Targeting the PI3K/AKT/mTOR pathway: Pre-clinical data II

Biomarkers in the pre-clinical setting

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Somatic Genomic Aberrations:

HER2 amplification
EGFR amplification/mutation/vIII
PIK3CA amplification/mutation
PTEN LOH
AKT amplification and mutations
p70S6K amplification

Hennessy BT et al *Nature Rev Drug Discovery* 2005
<table>
<thead>
<tr>
<th>Gene</th>
<th>No. of samples</th>
<th>Percentage</th>
<th>Gene</th>
<th>No. of samples</th>
<th>Percentage</th>
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<tr>
<td>P53</td>
<td>152</td>
<td>23.3</td>
<td>EGFR</td>
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<td>PTEN</td>
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<td>MYC</td>
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<td>MET</td>
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<td>TBX3</td>
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<td>2.6</td>
<td>PIK3R1</td>
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<td>0.3</td>
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<td>MAP3K13</td>
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<td>0.3</td>
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<tr>
<td>GNAQ</td>
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<td>NCOR1</td>
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<td>0.2</td>
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<tr>
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<td>1.7</td>
<td>FGFR3</td>
<td>1</td>
<td>0.2</td>
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<tr>
<td>APC</td>
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<td>RB1</td>
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</table>

Sinead Toomey, Aoife Carr, Yasir Elamin, John Crown
PIK3CA mutation frequency in different solid tumours

Sinead Toomey, Aoife Carr
Table. PI3K pathway Genetic Mutations and Other Aberrations in 1261 Breast, 332 Ovarian, 246 Endometrial Cancers (total 1839 cancers analyzed).

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>PIK3CA</th>
<th>PIK3CA</th>
<th>PIK3CB</th>
<th>AKT1</th>
<th>AKT1/2</th>
<th>PTEN</th>
<th>PTEN</th>
<th>INPP4B</th>
<th>PDK1</th>
<th>p70S6</th>
<th>RAS/RAF</th>
<th>HER2</th>
<th>p53</th>
<th>BRCA1</th>
<th>BRCA2</th>
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<tbody>
<tr>
<td></td>
<td>Mutations</td>
<td>Amplification</td>
<td>Amplification</td>
<td>Mutation</td>
<td>Amplification</td>
<td>Mutation</td>
<td>Protein loss</td>
<td>deletion</td>
<td>Amplification</td>
<td>Amplification</td>
<td>Mutation</td>
<td>Amplification</td>
<td>Mutation</td>
<td>Mutation</td>
<td>Mutation</td>
</tr>
<tr>
<td>Breast Total</td>
<td>339/1261 (26.9%)</td>
<td>5%</td>
<td>5%</td>
<td>27/1008 (2.6%)</td>
<td>5%</td>
<td>6/209 (2.3%)</td>
<td>25/110 (22.7%)</td>
<td>~20%</td>
<td>27/129 (20.9%)</td>
<td>30%</td>
<td>2/406</td>
<td>15%</td>
<td>46/121</td>
<td>8%</td>
<td>2%</td>
</tr>
<tr>
<td>Breast HR+</td>
<td>101/305 (33.1%)</td>
<td>6/232 (2.6%)</td>
<td>4/131 (3.4%)</td>
<td>10/69 (14.5%)</td>
<td>rare</td>
<td>16/79 (23.2%)</td>
<td>0</td>
<td>18/73 (24.6%)</td>
<td>0</td>
<td>100%</td>
<td>14/23 (60.9%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast HER2+</td>
<td>24/98 (24.5%)</td>
<td>0/75</td>
<td>0/33</td>
<td>2/18 (11%)</td>
<td>rare</td>
<td>5/19 (26.3%)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>Breast TN</td>
<td>21/262 (8.0%)</td>
<td>0/111</td>
<td>2/41 (4.9%)</td>
<td>11/21 (52%)</td>
<td>60%</td>
<td>2/15 (13.3%)</td>
<td>0</td>
<td>14/22 (63.6%)</td>
<td>0</td>
<td>100%</td>
<td>14/23 (60.9%)</td>
<td></td>
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<tr>
<td>Ovarian</td>
<td>2/332 (0.6%)</td>
<td>45%</td>
<td>5%</td>
<td>2/332 (0.6%)</td>
<td>5%</td>
<td>4/132 (3%)</td>
<td>40%</td>
<td>~20%</td>
<td>rare</td>
<td>rare</td>
<td>12/428 (2.8%)</td>
<td>8%</td>
<td>90/132 (68%)</td>
<td>31/235 (13.2%)</td>
<td>12/178 (6.7%)</td>
</tr>
<tr>
<td>Endometrial*</td>
<td>73/246 (30%)</td>
<td>low</td>
<td>low</td>
<td>3/150 (2.0%)</td>
<td>low</td>
<td>20/76 (26%)</td>
<td>&gt;50%</td>
<td>8.00%</td>
<td>rate</td>
<td>rare</td>
<td>44/206 (21%)</td>
<td>rare</td>
<td>9/96 (9%)</td>
<td>1/199 (0.5%)</td>
<td>2/199 (1%)</td>
</tr>
</tbody>
</table>

