PI3K/Akt/mTOR pathway

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Outline of the presentation

- Schematic representation of the pathway
- Structure of PI3K encoding genes
- Regulation of different PI3K isoforms
- Akt genes and regulation
- mTOR and downstream signalling
- Preclinical evidence of activity
- Drugs in development
- Clinical evidence
- Future direction
The PI3K/Akt/mTOR pathway

PI3Ks

PTEN

phosphatidyl-inositol
The PI3K/Akt/mTOR pathway

- **6SK1**
  - Phosphorylation at T389

- mTOR
  - Phosphorylation T37, T46, S65, T70
  - Formation of eIF4F complex
  - Translation of mRNAs involved in cell cycle control: cycD c-myc

- **4EBP1**
  - Phosphorylation
  - Members of translational machinery
  - Translation of RNA with 5' oligopyrimidin (TOP)

- **eIF4E**
  - Translation of mRNA involved in cell cycle control: cycD c-myc
Structure of different PI3K isoforms

## PI3K Complexity

<table>
<thead>
<tr>
<th>Class</th>
<th>Catalytic Subunit</th>
<th>Regulatory Subunit</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CLASS I</strong></td>
<td>p110a, PIK3CA</td>
<td>p85a, PIK3R1</td>
</tr>
<tr>
<td></td>
<td>p110b, PIK3CB</td>
<td>p85b, PIK3R2</td>
</tr>
<tr>
<td></td>
<td>p110d, PIK3CD</td>
<td>p87, PIK3R6</td>
</tr>
<tr>
<td></td>
<td>p110g, PIK3CG</td>
<td>p101, PIK3R5</td>
</tr>
<tr>
<td><strong>CLASS II</strong></td>
<td>PI3K-C2a, PIK3C2A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PI3K-C2b, PIK3C2B</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PI3K-C2g, PIK3C2G</td>
<td></td>
</tr>
<tr>
<td><strong>CLASS III</strong></td>
<td>VPS34, PIK3C3</td>
<td>VPS15</td>
</tr>
</tbody>
</table>
The PI3K/Akt pathway

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Fraction mutated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colon</td>
<td>74/234 (32%)</td>
</tr>
<tr>
<td>Brain</td>
<td>4/15 (27%)</td>
</tr>
<tr>
<td>Gastric</td>
<td>3/12 (25%)</td>
</tr>
<tr>
<td>Breast</td>
<td>1/12 (8%)</td>
</tr>
<tr>
<td>Lung</td>
<td>1/24 (4%)</td>
</tr>
</tbody>
</table>

From Samuels Y et al. Science 2004;304(5670):554-554. Reprinted with permission from AAAS
The PI3K/Akt pathway

- PI3Ks as monomers are rapidly degraded
- Binding of the catalytic subunit to the regulatory subunit stabilizes PI3Ks
- At the same time the p85 binding inactivates PI3Ks
The PI3K/Akt pathway

The PI3K/Akt pathway: Signals activating the pathway
The PI3K/Akt pathway:
Signals downstream the pathway - Akt

AKT1
AKT2
AKT3

T308 S473
T309 S474
T305 S472

Chr
14q32
19q13
1q44

EXPR
Ubiquitous
Insulin sens
Brain testis

RXRXX[S/T]B

More than 50 substrates
The PI3K/Akt pathway:
Signals downstream the pathway

Redrawn from www.bio1000.com/zt/celebrity/3626.html
The PI3K/Akt pathway: Signals downstream the pathway

Akt and cell growth

AKT

Phosphorylates TSC2

Phosphorylation at S2448

RHEB

mTOR
The PI3K/Akt pathway: Signals downstream the pathway

Akt and survival

Akt and survival

AKT → AKT-p

mdm2 → mdm2

p53

mdm2

FOXO

SEQ BY 14-3-3 PROTEINS

FOXO

BAD

SEQ BY 14-3-3 PROTEINS

BAD

CAS9

DECREASED PROTEOLYTIC CLEAVAGE

mdm2

p53

PUMA

NOXA

BAX

BIM

FasL

CAS9

P

P

P

The PI3K/Akt pathway: Signals downstream the pathway

Akt and cell proliferation

Diagram showing the interaction between various proteins and pathways:
- AKT
- GSK3
- CYC D
- cMYC
- mdm2
- m53
- p27
- p21
- Stability
Akt-independent PI3K signaling

- Evidence that an alternative PI3K-dependent, Akt independent pathway contributes to malignant transformation

- While almost invariably PTEN null cancer cells present increased phospho Akt levels, some cancer cell lines with activating mutations in PIK3CA gene have low levels of activated Akt
The PI3K/Akt pathway: Signals downstream the pathway

