Mechanisms of Resistance to Antineoplastic Agents

Miquel Taron, Rafael Rosell
Catalan Institute of Oncology
Hospital Germans Trias i Pujol

10th European Perspectives in Lung Cancer
Brussels 6-7 March 2009

Low ERCC1
Ovarian cancer
Better response
Dabholkar et al
J Clin. Invest

Low ERCC1
NSCLC
Longer survival
No differences in response
Lord et al CC
Rosell et al Oncogene

Low RRM1
Better than ERCC1
CC: 0.410 P < 0.001
Rosell et al CCR

Low ERCC1
NSCLC
Better response
Cobo et al JCO

Low ERCC1
Colorectal cancer
Longer survival
No differences in response
Shirota JCO

Low BRCA1
Differential modulator
Quinn et al Cancer Res

ERCC1 & RRM1 mRNA in gem/cis-treated NSCLC

Scagliotti et al Ann Oncol 2006
Rosell et al Oncogene 2003
Scagliotti et al Ann Oncol 2006

ERCC1 & RRM1 mRNA in gem/cis-treated NSCLC

Survival

Therapeutic benefit

RNA expression

Risk of death


ERCC1-based customized chemotherapy in NSCLC

Randomize

Control arm
- docetaxel / cisplatin

1:2

Genotypic arm
- ERCC1 levels
- low genotypic group: docetaxel / cisplatin
- high genotypic group: docetaxel / gemcitabine

Patient accrual: August 2001 – October 2005

Cobo et al. JCO 2007

RNA/DNA Isolation

FFPE tumor micro-dissection

RNA

DNA

PCR with TaqMan®

Data Analysis

**Treatment based on therapy-predictive markers**

- standard or ERCC1-customized cisplatin/docetaxel (Fossella et al. JCO 2003; Scagliotti et al. JCO 2008; Cobo et al. JCO 2007)
  - MS = 10-11 mo
  - TTP = 5.1-6.7 mo
  - 2-y survival = 14-21%

- RAP 80 / BRCA1-customized cisplatin-based chemotherapy (SLCG data; Rosell et al. ESMO 2008)
  - MS = NR
  - TTP = 14 mo

- customized erlotinib in EGFR-mutant lung cancer in 217 pts
  - MS = 27 mo
  - TTP = 14 mo
Reduction of cancer networks to abstract models for identification of therapy-predictive markers

Cancer Networks
- Angiogenesis
- Cell survival
- Proliferation

Prognostic Markers (BRCA1)

NOT Cured w/ Chemo

Chemosensitive

Targeted Therapy
- EGFR mutations
- HER2 mutations
- K-ras mutations
- IGF Inhibitors
- Antiangiogenics

Customized Chemo
- BRCA1 / RAP 80

HCCBR116: IC50 = 4.1 x 10^-6 M
HCCEV1: IC50 = 2.3 x 10^-7 M
HCCBR116: IC70 = 1.9 x 10^-9 M
HCCEV1: IC70 = 1.7 x 10^-5 M

cisplatin bleomycin etoposide

paclitaxel vinorelbine
BRCA1 – differential modulator of chemosensitivity

- In breast and ovarian cancer cells, inducible expression of BRCA1 enhances paclitaxel sensitivity (Mullan et al. Oncogene 2001)
- siRNA of BRCA1 led to paclitaxel and docetaxel resistance, and reconstitution of BRCA1 enhanced sensitivity to paclitaxel and vinorelbine (Quinn et al. Cancer Res 2003; Chabalier et al. Cell Cycle 2006; Quinn et al. CCR 2007)
- 3 retrospective studies in NSCLC and ovarian cancer (Taron et al. Hum Molec Genet 2004; Quinn et al. CCR 2007; Weberpals et al. IJC 2009)
  - low/intermediate BRCA1 → longer survival following platinum-based chemotherapy
  - high BRCA1 expression → survival increased following taxane-based therapy
### BRCA1 & ERCC1 in sporadic ovarian cancer

- **Image**: Diagram showing BRCA1 and ERCC1 levels.

### Response in 96 gem/doc-treated stage IV NSCLC according to BRCA1 mRNA levels

<table>
<thead>
<tr>
<th>BRCA1</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>OR</th>
<th>p</th>
<th>Multivariate OR</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>27%8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>13%8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>58.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.002</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.31</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.13</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.22</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.46</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### RRM1 in 96 gem/doc-treated stage IV NSCLC stratified by RRM1

<table>
<thead>
<tr>
<th>RRM1</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>N (%)</th>
<th>TTP max</th>
<th>p</th>
<th>N (%)</th>
<th>TTP max</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### PS in 96 gem/doc-treated stage IV NSCLC stratified by RRM1

<table>
<thead>
<tr>
<th>RRM1</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>N (%)</th>
<th>TTP max</th>
<th>p</th>
<th>N (%)</th>
<th>TTP max</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- **Image**: Diagram showing PS levels.

