ESTROGEN RECEPTOR AS A PREDICTICE FACTOR. 30 YEARS OF DEVELOPMENT IN ANALYTIC METHODS

Birgitte Bruun Rasmussen

DBCG, Pathology Board

TARGETED TREATMENT IN 1896

Classics in Oncology

George Thomas Beatson, M.D. (1848-1933)

Serena Stanford

"His paper is worth a complete reading by any surgeon who is concerned with the total treatment of breast cancer. It is also, we believe, a classic in the use of man's perceptive observations, based on indirectly associated problems at hand, in rational reconstruction and ultimate usage with either success or failure. His report is written in a lucid and beautifully narrative fashion. He intuitively developed a thesis which he then proceeded to explore in the laboratory. Guided by his clinical observations and knowledge of the disease process, he proceeded to apply a mode of treatment which has become a standard therapy in advanced breast carcinoma."

Charles F. MacMahon, M.D., and John L. Cahill, M.D.

Sir George Thomas Beatson, M.D., has been called the father of endocrine ablation in cancer management. His treatment of oophorectomy for advanced breast cancer is now standard therapy.

His classic paper, "On the Treatment of Inoperable Cases of Carcinoma of the Mamma: Suggestions for a New Method of Treatment, with Illustrative Cases," was

Three cases of metastatic breast cancer treated with oophorectomy. One"cured", two partial response.

A TWO-STEP MECHANISM FOR THE INTERACTION OF ESTRADIOL WITH RAT UTERUS*

By E. V. Jensen, T. Suzuki, T. Kawashima, W. E. Stumpf, P. W. Jungblut, and E. R. DeSombre

Ben May Laboratory for Cancer Research and Departments of Physiology and Biophysics, University of Chicago