Akt-independent PI3K signaling

- SGK protein isoforms are candidates downstream effectors for PI3K signaling independent of Akt
- SGK3, in particular, shares homology with Akt both at molecular level:
The PI3K/Akt pathway: Signals downstream the pathway

Akt-independent PI3K signaling

- And at downstream activation level:

mTOR activation

AKT

rictor

mTOR

Rapa insensitive

TorC 1

Rapamycin

TorC 2
Downstream mTOR

**6SK1**
- Phosphorylation in T389

**mTOR**
- Phosphorylation in T37, T46, S65, T70

**4EBP1**
- Translation of mRNA involved in cell cycle control

**eIF4E**
- eIF4F complex formation

**Components of translational apparatus**
- Translation of RNA with 5’ top
The PI3K/Akt pathway: Alterations in tumours

- PI3-kinase
- AKT-p
- PTEN
- Amplified/mutated
- Deleted
- SGK3
- BAD

AKT
- Mutated
- Amplified/mutated

- TSC 1/2
- RHEB
- mTOR
- mdm2
- p53
- Mutated
- Overexpr
- p27

mTOR
- 6SK, 4EBP1
- Amplified/overexpr
The PI3K/Akt pathway

- Tumours for which there is evidence of a prognostic role of PI3K/Akt/mTOR alterations

### pNET tumours

<table>
<thead>
<tr>
<th>Mutations in genes belonging to the pathway</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Akt activation</td>
<td>60</td>
</tr>
<tr>
<td>mTOR overexpression</td>
<td>65</td>
</tr>
<tr>
<td>PTEN and TSC2 alterations</td>
<td>85</td>
</tr>
</tbody>
</table>

Evidence that alterations in the pathways can associate with PFS and/or OS of patients with pNET

### RCC

- Rare mutations in PI3K genes and pathway components
- Loss of function of PTEN
- Loss of function of VHL (regulator of hypoxia response - HIF1alpha under control by mTOR)
The PI3K/Akt pathway

- Tumours for which there is evidence of a prognostic role of PI3K/Akt/mTOR alterations

**Breast cancer**

PI3K/akt/mTOR pathway has been implicated in endocrine and trastuzumab (in HER-2 overexp) resistance

**β-cell malignancies**

PI3Kδ isoform has a critical role in β-cells while PI3Kα gene and PTEN are rarely altered. Patients with CLL, MM, HL
The PI3K/Akt pathway: Inhibitors available

- ATP-competitive, allosteric, specific, selective
- Dual PI3K, mTOR inhibitors
- Rapalogues, dual TORC1 and TORC2 inhibitor (catalytic site)

Translation of genes involved in cell growth and survival
### PI3K/Akt/mTOR inhibitors in early phase (I/II) clinical studies

<table>
<thead>
<tr>
<th>Class</th>
<th>Example Inhibitors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pan PI3K inhibitors</td>
<td>XL147 (I), GDC0980 (II), WX037 (I), BGT226 (I/II), GSK1059615 (I)</td>
</tr>
<tr>
<td>PI3K SELECTIVE a</td>
<td>BYL719 (II), MLN1117 (I)</td>
</tr>
<tr>
<td>PI3K SELECTIVE d</td>
<td>TGR 1202 (I)</td>
</tr>
<tr>
<td>PI3K SELECTIVE d,g</td>
<td>IPI145 (II)</td>
</tr>
<tr>
<td>PI3K SELECTIVE a,d</td>
<td>BAY80-6946 (II)</td>
</tr>
<tr>
<td>PI3K SELECTIVE a,d,g</td>
<td>GDC0032 (II)</td>
</tr>
<tr>
<td>DUAL PI3K/mTOR</td>
<td>BEZ235 (I/II), DS-7423 (I), XL765 (I)</td>
</tr>
<tr>
<td></td>
<td>PF-05212384 (I/II), GSK2126458 (I), VS5584 (I)</td>
</tr>
<tr>
<td>AKT INHIBITORS</td>
<td>GSK2141795 (II), MK2206 (II), BAY1125976 (I)</td>
</tr>
<tr>
<td></td>
<td>GDC0068 (I/II), AZD 5363 (I/II), GSK690693 (I)</td>
</tr>
<tr>
<td></td>
<td>GSK2110183 (I/II), LY2780301 (I)</td>
</tr>
<tr>
<td>mTOR INHIBITORS</td>
<td>MLN 0128 (I/II), OSI 027 (II), INK128 (I/II), AZD 8055 (I)</td>
</tr>
</tbody>
</table>
# PI3K/Akt/mTOR inhibitors in advanced clinical studies

