

# Molekulare Testung: Welche Systeme werden klinisch relevant?

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# Disclosure of potential conflicts of interest

Potentielle Interessenkonflikte bestehen mit

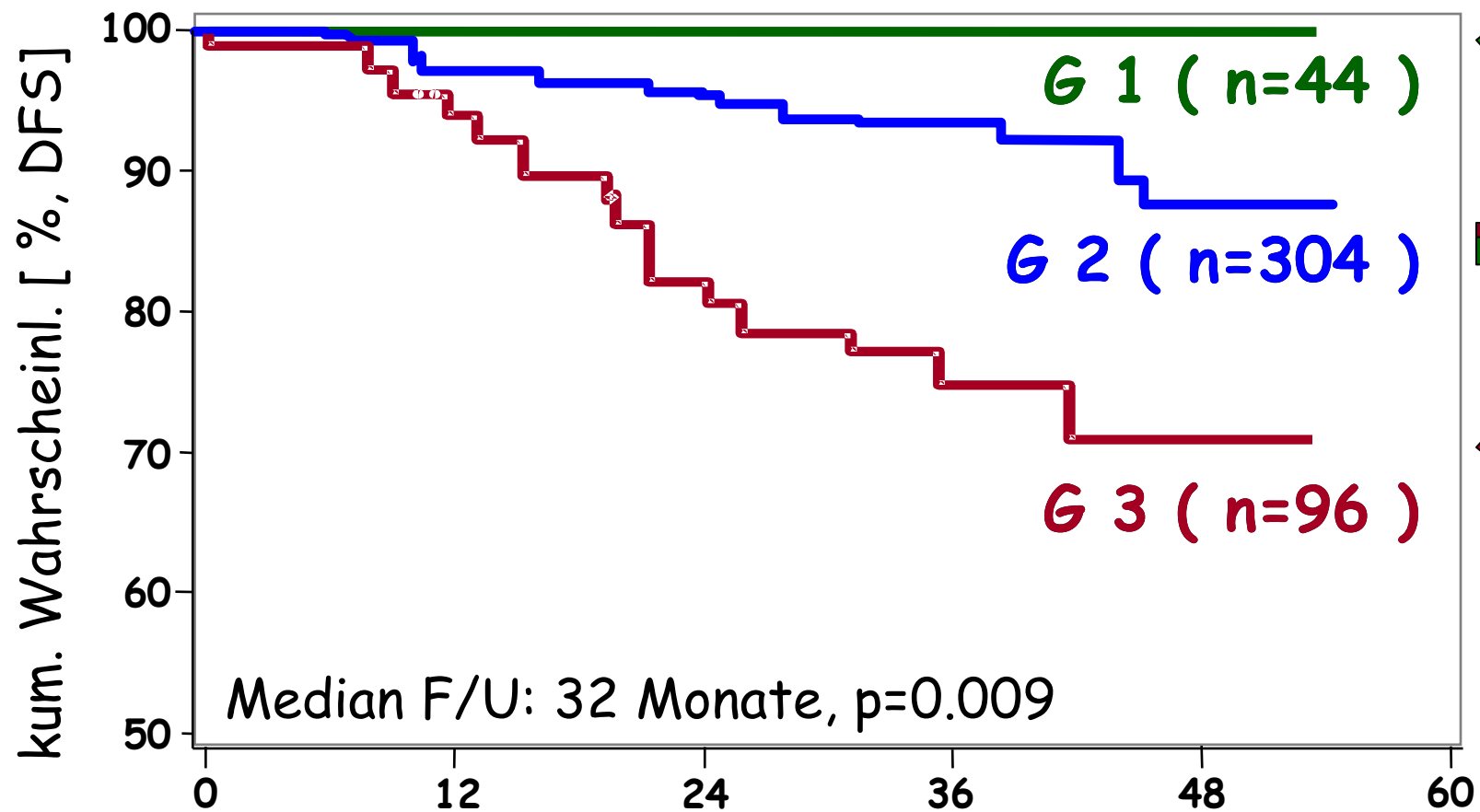
- Hoffmann-La Roche (H,A,F,T)
- Sanofi-Aventis (H,A,F,T)
- Pfizer (H,A,F,T)
- American Diagnostica (F)

