Discovery of GDC-0068: A Selective ATP-competitive Akt Inhibitor for the Treatment of Human Tumors

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Array BioPharma Inc, Boulder, CO

CHI Next Generation Kinase Inhibitors June 6–8, 2011
Akt is a major downstream target of PI3K:
- Central node in cell signaling of growth factors, cytokines, and other cellular stimuli. Akt can control key cellular processes by phosphorylating substrates involved in apoptosis, transcription, cell cycle progression, and translation (Manning and Cantley *Cell* 2007, 129, 1261-1274).
- Synonyms: PKB, RAC, EC 2.7.11.1, ...
- Three isoforms: ca. 90% identity in kinase domain; 97-100% in ATP binding site.
- Related Proteins: AGC kinases, e.g. PKA, PKC, PKG, RSK, p70S6K ...
  - < 55% identity in kinase domain; 60-80% in ATP site

Pathway can be up-regulated in a number of ways.
- Loss of PTEN activity.
- Oncogenic mutations of PI3K kinase.
- Akt over expression/amplification (especially Akt2).
- Growth factor signaling.
- Chemotherapy-induced pathway activation.
- Akt1 activating mutation (E17K) (breast-8%, colon-6%, ovarian-2%).
PI3K/Akt Signaling Pathway

The Genesis of Array’s Akt Lead

- Screened compound collection against full length activated (hyperphosphorylated) Akt1.
- Original HTS hit came from a library designed to target protein kinases.
  - 2960 members:

  ![Chemical Structures]

- The initial hit offered a relatively potent starting point with good physical properties.
  - The single enantiomers showed marked stereospecificity with respect to Akt1 activity.
Model of Active Enantiomer Docked Into Akt2 X-Ray Crystal Structure

- High sequence identity between Akt1/2/3 in ATP site.
- Docked active HTS hit and utilized binding mode to drive chemistry and SAR.
- Key binding features are hinge interaction with quinazoline ring, carboxylates of Glu236 and Glu279 with amine, and hydrophobic contacts between P-loop and benzyl substituent.

Akt1 IC$_{50}$ = 884 nM

Amino Amide Region

- Examined substitutions around the phenyl ring varying size and electronics.
  - 40-fold improvement in enzyme potency via 4-Cl substitution.
  - Cell IC$_{50}$ LNCaP p-PRAS40 = 1.5 μM.

<table>
<thead>
<tr>
<th>R</th>
<th>Akt1 Inhibition IC$_{50}$, nM</th>
<th>Akt2 Inhibition IC$_{50}$, nM</th>
<th>Akt3 Inhibition IC$_{50}$, nM</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>884</td>
<td>8,362</td>
<td>8,410</td>
</tr>
<tr>
<td>4-Cl</td>
<td><strong>20</strong></td>
<td><strong>118</strong></td>
<td><strong>179</strong></td>
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<tr>
<td>4-CN</td>
<td>284</td>
<td>4,371</td>
<td>2,718</td>
</tr>
<tr>
<td>4-F</td>
<td>118</td>
<td>1,288</td>
<td>1,122</td>
</tr>
<tr>
<td>4-OCH$_3$</td>
<td>150</td>
<td>1,485</td>
<td>2,056</td>
</tr>
<tr>
<td>4-CH$_3$</td>
<td>74</td>
<td>630</td>
<td>754</td>
</tr>
<tr>
<td>4-Br</td>
<td>30</td>
<td>279</td>
<td>363</td>
</tr>
<tr>
<td>2-Cl</td>
<td>2,346</td>
<td>26,500</td>
<td>25,110</td>
</tr>
<tr>
<td>3-Cl</td>
<td>595</td>
<td>5,586</td>
<td>6,079</td>
</tr>
<tr>
<td>2-,4-diCl</td>
<td>142</td>
<td>1,067</td>
<td>1,464</td>
</tr>
<tr>
<td>3-,4-diCl</td>
<td>51</td>
<td>433</td>
<td>485</td>
</tr>
<tr>
<td>3-,4-diF</td>
<td>81</td>
<td>872</td>
<td>666</td>
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<tr>
<td>4-CF$_3$</td>
<td>95</td>
<td>707</td>
<td>746</td>
</tr>
<tr>
<td>4-phenyl</td>
<td>2,883</td>
<td>37,826</td>
<td>13,916</td>
</tr>
<tr>
<td>3-,5-diF</td>
<td>690</td>
<td>5,027</td>
<td>6,073</td>
</tr>
<tr>
<td>3-,4-OCH$_3$</td>
<td>838</td>
<td>4,951</td>
<td>5,769</td>
</tr>
</tbody>
</table>