- **Table**: Data on response and survival outcomes stratified by RRM1.
Survival to neoadjuvant gem/cis in NSCLC according to BRCA1 mRNA expression

Survival (months)

<table>
<thead>
<tr>
<th>Group</th>
<th>Survival</th>
<th>60</th>
<th>50</th>
<th>40</th>
<th>30</th>
<th>20</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRCA1+</td>
<td>Gem/Cis</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>5</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>BRCA1-</td>
<td>Docetaxel</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>3</td>
<td>2</td>
</tr>
</tbody>
</table>

BRCA1-customized phase II study in stage IV NSCLC (non-squamous)

<table>
<thead>
<tr>
<th>EGFR Mutations</th>
<th>Erlotinib</th>
<th>Gem/Cis</th>
<th>Docetaxel/Cis</th>
<th>Docetaxel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>3.3%</td>
<td>16.7%</td>
<td>0%</td>
<td>2.5%</td>
</tr>
<tr>
<td>Intermediate</td>
<td>34.1%</td>
<td>58.3%</td>
<td>21.1%</td>
<td>37.5%</td>
</tr>
<tr>
<td>High</td>
<td>30.1%</td>
<td>8.3%</td>
<td>47.4%</td>
<td>17.5%</td>
</tr>
</tbody>
</table>

Overall response rate:
- All Patients: 43.6%
- EGFR Group: 90.9%
- BRCA1 Group: 25.0%
- Intermediate BRCA1 Group: 45.7%
- High BRCA1 Group: 41.9%

Survival:
- Median: 12 mos
- 1-year: 49.2%
- 2-year: 31.5%
- 28 months: 24.5%

Time to progression, mos:
- 6 mos: 24.5 mos
- 9 mos: 17.5 mos
- 12 mos: 15.0 mos
- 18 mos: 9.0 mos
- 24 mos: 6.0 mos

EGFR Group:
- Low: 12.2%
- Intermediate: 16.7%
- High: 15.8%
- Overall: 12.5%

BRCA1 Group:
- Low: 0%
- Intermediate: 47.4%
- High: 15.8%
- Overall: 12.5%

<table>
<thead>
<tr>
<th>Outcome</th>
<th>All Patients</th>
<th>EGFR Group</th>
<th>Low BRCA1 Group</th>
<th>Intermediate BRCA1 Group</th>
<th>High BRCA1 Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=123)</td>
<td>(n=12)</td>
<td>(n=38)</td>
<td>(n=40)</td>
<td>(n=33)</td>
</tr>
<tr>
<td>Complete response</td>
<td>3.3%</td>
<td>16.7%</td>
<td>0%</td>
<td>2.5%</td>
<td>3%</td>
</tr>
<tr>
<td>Partial response</td>
<td>54.1%</td>
<td>58.3%</td>
<td>21.1%</td>
<td>37.5%</td>
<td>36.4%</td>
</tr>
<tr>
<td>Stable disease</td>
<td>25.1%</td>
<td>8.3%</td>
<td>47.4%</td>
<td>17.5%</td>
<td>33.3%</td>
</tr>
<tr>
<td>Progressive disease</td>
<td>25.3%</td>
<td>0%</td>
<td>15.8%</td>
<td>38%</td>
<td>21.2%</td>
</tr>
<tr>
<td>Overall response rate</td>
<td>43.6%</td>
<td>90.9%</td>
<td>25%</td>
<td>45.7%</td>
<td>41.9%</td>
</tr>
<tr>
<td>Relapse free survival</td>
<td>37.4%</td>
<td>75%</td>
<td>21.1%</td>
<td>46%</td>
<td>38.5%</td>
</tr>
<tr>
<td>Survival</td>
<td>12 mos</td>
<td>12 mos</td>
<td>12 mos</td>
<td>12 mos</td>
<td>12 mos</td>
</tr>
<tr>
<td>Cancer death</td>
<td>89.2%</td>
<td>91.7%</td>
<td>87.6%</td>
<td>41.1%</td>
<td>42.8%</td>
</tr>
<tr>
<td>Progression free survival</td>
<td>37.4%</td>
<td>75%</td>
<td>21.1%</td>
<td>46%</td>
<td>38.5%</td>
</tr>
<tr>
<td>Time to progression</td>
<td>6 mos</td>
<td>14 mos</td>
<td>6 mos</td>
<td>9 mos</td>
<td>9 mos</td>
</tr>
</tbody>
</table>

