The ERβ story: how much to trust supplier validation

The Antibody Society Webcast series - Antibody Validation #2

Professor Cecilia Williams - Royal Institute of Technology, Stockholm
The ERβ story

1. Background: Interest for ERβ in breast cancer therapeutics
2. Problems
3. Antibody validation
4. Impact on the field
5. Lessons learned
Tamoxifen binds estrogen receptor

About half of patients develop resistance. A third of tumors are ER-negative.


Lancet, 2011

Meta-analysis: Adjuvant tamoxifen

Lowers recurrence

Nilsson et al., Trends in Endocrinology & Metabolism, 1998

Normal Breast cancer

ER-positive (≈70%) ER-negative (≈30%)

Nilsson et al., Trends in Endocrinology & Metabolism, 1998

Lancet, 2011

About half of patients develop resistance. A third of tumors are ER-negative.
Discovery of 2\textsuperscript{nd} estrogen receptor!

Kuiper et al. PNAS, 1996

- Intense research area (20 years)
- Implicated role in multiple tissues and diseases
- > 5,000 publications
- Commercial efforts
- Clinical trials

ERα and ERβ can be selectively targeted

- Estrogen-activated transcription factors, nuclear receptor super family class I
- Differ in ligand-binding domain
- Selective therapeutic targeting possible with receptor-selective agonists

O’Day and Lal, 2010

Cecilia Williams, KTH Royal Institute of Technology
# 2017: ERβ clinical breast cancer trials

<table>
<thead>
<tr>
<th>Status</th>
<th>Description</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recruiting</td>
<td>Adjuvant Endocrine Therapy for <strong>ERβ positive</strong> Triple Negative Breast Cancer.</td>
<td>China, Peking Union Medical College Hospital, Beijing</td>
</tr>
<tr>
<td>Recruiting</td>
<td>Evaluation of Tamoxifen's Efficacy for ER/PR Negative, <strong>ERβ positive</strong> Operable Breast Cancer Patients.</td>
<td>China, Guangdong Women and Children Hospital</td>
</tr>
<tr>
<td>Recruiting</td>
<td>CR1447 in Endocrine Responsive-HER2-neg and triple-negative AR-pos Breast Cancer <strong>ERβ targets outcome measure</strong>.</td>
<td>Switzerland, Universitätsspital Basel</td>
</tr>
<tr>
<td>Recruiting</td>
<td>Broccoli Sprout Extract in Treating Patients With Breast Cancer (<strong>ERβ outcome measure</strong>).</td>
<td>USA, Roswell Park Cancer Institute, Buffalo, New York</td>
</tr>
<tr>
<td>Active</td>
<td>Soy Isoflavones Supplementation in Treating Women at High Risk For or With Breast Cancer <strong>ERβ outcome measure</strong>.</td>
<td>USA, University of Southern California, Los Angeles</td>
</tr>
<tr>
<td>Completed</td>
<td>Flaxseed, Aromatase Inhibitors and Breast Tumor Characteristics (<strong>ERβ outcome measure</strong>).</td>
<td>USA, Roswell Park Cancer Institute, Buffalo, New York</td>
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<tr>
<td>Recruiting</td>
<td>S-equol in Women With Triple Negative Breast Cancer. (aim to upregulate and activate ERβ)</td>
<td>USA, San Antonio, Texas</td>
</tr>
<tr>
<td>Terminated</td>
<td>Estrogen for Triple Negative Breast Cancer (aim to activate ERβ)</td>
<td>USA, Madison, Wisconsin</td>
</tr>
</tbody>
</table>

Cecilia Williams, KTH Royal Institute of Technology
The ERβ story

1. Background: Why the interest for ERβ in breast cancer

2. Problems

3. Antibody validation

4. Impact on the field

5. Lessons learned
No ERβ mRNA in breast cancers?

<table>
<thead>
<tr>
<th>mRNA expression breast cancer (FPKM)</th>
<th>0.0-0.1</th>
<th>0.2-1</th>
<th>1.1-2.0</th>
<th>2.1-10.0</th>
<th>10.1-100</th>
<th>100-272</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ERα (average 40.2)</strong></td>
<td>55</td>
<td>149</td>
<td>35</td>
<td>86</td>
<td>637 (59%)</td>
<td>113 (10.5%)</td>
</tr>
<tr>
<td></td>
<td>(5%)</td>
<td>(13.9%)</td>
<td>(3.2%)</td>
<td>(8%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>ERβ (average 0.0)</strong></td>
<td>1020</td>
<td>54</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>(94.9%)</td>
<td>(5%)</td>
<td>(0.09%)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TCGA RNA-Seq data from 1075 breast tumors (FPKM values downloaded from proteinatlas.org)
Grey shade indicates average expression.