(Honorare (H), Advisory Boards (A), Forschungskooperation (F),  
Tagungsreisen (T))

# Prognose und Prädiktion: Molekulare Teste

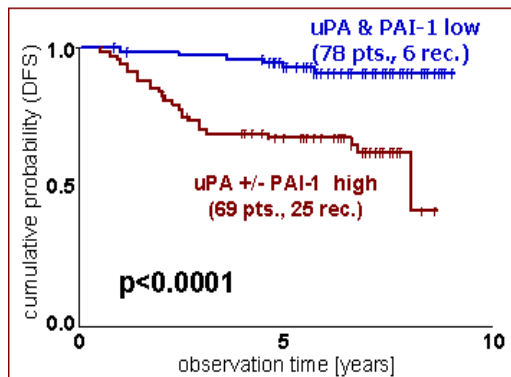
- Proteinassays:
  - uPA / PAI-1
- Proliferation assays:
  - Ki-67 (MIB-1)
  - TLI (Thymidine-  
Labelling Index)
  - S-Phase-Fraction
- Andere (Cycline, ...)
- Multigen-Profile:
  - 21-gene assay  
(Oncotype DX<sup>®</sup>)
  - 70-gene profile  
(Mammaprint<sup>®</sup>)
  - Genomic grading  
(GGI/MGI)
  - H/I (*HOXB3/IL17BR*)
- Methylation assays:
  - PITX2

# Grading wirklich brauchbar?



# uPA / PAI-1: Validation of clinical utility in node-negative breast cancer

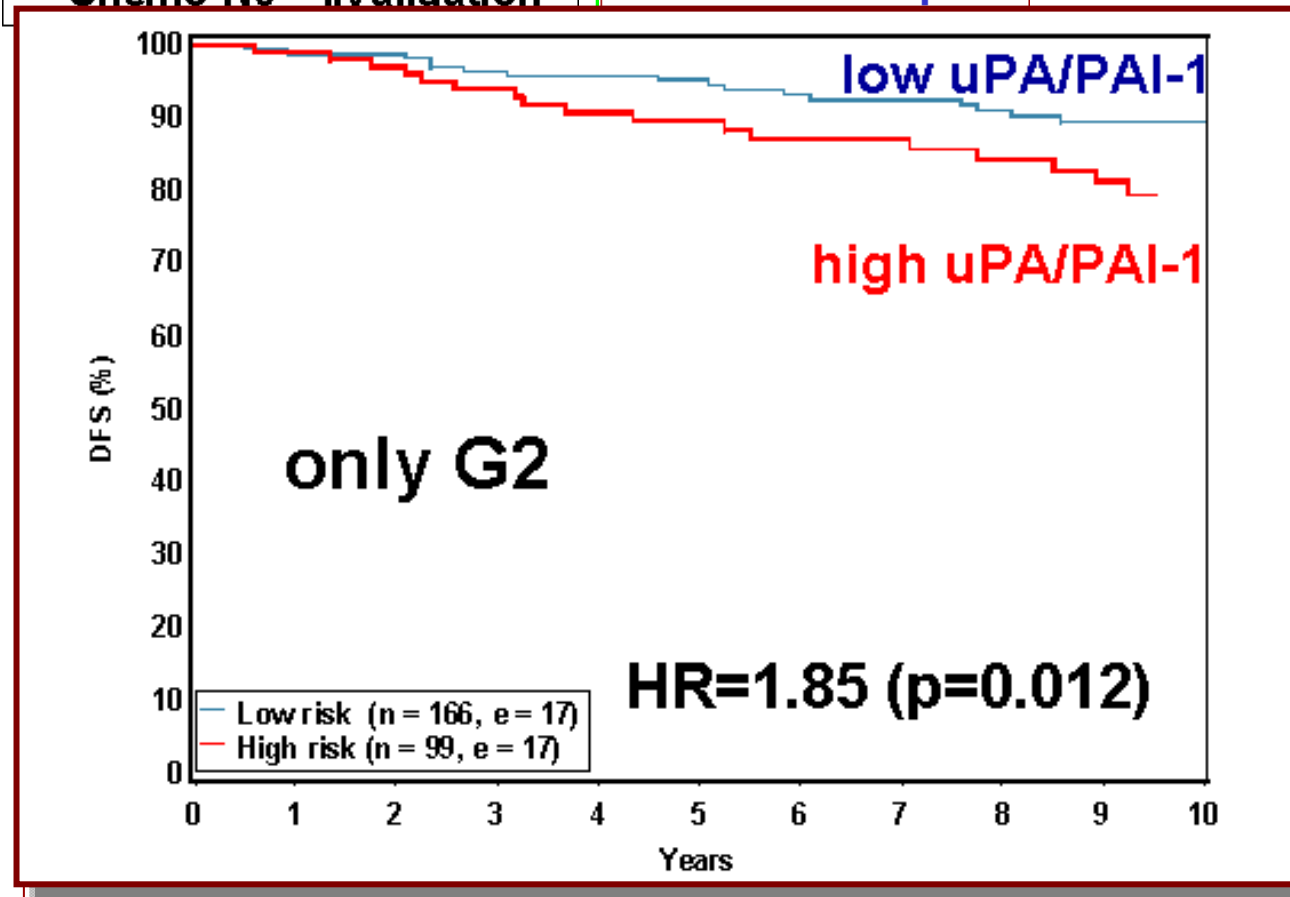
1987-91  
**MaCa I – „Pilot“**  
 unicenter (TU Munich)



