Predictors of Breast Cancer Recurrence (ProBeCaRe) study: Tamoksifenresistens hos præmenopausale kvinder med brystkræft

Deirdre Cronin Fenton
Associate Professor, Ph.D.
dc@clin.au.dk

DBCG Repræsentantskabsmøde, 23. january 2017
Overview of presentation

• Background to the Danish ProBeCaRe Premenopausal Study
• The ProBeCaRe dataset
• Project progress:
  1. Tumor block collection
  2. Validation study findings
  3. Drug-drug interaction results
• Ongoing work
Figure 1: Major metabolic pathways for tamoxifen. Bold type denotes the enzyme(s) primarily involved in each step. (Nx ER) = binding affinity to estrogen receptor relative to tamoxifen itself. [C] = plasma concentration of the metabolite, relative to tamoxifen’s concentration, after four months of tamoxifen therapy at 20 mg per day.

Cronin-Fenton et al., Future Oncology, 2014
Figure 1: Major metabolic pathways for tamoxifen. Bold type denotes the enzyme(s) primarily involved in each step. \( (N \times ER) \) = binding affinity to estrogen receptor relative to tamoxifen itself. [C] = plasma concentration of the metabolite, relative to tamoxifen’s concentration, after four months of tamoxifen therapy at 20 mg per day.

Cronin-Fenton et al., Future Oncology, 2014
Any CYP2D6*4 or *10

Cronin-Fenton et al, Future Oncology, 2014
New perspectives 3: Pre-menopausal women

1.0 (95% CI=0.76, 1.4)
7 studies
0–6% premenopausal
median 0%

1.7 (95% CI=1.2, 2.3)
13 studies
21–69% premenopausal
median 49%
Study aims

• Assess inhibition of tamoxifen metabolism via comprehensive genotyping & concurrent drug use, and risk of breast cancer recurrence
  – Examine genetic variants in 13 enzymes that catalyze the biotransformation of tamoxifen

• Assess competitive inhibition of tamoxifen through assay of oestrogen regulating enzymes
  – $17\beta$HSD1 & $17\beta$HSD2

• Assess interaction between inhibition of tamoxifen metabolism and ER$\beta$ expression

=> This is the first and largest study of premenopausal women
Network of Danish Registries

Danish Breast Cancer Group
- Cancer-directed treatment
- Clinical characteristics
- Patient characteristics
- Follow-up

Danish National Registry of Patients
- Comorbid diseases
- ProBeCaRe Cohort (DBCG data)
  - High quality clinical database
  - CPR number

Danish Civil Registry
- 1968+
  - Emigration
  - Vital status

Danish Pathology Registry
- FFPE tumor & normal tissue

National Prescription Registry:
- Prospectively collected prescription data:
  - CYP2D6, CYP3A4, CYP2C19 inhibiting medications

Network of Danish Registries
Pre menopausal women diagnosed 2002-2011 with stage I, II or III breast cancer reported to the Danish Breast Cancer Group (n=8,047)

- Estrogen receptor + tamoxifen use (4600)
  - Recurrence (396)
    - No Recurrence (4204)
  - No Recurrence (1573)
    - ER+/
      - TAM- = 1573
      - ER-/Tam+ = 73
      - ER missing/Endo tam not missing = 40
      - ER missing/Endo tam missing = 5
      - ER not missing/Endo tam missing = 393

- Estrogen receptor - No tamoxifen (1359)
  - Recurrence (216)
    - No Recurrence (1143)

All others excluded (n=2088)
<table>
<thead>
<tr>
<th>Patient and tumour characteristics</th>
<th>ER+/TAM+</th>
<th></th>
<th>ER-/TAM-</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td><strong>Age at diagnosis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;35</td>
<td>222</td>
<td>4.8</td>
<td>182</td>
<td>13.4</td>
</tr>
<tr>
<td>35-49</td>
<td>487</td>
<td>10.6</td>
<td>229</td>
<td>16.9</td>
</tr>
<tr>
<td>40-44</td>
<td>1123</td>
<td>24.4</td>
<td>321</td>
<td>23.6</td>
</tr>
<tr>
<td>45-49</td>
<td>1668</td>
<td>36.3</td>
<td>385</td>
<td>28.3</td>
</tr>
<tr>
<td>50+</td>
<td>1100</td>
<td>23.9</td>
<td>242</td>
<td>17.8</td>
</tr>
<tr>
<td><strong>Stage at diagnosis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage I</td>
<td>1184</td>
<td>25.7</td>
<td>402</td>
<td>29.6</td>
</tr>
<tr>
<td>Stage II</td>
<td>2476</td>
<td>53.8</td>
<td>702</td>
<td>51.7</td>
</tr>
<tr>
<td>Stage III</td>
<td>917</td>
<td>19.9</td>
<td>246</td>
<td>18.1</td>
</tr>
</tbody>
</table>
1. Cooperation from Danish pathology departments