Potency vs. 4-Phenyl Substitution

### Core Exploration

- Of the 6,6- and 6,5-fused cores prepared, the pyrrolopyrimidine possessed the best potency.
- 4-F, coupled with C5-methyl core substitution yielded a compound that would be suitable for proof-of-concept studies.

<table>
<thead>
<tr>
<th>Core</th>
<th>Akt1 IC₅₀ (nM)</th>
<th>p-PRAS40 IC₅₀ (nM)</th>
<th>Caco-2 AB·10⁻⁶cm/s (BA/AB)</th>
<th>Human Hepatocyte CL mL/min/kg (ER %)</th>
<th>3A4 inhibition 10µM testosterone/midazolam</th>
</tr>
</thead>
<tbody>
<tr>
<td>46</td>
<td>8,122</td>
<td>46</td>
<td>6.7 (4.4)</td>
<td>9.0 (45%)</td>
<td>20%/78%</td>
</tr>
<tr>
<td>8,122</td>
<td>22,920</td>
<td>114</td>
<td>6.8 (3.8)</td>
<td>3.4 (17%)</td>
<td>56%/35%</td>
</tr>
<tr>
<td>22,920</td>
<td></td>
<td>2,048</td>
<td>7.0 (3.2)</td>
<td>8.0 (40%)</td>
<td>81%/60%</td>
</tr>
<tr>
<td>Akt1 IC₅₀ (nM)</td>
<td>5</td>
<td>104</td>
<td>4.7 (4.8)</td>
<td>9.0 (45%)</td>
<td>65%/60%</td>
</tr>
<tr>
<td>8</td>
<td>8</td>
<td>8</td>
<td>12.9 (1)</td>
<td>11.1 (56%)</td>
<td>69%/61%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>R-group</th>
<th>4-Cl</th>
<th>4-F</th>
<th>4-CF₃</th>
<th>4-CH₃</th>
<th>3,4-diCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Akt1 IC₅₀ nM</td>
<td>1.2</td>
<td>2.5</td>
<td>2.9</td>
<td>4.2</td>
<td>0.8</td>
</tr>
<tr>
<td>p-PRAS40 IC₅₀ nM</td>
<td>39</td>
<td>160</td>
<td>209</td>
<td>119</td>
<td>103</td>
</tr>
<tr>
<td>Caco-2 AB·10⁻⁶cm/s (BA/AB)</td>
<td>6.7 (4.4)</td>
<td>6.8 (3.8)</td>
<td>7.0 (3.2)</td>
<td>4.7 (4.8)</td>
<td>12.9 (1)</td>
</tr>
<tr>
<td>Human Hepatocyte CL mL/min/kg (ER %)</td>
<td>9.0 (45%)</td>
<td>3.4 (17%)</td>
<td>8.0 (40%)</td>
<td>9.0 (45%)</td>
<td>11.1 (56%)</td>
</tr>
<tr>
<td>3A4 inhibition 10µM testosterone/midazolam</td>
<td>20%/78%</td>
<td>56%/35%</td>
<td>81%/60%</td>
<td>65%/60%</td>
<td>69%/61%</td>
</tr>
</tbody>
</table>
Effect of Prototype Compound on p-PRAS40 in Mouse Xenograft Model

- U87MG xenograft (glioblastoma, PTEN null) in female nude mice (N=4).
- Dosed at 20 mg/kg IP.
- Concentration dependent inhibition of p-PRAS40.
  - >90% inhibition at 1h, still robust at 4h time point.
Poor Kinase Selectivity Correlates with Poor Tolerability

Analogs that weren’t tolerated exhibited a poor selectivity profile and precluded moving potent analogs into POC efficacy study.

Critical to improve overall selectivity.

