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

ANTI
BODY
SOCI
.ETY

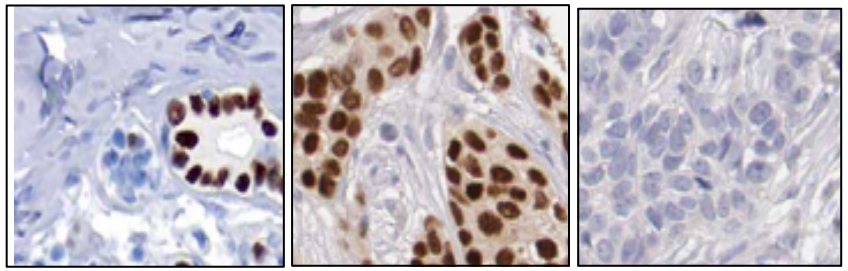
The ERβ story

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

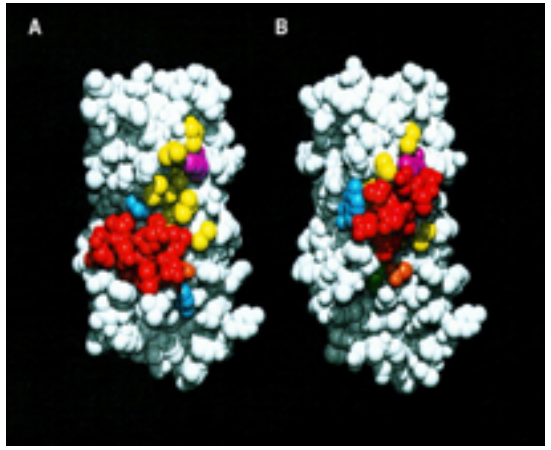
Normal

Breast cancer



ER-positive (≈70%)

ER-negative (≈30%)