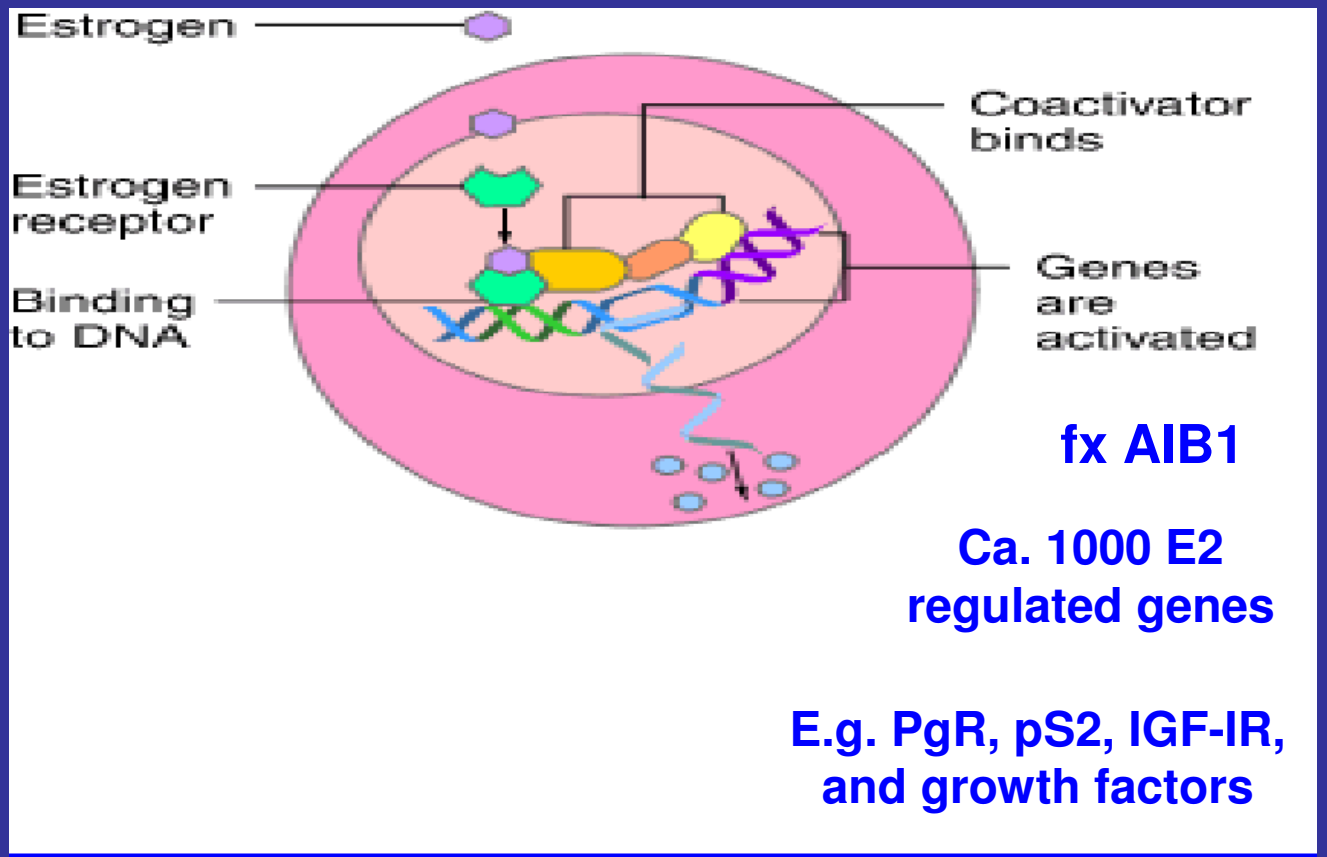
Proc Natl Aca Sci USA 49; 632, 1968

ESTROGEN RECEPTOR PROTEIN

Located in the nucleus.
Molecular weight of app. 200.000 KD
387 amino acids
Coded for by *ESR1*, located at 6q

Two forms:
ERα and ERβ

Estrogen receptor (ER)



fx AIB1

Ca. 1000 E2 regulated genes

E.g. PgR, pS2, IGF-IR, and growth factors

Hormone-receptors (HR)

- Nuclear-bound
- Positive in app. 80% of breast carcinomas

  ER-pos./PgR-pos.

  ER-pos./PgR-neg.

  ER-neg./PgR-pos.

  ER-neg./PgR-neg.

- App. 60% of HR-positive patients respond to endocrine treatment

ESTROGEN RECEPTOR ASSESSMENT

EXTRACTION ASSAYS:

DCC
ELISA

IMMUNOHISTOCHEMISTRY

Fresh Frozen Tissue
Paraffin Embedded Tissue

EXTRACTION METHODS

DCC:
Dextran-Charcoal-Coated method
Radioactive conjugated estrogen
Indirect method

