Targeting Src to treat antiestrogen resistant breast cancer

Joyce Slingerland

Braman Family Breast Cancer Institute
Sylvester Comprehensive Cancer Center
U of Miami Miller School of Medicine
p27- key mediator of G1 arrest

- binds and inhibits cyclin E-Cdk2
- levels fall as cells move through G1
- links extracellular growth regulators and the cell cycle
- reduced in up to 60% human cancers
**Estrogens cause loss of p27 and G1 progression**

<table>
<thead>
<tr>
<th>Time (hrs)</th>
<th>0</th>
<th>3</th>
<th>6</th>
<th>9</th>
<th>12</th>
<th>16</th>
<th>22</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>92</td>
<td>92</td>
<td>93</td>
<td>92</td>
<td>78</td>
<td>68</td>
<td>44</td>
</tr>
<tr>
<td>S</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>16</td>
<td>26</td>
<td>44</td>
</tr>
<tr>
<td>G2/M</td>
<td>5</td>
<td>6</td>
<td>5</td>
<td>5</td>
<td>6</td>
<td>6</td>
<td>12</td>
</tr>
</tbody>
</table>

![p27 blot](image.png)
p27 protein is reduced in 60% of Breast Ca

Lymph node negative breast cancer
p27 prognostic for poor DFS

- p27 > 25%
- p27 < 25%

n = 1015
P < 0.0001
# Disease Free Survival

## Node negative breast ca n=1015

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Hazard Ratio (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Decreased p27 level</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(&lt;25% vs &gt;25%)</td>
<td>1.53 (1.05, 2.23)</td>
<td>0.03</td>
</tr>
<tr>
<td><strong>Tumor grade</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(grade 2-3 vs 1)</td>
<td>3.37 (1.77, 6.39)</td>
<td>0.0002</td>
</tr>
<tr>
<td><strong>Tumor size</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;5 cm vs &lt;2 cm</td>
<td>3.01 (1.52, 5.97)</td>
<td>0.002</td>
</tr>
<tr>
<td>2-5 cm vs &lt;2 cm</td>
<td>1.78 (1.2, 2.64)</td>
<td>0.004</td>
</tr>
<tr>
<td><strong>Lymphatic invasion</strong></td>
<td>1.76 (1.17, 2.65)</td>
<td>0.007</td>
</tr>
<tr>
<td><strong>Estrogen receptor</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(negative vs positive)</td>
<td>1.13 (0.67, 1.89)</td>
<td>0.36 NS</td>
</tr>
<tr>
<td><strong>Progesterone receptor</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(negative vs positive)</td>
<td>1.12 (0.71, 1.77)</td>
<td>0.63 NS</td>
</tr>
</tbody>
</table>
p27 degradation promotes S phase entry
3 tyrosines in p27 interact with Cdk2

cSrc phosphorylates p27 in vitro
Src induction increases cellular pY74 p27
Effect of pY88 on binding of p27Kip1 to Cdk2

Y88 interacts with hydrophobic aa V64, F80, F82, L83 and L134

pY88 ejected from hydrophobic pocket due to unfavorable thermodynamic interactions – destabilizes p27-Cdk2
pY-p27 has reduced inhibitory activity toward cyclin E-Cdk2
Effect of pY74 on binding of p27 to Cdk2

Y74 forms hydrophobic interactions with aa L25, V30, L67 and V79

In pY74 hydrophobic interactions lost: destabilizes Cdk2-p27
pY-p27 has reduced binding to Cyclin E-Cdk2 in vitro

Src also reduces p27-bound Cyclin E-Cdk2 in cells
Src siRNA or inhibitors increase p27

<table>
<thead>
<tr>
<th>siRNA</th>
<th>AZD0530</th>
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<tbody>
<tr>
<td>NS</td>
<td>-</td>
</tr>
<tr>
<td>cSrc</td>
<td>-</td>
</tr>
<tr>
<td>cSrc</td>
<td>+</td>
</tr>
<tr>
<td>p27</td>
<td></td>
</tr>
<tr>
<td>p27</td>
<td></td>
</tr>
<tr>
<td>β-actin</td>
<td></td>
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<tr>
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</table>

Chu Cell 2007
**Src inhibition increases p27 t1/2**

Hrs after CHX  | 0  | 0.5 | 1  | 2  | 4  | 8  
---|---|---|---|---|---|---
p27 | control | control | p27 | control | control | p27 |

**Src induction decreases p27 t1/2**

![Graph showing p27 half life](#)
p27: inhibitor & substrate of Cyclin E-Cdk2
Phosphorylation by Src promotes p27 degradation

- Src phosphorylates p27
- Src-phosphorylated p27 is a poor Cdk2 inhibitor
- pYp27 has reduced binding to cyclin E-Cdk2
- Src induction increases p27pT187
- Src siRNA or inhibitors increase p27 levels
- Src induction decreased p27 t1/2

Does Src promote p27 loss in cancers?
Activated Src correlates with low p27 in breast cancers

