WELCOME TO IMPAKT

IMPROVING CARE AND KNOWLEDGE THROUGH TRANSLATIONAL RESEARCH IN BREAST CANCER

Brussels, Belgium 7-9 MAY 2015

IMPAKT Chairs: Nicholas Turner London, UK and Carsten Denkert, Berlin, Germany
PI3 kinase/mTOR inhibition increases sensitivity of ER positive breast cancers to CDK4/6 inhibition by blocking cell cycle re-entry driven by cyclinD1 and inducing apoptosis

Maria Teresa Herrera-Abreu
Uzma Asghar, Ros Cutts, Isaac Garcia-Murillas, Alex Pearson, Richard Elliott, Michelle A Nannini, Amy Young, Deepak Sampath, Sunil Pancholi, Mitch Dowsett, Lesley Ann Martin, and Nicholas C. Turner

ESMO IMPAKT 2015
Michelle Nannini, Amy Young and Deepak Sampath are employees of Genentech.

Nicholas Turner reports receiving advisory board honoraria from Pfizer and Roche.

MT Herrera-Abreu, Uzma Asghar, Ros Cutts, Isaac Garcia-Murillas, Alex Pearson, Richard Elliott, Lesley Ann Martin and Nicholas Turner are employees of the Institute of Cancer Research, which has a commercial interest in the development of PI3K inhibitors, including GDC-0941.
Proliferation ER positive breast cancer is driven by cyclin D1-CDK4/6

ER targeting (Fulvestrant, Letrozole)

CDK4/6 inhibition (Palbociclib)

M Aarts, S Linardopoulos, and NC Turner
Current Opinion in Pharmacology 2013, 13:529–535
Aims:

• To study the molecular mechanisms limiting sensitivity to CDK4/6 inhibitors (palbociclib)

• To identify novel therapeutic combinations with CDK4/6 inhibition in breast cancer, which could potentially progress to the clinic

• Study acquired resistance to CDK4/6 inhibitors
CDK4/6 inhibition with palbociclib fails to induce a durable cell cycle arrest and leads to cyclin D1 accumulation

CDK4/6-CyclinD1

E2F induce expression of cell cycle mediators

Cell cycle progression G1/S phase

palbociclib

Early adaptability to single agent palbociclib in ER+ sensitive cell lines

Relative BrdU incorporation

MCF-7

0 24 48 72h Palbo

P-RB1 S807/811
RB1
Cyclin E2
Cyclin D1
CDK4
actin

* vehicle or drug added every 24h

vehicle
Palbo
Palbo
Palbo
Palbo

24 24 72 72

vehicle
Palbo

Relative Brdu incorporation

0.0 0.5 1.0

vehicle
Palbo

* vehicle or drug added every 24h
Inhibition of the PI3 kinase-mTOR signaling pathway sensitizes ER+ breast cancer cell lines to CDK4/6 inhibition

Drug screen in MCF-7 and T47D cell lines
3520 compound library

Outputs
Survival effect
Sensitivity to palbociclib

Treatments
Control
Palbociclib
MK2206
Palbo+ MK2206
Everolimus
Palbo+ Everolimus
GDC-0941
Palbo+ GDC-0941

Cell line: MCF-7
Z score
Palbociclib sensitivity

PI3K/mTOR, PDK, AKT inhibitors
Tamoxifen
IGFR inhibitor

Uzma Asghar
Combination of CDK4/6 and PI3 kinase inhibitors (palbociclib + GDC0941) prolong cell cycle arrest and induced apoptosis

**Apoptotic effect with the combination**

96 hours treatment
PI3 kinase and CDK4/6 inhibitors combinations are efficacious \textit{in vivo}

\begin{enumerate}
\item[A.] Tumor regression
\begin{itemize}
\item Vehicle PO, QD
\item GDC-0941 75 mg/kg PO, QD
\item Palbociclib 50 mg/kg PO, QD
\item Palbociclib 50 mg/kg + GDC-0941 75 mg/kg PO, QD
\end{itemize}