**EORTC RBG  
 Pooled analysis**

1993-98  
**Chemo-N0 – „Validation“**

2003 - 2009  
**NNBC 3-Europe**



Summary	Not Recommended	Recommended
P53	Screening, diagnosis, staging, prognosis, surveillance, or monitoring.	
Cathepsin D	Screening, diagnosis, staging, prognosis, surveillance, or monitoring.	
<b>uPA and PAI-1</b>	Screening, diagnosis, staging, surveillance, or monitoring.	To determine prognosis. For treatment planning. To guide use of CMF-based adjuvant chemotherapy.

- Cyclin E Fragments
- Proteomic Analysis



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in der DGGG e.V.  
sowie  
in der DKG e.V.  
Guidelines Breast  
Version 2009.1.0

Further Information

References

FORSCHEN  
LEHREN  
HEILEN

## Prognostic Factors in Node-Negative Breast Cancer

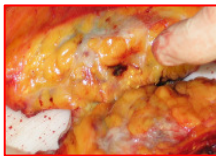
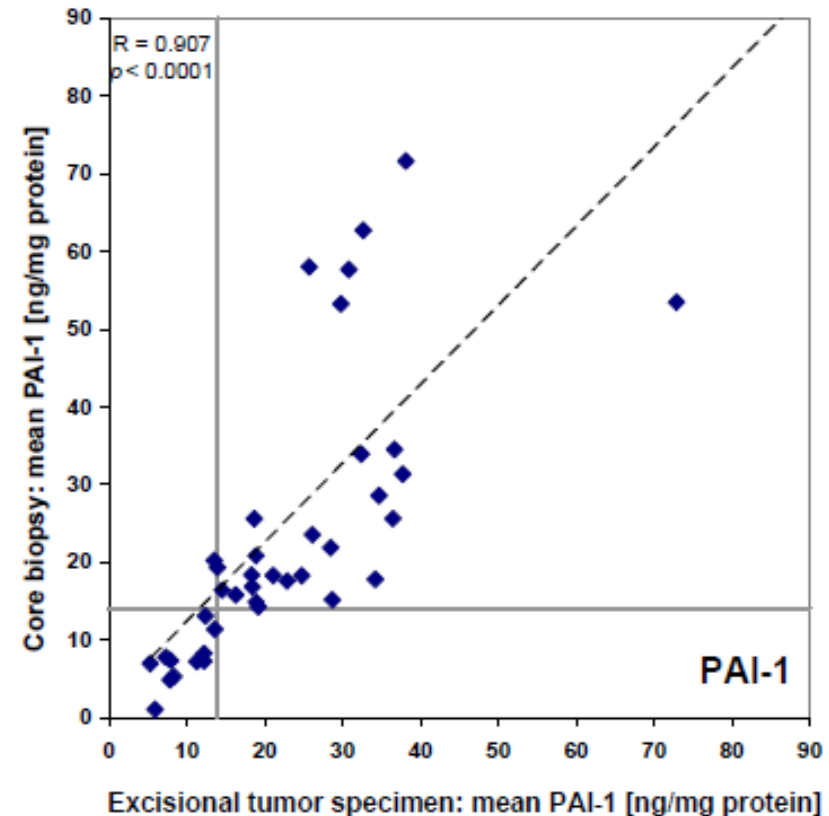
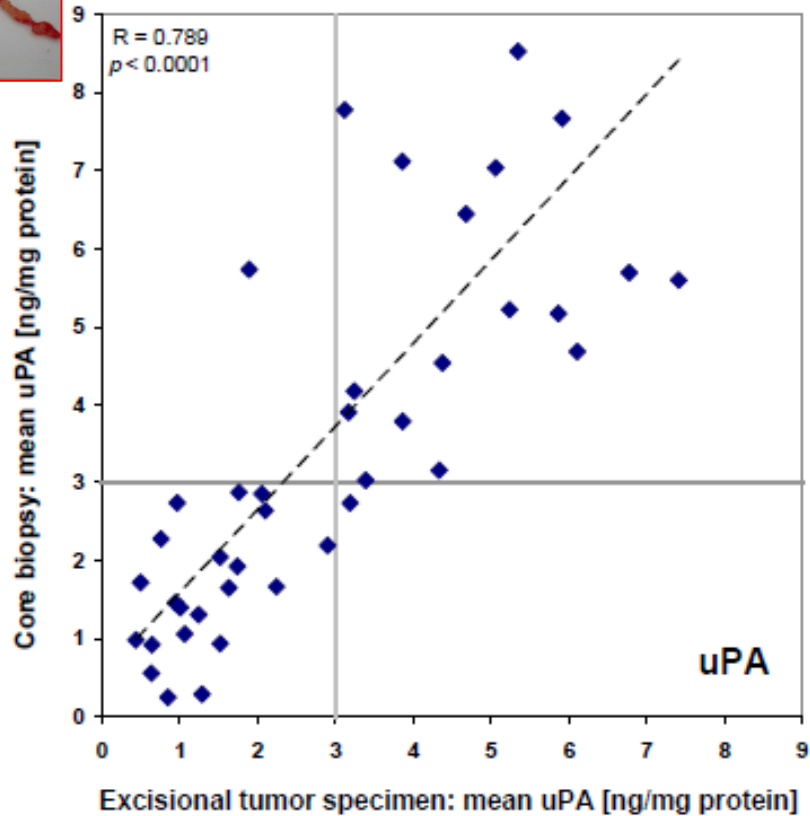
Factor	Oxford / LoE	AGO / GR	Patients with node-negative breast cancer who will receive adjuvant tamoxifen and endocrine therapy (ET) (usually CMF).
➤ Grade	2b B	++	
➤ Tumor size	2b B	+	
➤ Age	2b B	+	
➤ uPA / PAI-1 (ELISA)	1a A	+	
➤ Proliferation (SPF, TLI, Ki-67)	2b C	+/-	
➤ Oncotype DX™	2b B	+/-*	
➤ Mammaprint™	2b B	+/-*	