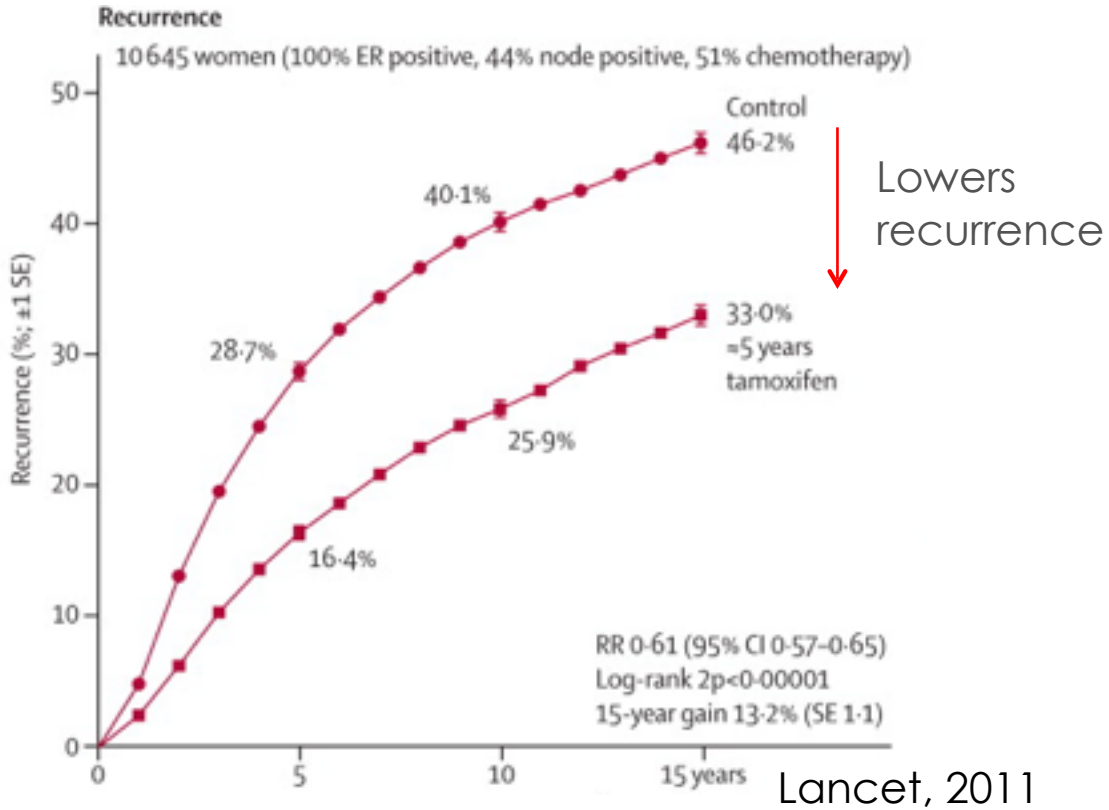


Ligand-binding domain

17β-estradiol Raloxifen

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

Meta-analysis: Adjuvant tamoxifen



Lowers recurrence

Lancet, 2011

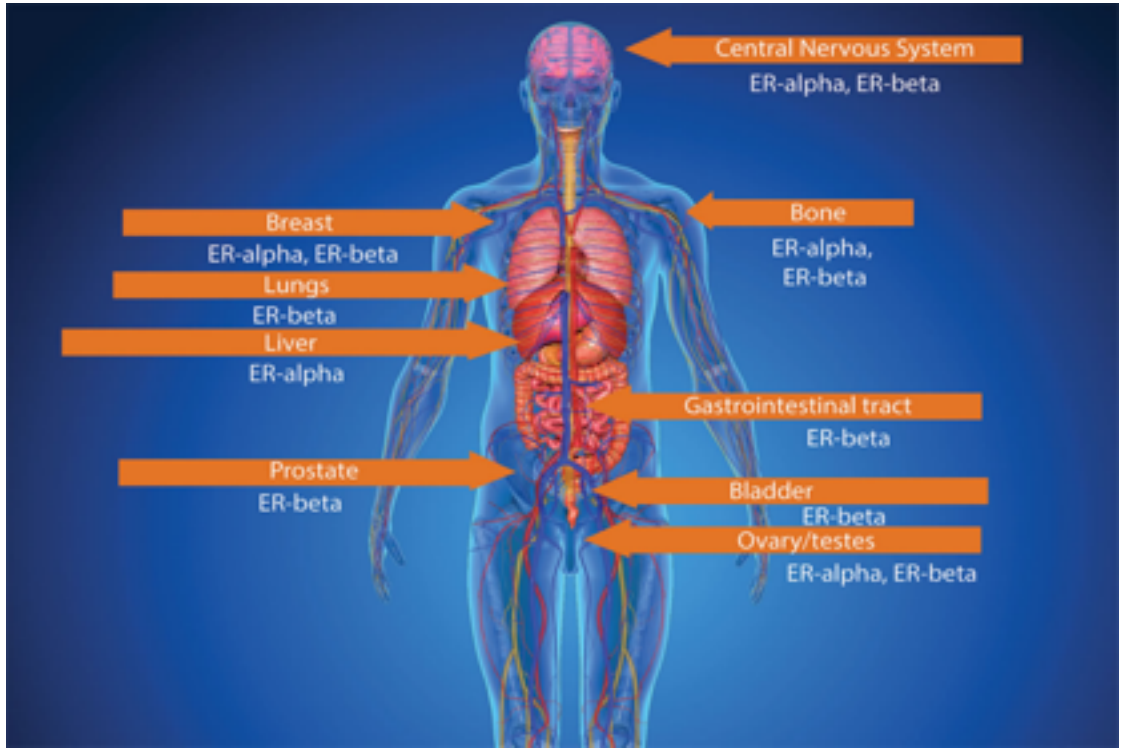
About half of patients develop resistance.
A third of tumors are ER-negative

Reviewed in Williams and Lin, Ecancermedicalscience 7, 2013

ANTIBODY SOCIETY

Discovery of 2nd estrogen receptor!

Kuiper *et al.* PNAS, 1996



Hewitt, Winuthayanon, Korach. J Mol Endocr (2016)

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

nature REVIEWS CANCER

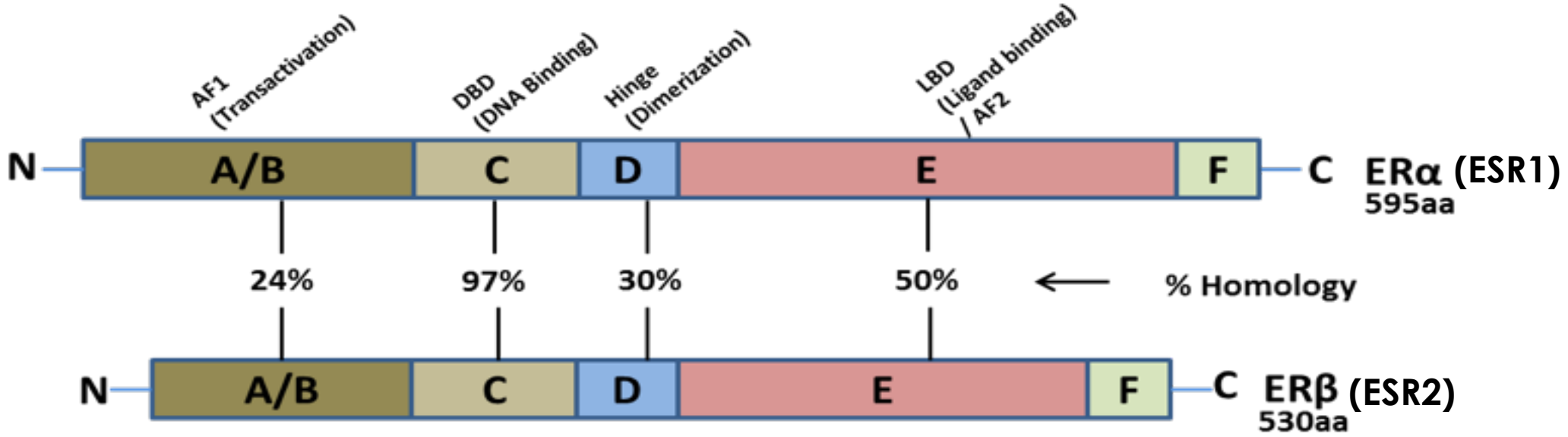
Nature Reviews Cancer **11**, 597-608 (August 2011) | doi:10.1038/nrc3093

The different roles of ER subtypes in cancer biology and therapy

Christoforos Thomas & Jan-Åke Gustafsson

ANTIBODY SOCIETY

ERα and ERβ can be selectively targeted



O'Day and Lal, 2010

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

2017: ERβ clinical breast cancer trials

U.S. National Library of Medicine ClinicalTrials.gov

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

ANTIBODY SOCIETY

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?