ELISA
Enzyme-Linked Sorbent Assay
Monoclonal antibody against ER
Direct method

EXTRACTION METHODS

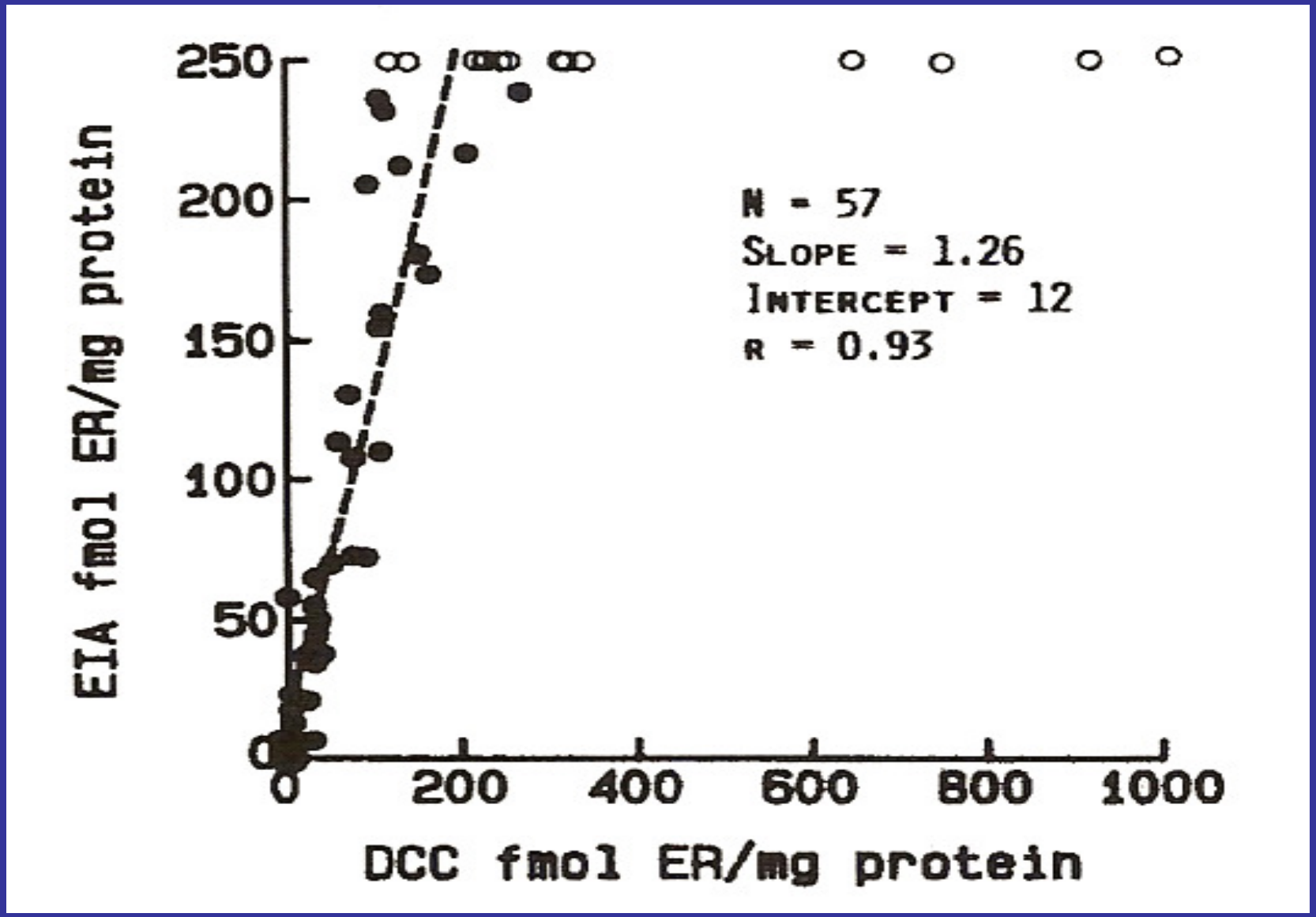
DCC – ELISA

Requirement:
Fresh , crushed tissue

Results quantitative, in fmol/mg protein

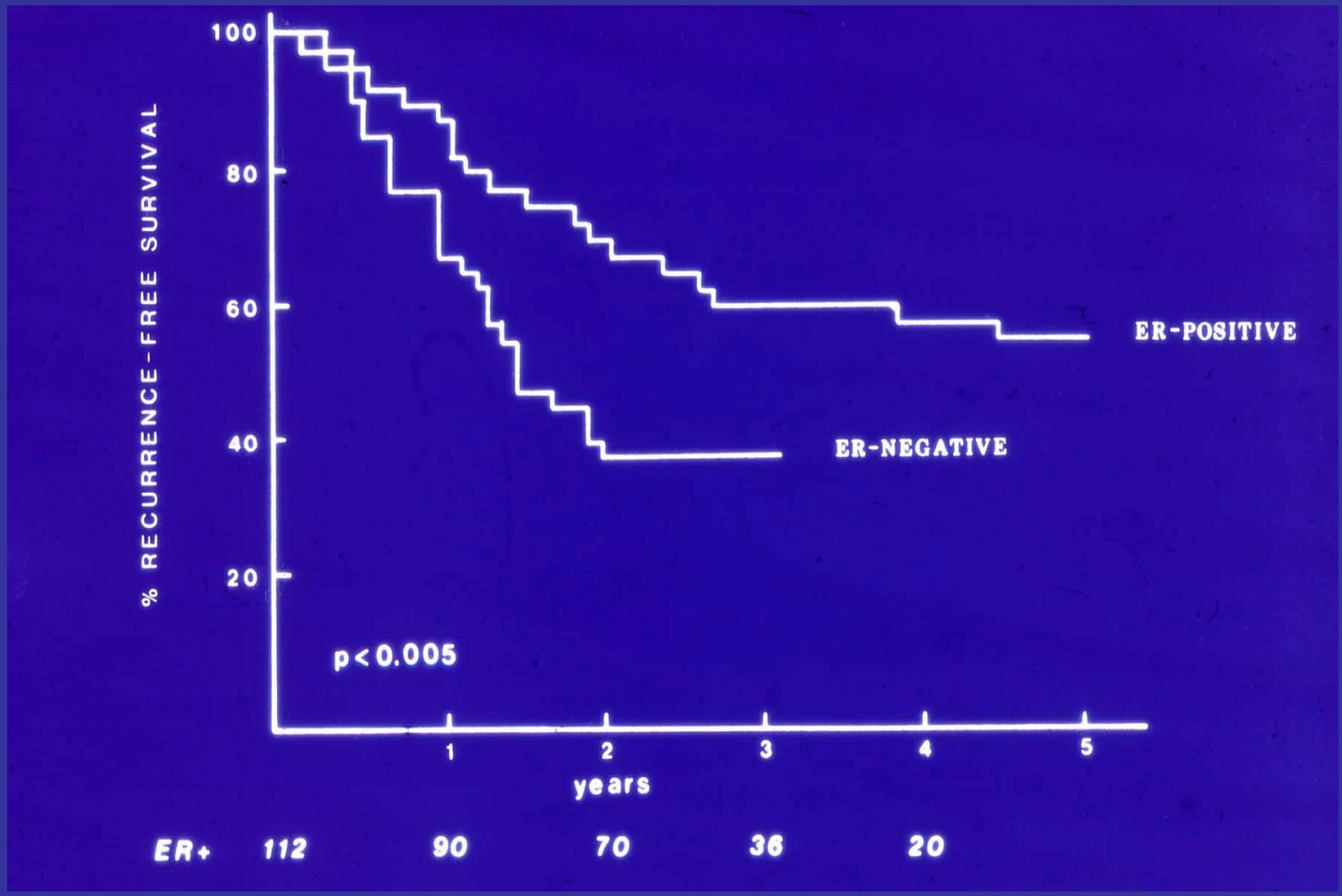
Positive reaction: ≥10 fmol/mg protein