- First letter requesting blocks sent April 2014
- End of block collection August 2015 for majority of pathology departments
- N=5,500 FFPE blocks received

Thank you!
2. ProbeCaRe Validation Study Aims

Compare DBCG data used in the ProBeCaRe study with medical records as a gold standard

**Aims:**

To validate:

- Changes in menopausal status during follow-up
- Changes in endocrine therapy during follow-up
- Breast cancer recurrence
Study sampling criteria

- 50 patients each hospital: Aarhus, Aalborg, Odense University Hospitals
- 2002-2006 & 2007-2011
- ER/TAM status
- Stage I, II, III
- 36 strata
- Random numbers to each patient within each stratum
- Selected 4-5 patients from each stratum

=> 151 patients in total
Results

~100% agreement between registry and medical records for clinical, demographic and treatment characteristics
Results: Menopausal transition

<table>
<thead>
<tr>
<th>DBCG Registry Menopausal transition (n=151 patients)</th>
<th>Medical Record Menopausal transition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency</td>
<td>No</td>
</tr>
<tr>
<td>No</td>
<td>78</td>
</tr>
<tr>
<td>Yes</td>
<td>11</td>
</tr>
<tr>
<td>Total</td>
<td>89</td>
</tr>
<tr>
<td>PPV = 61% (42%, 77%)</td>
<td></td>
</tr>
</tbody>
</table>
Results: Changes in endocrine therapy

| Registry Change in endocrine therapy among ER+ patients only (n=77 patients) | Medical Record Change in endocrine therapy |
|---|---|---|
| No change from tamoxifen | No | Yes | Total |
| 48 | 3 | 51 |
| Change from tamoxifen to aromatase inhibitor | 1 | 25 | 26 |
| Total | 49 | 28 | 77 |

PPV = 96% (83%, 100%)
Results: Breast cancer recurrence

<table>
<thead>
<tr>
<th>Registry Recurrence (n=151 patients)</th>
<th>Medical Record Recurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No recurrence</td>
</tr>
<tr>
<td>No recurrence</td>
<td>131</td>
</tr>
<tr>
<td>Yes recurrence</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>131</td>
</tr>
</tbody>
</table>

PPV = 100%
Implications of the validation study

• Changes in endocrine therapy can be incorporated as time-varying covariates

• Changes in menopausal status were difficult to validate
  – Other variables (e.g., prescription drugs) may be more robust as time-varying variables
  – The medical record may be a poor gold standard for menopausal transition

• Recurrence may be missing for some patients but this will not bias most ratio measures of association
3. Drug-interaction study

Tamoxifen biotransformation chiefly catalyzed by CYP2D6, CYP2C19, and CYP3A4

Aim:

• To evaluate whether tamoxifen-treated premenopausal breast cancer patients have a higher recurrence rate if concomitantly exposed to a metabolism-impairing drug
Study Population

• Stage I-III premenopausal breast cancer patients in Denmark (n=5,959)