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

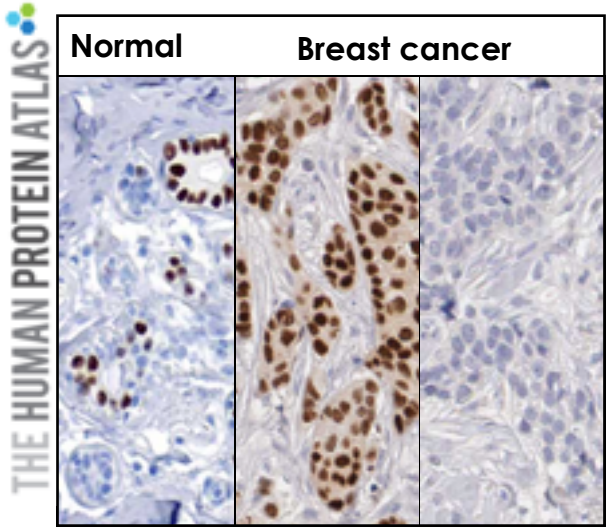
THE CANCER GENOME ATLAS
National Cancer Institute
National Human Genome Research Institute

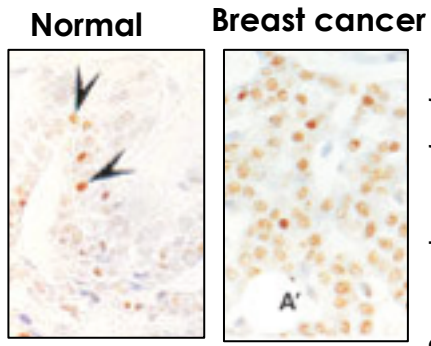
TCGA RNA-Seq data from 1075 breast tumors (FPKM values downloaded from proteinatlas.org)
Grey shade indicates average expression.

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

No ERβ mRNA in breast tissue?

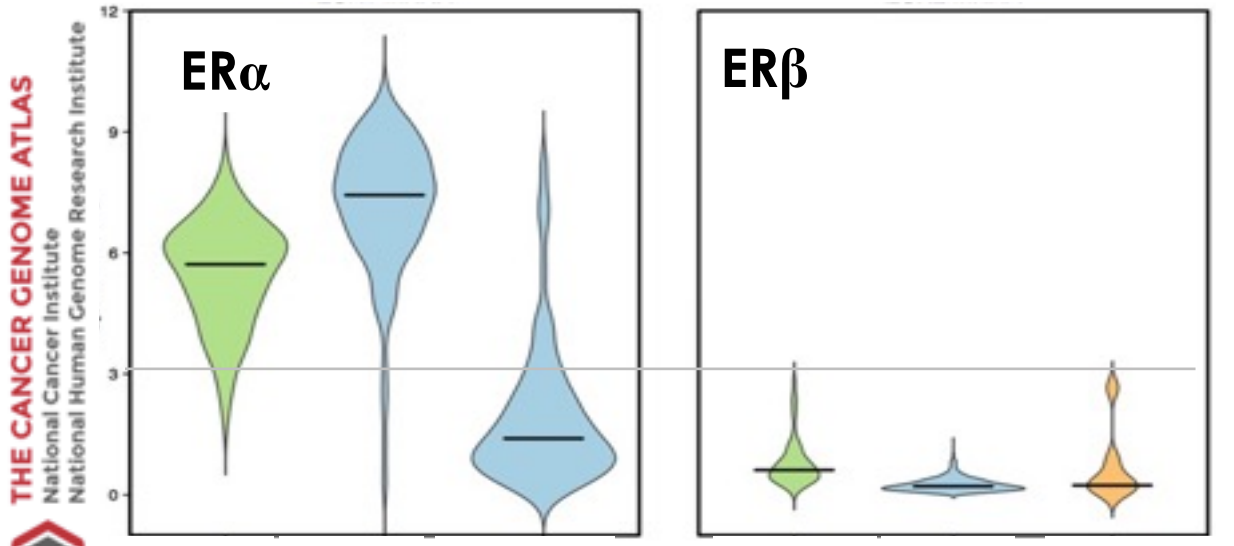
IHC



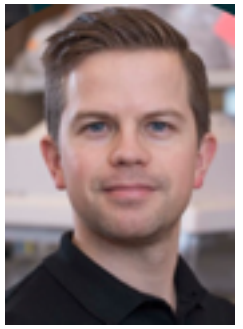


Saunders *et al.*, Br J Cancer (2002)

RNA-seq



Andersson *et al.*, Nat Comm (2017)