RT-PCR of NCI60 panel breast cancer cell lines: negative for ERβ (Holbeck et al., Mol. Endo. 2010)

Cecilia Williams, KTH Royal Institute of Technology
No ERβ mRNA in breast tissue?

Philip Jonsson, Memorial Sloan Kettering Cancer Center

Andersson et al., Nat Comm (2017)

Saunders et al., Br J Cancer (2002)

RNA-seq

IHC

Normal breast n=98  ER-pos n=792  ER-neg n=292

Normal breast n=98  Breast tumor n=906  Metastasis n=7

Saunders et al., Br J Cancer (2002)

Philip Jonsson, Memorial Sloan Kettering Cancer Center
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ERβ antibody validation

13 antibodies (epitopes mapped)

Andersson et al., Nature Communications (2017)

Anna Asplund
Uppsala University

Cecilia Williams, KTH Royal Institute of Technology
Validation Scheme

- Immunohistochemistry
- Western blotting
- Immunoprecipitation and Mass spectrometry

Formalin fixation
Denaturated (SDS)
Native

12 of 13 ERβ antibodies inadequate

Andersson et al., Nature Communications (2017)

Cecilia Williams, KTH Royal Institute of Technology
Trusted antibodies bind other proteins

Andersson et al., Nat Comm (2017)

- IHC
- WB
- IP-MS
- IHC Human Protein Atlas
- RNA-seq correlation

Not used in clinical cohorts

Standard antibodies for biomarker determination in clinical trials
# Tissue ERβ: IHC & RNA-seq

<table>
<thead>
<tr>
<th>Normal Tissue</th>
<th>RNA (FPKM)</th>
<th>PPZ</th>
<th>IHC 14C8</th>
<th>PPG</th>
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<tbody>
<tr>
<td>Testis</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adrenal gland</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ovary</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stomach</td>
<td>1</td>
<td></td>
<td></td>
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<tr>
<td>Appendix</td>
<td>1</td>
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<td></td>
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<tr>
<td>Colon</td>
<td>1</td>
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<td></td>
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<tr>
<td>Rectum</td>
<td>1</td>
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<td></td>
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</tr>
<tr>
<td>Urinary bladder</td>
<td>1</td>
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<tr>
<td>Adipose tissue</td>
<td>1</td>
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<tr>
<td>Lymph node</td>
<td>1</td>
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<tr>
<td>Tonsil</td>
<td>1</td>
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<td></td>
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<tr>
<td>Spleen</td>
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<table>
<thead>
<tr>
<th>Tissue</th>
<th>RNA (FPKM)</th>
<th>PPZ</th>
<th>IHC 14C8</th>
<th>PPG</th>
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<tbody>
<tr>
<td>Liver</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Gallbladder</td>
<td>0</td>
<td></td>
<td></td>
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<tr>
<td>Pancreas</td>
<td>0</td>
<td></td>
<td></td>
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<tr>
<td>Salivary gland</td>
<td>0</td>
<td></td>
<td></td>
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<tr>
<td>Esophagus</td>
<td>0</td>
<td></td>
<td></td>
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<tr>
<td>Duodenum</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Small intestine</td>
<td>0</td>
<td></td>
<td></td>
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<tr>
<td>Kidney</td>
<td>0</td>
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<tr>
<td>Prostate</td>
<td>0</td>
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<td>Breast</td>
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<td>Endometrium</td>
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<tr>
<td>Fallopian tube</td>
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<tr>
<td>Placenta</td>
<td>0</td>
<td></td>
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<td></td>
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<tr>
<td>Skin</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>0</td>
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<tr>
<td>Smooth muscle</td>
<td>0</td>
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<tr>
<td>Bone marrow</td>
<td>0</td>
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<tr>
<td>Cerebral cortex</td>
<td>0</td>
<td></td>
<td></td>
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<tr>
<td>Thyroid gland</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart muscle</td>
<td>0</td>
<td></td>
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</tr>
</tbody>
</table>