<table>
<thead>
<tr>
<th>Approved</th>
<th>Phase III</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAN PI3K INHIBITORS</td>
<td>BKM120 HR+, HER2-, Locally Advanced or Metastatic Breast Cancer</td>
</tr>
<tr>
<td>PI3K SELECTIVE</td>
<td>GS1101 relapsed CLL, follicular B-cell NHL and small lymphocytic lymphoma</td>
</tr>
<tr>
<td>d</td>
<td>GS1101 in CLL, iNHL</td>
</tr>
<tr>
<td>PI3K SELECTIVE</td>
<td>IPI145, CLL SLL</td>
</tr>
<tr>
<td>a</td>
<td></td>
</tr>
<tr>
<td>d,g</td>
<td></td>
</tr>
<tr>
<td>PI3K SELECTIVE</td>
<td></td>
</tr>
<tr>
<td>a,d</td>
<td></td>
</tr>
<tr>
<td>PI3K SELECTIVE</td>
<td></td>
</tr>
<tr>
<td>a,d,g</td>
<td></td>
</tr>
<tr>
<td>DUAL PI3K/mTOR</td>
<td></td>
</tr>
<tr>
<td>AKT INHIBITORS</td>
<td>PERIFOSINE MM, CRC</td>
</tr>
<tr>
<td>mTOR INHIBITORS</td>
<td>EVEROLIMUS hormone receptor+, HER2- breast cancer, RCC, PNET</td>
</tr>
<tr>
<td>TEMSIROLIMUS RCC</td>
<td>RIDAFAFOROLIMUS metastatic sarcoma</td>
</tr>
</tbody>
</table>
PI3K/Akt/mTOR inhibitors: Preclinical evidence – sensitivity predictors

Activity of PI3Ka specific inhibitor BYL719 against 474 human cancer cell lines

Reprinted from Fritsch C et al. Mol Cancer Ther 2014;13(5):1117-1129, with permission from AACR
PI3K/Akt/mTOR inhibitors: Preclinical evidence – sensitivity predictors

Activity of pan PI3K inhibitor GDC-0941 against human NSCLC cell lines

PI3K/Akt/mTOR inhibitors: Preclinical evidence – sensitivity predictors

Activity of the pan PI3K inhibitor GDC-0941 and of the dual PI3K/mTOR inhibitor GDC-0980 against human NSCLC cell lines

PI3K/Akt/mTOR inhibitors: Clinical evidence

- **Clinical evidence of activity**
  - Phase III RADIANT-3 study: Everolimus in pNET
    - Randomised trial everolimus vs. placebo
    - Everolimus doubled PFS with low toxicity

- **Clinical evidence of activity**
  - Phase III RECORD-1 study: Everolimus in RCC
    - Randomised trial everolimus vs. placebo
    - Everolimus doubled PFS (4 vs. 1.9 mo)

- **Clinical evidence of activity**
  - Phase III study: Temsirolimus in RCC
    - Randomised trial everolimus vs. interferon
    - Everolimus increased OS (10.9 vs. 7.3 mo)

- **Clinical evidence of activity**
  - Phase III study: GS101 in CLL
    - Randomised trial GS101 + rituximab vs. placebo + rituximab
    - GS101 significantly improved RR and OS
PI3K/Akt/mTOR inhibitors: Clinical evidence

- Clinical activity of everolimus in ER+ metBC phase III - BOLERO-2
  - Randomised trial Eve plus exemestane vs. placebo + exemestane
  - Increased PFS in hormone refractory pts

- Clinical activity of everolimus in HER+, trastuzumab resistant BC phase III - BOLERO-3
  - Randomised trial Eve plus trastuzumab and vrb vs. placebo + trastuzumab/vrb
  - Increased PFS by Eve (7 vs. 5.8 mo)
  - Increased AE

- Clinical activity of BEZ235 IN BC, RCC, pNET
  - Lack of significant activity either alone or in combination in several trials – lack of acceptable safety profile - discontinued
## PI3K/Akt/mTOR inhibitors in clinical studies: Dose limiting toxicities