Events that occur in 20% or more of patients are shown in bold.

3. Hennessy BT,......Mills GB. *Cancer Research* 2009
4. Gonzalez AM...Hennessy BT. *CCR* 2009
The frequency of activating PI3K pathway genetic aberrations in cancer suggests that the pathway will be a useful target in many human tumors.
Defining the functional effects of PI3K pathway mutations and other aberrations in cancer:

What specific isoforms and downstream effectors mediate effects?
PTEN mutations
Figure 1 | Targets in the signalling network and their role in tumour biology. This diagram shows a highly simplified scheme of the signalling pathway leading from phosphoinositide 3-kinase (PI3K), to AKT, to mammalian target of rapamycin (mTOR). The four isoforms of class I PI3K are shown in orange boxes. The cancer-cell-intrinsic functions of the isoforms are illustrated above: the PI3K catalytic isoform p110α (encoded by PIK3CA) is a frequent genetic driver (PIK3CA mutations); basal activity of p110β is implicated in tumours with loss of phosphatase and tensin homolog (PTEN); and p110δ has a fundamental role in the survival of normal B cells and is implicated in malignancies of this lineage. PI3K and mTOR drive tumour metastasis by promoting cell motility and epithelial–mesenchymal transition (EMT). The bold arrows represent cell-extrinsic functions of various components in the network. p110α drives angiogenesis; p110γ, p110δ and p110β have important functions in inflammatory cells; and p110δ and mTOR control key aspects of adaptive immunity, including lymphocyte activation, differentiation and tolerance. Drugs in clinical development that target the nodes in this network are listed in Supplementary information S1 (table).
Structure of the p110 alpha subunit of PI3K (PIK3CA)

Huang CH et al. Science 2007
PIK3CA mutations do not impact HR-positive breast cancer patient outcomes after treatment with adjuvant tamoxifen.

Hale Hennessy BT. Cancer Research 2008
In contrast to PTEN loss, PIK3CA mutations do not lead to consistent activation of AKT in HR-positive breast cancers or cell lines.
implicate a novel AKT-independent and PDK1-dependent oncogenic signal that may help us to refine new targeted therapeutic opportunities in tumors defined by PIK3CA mutations

PIK3CA mutations: Potential substrates:

Overlap between:

- synthetic lethality candidates identified in a shRNA screen in a PIK3CA mutant cell line
- known PDK1 substrates

PIK3CA mutations

PTEN mutations

NO ONE SIZE FITS ALL

Hennessy et al. Nature Reviews Drug Discovery 2005
PI3K PATHWAY DRUGS IN DEVELOPMENT