\*Study participation recommended

# Biomarker determination in core needle biopsies (uPA/PAI-1, n=42)



Core Biopsy Specimen



Excisional Tumor Specimen

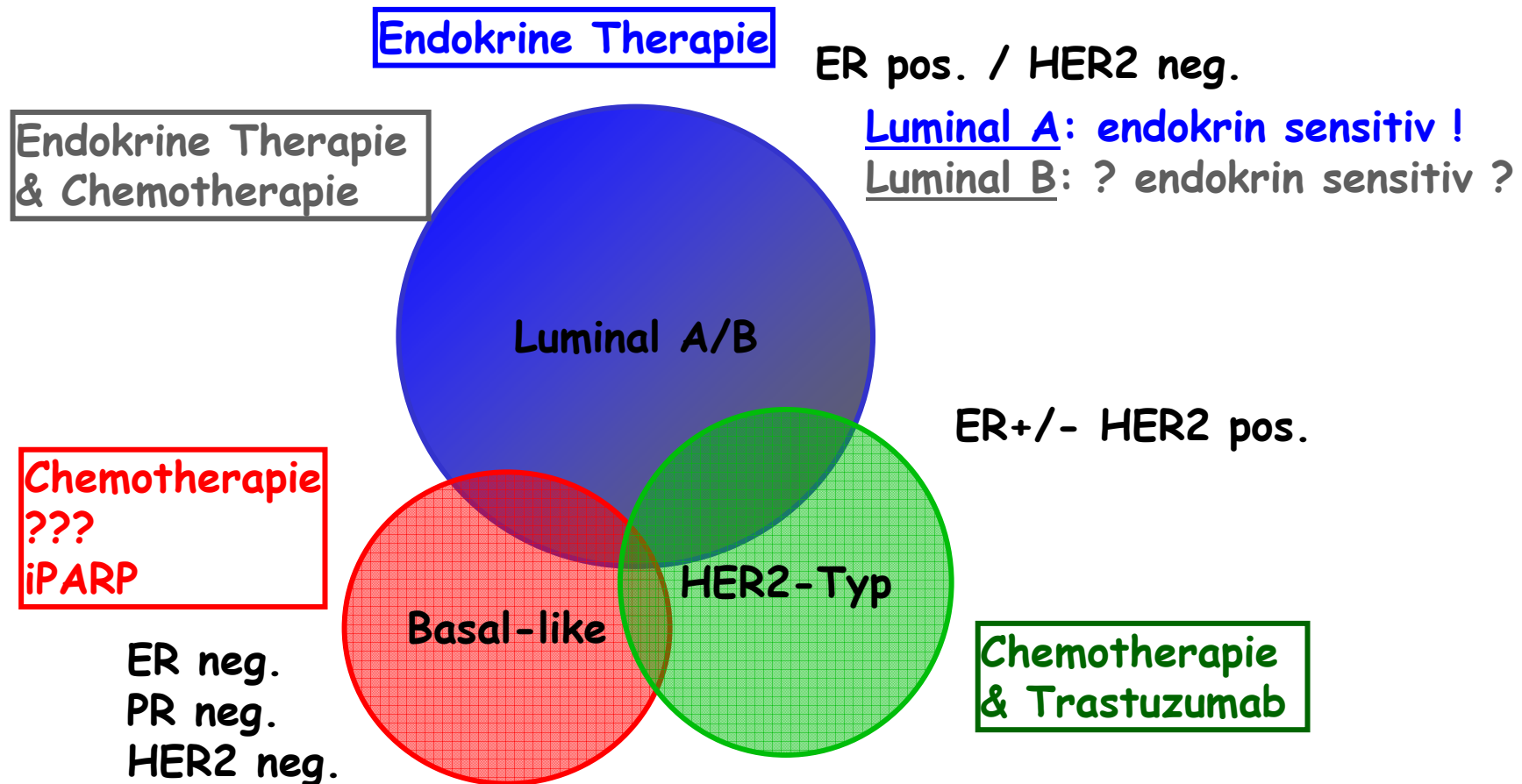
# Validierung uPA/PAI-1

- Biologisches Modell: Proteolyse, Invasivität
- Assay: stabil, QS ok; Probenasservierung aufwendig
- Analyse-Randbedingungen (Stanze, Tumorgewebe-Heterogenität, Asservierungsbedingungen): untersucht
- Pilotstudie: prospektiv
- Validierung: retrospektive und prospektive Studien
- Korrelation: unabhängig
- Anteil „low risk“: bis 55%
- Therapiekonsequenz: Chemosensitivität
- Meta-Analyse: Bestätigung