• Diagnosed 2002-2011, registered in DBCG

• Follow-up for breast cancer recurrence in the DBCG registry

• 10 years of follow-up or through 01/07/2013
Prescription drugs

Exposure drugs:
• >=1 prescription each year, updated daily & lagged by one year

Statistical analyses:
• Crude and adjusted Cox proportional hazards regression models with time-varying drug exposure updated yearly & lagged by one year
• Sensitivity analyses altering the definition of drug exposure
<table>
<thead>
<tr>
<th></th>
<th>Adjusted HR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CYP2D6 weak inhibitors</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ER-</td>
<td>1.71</td>
<td>(1.17, 2.50)</td>
</tr>
<tr>
<td>ER+</td>
<td>1.00</td>
<td>(0.74, 1.36)</td>
</tr>
<tr>
<td><strong>CYP2D6 strong inhibitors</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ER-</td>
<td>0.52</td>
<td>(0.19, 1.41)</td>
</tr>
<tr>
<td>ER+</td>
<td>0.62</td>
<td>(0.34, 1.13)</td>
</tr>
<tr>
<td><strong>CYP2D6 any inhibitors</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ER-</td>
<td>1.44</td>
<td>(0.99, 2.09)</td>
</tr>
<tr>
<td>ER+</td>
<td>0.98</td>
<td>(0.74, 1.30)</td>
</tr>
<tr>
<td><strong>CYP3A4 inhibitors</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ER-</td>
<td>0.67</td>
<td>(0.25, 1.82)</td>
</tr>
<tr>
<td>ER+</td>
<td>1.82</td>
<td>(1.12, 2.96)</td>
</tr>
<tr>
<td><strong>CYP2C19 inhibitors</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ER-</td>
<td>1.10</td>
<td>(0.67, 1.82)</td>
</tr>
<tr>
<td>ER+</td>
<td>0.99</td>
<td>(0.71, 1.38)</td>
</tr>
</tbody>
</table>
Conclusion: Drug-drug interaction study

- Positive association for CYP3A4 inhibition was specific to ER+/TAM+ women, as expected for a predictive marker

- The short-term use of CYP3A4-inhibiting drugs (antifungals and antibiotics) would not overlap much with five years of tamoxifen duration, so this association merits further investigation

- All associations warrant study with incorporation of functional variants in the genes encoding these enzymes
Ongoing work

• DNA extraction for comprehensive genotyping

• Optimising the analytic approach for statistical analyses

• Developing tissue microarrays for biomarker analyses

=> Collaborative projects?
Acknowledgements & Funding

• DBCG

• Danish breast cancer pathologists, pathology departments & staff

• US National Cancer Institute, R01CA166825
Thank you for your attention
Extra slides
ProBeCaRe: Premenopausal Breast Cancer Cohort

**Figure 2: Design Summary**

**Source Population**
- Pre-menopausal women
- Stage I, II, III breast cancer
- Diagnosed 2002 to 2010
- Reported to the Danish Breast Cancer Cooperative Group (DBCG)

**ER+a/T+ cohort**
- Estimate 3600 patients, 25,000 person-years
- Followed maximum 10 years

**Baseline data**
- stage, grade, histology, surgery, radiation therapy, chemotherapy, ER status, tamoxifen therapy

**ER+a/T− cohort**
- Estimate 2600 patients, 18,000 person-years
- Followed maximum 10 years

**Time-varying data**
- menopausal status, tamoxifen adherence, SSRI use, comorbidity

**Estimate**
- 660 recurrences
- 580 recurrences
The Few: Have no information on recurrence

- DBCG Dx: July 2010
- DBCG: Last follow-up: October 2011
- MRR: Recurrence April 2012 (missing in DBCG)
- DBCG via CPR Death: August 2012
Exposure drugs

**CYP2D6:**
- *Weak inhibitors:* mirtazapin, amitriptyline, propranolol, pindolol, zuclopenthixol, amiodarone, celecoxib, cimetidine, venlafaxine, diltiazem, diphenhydramine, citalopram, escitalopram, febuxostat, gefitinib, hydralazine, hydroxychloroquine, imatinib, methadone, propafenone, ranitidine, ritonavir, sertraline, verapamil, metoclopramide.
- *Strong/Moderate inhibitors:* fluoxetine, paroxetine, buproprion, quinidine, terbinafine, levomepromazine, duloxetine, moclobemide.

**CYP2C19:**
- *Strong/Moderate inhibitors:* Fluconazole, fluvoxamine (PPIs: omeprazole, esomeprazole are typically moderate inhibitors)

**CYP3A4:**
- *Strong inhibitors:* ketokonazole, itraconazole, posaconazole, voriconazole, clarithromycin, ritonavir, nelfinavir, saquinavir, telaprevir, indinavir, cobicistat
Tamoxifen and primary metabolites completely inhibited cell growth regardless of the CYP2D6 genotype in all cell lines.
New perspectives 1: Comprehensive genotyping