Philip Jonsson, Memorial Sloan Kettering Cancer Center

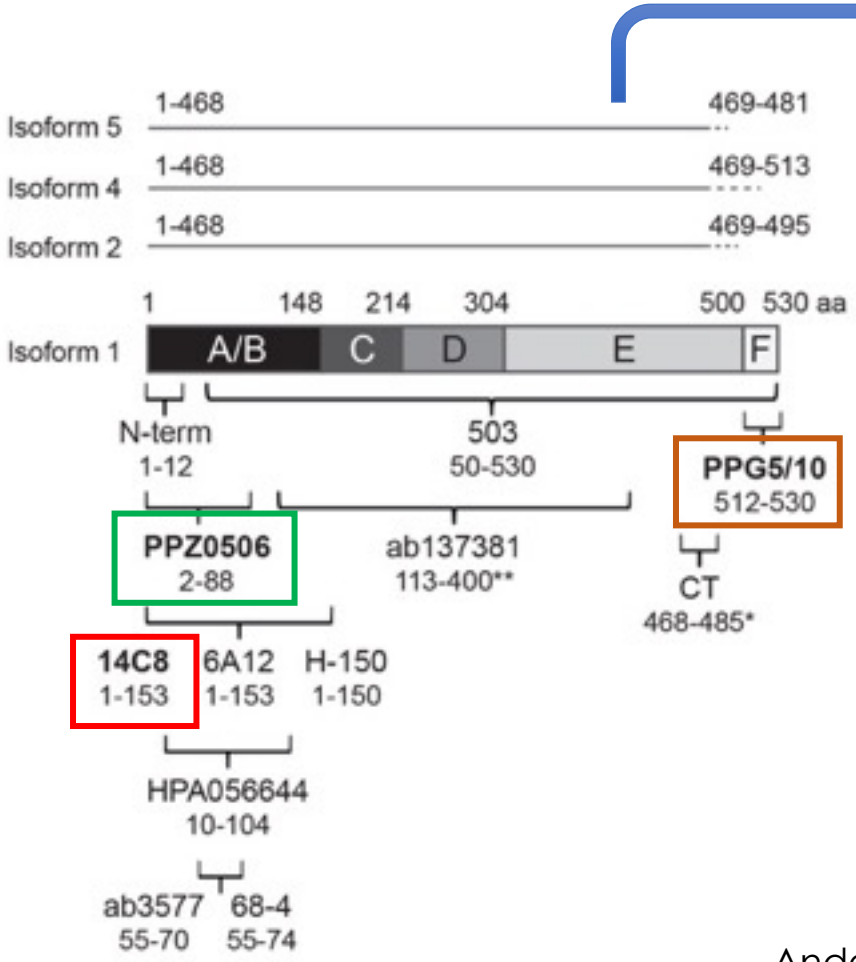
ANTIBODY SOCIETY

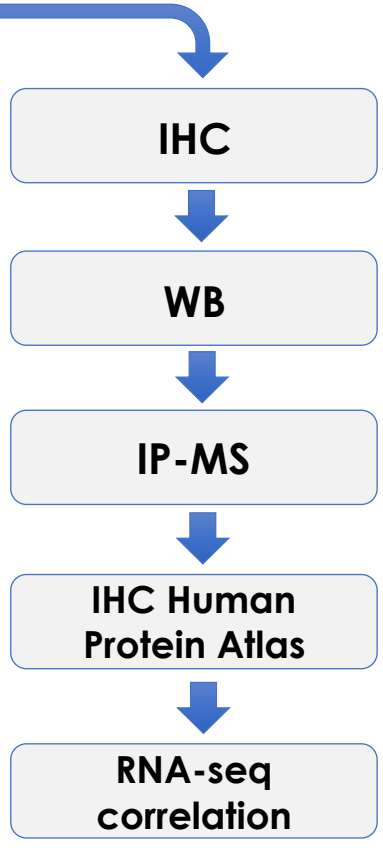
The ERβ story

1. Background: Why the interest for ERβ in breast cancer
2. Problems
3. Antibody validation
4. Impact on the field
5. Lessons learned

ERβ antibody validation

13 antibodies (epitopes mapped)





THE HUMAN PROTEIN ATLAS

Anna Asplund
Uppsala University

Andersson *et al.*, Nature Communications (2017)