\item[B.] Well tolerated
\begin{itemize}
\item Vehicle PO, QD
\item GDC-0941 75 mg/kg PO, QD
\item Palbociclib 50 mg/kg PO, QD
\item Palbociclib 50 mg/kg + GDC-0941 75 mg/kg PO, QD
\end{itemize}
\end{enumerate}
**Mechanism of early adaptation**

Cyclin D1 and CDK2 promote cell cycle entry despite CDK4/6 inhibition

**Working model:**

\[ \text{ER} \quad \text{Cyclin D1-CDK4/6} \quad \text{Proliferation} \]

\[ \text{RTK/PI3K} \quad \text{Cyclin D1-CDK2} \quad \text{Proliferation} \]

\[ \text{Early adaptability} \]

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**Co-precipitation**

<table>
<thead>
<tr>
<th>IP: IgG</th>
<th>IP: CDK2</th>
<th>IP: CyclinD1</th>
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<tbody>
<tr>
<td>vehicle</td>
<td>vehicle</td>
<td>Pabliciclib GDC0941</td>
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<tr>
<td>vehicle</td>
<td>vehicle</td>
<td>Pabliciclib GDC0941</td>
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**Relative Brdu incorporation**

MCF-7

- Vehicle
- Palbo 48h
- Palbo 96h

P<0.001

0.0
0.5
1.0

siCON2
siCyclinD1
Vehicle
Palbo 48h

MCF-7

- Vehicle
- Palbo 96h

P<0.001

0.0
0.5
1.0

siCON2
siCDK2
The triplet combination of fulvestrant, palbociclib, and GDC-0941 has greater efficacy than either doublet

10 days treatments + 7 days after washout

Vehicle  Palbociclib  GDC0941  Fulvestran

MCF-7

Palbo+ 0941  Palbo+ Fulv  0941+ Fulv  0941+ Fulv+Palbo
Mechanism of acquired resistance:
Acquired palbociclib resistance reflects loss of cyclin D1 dependence

Parental MCF-7 and T47D
CDK4/6-CyclinD1 dependence

Resistant MCF-7pR and T47pR
RB1 loss
CyclinE1-CDK2

<table>
<thead>
<tr>
<th></th>
<th>MCF-7</th>
<th>MCF-7pR</th>
<th>T47D</th>
<th>T47DpR</th>
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<tbody>
<tr>
<td>Palbo</td>
<td>-</td>
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<tr>
<td>P-RB1 S807/811</td>
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<td>RB1</td>
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<tr>
<td>Cyclin E1</td>
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<td>actin</td>
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Reduced number of cells or cells arrested
MCF-7pR cells switch to cyclin E1-CDK2 dependency

Increasing copy number MCF-7pR

Relative BrdU incorporation

MCF-7 pR cells switch to cyclin E1-CDK2 dependency

CDK4/6-CyclinD1 ↔ CDK2-CyclinE1

Proliferation
Conclusions:

**Early adaptation to CDK4/6 inhibition:**
- caused by low level cell cycle re-entry due to cyclin D1 accumulation (CDK2 interaction) induced by PI3K signaling
- Combination CDK4/6 and PI3K inhibition prolong cell cycle arrest and is efficacious in vitro and in vivo in ER positive breast cancer cell lines
- PI3K inhibition converts the cytostatic effect of palbociclib into cytotoxic

**Acquired resistance to CDK4/6 inhibition:**
- caused by loss of cyclin D1 dependency (through RB1 loss or cyclin E1 amplification)
- Resistant cell lines do not benefit from the combination palbociclib-GDC0941.
- New therapeutict strategies are needed for the treatment of palbociclib resistant cancers. E.g. CDK2+CDK4/6 inhibition in cell lines with cyclin E1 amplification
Acknowledgments

The Institute of Cancer Research

Nicholas Turner
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