ERCC-1 and response to chemotherapy

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Institut Gustave Roussy
Cancer and Chemotherapy: the exemple of Lung Cancer

RT = radiotherapy
CT = chemotherapy

- Surgery ± CT
- RT ± CT
- CT + supportive care

Patients (%)

- Localised
- Regional
- Distant
Platin-based chemotherapy is the mainstay of first-line treatment for NSCLC.

Getting the right drug into the right patient

Pharmacogenomics will help explain why drugs work better in some patients than in others. It also presents numerous commercial opportunities for both startups and established biotechnology companies.

Current state of drug development research

Proportion of patients who respond to drug

Patients receiving drug
Platinum derivatives and DNA repair pathway

- Platinum cytotoxic effects are related to DNA binding and DNA adducts.
- Nucleotide excision repair (NER) plays a central role in DNA repair pathways.
- ERCC1 enzyme plays a rate-limiting role in the NER pathway.
- *In vitro* and clinical studies suggest a relation between ERCC1 mRNA and response to cisplatin.
ERCC1 is a rate-limiting partner in the NER pathway

Platinum resistance in vitro

- ERCC1 mRNA or protein expression levels correlate with cisplatin resistance in human cancer cell lines

<table>
<thead>
<tr>
<th>Cancer cell lines</th>
<th>ERCC1 expression</th>
<th>Phenotypic effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>ovarian</td>
<td>mRNA (3-fold ↑)</td>
<td>Cisplatin resistance</td>
</tr>
<tr>
<td>ovarian</td>
<td>mRNA and protein (2-fold ↓)</td>
<td>↓ Repair of cisplatin-DNA adducts</td>
</tr>
<tr>
<td>ovarian</td>
<td>ERCC1 anti-sense mRNA</td>
<td>Restored sensitivity to cisplatine</td>
</tr>
<tr>
<td>ovarian</td>
<td>ERCC1 SiRNA</td>
<td>↑ &gt; 53-fold in cisplatin sensitivity</td>
</tr>
<tr>
<td>cervical</td>
<td>mRNA</td>
<td>Positively correlated with oxaloplatine resistance</td>
</tr>
<tr>
<td>testis</td>
<td>protein</td>
<td>Low levels of ERCC1 compared with other cell lines</td>
</tr>
<tr>
<td>lung</td>
<td>ERCC1 anti-sense mRNA</td>
<td>Decreased the repair capacity</td>
</tr>
</tbody>
</table>

Gossage et al, Cancer Treat Rev. 2007
<table>
<thead>
<tr>
<th>Cancer type</th>
<th>Number of patients</th>
<th>Treatment</th>
<th>ERCC1 expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resected NSCLC</td>
<td>761</td>
<td>Adjuvant cisplatin-based therapy</td>
<td>Protein</td>
</tr>
<tr>
<td>Resected NSCLC</td>
<td>51</td>
<td>Majority received no chemotherapy</td>
<td>mRNA</td>
</tr>
<tr>
<td>Advanced NSCLC</td>
<td>70</td>
<td>G(gemcitabine)/C or C(cisplatin)</td>
<td>mRNA</td>
</tr>
<tr>
<td>Advanced NSCLC</td>
<td>&gt;400</td>
<td>Randomized to D/C, or D/C if ↓ ERCC1 or D/C if ↑ ERCC1</td>
<td>mRNA</td>
</tr>
<tr>
<td>Advanced NSCLC</td>
<td>56</td>
<td>G/C</td>
<td>mRNA</td>
</tr>
<tr>
<td>Advanced colorectal cancer</td>
<td>50</td>
<td>5FU/oxaliplatin</td>
<td>mRNA</td>
</tr>
<tr>
<td>Advanced colorectal cancer</td>
<td>33</td>
<td>Irinotecan</td>
<td>mRNA</td>
</tr>
<tr>
<td>Advanced gastric cancer</td>
<td>64</td>
<td>Oxaliplatin/5FU</td>
<td>Protein</td>
</tr>
<tr>
<td>Operable gastric cancer</td>
<td>38</td>
<td>Neoadjuvant C/F</td>
<td>mRNA</td>
</tr>
<tr>
<td>Oesophageal cancer</td>
<td>99</td>
<td>Neoadjuvant CRT (chemoradiotherapy) (C/F)</td>
<td>mRNA</td>
</tr>
<tr>
<td>Oesophageal cancer</td>
<td>36</td>
<td>Neoadjuvant CRT (C/F)</td>
<td>mRNA</td>
</tr>
<tr>
<td>Ovarian cancer</td>
<td>26</td>
<td>Platinum based therapy</td>
<td>mRNA</td>
</tr>
<tr>
<td>Ovarian cancer</td>
<td>28</td>
<td>Platinum based therapy</td>
<td>mRNA</td>
</tr>
<tr>
<td>Advanced bladder cancer</td>
<td>57</td>
<td>G/C or G/C/P</td>
<td>mRNA</td>
</tr>
</tbody>
</table>

Gossage et al, Cancer Treat Rev. 2007
ASCO 2003: International Adjuvant Lung Trial

- 1867 patients with completely resected NSCLC I-II-IIIA
- Absolute benefit: 4.1% improvement of 5 year OS

HR = 0.86 [0.76-0.98]
p < 0.03

The IALT Collaborative Group, NEJM 2004
ASCO 2006: IALT-bio study

ERCC1: a predictor of chemotherapy benefit?