CORRELATION BETWEEN DCC AND ELISA



Andersen et al. Eur j cancer clin oncol 24: 377, 1988

DCC-ASSAY AS A PROGNOSTIC FACTOR



DCC-ASSAY AS A PREDICTIVE FACTOR

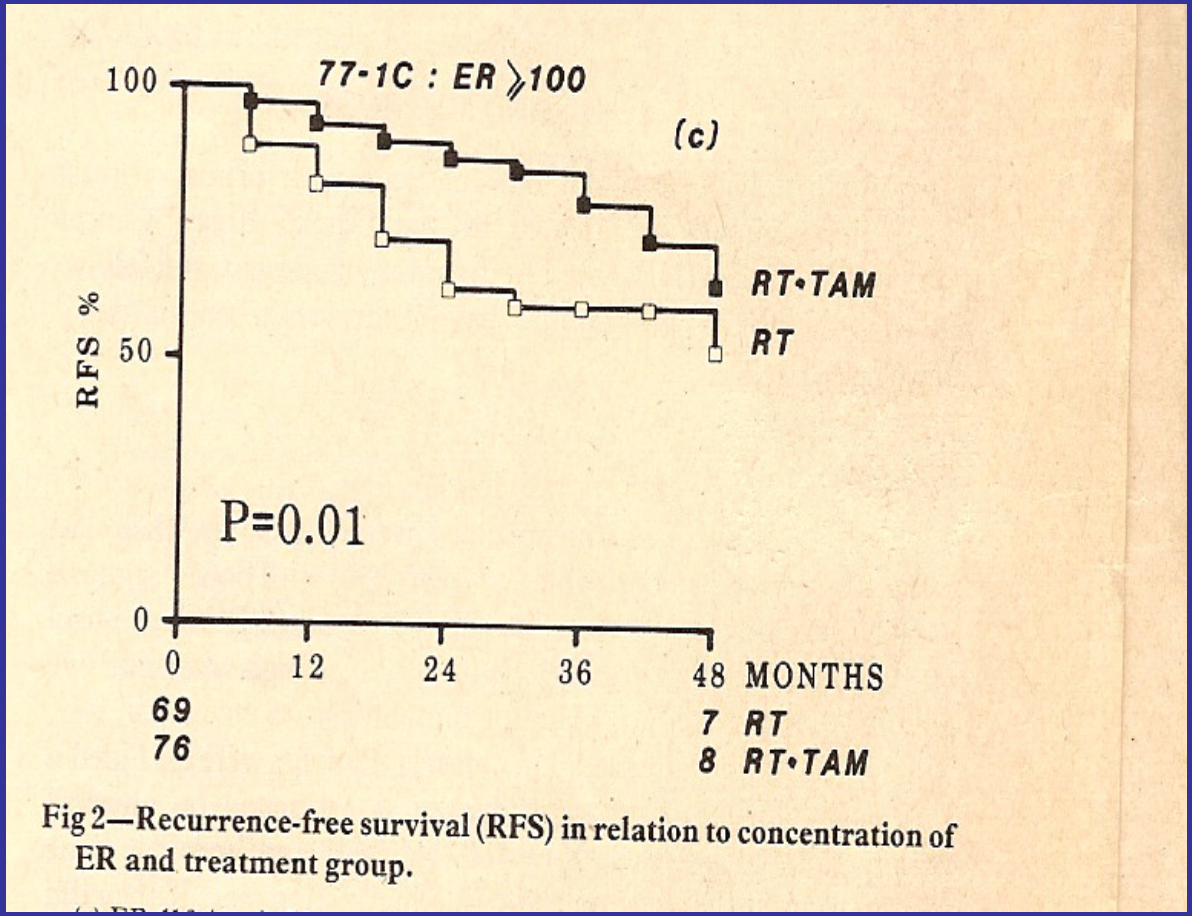


Fig 2—Recurrence-free survival (RFS) in relation to concentration of ER and treatment group.