⇒ **Transfer in die Routine möglich**      Kosten: ca. 200 €

# Multigen-Profile

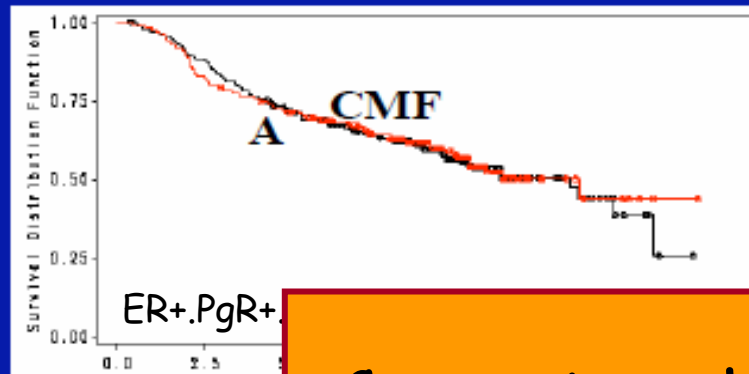
# Modell: Molekularbiologische Klassifikation und adjuvante Therapie



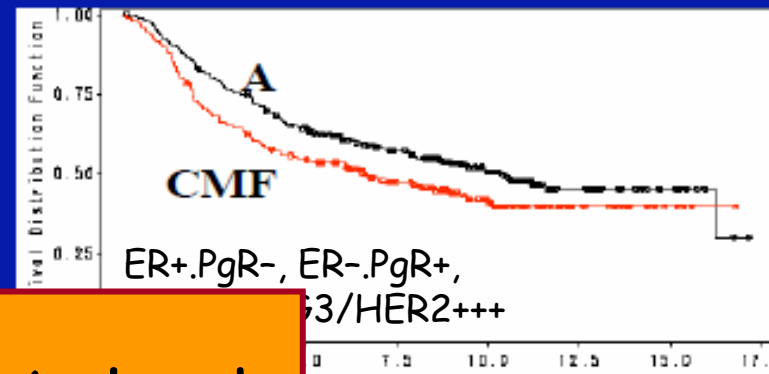
# ER / PgR / HER2: Anthrazykline

Meta-Analyse (n=1,994/ca.3,500)  
(Bordet / DBCG 89D / MA.5 / NEAT)

