibits significant efficacy in PTEN- and PI3K mutant xenograft models

**LNCaP**  
(PTEN-, Androgen-dep prostate)

**KPL-4**  
(PI3K H1047R, Her2+. ER- breast)

![Graph showing tumor volume over days for LNCaP and KPL-4](image-url)
IHC/IF and RPPA: Complementary platforms to demonstrate PD changes

• GDC-0068 reduces pS6 and peIF4G levels in BT474-Tr xenografts
IHC/IF: Akt Inhibitor GDC-0068 relocalizes FOXO3a to nucleus

- Subcellular localization of FOXO3a regulated by phosphorylation by Akt
- Nuclear FOXO3a controls transcription of pro-apoptotic and cell cycle inhibitory genes

**Vehicle**

**GDC-0068**

GDC-0068 treated BT474-Tr xenografts
RPPA analyses demonstrate feedback upregulation of HER3 and pERK induced by the Akt Inhibitor GDC-0068

@ Zimmermann and Moelling, *Science*, 1999

GDC-0068 treated BT474-Tr xenografts
**Dose:** oral daily x 21 days on/ 7 days off

**Schema:**
- Single PK dose in week 1, then 21/28 day dosing
- Dose GDC-0068 in am (post O/N fast) and fast 2 hrs post dose
- Standard 3+3 design

**PD:**
- Surrogate tissue: AKT pathway evaluation in platelet-rich plasma
- pre- and on-treatment skin biopsy of all patients
- Tumor biopsy when ≥ 50% pathway knockdown achieved in surrogate tissue
Preliminary evidence for pathway knockdown in tumors

- Tumor biopsies were obtained from patients during screening (baseline) and once during Cycle 1 (between Days 15 and 21).

- Needle core biopsies were snap-frozen and evaluated by reverse phase protein array for epitopes, including pPRAS40.

- Decreases of 60%–70% in pPRAS40 (compared with baseline) were demonstrated in all 3 patients treated at 400 mg once daily.

- All 3 patients in 400 mg cohort show >60% inhibition.
Conclusions

• GDC-0068 is a novel, oral, selective ATP-competitive AKT inhibitor

• Preclinical activity is most pronounced in models driven by PI3K kinase domain mutations, PTEN loss and HER2 amplification

• GDC-0068 treatment resulted in pronounced PD effects in tumor xenograft models as measured by RPPA and/or IHC, including dose-dependent suppression of P-S6 and P-eIF4G, as well as induction of FOXO nuclear localization

• GDC-0068 is currently in phase I development

• At well-tolerated doses, GDC-0068 results in >60% pathway knockdown in surrogate and tumor tissue
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