- mTOR
- FRAP
- p70S6
- 4eBP
- p70S6
- 4eBP
- PI3K
- PDK1
- AKT1,2,3
- BCR/Ab1
- SRC
- P85
- P110
- ILK
- INTEGRINS
- av
- b3
- FTI
- STI571
- C225
- Herceptin
- Iressa
- STI571
- C225
- Semaphore, Genentech, Lilly, Echelon, Iconix, Icos, Baxter, Cancer Research UK
- Piramed, Wyeth, Novartis
- Rapamycin
- Wyeth CCI 779
- Novartis Rad OO1
- Ariad AP23573
- Vertex, QLT, Celgene, Bioimage
- BMS, Novartis, Pfizer, Wyeth
- Aria, Astrazeneca, BMS, Novartis, Pfizer, Wyeth
- ICOS
- Berlex Vertex
- Rapamycin
- Wyeth CCI 779
- Novartis Rad OO1
- Ariad AP23573
- Vertex, QLT, Celgene, Bioimage
- BMS, Novartis, Pfizer, Wyeth
Cell line sensitivity to PI3K pathway inhibition

A

B

C

PD173074
CGC-11047
Cisplatin
Docetaxel
Etoposide
VX-680
GSK1070916
Erlotinib
AG1478
Gefitinib
BIBW2992
Lapatinib
17-AAG
PI3K
mTOR
FIDACs
Vorinostat
Trichostatin-A
Fascaplysin

Heiser LM et al. PNAS 2009
PIK3CA-mutated cancers

- p110 alpha-specific inhibitors demonstrate particular preclinical antitumour activity
  - including tumor growth arrest and significant regression in xenograft models

Olivero AG; Genentech team. AACR 2008 (GDC0032)
Liu N et al. Mol Cancer Ther 2013 (BAY80-6946)
AKT phosphorylation by PI3Kα compared with PI3Kβ in cells. BAY 80-6946 showed superior antitumor activity (>40-fold) in PIK3CA mutant and/or HER2 overexpression as compared with HER2-negative and wild-type PIK3CA breast cancer cell lines. In addition, BAY 80-6946 revealed potent activity to induce apoptosis in a subset of tumor cells with aberrant activation of PI3K as a single agent. In vivo, single intravenous
PIK3CA-mutated cancers

- p110 alpha-specific inhibitors demonstrate particular preclinical antitumour activity
  - including tumor growth arrest and significant regression in xenograft models
    Olivero AG; Genentech team. AACR 2008 (GDC0032)
    Liu N et al. Mol Cancer Ther 2013 (BAY80-6946)

- However, persistent mTORC1 signaling a/w preclinical resistance to p110 alpha inhibitors
  Elkabets M et al. Sci Transl Med 2013
PTEN-deficient cancers

- Some studies suggest that p110 beta activity is essential in cancer cells lacking PTEN (particularly in prostate and breast cancers)

- Thus, p110 beta inhibitors may be more effective than p110 alpha inhibitors in PTEN-deficient tumours

- However, this may be tumor type- and context-dependent

  Wee S et al. PNAS 2008

  Berenjeno IM et al. Biochem J 2012
Role for PI3K pathway aberrations in treatment resistance:

For example, PIK3CA mutations and PTEN loss are associated with trastuzumab resistance in HER2-amplified breast cancer:

Nagata, Y et al. Cancer Cell 2004

Berns (1st), Horlings (1st), Hennessy et al. Cancer Cell 2007
We are also working on novel methods for identification of PD biomarkers indicating benefit from PI3K pathway inhibitors

- **PD biomarkers:** quantitative protein assays such as high throughput reverse phase protein arrays (RPPA) can objectively and reproducibly quantitate the activity of several proteins and pathways simultaneously in tumors

RPPA: Pharmacodynamic biomarkers for antitumor efficacy of perifosine, a PH domain inhibitor of AKT

In summary

- PI3K pathway aberrations are very common in solid cancers

- PI3K pathway aberrations are associated with targeted and cytotoxic therapy resistance

- PI3K pathway inhibitors have potential clinical utility but the optimal inhibitors and combinations in different settings still requires clear definition

- Careful attention to clinical trial design and correlative studies required to define those PI3K pathway inhibitors that result in optimal pathway modulation in human tumors along with the specific biomarkers a/w benefit from treatment
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