## Highly hormono-sensitive

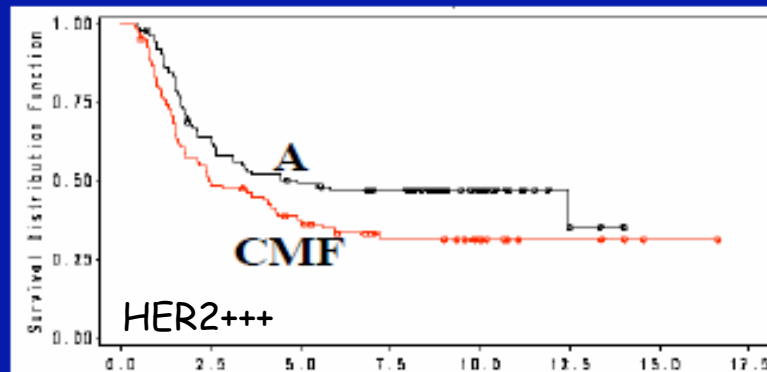


## Moderately hormono-sensitive

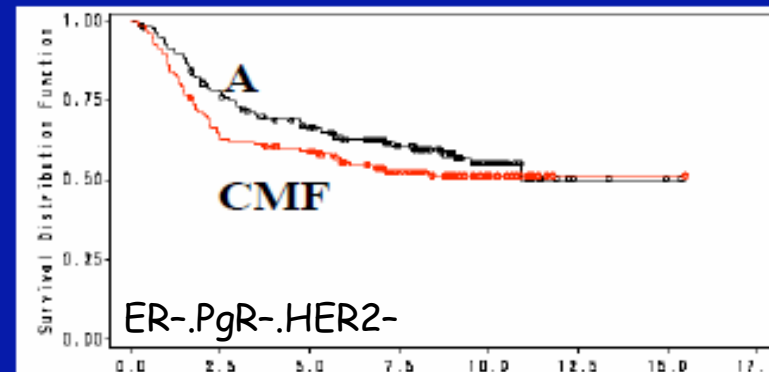


**Cave: retrospektive Analysen!**

## HER-2+

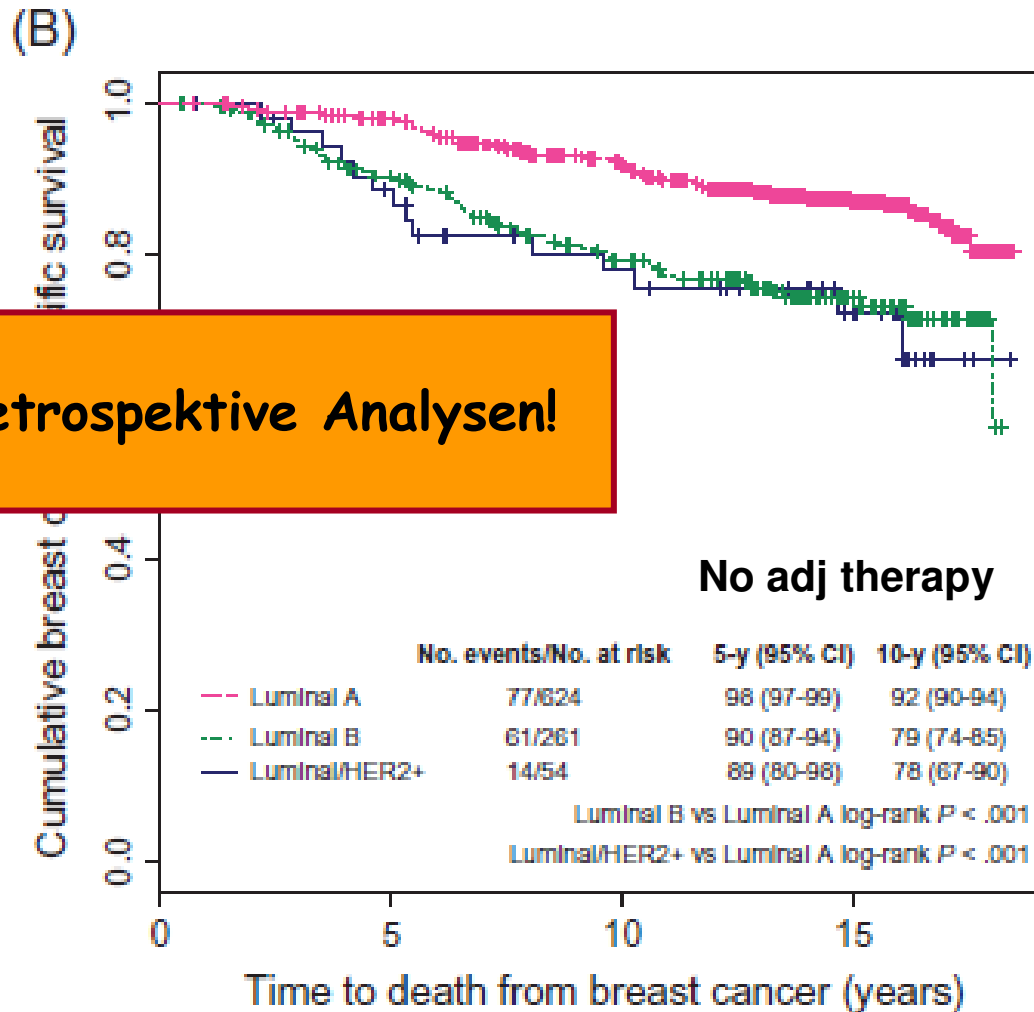