<table>
<thead>
<tr>
<th>Target</th>
<th>Agents</th>
<th>DLTs</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI3K-mTOR</td>
<td>GSK2126458</td>
<td>Diarrhea</td>
</tr>
<tr>
<td></td>
<td>GDC-0980</td>
<td>Rash, ↑BS</td>
</tr>
<tr>
<td></td>
<td>XL765</td>
<td>↑LFTs, N/V, rash, fatigue</td>
</tr>
<tr>
<td></td>
<td>BEZ235</td>
<td>Mucositis, fatigue</td>
</tr>
<tr>
<td></td>
<td>PF-04691502</td>
<td>Fatigue, rash</td>
</tr>
<tr>
<td></td>
<td>PF-05212384</td>
<td>↑LFTs, ↑BS, rash, mucositis</td>
</tr>
<tr>
<td></td>
<td>SF1126</td>
<td>Diarrhea</td>
</tr>
<tr>
<td>pan-PI3K</td>
<td>GDC-0941</td>
<td>Rash, ↑BS, Twi, ↓PLT</td>
</tr>
<tr>
<td></td>
<td>BKM120</td>
<td>Mood alteration, rash, ↑BS</td>
</tr>
<tr>
<td></td>
<td>XL147</td>
<td>Rash, hypersensitivity</td>
</tr>
<tr>
<td></td>
<td>BAY80-6946</td>
<td>↑BS, liver/renal failure</td>
</tr>
<tr>
<td></td>
<td>PX-866</td>
<td>↑LFTs, diarrhea</td>
</tr>
<tr>
<td>Isoform-specific/sparing</td>
<td>BYL719 (α-specific)</td>
<td>↑BS, nausea, vomiting</td>
</tr>
<tr>
<td></td>
<td>GDC-0032 (β-sparing)</td>
<td>↑BS, fatigue, renal failure</td>
</tr>
<tr>
<td></td>
<td>GS-1101 (δ-specific)</td>
<td>↑LFTs</td>
</tr>
</tbody>
</table>

DLT = dose limiting toxicity; BS = blood sugar; N/V = nausea/vomiting; Twi = T-wave inversion; LFTs = transaminases; PLT = platelet

Bedard P. ESMO Signaling Pathways in Cancer 2014
## PI3K/Akt/mTOR inhibitors in clinical studies: Dose limiting toxicities

<table>
<thead>
<tr>
<th>Target</th>
<th>Agents</th>
<th>DLT</th>
</tr>
</thead>
<tbody>
<tr>
<td>mTOR</td>
<td>Temsirolimus</td>
<td>Stomatitis, mood, fatigue, ↓PLT</td>
</tr>
<tr>
<td></td>
<td>Everolimus</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ridaforolimus</td>
<td>Stomatitis, ↑BS, ↓PMN</td>
</tr>
<tr>
<td></td>
<td>MLN0128</td>
<td>Mucositis</td>
</tr>
<tr>
<td></td>
<td>CC-223</td>
<td>↑BS, rash, anemia</td>
</tr>
<tr>
<td></td>
<td>AZD2014</td>
<td>↑BS, rash, mucositis, fatigue</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mucositis, fatigue</td>
</tr>
<tr>
<td>AKT</td>
<td>MK2206</td>
<td>Stomatitis, rash</td>
</tr>
<tr>
<td></td>
<td>GDC-0068</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GSK2141795</td>
<td>Fatigue</td>
</tr>
<tr>
<td></td>
<td>AZD5363</td>
<td>↑BS, ↓BS, stomatitis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>↑BS, rash, diarrhea, hypoxemia</td>
</tr>
</tbody>
</table>

DLT = dose limiting toxicity; BS = blood sugar; PMN = neutrophil; PLT = platelet

Bedard P. ESMO Signaling Pathways in Cancer 2014
PI3K/Akt/mTOR inhibitors

- PI3K pathway aberrations are very frequent events in solid tumours. The most common mutations affect PIK3CA gene. Some clinical evidence suggesting that the presence of mutations can be associated with response to inhibiting agents.

- Preclinical studies demonstrated that PI3K pathway inhibitors have a strong activity against cell lines harbouring mutations in genes belonging to the pathway.

- PI3K pathway inhibitors are being tested in the clinical setting but the results seem to be below expectation.

- Further studies are mandatory to optimise their use:
  - To find optimal dose able to inhibit the pathway
  - To reduce toxicity
  - To define patient population likely to respond
  - To find optimal combinations due to the low activity of single agents.
PI3K/Akt/mTOR inhibitors: Challenges

- Define in which setting selective or pan inhibitors of the pathway have a better chance to achieve a response
- Define specific biomarkers to select patients for specific inhibitors
- Define which level of the pathway is better to target (PI3K, AKT, mTOR)
- Explore new PI3K inhibitors directed against class II genes
- Optimise the use of PI3K mTOR dual inhibitors
- Find new combinations that increase the activity but not toxicity (synthetic lethality)
- Identify mutant specific inhibitors
THANK YOU!