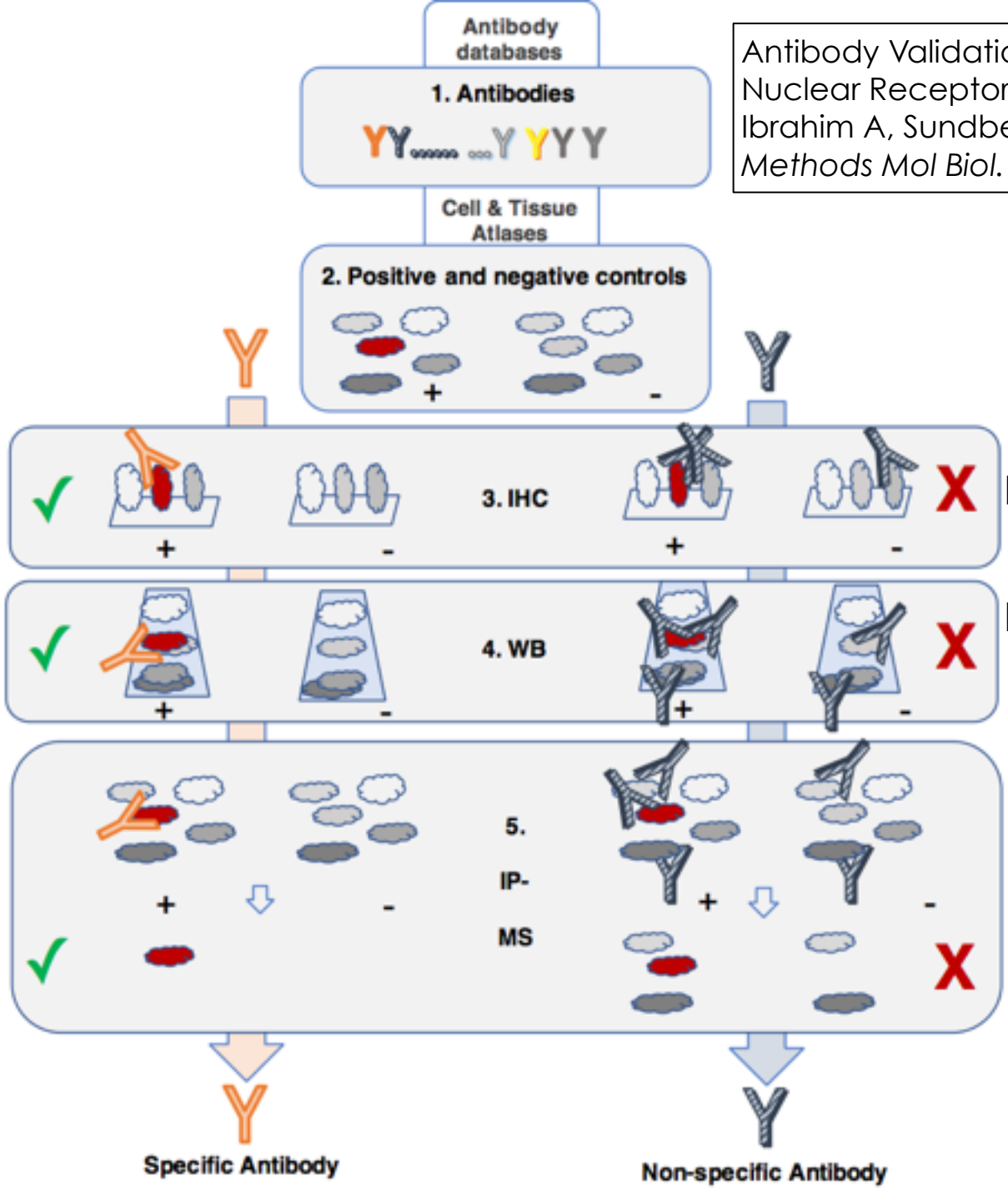
ANTIBODY SOCIETY

Validation Scheme

Immunohistochemistry

Western blotting

Immunoprecipitation
and
Mass spectrometry



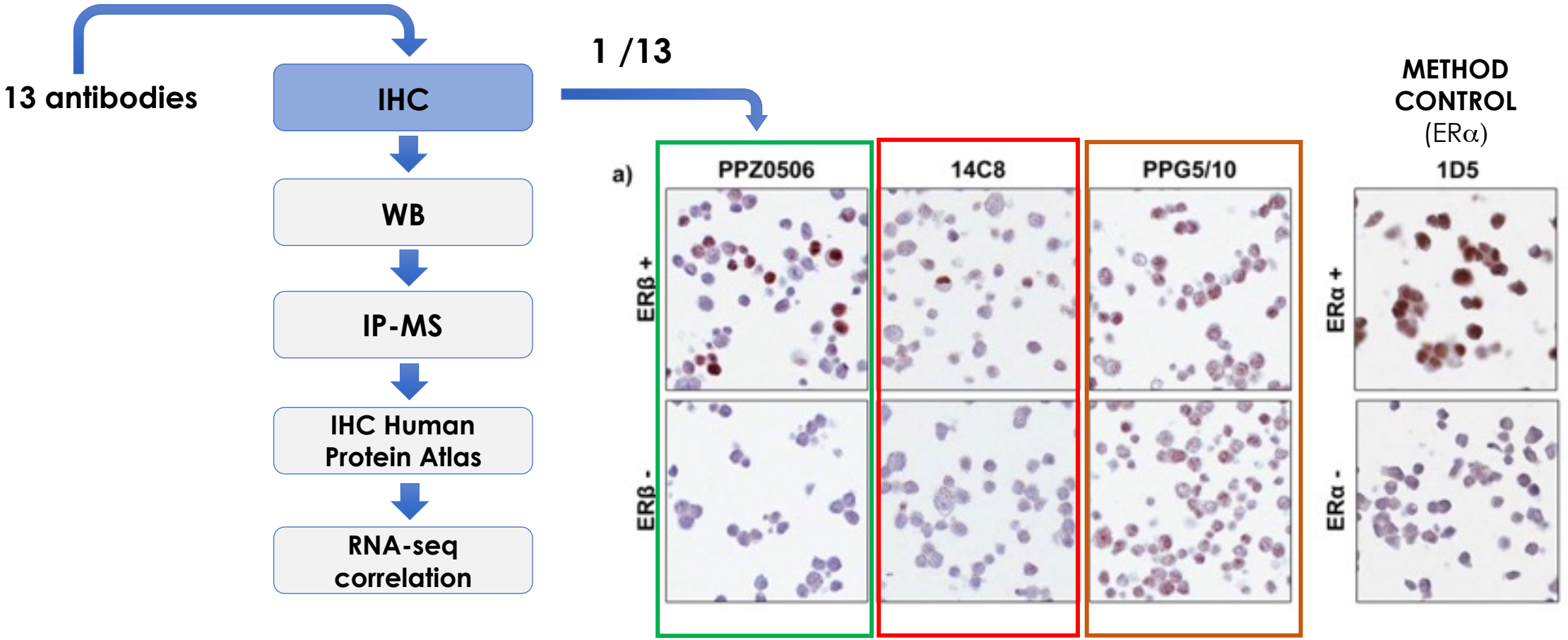
Antibody Validation Strategy for Nuclear Receptors. Katona B, Ibrahim A, Sundberg M, Williams C. *Methods Mol Biol.* 2019;1966:79-99.

Formalin fixation

Denaturated (SDS)