Rose C et al. Lancet 1985: 16-19

INTERLABORATORY VARIATION

Tabel 2: Biokemisk receptoranalyse.

	Receptor-negativ	Receptor-positiv	Total
Afdeling	N(%)	N(%)	N
Fibiger	328 (20)	1344 (80)	1672
Århus	81 (25)	242 (75)	323
Aalborg	42 (13)	283 (87)	325
Total	451 (19)	1869 (81)	2320

EXTRACTION METHODS

- ADVANTAGES
- Quantitative results
- Fresh tissue banking

- DISADVANTAGES
- No morphology
- Large amounts of tissue

MONOCLONAL ANTIBODIES

Greene GL, Nolan C, Engler JP, Jensen EW: Proc Natl Acad SCi USA, 77: 5115, 1980

<u>METHODS:</u>
ELISA
Immunohistochemistry (IHC)- frozen tissue
IHC- paraffinembedded tissue

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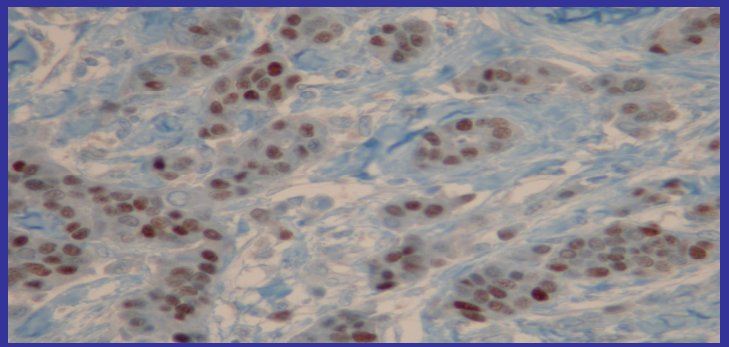


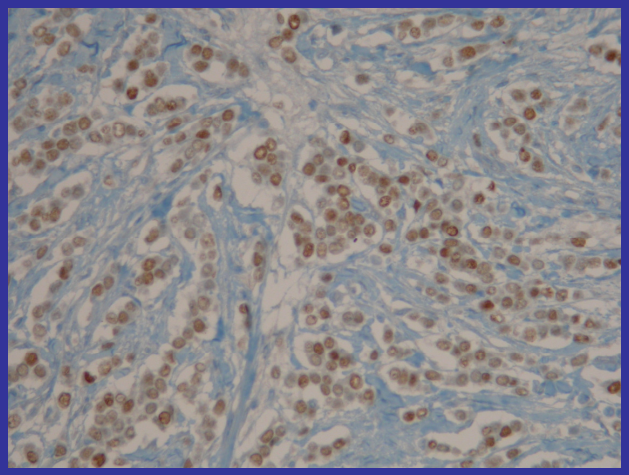
ASSESSMENT METHODS

- **PERCENTAGE POSITIVE CELLS**
- **Positive ≥ 10%**

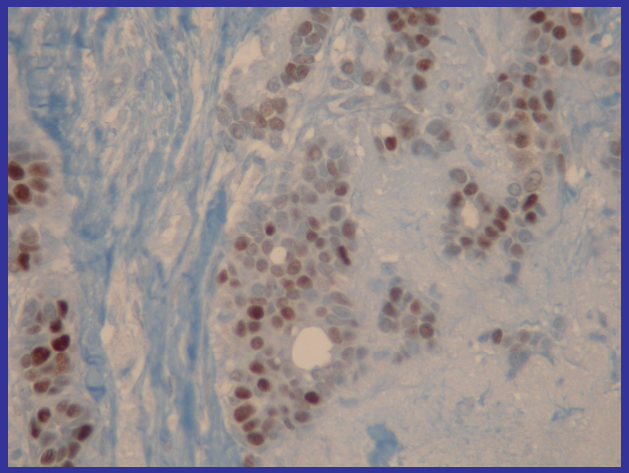
- **ALLRED SCORE:**
- **Percentage positive cells + intensity score**
  0, 1(<10%), 2(10-30%), 3(30-60%), 4(>60%)
  0, 1 weak, 2 intermediate, 3 strong
- **Points from 0 -8, positive ≥ 2**

- **H-SCORE:**
- **(intensity +1) x percentage positive cells.**
  $\Sigma P_i (i+1)$
- **Points from 0-500, positive ≥ 75**