Taron et al. Hum Mol Genet 2004

Advanced NSCLC

Cisplatin/Gemcitabine

EGFR

EGFR mutations

Low EGFR

Intermediate EGFR

High EGFR

Docetaxel/Cis

Docetaxel

STAGE IV NSCLC
BRCA1 assembly line of DNA damage response to DSBs

Requirement of RAP80 for damage-induced BRCA1 focus formation

Median survival according to levels of BRCA1 & RAP80

<table>
<thead>
<tr>
<th>RAP80 LEVEL</th>
<th>BRCA1 Levels</th>
<th>N</th>
<th>Median Survival (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>≤0.79</td>
<td>11</td>
<td>9 (5.8-14.4)</td>
<td>0.13</td>
</tr>
<tr>
<td>Median</td>
<td>0.79-1.41</td>
<td>16</td>
<td>14 (12.0-15.9)</td>
<td>0.15</td>
</tr>
<tr>
<td>High</td>
<td>&gt;1.41</td>
<td>12</td>
<td>11 (8.2-13.8)</td>
<td>0.11</td>
</tr>
</tbody>
</table>

RAP80 by itself is able to translocate to IRIF in HCC1937 cells expressing truncated BRCA1 unable to migrate to IRIF

RAP80 alone could replace the BRCA1 function in cells lacking BRCA1 (Yan et al. Cancer Res 2007)
Survival in patients with low BRCA1 according to RAP80 levels

<table>
<thead>
<tr>
<th>RAP80 LEVELS</th>
<th>≤0.79</th>
<th>0.79-1.41</th>
<th>&gt;1.41</th>
</tr>
</thead>
<tbody>
<tr>
<td>N mo (95% CI)</td>
<td>14 (5-22.9)</td>
<td>9 (4.9-15.5)</td>
<td>6 (3.1-8.9)</td>
</tr>
<tr>
<td>P</td>
<td>0.006</td>
<td>0.42</td>
<td>0.08</td>
</tr>
</tbody>
</table>

PFS according to RAP80 levels

<table>
<thead>
<tr>
<th>RAP80 LEVELS</th>
<th>Low</th>
<th>Intermediate</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>N mo (95% CI)</td>
<td>14 (5-22.9)</td>
<td>9 (4.9-15.5)</td>
<td>6 (3.1-8.9)</td>
</tr>
<tr>
<td>P</td>
<td>0.006</td>
<td>0.42</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Correlation between expression levels of nine genes
New international BREC (BRCA1 Expression Customization)

CONTROL

Docetaxel/Cis

Advanced NSCLC

T1 RAP80 (T1-T3 BRCA1)

T2-T3 RAP80 (T1-T2 BRCA1)

T2-T3 RAP80 (T2 BRCA1)

Gem/Cis

EXPERIMENTAL

Docetaxel/Cis

Docetaxel

216 patients per arm – total: 432

Potential mechanisms of resistance in the BREC trial

IL-6

ILK

EGF

STAT3

BGC1L

survivin

Hep70

cyclin D1/CDK4

c-Myc

Mcl-1

EGFR

G1183

JAK

Shp2

PI3K

Ras

Akt

Erk

mTOR

The hallmarks of cancer – the BREC trial

Survival

Longer

Shorter

Shortest

BREC customization

Low RAP80 & any
BRCA1

Intermediate RAP80 &
low/intermediate
BRCA1

High RAP80 &
BRCA1

Drug response

Cisplatin +++
Antimicrotubules

Cisplatin +
Antimicrotubules ++

Cisplatin-
Antimicrotubules +++

Drug resistance

IL-6

EGFR

IGF-1R

HER2

Survivin

STAT3

Prognosis

cyclin D1/CDK4

CRKL

Dear Readers,
Conclusions

• Customized chemotherapy based on RAP 80 / BRCA1 can improve PFS and overall survival

• Histology by itself is not predictive of pemetrexed efficacy in NSCLC. The levels of BRCA1 and RAP80 can accurately select adenocarcinomas and squamous cell carcinomas that are more sensitive to pemetrexed-platinum or other platinum-based combinations.

• Customizing EGFR TKIs based on EGFR mutations is the paradigm of targeted therapy in NSCLC