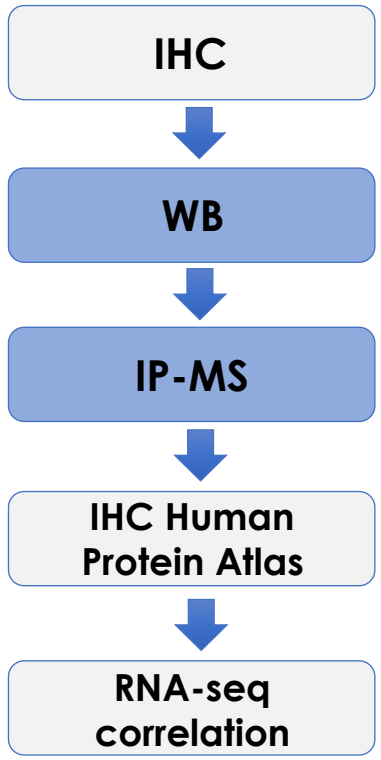
Native

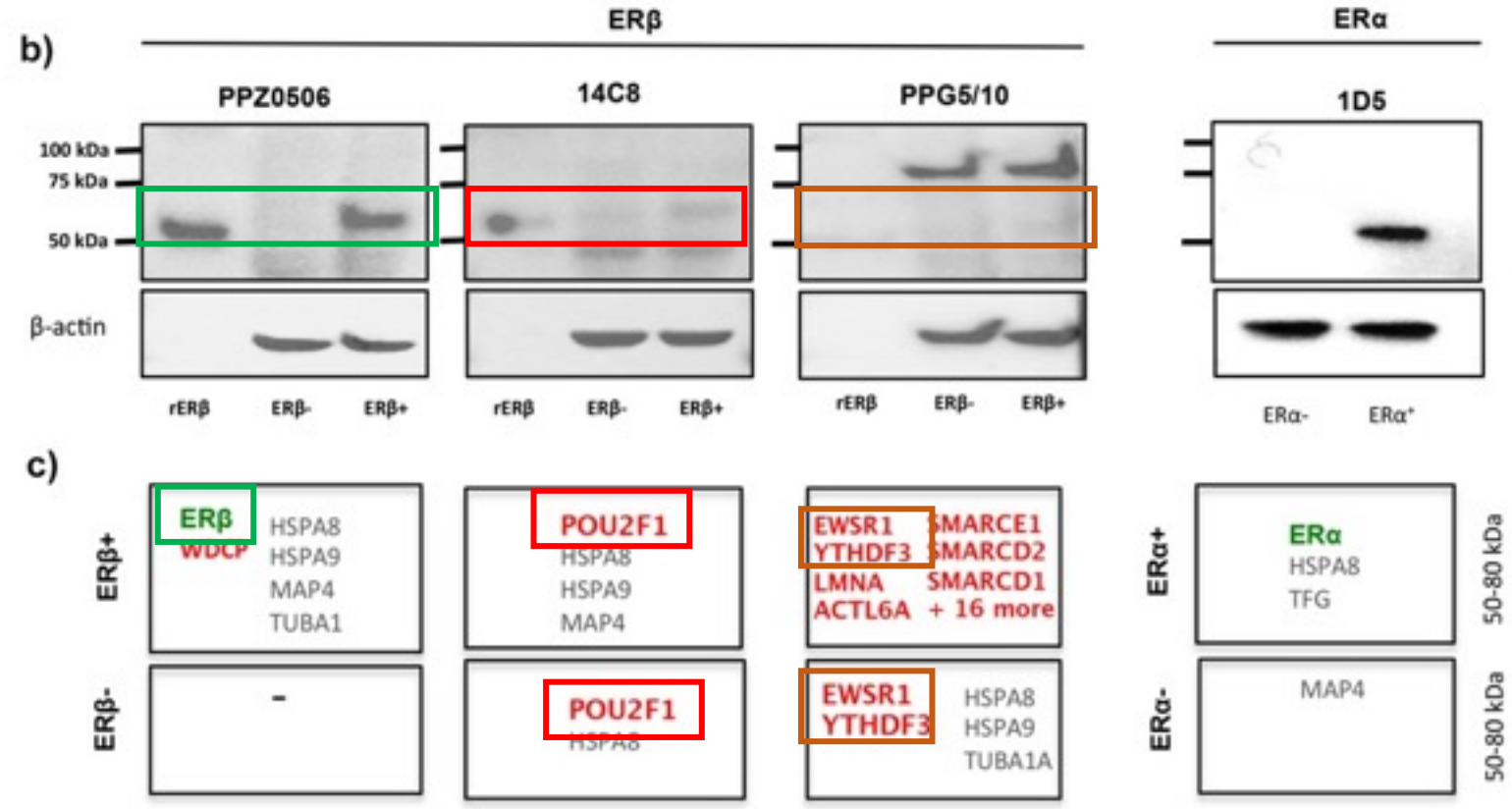
12 of 13 ERβ antibodies inadequate



Andersson *et al.*, Nature Communications (2017)

Trusted antibodies bind other proteins





Not used in clinical cohorts

Standard antibodies for biomarker determination in clinical trials

Andersson *et al.*, Nat Comm (2017)

ANTIBODY SOCIETY

Tissue ERβ: IHC & RNA-seq

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

NORMAL TISSUE	RNA (FPKM)	IHC PPZ	IHC 14C8	IHC PPG
Testis	5			
Adrenal gland	4			
Ovary	3			
Stomach	1			
Appendix	1			
Colon	1			
Rectum	1			
Urinary bladder	1			
Adipose tissue	1			
Lymph node	1			
Tonsil	1			
Spleen	1			

THE HUMAN PROTEIN ATLAS

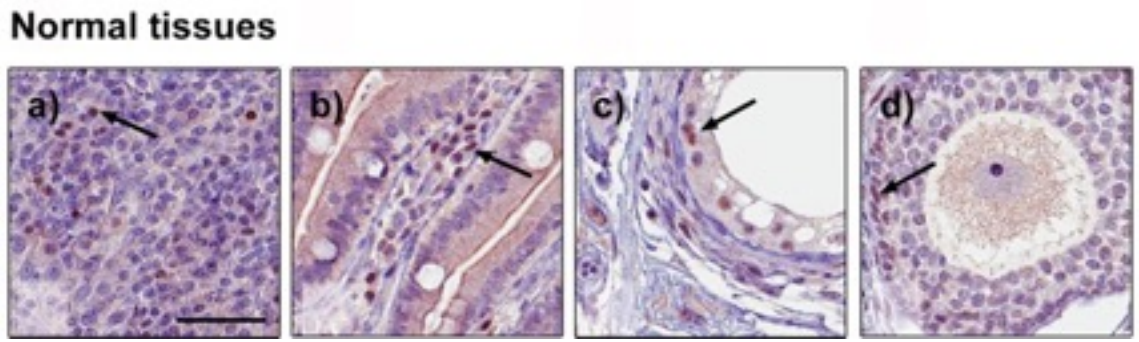
44 normal tissue types
20 cancer types
IHC
RNA-Seq