All IALT centers
1867 pts

IALT Centers > 10 patients
1045 pts

867 blocks received

783 exploitable NSCLC (after pathological review)

761 patients evaluable for ERCC1
Methods: ERCC1 immunohistochemistry

- Immunohistochemical analysis
- Standardized antigen retrieval
- ERCC1 monoclonal antibody (NeoMarkers)
- Evaluation of staining by two independent investigators, blinded to clinical data
  - Internal controls (normal tissue)
  - Staining intensity and percentage of positive cells (H-score)
Test of interaction ERCC1 treatment: $p = 0.009$

<table>
<thead>
<tr>
<th></th>
<th>Chemotherapy n=389</th>
<th>Control group n=372</th>
<th>Hazard ratio for death CT vs. no CT</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERCC1 negative tumors n=426</td>
<td>47% [40%-55%] 56 months</td>
<td>39% [32%-47%] 42 months</td>
<td>0.65 [0.50-0.86] p = 0.002</td>
</tr>
<tr>
<td>ERCC1 positive tumors n=335</td>
<td>40% [32%-49%] 50 months</td>
<td>46% [37%-55%] 55 months</td>
<td>1.14 [0.84-1.55] p = 0.40</td>
</tr>
</tbody>
</table>

Gain of 14 months of overall survival from adjuvant chemotherapy in patients with ERCC1 negative tumor
Effect of adjuvant chemotherapy on overall survival in pts with ERCC1 negative tumor

Adjusted HR = 0.65, 95% CI [0.50-0.86], p = 0.002
Effect of adjuvant chemotherapy on overall survival in pts with ERCC1 positive tumor

Adjusted HR = 1.14, 95% CI [0.84-1.55], P = 0.40
In the control group, ERCC1 positive patients have a favorable prognosis.

<table>
<thead>
<tr>
<th></th>
<th>HR *</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control group</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ERCC1 negative</td>
<td>1</td>
<td>[0.49-0.90]</td>
<td><strong>0.009</strong></td>
</tr>
<tr>
<td>ERCC1 positive</td>
<td>0.66</td>
<td>[0.49-0.90]</td>
<td></td>
</tr>
<tr>
<td><strong>Chemotherapy</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ERCC1 negative</td>
<td>1</td>
<td>[0.86-1.56]</td>
<td>0.34</td>
</tr>
<tr>
<td>ERCC1 positive</td>
<td>1.16</td>
<td>[0.86-1.56]</td>
<td></td>
</tr>
<tr>
<td><strong>All patients</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ERCC1 negative</td>
<td>1</td>
<td>[0.71-1.10]</td>
<td>0.26</td>
</tr>
<tr>
<td>ERCC1 positive</td>
<td>0.88</td>
<td>[0.71-1.10]</td>
<td></td>
</tr>
</tbody>
</table>
Results

ERCC1 negative

ERCC1 positive

Benefit of Cisplatin-based CT in ERCC1 NEGATIVE patients

DNA Synthesis and Repair Genes **RRM1** and **ERCC1** in Lung Cancer

Zhong Zheng, M.D., Ph.D., Tingan Chen, M.D., Ph.D., Xueli Li, M.D., Eric Haura, M.D., Anupama Sharma, M.D., and Gerold Bepler, M.D., Ph.D.

**Figure 5.** Disease-free Survival and Overall Survival among 184 Patients with AQUA Scores for RRM1 and ERCC1.

**Prognostic value of ERCC1 and RRM1 in stage I NSCLC patients**
GILT: the first ERCC1-based customized chemotherapy

Control arm
docetaxel / cisplatin

Cisplatin 75 mg/m² day 1
Docetaxel 75 mg/m² day 1

Experimental arm
ERCC1 levels
RT-PCR

docetaxel / cisplatin
(low ERCC1 mRNA)
Cisplatin 75 mg/m² day 1
Docetaxel 75 mg/m² day 1

gemcitabine / docetaxel
(high ERCC1 mRNA)
Docetaxel 40 mg/m² day 1, 8
Gemcitabine 1000 mg/m² day 1, 8

• Advanced NSCLC
• Microdissection
then RT-PCR
Projected accrual 297

342 patients randomized: 283 patients ‘enrolled’
→ 17% dropped out!
→ Of which 57% due to insufficient tissue

An additional 102 patients were included

Total randomized 444 and 366 ‘enrolled’
→ Still 17% dropped out
GILT: ERCC1-based customized chemotherapy

- **Response rate**
  - Statistical difference (p=0.02)
  - 39% in control vs. 51% in genotypic
    - OR in ERCC1 low: 53.7%
    - OR in ERCC1 high: 47.2%

- **No difference in complete response rate**
  - 4.3% in control vs 3.1% in genotypic

- **No differences in OS or PFS**
GILT: ERCC1-based customized chemotherapy

- Trial initiated in the early 2000s: visionary and extremely audacious!