39% of all cancers and 37% of ER+ cancers show Src activation

n=482
p=0.02
Chu Cell 2007
Antiestrogen Resistance in ER+ Breast Cancer

- Estrogens activate mammary cell proliferation
- 2/3 new breast cancers express ER protein
- >15 million women worldwide on tamoxifen or aromatase inhibitors

PROBLEM: RESISTANCE
p27 is required for antiestrogen mediated G1 arrest

- Estrogen stimulates cell cycle by decreasing p27
- Tam and fulvestrant cause G1 arrest by increasing p27-cyclin E-cdk2
- Antisense p27 abrogates Tam arrest

Cariou et al PNAS 2000
Since p27 is required for G1 arrest by tamoxifen and since Src activates p27 loss...... does Src mediate antiestrogen resistance?
**Src inhibitor restores G1 arrest by Tamoxifen**

![Graph showing the effect of different treatments on MDA-MB-361 cells.](chub-cell-2007.png)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>C</th>
<th>TAM</th>
<th>AZD</th>
<th>TAM</th>
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<tbody>
<tr>
<td>AZD0530</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>TAM</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cyclin E</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Cdk 2</td>
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<td></td>
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</tr>
<tr>
<td>p27</td>
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**IP Cyclin E**

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<tr>
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<td>p27</td>
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Chu Cell 2007
Anastrozole

- nonsteroidal aromatase inhibitor used for ER/PR + breast cancer
- blocks conversion of androstenedione to estrogen
- reduces estrogen by >90% in postmenopausal women
AZD0530 and anastrozole cause G1 arrest
### Drug effects on signaling kinases

<table>
<thead>
<tr>
<th></th>
<th>+ Androstendione</th>
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<tbody>
<tr>
<td></td>
<td>no drug</td>
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<tr>
<td>p27</td>
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<tr>
<td>pSrc</td>
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<tr>
<td>Src</td>
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<tr>
<td>pMAPK</td>
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<tr>
<td>MAPK</td>
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</table>
AI & AZD0530 increase p27 binding to cyclin E-Cdk2
Evidence for synergy between AZD0530 and anastrozole
Molecular markers of tumor response

**p27**
- No drug
- Both drugs

**% nuclei (+)**

- Andro
- SI
- Ana
- Ana+SI

**Ki 67**
- No drug
- Both drugs

**% nuclei (+)**

- Andro
- SI
- Ana
- Ana+SI
Drug effects on Src:
- no inhibition in resistant tumors
- anastrozole alone activates Src
- combination inhibits Src
Proteomic data

Resistance mechanism:

MEK pathway activation
Resistance mechanism: PI3K pathway activation
Rationale for AZD0530/anastrozole trials

- p27 mediates G1 arrest by AIs
- Src phosphorylates p27 to promote p27 loss
- AZD0530 cooperates with anastrozole in xenograft tumors
- p27 increase and Ki67 loss may predict response
- Anastrozole alone stimulates Src
- Rapid emergence of resistance to AZD0530 not seen with combination
- AZD0530 resistant tumors had MEK and PI3K activation
**Src inhibitor plus AI clinical trial**

- **Phase I trial:** drug combination in metastatic disease

- **Phase II trial:** AZD0530/anastrozole for postmenopausal LABC with pre-post biopsies:
  - Tumor size and vascularity by MRI
  - IHC of p27 pSrc pMAPK pAkt Ki67
  - RPPA Proteomic profiles
  - Src activation genomic profile
  - Mammosphere and TIC assays
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Angel Arnaout (U Toronto)
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NCI R01  Avon/AACR
BCRF
Doris Duke Foundation
Evidence for synergy between anastrozole and AZD0530

<table>
<thead>
<tr>
<th>FTV</th>
<th>FTV</th>
<th>Combination</th>
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</thead>
<tbody>
<tr>
<td>Anastrozole</td>
<td>AZD0530</td>
<td>Expected FTV&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.66</td>
<td>1.07</td>
<td>0.71</td>
</tr>
</tbody>
</table>

<sup>a</sup> FTV, fractional tumor volume (mean final tumor volume experimental)/(mean final tumor volume control).

<sup>b</sup> Expected FTV = (mean FTV of Anastrozole)× (mean FTV of AZD0530)

<sup>c</sup> Observed FTV = final tumor vol combined therapy/final tumor vol androstendione alone

<sup>d</sup> Combination Ratio = Expected FTV/ Observed FTV. A ratio of > 1 indicates synergy
In vitro synergy between Src Inhibitor and anastrozole

![Graph showing in vitro synergy between Src Inhibitor and anastrozole.](image)

- **Anastrozole**
- **Anastrozole + AZD0530**
Crystal structure of cyclin A-Cdk2-p27

Loss of p27 abrogates G1 arrest by Tamoxifen

Control (lipid only) cells remain arrested

Loss of p27 in the ASp27 transfectants leads to S phase entry

Control (MSMp27) cells remain arrested

Cariou et al PNAS 2000