NORMAL TISSUE	RNA (FPKM)	IHC PPZ	IHC 14C8	IHC PPG
Liver	0			
Gallbladder	0			
Pancreas	0			
Salivary gland	0			
Esophagus	0			
Duodenum	0			
Small intestine	0			
Kidney	0			
Prostate	0			
Breast	0			
Endometrium	0			
Fallopian tube	0			
Placenta	0			
Skin	0			
Skeletal muscle	0			
Smooth muscle	0			
Bone marrow	0			
Cerebral cortex	0			
Thyroid gland	0			
Lung	0			
Heart muscle	0			

Andersson *et al.*, Nature Communications (2017)

ANTIBODY SOCIETY

FEW tissues express ERβ – NOT breast



Tonsil
- **Spleen**
- **Lymph node**

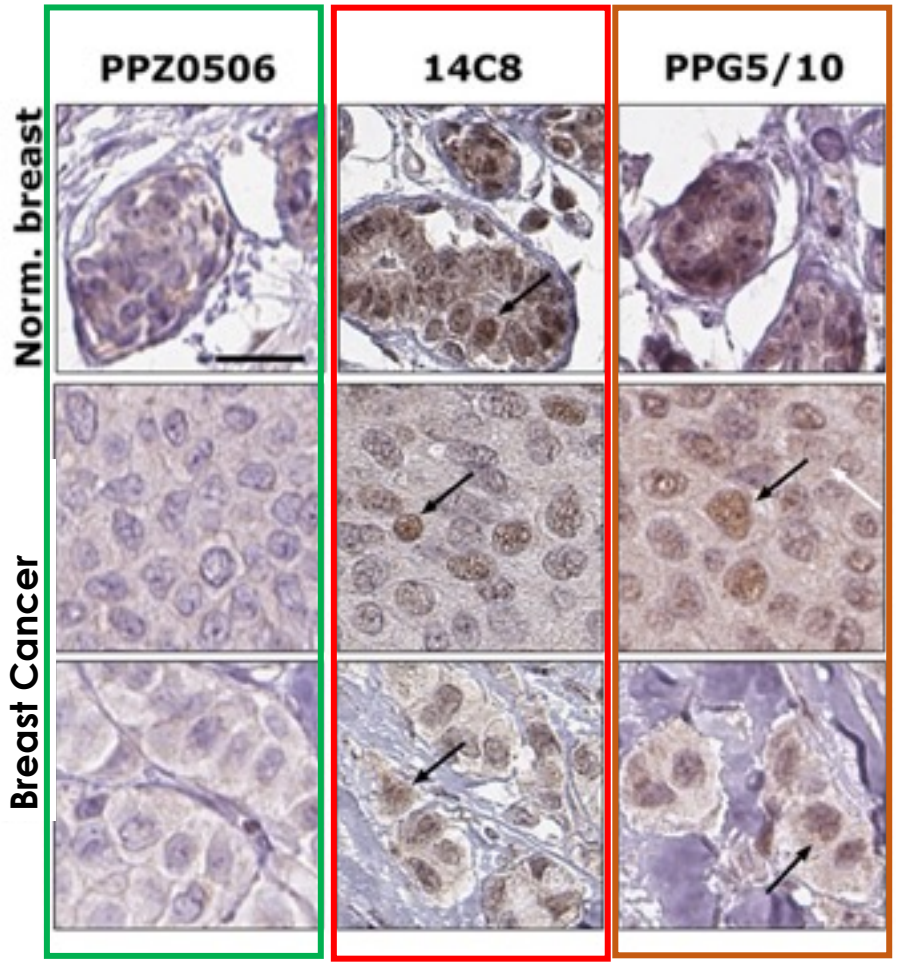
Peripheral Lymphocytes

Testis

Ovary

ERβ validated antibody PPZ0506

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)