- Why is this trial negative? Did ERCC1 failed in identifying patients who should receive cisplatin based chemotherapy?
  - Docetaxel-gemcitabine the best non-platinum combination?
  - The control arm received the same chemotherapy as the low ERCC1 and no data on ERCC1 are known in the control arm
  - It would have been better to prospectively confirm that low ERCC1 expressors respond better than high ERCC1 expressors to one platinum combination

- Methodological and technological issues complicate the interpretation of the study
  - Reproductibility
  - high drop out rate
Predictive biomarkers of Ct efficacy

- Cisplatin
- Gemcitabine
- Pemetrexed
- Paclitaxel
- Docetaxel

- ERCC1
  - RRMI ?
- RRM1 ?
- FPGS ?
- MAPtau ?
- Bcl2 ?
Clinical questions
- Validation of ERCC1 predictive value
- Molecular and clinical characterization ERCC1 pos/neg pts
- Future clinical trials
- Other tumor types

Biological questions
- ERCC1 partners (XPF, XPA, RPA…)
- Biological function of ERCC1
- Regulation of ERCC1 gene expression

Methodological perspectives
External validation of ERCC1 expression predictive/prognostic value

Assuming 200 patients for BR10 and 400 patients for Anita
Clinical characterization of ERCC1 neg/pos pts

Brain metastasis are increased in ERCC1 NEG patients treated by CT (non squamous histology, n=335)

Brain metastasis occurrence according to treatment

Besse et al. ASCO 2007
Future clinical trials

- Design and implement a customized trial of adjuvant Ct integrating ERCCI data
  - should ERCCI be associated with other markers (EGFR mut/FISH ?)
  - to which compounds ERCCI positive and negative patients are the most likely to respond (beyond cisplatin)
  - should ERCCI positive patients get a treatment?
### SWOG Pilot Study: Pharmacogenomic-directed Adjuvant Therapy of NSCLC

**Eligibility Criteria:**
- **NSCLC**
  - pT1 (x>2cm)
  - pT2N0M0
  - R0 resection
- **Age:** 18-75
- **PS:** 0-1
- **N~TBD**

**Treatment Regimens:**
- **RRMI ≥ 40.5 AND ERCC1 > 66.0**
  - **Active Monitoring**
- **All Others**
  - (RRMI < 40.5 OR ERCC1 < 66.0)
  - **Cisplatin-Gemcitabine**

**PI:** Bepler
French adjuvant lung cancer study (IFCT)

ARM A (Standard)
CDDP doublet

ARM B
Customized Bio - Chemo

ERCC1+
EGFR+
Erlotinib

EGFR-
No treatment

ERCC1-
EGFR+
CDDP doublet followed by erlotinib

EGFR-
CDDP doublet
Clinical questions

- Validation of ERCC1 predictive value
- Molecular and clinical characterization ERCC1 pos/neg pts
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- Other tumor types

Biological questions

- ERCC1 partners (XPF, XPA, RPA…)
- Biological function of ERCC1
- Regulation of ERCC1 gene expression

Methodological perspectives
ERCC1 partners in cancer

XPF

RPA
Possible ERCC1 target sites

Protein-Protein
XPF/ERCC1

Protein-Protein
XPA/ERCC1

Protein-DNA

5'→3'
cleavage site

upstream

ERCC1

XPF

DNA binding site

XPA binding site

ERCC1 gene expression regulation

- AP1: 2 sites
  - c-Fos
  - c-Jun
  - ATF2
  - CBP
- Ets-1: 2 sites
  - Ets-1 (AP1 => TIMP-1)
  - CREB
- GATA-1: 1 site
- HMG2
- Antisens codant pour CD3-epsilon AP

La Phosphorylation de c-jun sur Ser63 et Ser73
Permet l’Interaction avec CBP et l’Ouverture de la Chromatine

(O. Coqueret)
Conclusions

- ERCC1 expression is a predictive factor of chemotherapy benefit in patient treated by cisplatin-based adjuvant chemotherapy

- ERCC1 expression is a prognostic factor in patient not treated by chemotherapy

- ERCC1 plays a fundamental function in drug resistance and cancer susceptibility (Janus-faced of ERCC1)
Personalized medicine is one of the strongest expectations from patients and health-care takers.

The next step:
- Integration of the best biomarkers in prospective trials
- Implementation of pharmacogenomic-based clinical trials
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- Benjamin Besse
- Fabrice André

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