PKA is closely related structurally and shows the least selectivity.
PKA Selectivity: Amino Amide

- Improving selectivity over PKA should lead to improved selectivity over more distantly related kinases as well.

- The class of amino amide (α, β, or γ) has little effect on its selectivity profile:

<table>
<thead>
<tr>
<th>AA</th>
<th>Akt1 IC&lt;sub&gt;50&lt;/sub&gt; (nM)</th>
<th>PKA IC&lt;sub&gt;50&lt;/sub&gt; (nM)</th>
<th>PKA / Akt1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.2</td>
<td>1.5</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>0.2</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>3.6</td>
<td>0.6</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>2.8</td>
<td>0.4</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>4.8</td>
<td>0.5</td>
<td>0.1</td>
</tr>
</tbody>
</table>

- Substitution of the benzyl amine also has no significant effect:

<table>
<thead>
<tr>
<th>R</th>
<th>4-F</th>
<th>4-CH₃</th>
<th>4-CF₃</th>
<th>4-OCH₃</th>
<th>4-CN</th>
<th>2,4-diCl</th>
<th>3,4-diCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Akt1 IC&lt;sub&gt;50&lt;/sub&gt; (nM)</td>
<td>2.5</td>
<td>4.2</td>
<td>2.9</td>
<td>2.1</td>
<td>1.9</td>
<td>1.2</td>
<td>0.8</td>
</tr>
<tr>
<td>PKA IC&lt;sub&gt;50&lt;/sub&gt; (nM)</td>
<td>6.2</td>
<td>2.1</td>
<td>0.6</td>
<td>0.8</td>
<td>0.5</td>
<td>0.4</td>
<td>0.2</td>
</tr>
<tr>
<td>PKA / Akt1</td>
<td>2.5</td>
<td>0.5</td>
<td>0.2</td>
<td>0.4</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
</tbody>
</table>
Akt1 vs. PKA Structure

- Narrower, less polar cavity in PKA (magenta) principally due to several differences near the hinge that include:
  - Bound water observed in Akt1 near Ala230, coordinated by the backbone and side chain of Glu228. No room for water in PKA.
  - For an aligned set of 470 human kinase sequences, only 5% have an alanine corresponding to Ala230 of Akt1, only 11% have a threonine corresponding to Thr211, and only Akt has both.
  - Hydrophilic substituents in the vicinity of Thr211 and the bound water would be expected to improve selectivity over not only PKA, but over most other kinases as well.
  - Though potency decreases with 5,6-dimethyl pyrrolopyrimidine core, selectivity improves.

<table>
<thead>
<tr>
<th>Residue</th>
<th>Akt1</th>
<th>PKA</th>
<th>ROCK1</th>
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<tr>
<td>211</td>
<td>Thr</td>
<td>Val</td>
<td>Val</td>
</tr>
<tr>
<td>230</td>
<td>Ala</td>
<td>Val</td>
<td>Met</td>
</tr>
<tr>
<td>281</td>
<td>Met</td>
<td>Leu</td>
<td>Leu</td>
</tr>
</tbody>
</table>

PKA Akt1

PKA Akt1
Substitution of methyl at C5 of the sulfide core afforded a 5X increase in Akt1 potency, relative to des methyl core.

Sulfide core retains cell potency of pyrrolopyrimidine class, and exhibits much greater selectivity profile.

- From a panel of 40 related kinases at 1 μM, the sulfide core inhibited only three kinases at greater than 50% (PKA, p70S6K and PKC\(\eta\)).

**Sulfide Core U87MG and PC3 Xenografts**

- Compound dosed QD at 25, 50, 100, and 200 mg/kg PO for 14 days in PC3 (PTEN null) prostate tumor bearing mice (N = 10).
- Dose dependent inhibition of p-PRAS40.

- Sulfide was found to be well tolerated.
- Demonstrated a dose-dependent reduction in tumor growth.
- High %F, but also high clearance.
- Would prefer higher selectivity.
Dihydrocyclopenta[d]pyrimidine Core SAR

- Examined relatively small number of substitutions around the dihydrocyclopenta ring, based on earlier X-ray structures.
  - Exquisite selectivity obtained with R² OH substitution.
  - Testing against a broad panel of 230 kinases (Upstate) found the compound only inhibited 3 kinases by >70% at 1 μM concentration (PRKG1α, PRKG1β, and p70S6K, with IC₅₀s determined to be 98, 69, and 860 nM, respectively).