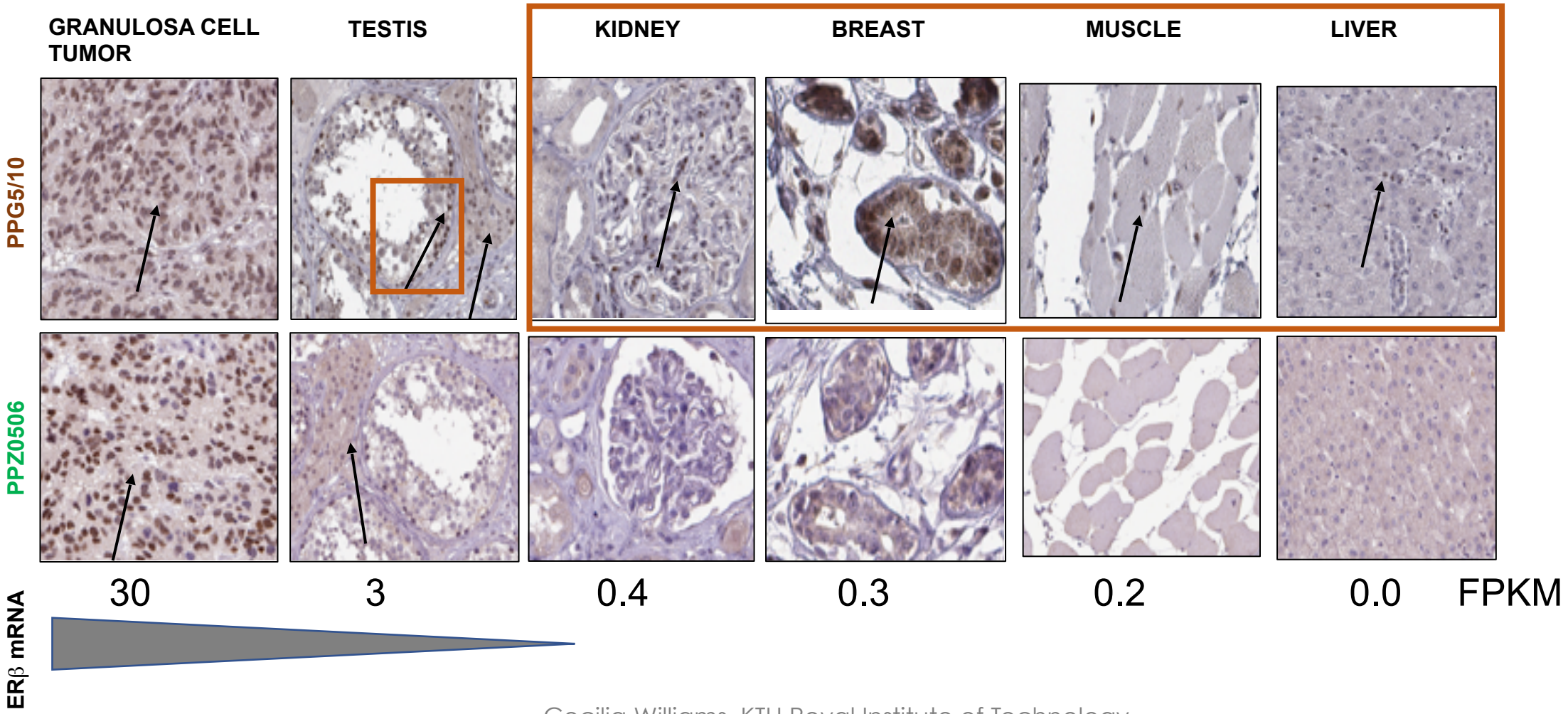
The ERβ story

1. Background: Why the interest for ERβ in breast cancer
2. Problems
3. Antibody validation
4. Impact on the field
5. Lessons learned

Antibody validation challenges our understanding of estrogen biology

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

Previously trusted antibodies - cannot be trusted



Why clinical trials have failed

BREAST

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,[7] Josephine Harter,[1,8] Mark E. Burkard[1,2]

Clinical Breast Cancer August 2016

Estrogen for Triple Negative Breast Cancer

Sponsor:

ier: NCT01083641 University of Wisconsin, Madison

Terminated (did not meet efficacy goals after interim analysis)

10, 2010

: March 7, 2017

: April 4, 2017

PROSTATE

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 Roehrborn[1], ME Spann[2], SL Myers[2], CR Serviss[2], L Hu[2] and Y Jin[2]

[1]UT Southwestern Medical Center at Dallas, Dallas, TX, USA ;
[2]Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN, USA.

ANTIBODY SOCIETY

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

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!

Acknowledgements

Previous / Current Group members:

Philip Jonsson, PhD
Ahmed Ibrahim, PhD
Amena Archer, PhD
Hakim Mohammed, PhD
Linnea Pettersson, MS
Rajitha Indukuri, MS
Dandan Song
Madeleine Birgersson

Uppsala University

Anna Asplund, PhD
Sandra Andersson, PhD
Borbala Katona
Margareta Ramström, PhD
Mårten Sundberg, PhD
Ola Söderberg, PhD
Nusa Pristovsek
Carl-Magnus Clausson
Agata Zieba
Fredrik Pontén, MD, PhD

KTH

Emma Lundberg, PhD
Mathias Uhlén, PhD

University of Houston

Jan-Åke Gustafsson, MD, PhD
Anders Ström, PhD
Christoforos Thomas
Maria Bondesson, PhD
Chin-Yo Lin, PhD
Preethi Gunaratne, PhD
Dan Frigo, PhD

Karolinska Institutet

Karin Dahlman-Wright, PhD
Chunyan Zhao, PhD
Lars-Arne Haldosén, PhD
Johan Hartman, MD, PhD

Funding:

Antibodies and the reproducibility crisis

The Antibody Society Webcast series – Antibody Validation #2

Glenn Begley — Biocurate, Melbourne

Cecilia Williams — KTH and Karolinska, Stockholm

ANTIBODY SOCIETY

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 ?

ANTIBODY SOCIETY

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

Writen by Simon Goodman

https://www.antibodysociety.org/

Validation of Commercial Tool Antibodies

An Antibody Society Webcast series

https://www.antibodysociety.org/

Administrative Support: Dr. Fran Breden and Dr. Mini Muralidharan

Executive Director: Dr. Jan Reichert

This series would be impossible without the generous financial support of our Corporate Sponsors:

ABLEXIS
ADIMAB
aldevron
AlivaMab DISCOVERY SERVICES
Antibody Solutions
biotechne
Boehringer Ingelheim
Bristol-Myers Squibb
Fusion
geneious biologics
Genentech A Member of the Roche Group
gsk GlaxoSmithKline
GRIFOLS
IMMUNOPRECISE
IONTAS
Merus
MOLECULAR partners
morphosys
NBE therapeutics innovating medicines
OmniAb
Pfizer
Roche
SANOFI
SeattleGenetics
SPARRING bioconsult
Specifica
TRIANNI
TWIST BIOSCIENCE
ucb
xencor
YUMAB

Contact us at info@antibodysociety.org or +1 (508) 808-8311 to become a corporate sponsor!

ANTIBODY SOCIETY