ASSESSMENT METHODS



- 100% positive cells
- Allred score: 6



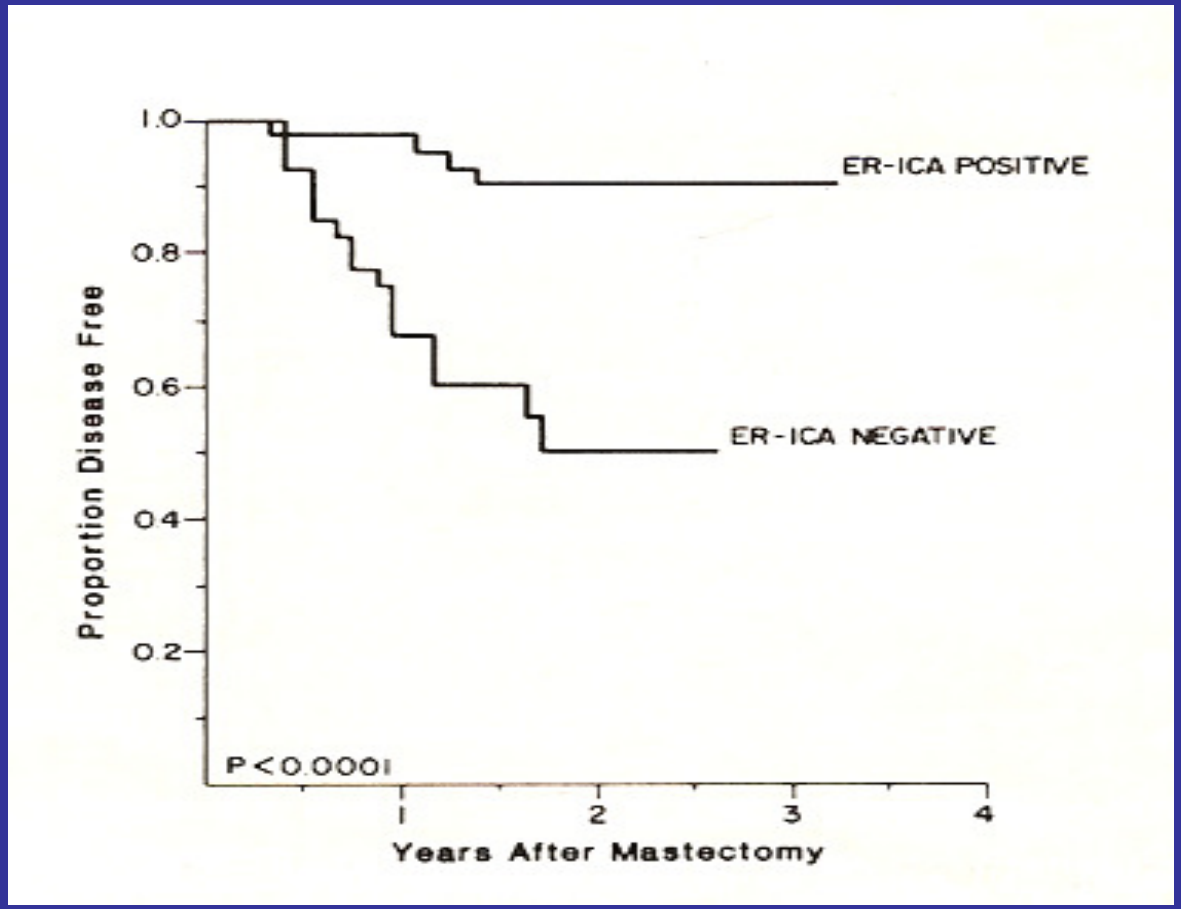
- 70% positive cells
- Allred score: 6

CONCORDANCE, IHC/ BIOCHEMISTRY

Table 2. Biochemical (BCA) and immunohistochemical (IHC) ER analysis in 2364 tumours			
BCA\IHC	**ER Positive (%)**	**ER Negative (%)**	**Total (%)**
ER positive	1560 (82)	343 (18)	1903 (80)
ER negative	48 (10)	413 (90)	461 (19)
Total	1608 (68)	756 (32)	2364 (100)