44 normal tissue types
20 cancer types
IHC
RNA-Seq

Andersson et al., Nature Communications (2017)

Cecilia Williams, KTH Royal Institute of Technology
FEW tissues express ERβ – NOT breast

Breast tissue has frequently been used as positive control (based on literature) -> led to the PPZ0506 antibody being disregarded

Andersson et al., Nature Communications (2017)
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Antibody validation challenges our understanding of estrogen biology

Our body of knowledge – and available literature – has been acquired with antibodies that were unspecific

Cecilia Williams, KTH Royal Institute of Technology
Previously trusted antibodies cannot be trusted

Cecilia Williams, KTH Royal Institute of Technology
Why clinical trials have failed

Targeting Estrogen Receptor Beta in a Phase 2 Study of High-Dose Estradiol in Metastatic Triple-Negative Breast Cancer: A Wisconsin Oncology Network Study

Kari B. Wisinski,1,2 Wei Xu,1,3 Amye J. Tevaarwerk,1,2 Sandeep Saha,4 KyungMann Kim,1,4 Anne Traynor,1,2 Leah Dietrich,5 Robert Hegeman,1,2 Dhimant Patel,6 Jules Blank,5 Josephine Harter,1,8 Mark E. Burkard1,2

Clinical Breast Cancer August 2016

Estrogen for Triple Negative Breast Cancer

Sponsor: University of Wisconsin, Madison

Terminated (did not meet efficacy goals after interim analysis)
10, 2010
1: March 7, 2017
2: April 4, 2017

Prostate Cancer and Prostatic Disease (2015) 18, 43–48

ORIGINAL ARTICLE

Estrogen receptor beta agonist LY500307 fails to improve symptoms in men with enlarged prostate secondary to benign prostatic hypertrophy

CG Roehrborn1, ME Spann2, SL Myers2, CR Serviss2, L Hu2 and Y Jin2

1UT Southwestern Medical Center at Dallas, Dallas, TX, USA
2Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN, USA.
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Cecilia Williams, KTH Royal Institute of Technology
Lessons learned

• Antibody validation is not simple
• Literature and company validation cannot be taken at face value
• The need for antibody validation cannot be overestimated
• Extremely high cost of inadequate ERβ antibody validation
  • 20 years’ of research in tissues/diseases where target may not be expressed
  • Misdirected and costly efforts by small and large companies
  • Multiple clinical trials run on incorrect basis

Costs (time; money; life) have been high!

Cecilia Williams, KTH Royal Institute of Technology
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Antibodies and the reproducibility crisis

The Antibody Society Webcast series – Antibody Validation #2

Glenn Begley  Biocurate, Melbourne
Cecilia Williams  KTH and Karolinska, Stockholm
Next Webcast in Antibody Validation: a 9-part series

| 1. | Andreas Pluckthun | The different antibody formats |
| 2. | Glenn Begley | Antibodies and the reproducibility crisis in biological science |
|     | Cecilia Williams | The Erß story – is your antibody like this? |
| 3. | Jan Voskuil | Beware the supplier OEM |
|     | Andy Chalmers | Finding antibodies in the Antibody Databases |
| 4. | Anita Bardowski | Which antibody are you looking for? The RRID |
|     | Jan Voskuil | Points to note on the supplier datasheets |
| 5. | Giovanna Roncador | Correct positive and negative controls in validation |
| 6. | Aldrin Gomes | Standard technology: “even” Western blots are non-trivial |
|     | Jim Trimmer | IHC issues in brain sciences |
| 7. | Travis Hardcastle | Cell KO technology |
|     | Alejandra Solache | Validating Antibodies with KO technology |
| 8. | Mike Taussig | Validating antibodies using array technologies |
|     | Fridjhof Lund-Johansen | Mass spectroscopy for mass validation |
| 9. | Andrew Bradbury | Why publish sequences? |
|     | Andreas Pluckthun | What are the coming alternatives? |
Validation of Commercial tool Antibodies

Antibodies and the reproducibility crisis
The Antibody Society Webcast series – Antibody Validation #2

Presented by Cecilia Williams and Glenn Begley
Produced and Directed by Simon L. Goodman
Production Manager Fran Breden
Written by Simon Goodman

https://www.antibodysociety.org/
Validation of Commercial Tool Antibodies

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