Phospho-AKT: a potential resistance marker to chemotherapy and therapy-target to restore sensitivity in pancreatic cancer

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Pancreatic Cancer “Actionable Genome”

- NOTCH1 Dependent (3%)
- MET Dependent (3%)
- MYC Dependent (4%)
- JAK1/2 Dependent (2%)
- mTOR Mutant (2%)
- BRAF Mutant (1%)
- ROS1 Mutant (1%)
- NTRK Dependent (6%)
- c-KIT Mutant (1%)
- PDGFR Mutant (1%)
- FGFR Dependent (6%)
- AKT Dependent (9%)
- PI3K Dependent (6%)
- RNF43 Mutant (7%)
- DDR Defective (24%)
- BRCA1/BRCA2/PALB2 mutations
- Unstable genome
- BRCA mutational signature

- LY3039478
- LY2875358
- INC280
- Crizotinib
- GSK525762
- MET Dependent
- RXDX-101
- LOXO-101
- Dovitinib
- Dovitinib
- BGJ398
- CH5183284
- FGFR Dependent
- Dovitinib
- Astra Zeneca

- AZD5363
- PI3K Dependent
- BYL719
- GDC-0032
- V5584
- LY3023414
- BKM120
- CLR457
- BRAF Mutant
- LY3023414
- Everolimus
- Vemurafenib
- Ruxolitinib

- DDR Defective
- BRCA1/BRCA2/PALB2 mutations
- Unstable genome
- BRCA mutational signature

- Platinum
- PARPi
- KRAS WT (7%)
- EGFRi
- HER2 Amplified (2%)
- Herceptin
- HKI-272

The protein expression of phospho-Akt was successfully evaluated by IHC in 100 human PDACs.

Patients were categorized according to their high vs. low phospho-Akt expression compared to the median value (30 a.u.).
CLINICAL DATA

- High phospho-Akt vs. Low phospho-Akt for Survival (%)
- Very-high phospho-Akt vs. others for Survival (%)
- Low phospho-Akt vs. High phospho-Akt for PFS (%)
- Very-high phospho-Akt vs. others for PFS (%)
**AKT1 mRNA expression**

![Graph showing AKT1 mRNA expression in various cell lines, primary tumor cultures, and tissues. The graph illustrates the expression levels of AKT1 mRNA, with a ratio relative to β-actin, across different cell lines and tissue types. Notably, LPC028 and PANC-1 are highlighted with high pAKT expression, while LPC006 and CFPAC exhibit low pAKT expression.]

- **LPC028**: High pAKT
- **LPC006**: Low pAKT
- **CFPAC**: Low pAKT
- **PANC-1**: High pAKT
PERIFOSINE INHIBITS CELL PROLIFERATION IN PDAC CELLS

LPC028

% Cell growth (vs. control)

Drug (nM)

LPC006

% Cell growth (vs. control)

Drug (nM)

Combination index

Antagonism

Synergism

LPC028

PANC-1

CFPAC-1

LPC006

Combination index

0.0

0.2

0.4

0.6

0.8

1.0

1.2

1.4

0.01

1

10

100

1000

10000
PERIFOSINE AND ITS COMBINATION WITH GEMCITABINE REDUCE THE SIZE OF PDAC SPHEROIDS

Control

Treated with Perifosine and gemcitabine

![Control](image1)

![Treated](image2)

<table>
<thead>
<tr>
<th>Group</th>
<th>Δ Volume Day3-Day0 (mm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gemcitabine</td>
<td>LPC006: 70, LPC028: 70</td>
</tr>
<tr>
<td>Perifosine</td>
<td>LPC006: 80, LPC028: 80</td>
</tr>
<tr>
<td>Perifosine and gemcitabine</td>
<td>LPC006: 90, LPC028: 95</td>
</tr>
</tbody>
</table>

* indicates a significant difference compared to the control group.
Perifosine reduces the expression of p-Akt in LPC028, CFPAC-1 and PANC-1

P-Akt levels in LPC006 are not affected
PERIFOSINE AND ITS COMBINATION WITH GEMCITABINE INHIBIT CELL MIGRATION/INVASION

Graphs showing the percentage of cell migration over time for LPC028 and LPC006 cell lines with different treatments:

- LPC028:
  - Control
  - Perifosine
  - Gemcitabine
  - Perifosine and Gemcitabine

- LPC006:
  - Control
  - Perifosine
  - Gemcitabine
  - Perifosine and Gemcitabine

Bar graphs showing the percentage of cell migration for CFPAC-1 cell line with different treatments:

- Control
- Perifosine
- Gemcitabine
- Perifosine and Gemcitabine

Legend:
- Control
- Perifosine
- Gemcitabine
- Perifosine and Gemcitabine
PERIFOSINE AND ITS COMBINATION WITH GEMCITABINE INDUCE APOPTOSIS

% Apoptosis (as detected by Annexin V assay)

LPC028

- Control
- Gemcitabine
- Perfosine
- Combination

LPC006

- Control
- Gemcitabine
- Perfosine
- Combination

Late
Early
PERIFOSINE AND ITS COMBINATION WITH GEMCITABINE ACTIVATE CASPASES AND PROAPOPTOTIC FACTORS
Glucose → Glycolysis → Pyruvate → Glut1

Glut1

PI3K → AKT

AKT → E-cadherin

CASPASE 3 → Bad, Bcl-2

CASPASE 6, 9 → NFkB

GEMCITABINE (dFdC)

PERIFOSINE

Gemcitabine (dFdC) → RR → dFdC → dFdCM → dFdCDP → dFdCTP

Glucose

Pyruvate

E-cadherin

CASPASE 3, 6, 9

NFkB

Bad, Bcl-2

Glucose

CASPASE 3, 6, 9

NFkB

Bad, Bcl-2

MIGRATION

APOPTOSIS

CYTOTOXICITY
Glut1 mRNA levels were significantly reduced after treatment with perifosine alone and in combination with gemcitabine in LPC0028.
INHIBITION OF GLUT1 BY THE NOVEL SPECIFIC COMPOUND PGL13

Cell growth (% vs. control)

- DMSO
- PGL-13

Inhibitors:
- Gemcitabine (IC50)
- Perifosine (IC50)
- Combination
- GLUT-1 inhibitor (10 uM)

Chemical structure of PGL13
CONCLUSIONS

- Phospho-Akt expression emerges as both a prognostic biomarker
- Perifosine has a synergistic interaction with gemcitabine
- Phospho-Akt levels influence the antitumor activity of perifosine
- Inhibition of Glut1 overcomes resistance to this combination treatment
- Inhibition of Glut1 might represent a new therapeutic approach with Akt inhibitors in PDAC