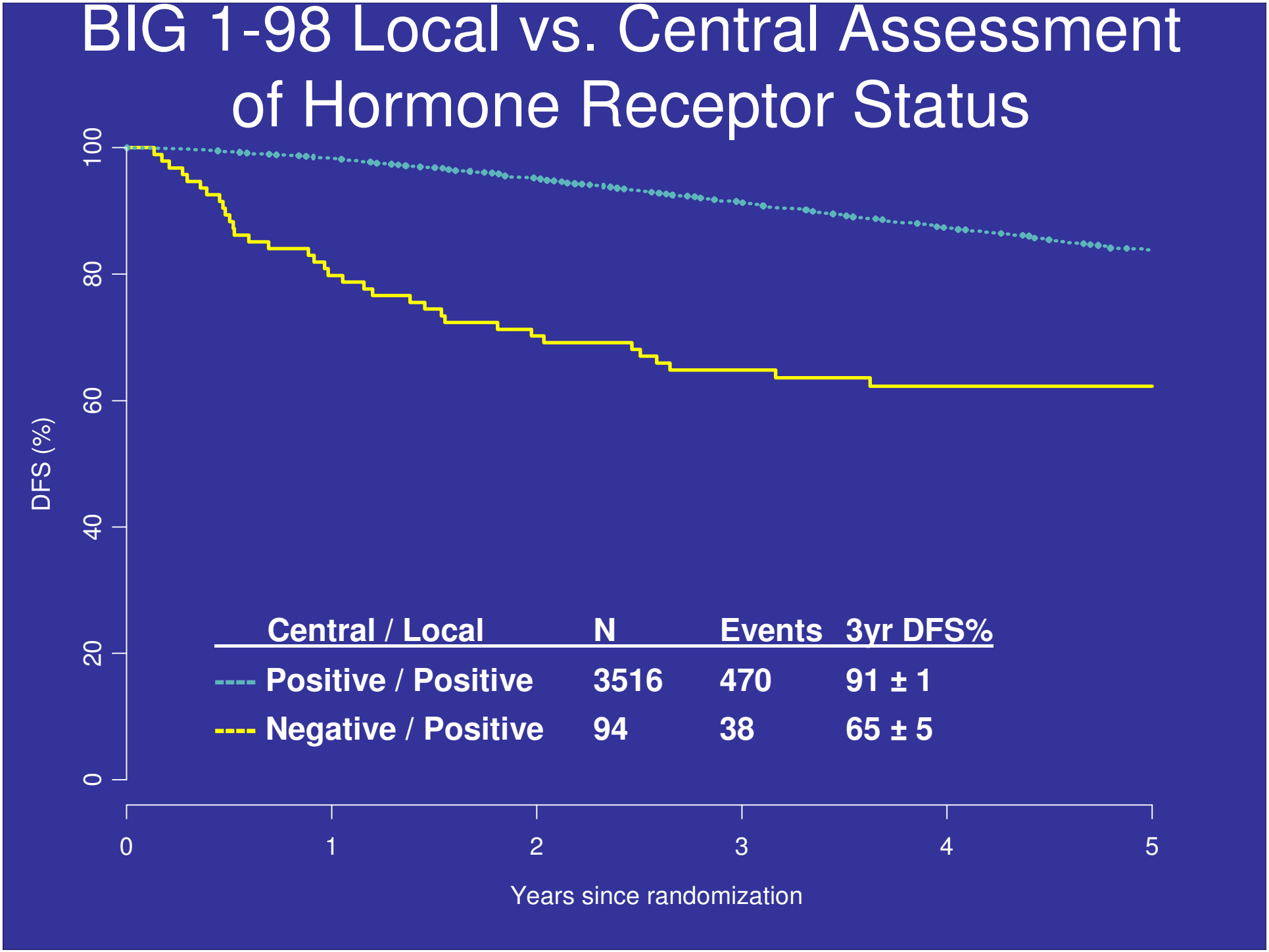
Talman M-J et al. Acta Oncol epub 2008

IHC ASSAY AS A PROGNOSTIC FACTOR



DeSombre ER et al Cancer Res (Suppl) 46, 4256; 1986

BIG 1-98 Local vs. Central Assessment of Hormone Receptor Status



METHODOLOGY
IHC ON FFPE-TISSUE

No standardization of methods – ”home brew” protocols

No standardization of assessment method
- **% positive cells**
- **Allred-score**
- **H-score**

No standardization of antibodies used – min. 70 clones
- **ID5**
- **SPI 1**
- **6F11**