<table>
<thead>
<tr>
<th>R¹</th>
<th>R²</th>
<th>Akt1 Inhibition IC₅₀, nM</th>
<th>Akt2 Inhibition IC₅₀, nM</th>
<th>Akt3 Inhibition IC₅₀, nM</th>
<th>Akt p-PRAS40 LNCaP IC₅₀, nM</th>
<th>PKA Inhibition IC₅₀, nM</th>
</tr>
</thead>
<tbody>
<tr>
<td>(R)-CH₃</td>
<td>H</td>
<td>6</td>
<td>12</td>
<td>5</td>
<td>287</td>
<td>33</td>
</tr>
<tr>
<td>dimethyl</td>
<td>H</td>
<td>&gt; 2000</td>
<td>&gt; 2000</td>
<td>&gt; 2000</td>
<td>ND</td>
<td>&gt; 10,000</td>
</tr>
<tr>
<td>(S)-vinyl</td>
<td>H</td>
<td>2</td>
<td>6</td>
<td>1</td>
<td>176</td>
<td>10</td>
</tr>
<tr>
<td>(R)-CH₂OH</td>
<td>H</td>
<td>81</td>
<td>356</td>
<td>83</td>
<td>846</td>
<td>957</td>
</tr>
<tr>
<td>(R)-CH₃</td>
<td>(R)-OH</td>
<td>5</td>
<td>18</td>
<td>8</td>
<td>157</td>
<td>3100</td>
</tr>
<tr>
<td>(R)-CH₃</td>
<td>(S)-OH</td>
<td>68</td>
<td>249</td>
<td>73</td>
<td>740</td>
<td>1552</td>
</tr>
<tr>
<td>(R)-CH₃</td>
<td>(R)-F</td>
<td>4</td>
<td>14</td>
<td>10</td>
<td>152</td>
<td>17</td>
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<tr>
<td>(R)-CH₃</td>
<td>(S)-F</td>
<td>12</td>
<td>35</td>
<td>23</td>
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<td>541</td>
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<tr>
<td>(R)-CH₃</td>
<td>diF</td>
<td>1169</td>
<td>4177</td>
<td>4160</td>
<td>8548</td>
<td>&gt; 10,000</td>
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<tr>
<td>(R)-CH₂F</td>
<td>H</td>
<td>3</td>
<td>6</td>
<td>5</td>
<td>123</td>
<td>35</td>
</tr>
</tbody>
</table>
2.0 Å X-Ray Crystal Structure of GDC-0068 Bound to Akt1

- The hydroxyl donates a hydrogen bond to the backbone carbonyl of Glu228 (O-O distance is 2.68 Å).
- Pyrimidine ring interacts via a hydrogen bond to the amide NH of Ala230 (N-N distance is 2.98 Å).
- Possible interaction with bound water adjacent to Glu228.
- The isopropyl amine side chain interacts in the carbonyl-rich region with the carboxylate side chains of Glu234 (3.03 Å) and Glu278 (2.72 Å).
- The 4-Cl phenyl group occupies a small hydrophobic pocket under the P-loop that is formed when Phe161 is displaced toward the C-helix.
<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Akt1/2/3 IC₅₀ (nM)</td>
<td>5/18/8</td>
</tr>
<tr>
<td>PKG1α/β IC₅₀ (nM)</td>
<td>98/69</td>
</tr>
<tr>
<td>p70S6K IC₅₀ (nM)</td>
<td>860</td>
</tr>
<tr>
<td>PKA IC₅₀ (nM)</td>
<td>3100</td>
</tr>
<tr>
<td>LNCaP p–PRAS40 IC₅₀ (nM)</td>
<td>157</td>
</tr>
<tr>
<td>PC3 p–PRAS40 IC₅₀ (nM)</td>
<td>197</td>
</tr>
<tr>
<td>BT474M1 p–PRAS40 IC₅₀ (nM)</td>
<td>208</td>
</tr>
<tr>
<td>Caco-2 AB ·10⁻⁶ cm/s (BA/AB)</td>
<td>7 (5)</td>
</tr>
<tr>
<td>% Plasma Protein Binding – H/C/D/R/M</td>
<td>39/44/86/55/56</td>
</tr>
<tr>
<td>CYP inhibition IC₅₀ (µM)</td>
<td>&gt; 25 µM</td>
</tr>
<tr>
<td>Hepatocyte CL H/C/R/M (mL/min/kg)</td>
<td>5/17/6/11</td>
</tr>
<tr>
<td>Solubility 1.2/6.5/7.4</td>
<td>&gt; 10 mg/mL</td>
</tr>
<tr>
<td>logD pH7.4</td>
<td>1.2</td>
</tr>
<tr>
<td>PSA (QikProp Å²)</td>
<td>80.3</td>
</tr>
<tr>
<td>MDS Pharma Lead Panel</td>
<td>No hits @ 10 µM</td>
</tr>
<tr>
<td>hERG Patch Clamp IC₅₀ (free drug)</td>
<td>23.7 µM</td>
</tr>
<tr>
<td>TDI (% loss @ 10 µM)</td>
<td>Negative</td>
</tr>
</tbody>
</table>
GDC-0068 Female Nude Mouse PK