**Figure 1:** Major metabolic pathways for tamoxifen. Bold type denotes the enzyme(s) primarily involved in each step. (N x ER) = binding affinity to estrogen receptor relative to tamoxifen itself. [?] = plasma concentration of the metabolite, relative to tamoxifen’s concentration, after four months of tamoxifen therapy at 20 mg per day.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Step(s) (See Figure 1)</th>
<th>Number of selected functional variants</th>
<th>Inhibitor comedications</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CYP2D6</strong></td>
<td>A, B, D</td>
<td>19</td>
<td><strong>bupropion, cinacalcet, fluoxetine, paroxetine, quinidine, duloxetine, sertraline, terbinafine, amiodarone, cimetidine</strong></td>
</tr>
<tr>
<td><strong>CYP3A4</strong></td>
<td>A, B, C</td>
<td>3</td>
<td><strong>indinavir, nelfinavir, ritonavir, clarithromycin, itraconazole, ketoconazole, nefazodone, saquinavir, telithromycin, aprepitant, erythromycin, fluconazole, verapamil, diltiazem, cimetidine, variconazole</strong></td>
</tr>
<tr>
<td><strong>CYP3A5</strong></td>
<td>A, C</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td><strong>CYP2C9</strong></td>
<td>A, B</td>
<td>9</td>
<td><strong>fluconazole, amiodarone, variconazole</strong></td>
</tr>
<tr>
<td><strong>CYP2C19</strong></td>
<td>A, B</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td><strong>CYP1A1</strong></td>
<td>A</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td><strong>CYP2B6</strong></td>
<td>B</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><strong>SULT1A1</strong></td>
<td>E</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td><strong>SULT1E1</strong></td>
<td>E</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td><strong>UGT1A8</strong></td>
<td>F</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td><strong>UGT1A10</strong></td>
<td>F</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><strong>UGT2B7</strong></td>
<td>F</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><strong>UGT2B15</strong></td>
<td>F</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>
New perspectives 2: Comprehensive biomarkers

• Tamoxifen transport
  – TAM metabolites are substrates of ABC-transporters
  – Polymorphisms in transporter genes mediate TAM resistance?

• ER-beta
  – ERβ opposes ERα-mediated proliferation by heterodimerizing with it
  – This heterodimer does not stimulate proliferation equivalent to the ERα/ERα homodimer
  – Tumors that express both ERα and ERβ are therefore less aggressive than tumors that express only ERα

• Hydroxy-steroid dehydrogenase enzymes
  – Balance host estrogen concentration
Collaborators

• Aarhus University: Henrik T. Sørensen, Stephen Hamilton-Dutoit, Lars Pedersen, Sinna Ulrichsen, Anders Kjærsgaard, Anne Ording, Ylva Hellberg, Marco Mele, Deirdre Cronin Fenton
• DBCG: Peer Christiansen, Bent Ejlertsen
• Odense University: Per Damkier, Marianne Ewertz
• Emory University: Tim Lash, Mike Zwick
• Boston University: Rebecca Silliman
• University of Vermont: Thomas Ahern
• University of Louisville: Carolyn Klinge
• Stavanger University: Emiel Janssen, Kristin Jonsdottir, Nina Granlund, Håvard Søiland
• University of Bergen: Ernst Lien
New perspectives 2: Comprehensive biomarkers

• Tamoxifen transport
  – TAM metabolites are substrates of ABC-transporters
  – Polymorphisms in transporter genes mediate TAM resistance?

• ER-beta
  – ERβ opposes ERα-mediated proliferation by heterodimerizing with it
  – This heterodimer does not stimulate proliferation equivalent to the ERα/ERα homodimer
  – Tumors that express both ERα and ERβ are therefore less aggressive than tumors that express only ERα

• Hydroxy-steroid dehydrogenase enzymes
  – Balance host estrogen concentration
1. **Collection of pathology blocks**

**KEA**
1. Patient list and send to Institute of Pathology
2. Request blocks from pathology departments in Dk
3. Receive & register pathology blocks from departments in Dk
4. Create tracking database for blocks and slides

**Aarhus University: Institute of Pathology**
1. Select appropriate blocks based on tissue quantity and quality
2. Receive blocks from pathology departments in DK
3. Cut FFPE sections for DNA, RNA extraction
4. Mark slides for tissue microarray generation
5. Biomarker assays

**DBCG**
1. Dataset of premenopausal women with stage I-III breast cancer diagnosed 2002-2011

**Danish Pathology Institutes**
Breast cancer pathology departments