QUALITY ASSURANCE

- UK-Nequas
- NordicQ
- Optimal good borderline poor

  52% 32% 15% 1%

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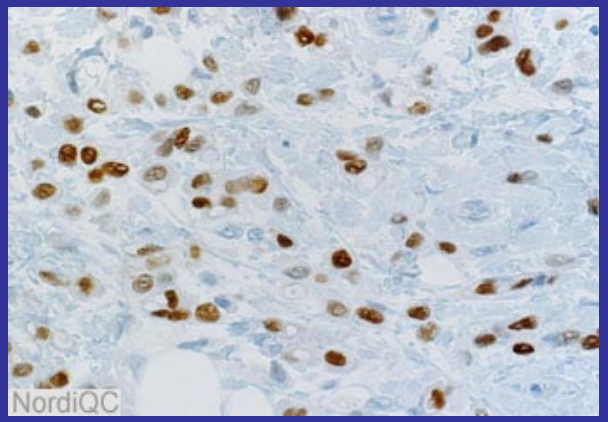
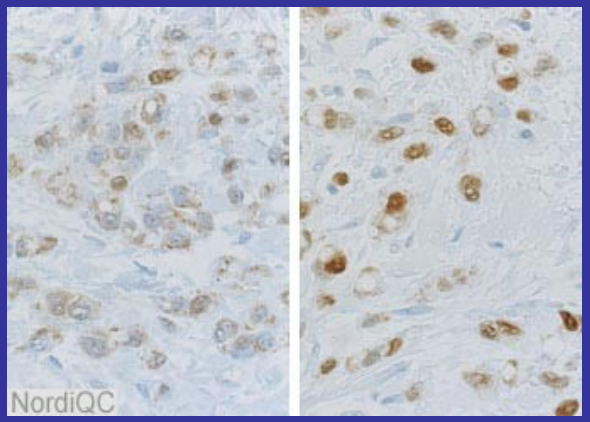




IHC

ADVANTAGES

- Preserved morphology
- Small pieces of tissue (core biopsies, metastases)
- Decentral analysis
- Fast

DISADVANTAGES

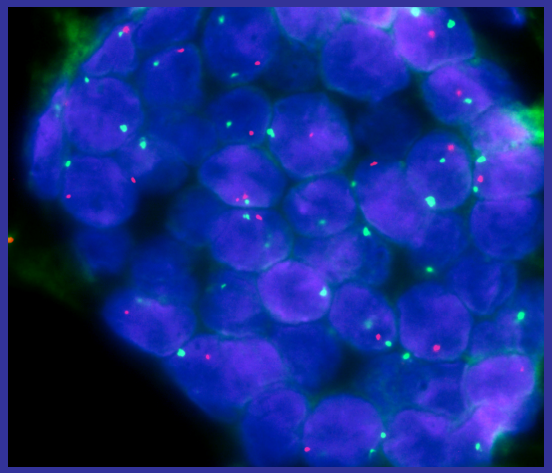
- Subjective evaluation
- Semiquantitative –at best
- Inter –and intra laboratory variation

ER CODING GENE *ESR1*

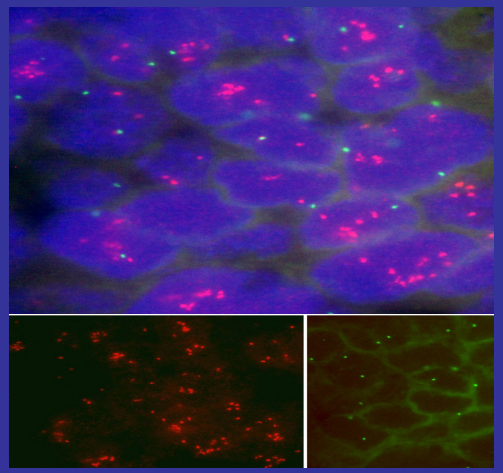
- Located on chromosome 6q
- Detection by FISH
- Normal gene copies, amplifications, deletions
- Amplification: ratio gene/chromosome ≥ 2.0
- Deletion: ratio gene/chromosome ≤ 0.8

ER CODING GENE *ESR1*

- Located on chromosome 16q
- Detection by FISH
- Normal gene copies,amplifications,deletions
- Amplification: ratio gene/chromosome ≥ 2.0
- Deletion: ratio gene/chromosome ≤ 0.8



***ESR1* deletion**



***ESR1* amplification**

CONCORDANCE BETWEEN *ESR1* AND IHC

Central ER	*ESR1* status						
	Deleted		Normal		Amplified		Total
< 10%	117	(75%)	254	(62%)	5	(63%)	376
≥ 10%	39	(25%)	155	(38%)	3	(38%)	197
Total	156		409		8		573

DISTRIBUTION OF DELETED, NORMAL AND AMPLIFIED CASES IN PATIENTS WITH EARLY AND LATE RECURRENCE

ESR1	Early recurrence e.g. within 4 years	Disease-free 7 years or more	Total
Deletion	2 (4%)	2 (5%)	4 (4%)
Normal	39 (75%)	38 (90%)	77 (82%)
Amplified	11 (21%)	2 (5%)	13 (14%)
Total	52	42	

SUMMARY

- Estrogen receptor target for endocrine treatment since 1968
- Development in analytical methods – indirect – direct (monoclonal antibodies)- genomic methods (FISH).
- From central biochemistry to decentral immunohistochemistry (IHC).
- IHC standard method in pathology departments. Part of diagnostic routine report in breast cancer

FUTURE

- Detection of gene abberations a better predictor?
- Other endocrine markers?