- Dosed as aqueous solution.
- Moderate to high bioavailability at higher doses (> 10 mg/kg).
- Non linear increase in exposure from low to high dose.
  - Increase in exposure is linear at higher doses.
- Increase in %F at higher concentrations.

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>AUC∞ (μM·h)</th>
<th>Cmax (μM)</th>
<th>t½ (h)</th>
<th>%F</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>9.2</td>
<td>2.1</td>
<td>2.3</td>
<td>87</td>
</tr>
<tr>
<td>50</td>
<td>35.8</td>
<td>7.4</td>
<td>1.8</td>
<td>168</td>
</tr>
<tr>
<td>150</td>
<td>94.8</td>
<td>13.8</td>
<td>3.2</td>
<td>148</td>
</tr>
</tbody>
</table>
Tumor ratios of p-PRAS40 to total PRAS40 (tPRAS40) were determined in female nude mice bearing PC3 prostate tumor xenografts.

Samples were collected 3 h following administration of 12.5, 25, and 100 mg/kg doses (free base equivalents formulated in 0.5% methylcellulose/0.2% Tween-80). The % inhibition of p-PRAS40/tPRAS40 is based on comparison to vehicle control.
High Akt Activity Predicts Sensitivity to GDC-0068

- 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.

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

- GDC-0068 demonstrated robust antitumor activity in a range of cancer xenograft models, especially those with activation of the PI3K-Akt-mTOR pathway.
Scheduling of GDC-0068

- Dosing in MCF7-neo/HER2 tumor xenografts.
  - Breast cancer cell line PI3K mutant, Her2+, ER positive.
  - Scheduling suggests AUC is key driver of efficacy.
  - No significant weight loss.
GDC-0068 Summary

- GDC-0068 is a novel, potent, oral, small molecule ATP-competitive Akt inhibitor, with high selectivity over more than 200 screened kinases.
- It demonstrates particularly robust activity in tumors with activation of the PI3K-Akt-mTOR pathway, enabling a straightforward strategy to identify patients most likely to benefit.
- GDC-0068 treatment resulted in pronounced PD effects in tumor xenograft models including dose-dependent suppression of p-PRAS40.

- Its predictable pharmacokinetic and favorable safety profiles support the use of GDC-0068 in combination with chemotherapy and targeted anticancer therapies.
- In the clinic, GDC-0068 has been well tolerated up to 400 mg once daily, with mild, manageable hyperglycemia, and no DLTs.
- GDC-0068 has low (~ 20%) inter-patient variability for exposure and half-life (ca. 24 h) suitable for once-daily dosing.
- Rapidly absorbed with \( t_{\text{max}} \) of 0.5-2 h.
- Generally dose proportional increase in \( C_{\text{max}} \) and AUC.
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Leo Berezhkovskiy
Leanne Berry
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Yingqing Ran
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Tony Morales
Tyler Risom
Keith Spencer
Francis Sullivan
Guy Vigers
Eli Wallace
Corey Williams
Jim Winkler
Richard Woessner
Wen-I Wu
Dengming Xiao
Rui Xu
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