## Triple negative

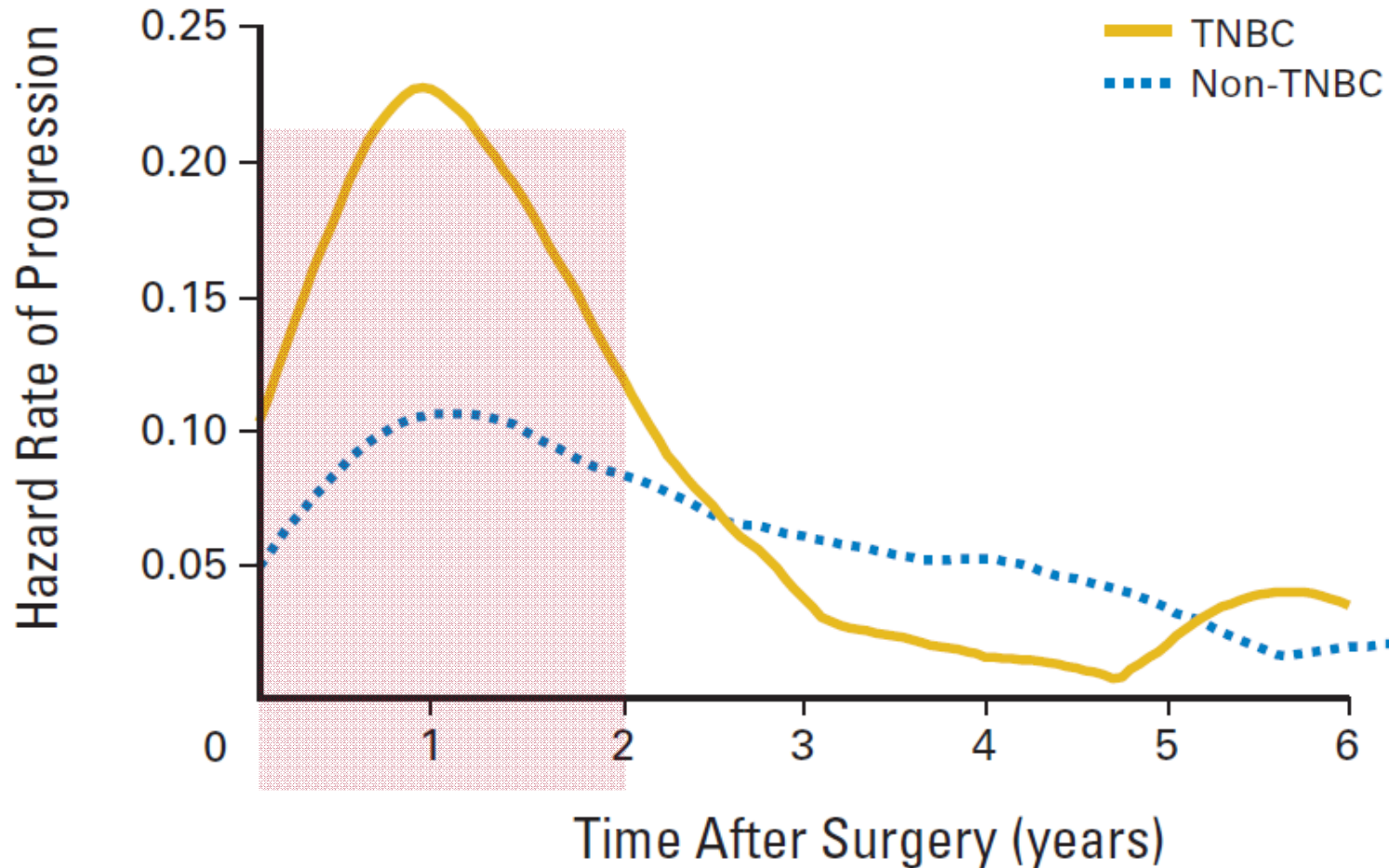


di Leo A et al. SABCS 2008, abstr. 705

# Luminal A/B (ER+) by Ki-67 & HER2



# Triple negatives Karzinom (ER<sup>-</sup> PgR<sup>-</sup> HER2<sup>-</sup>)

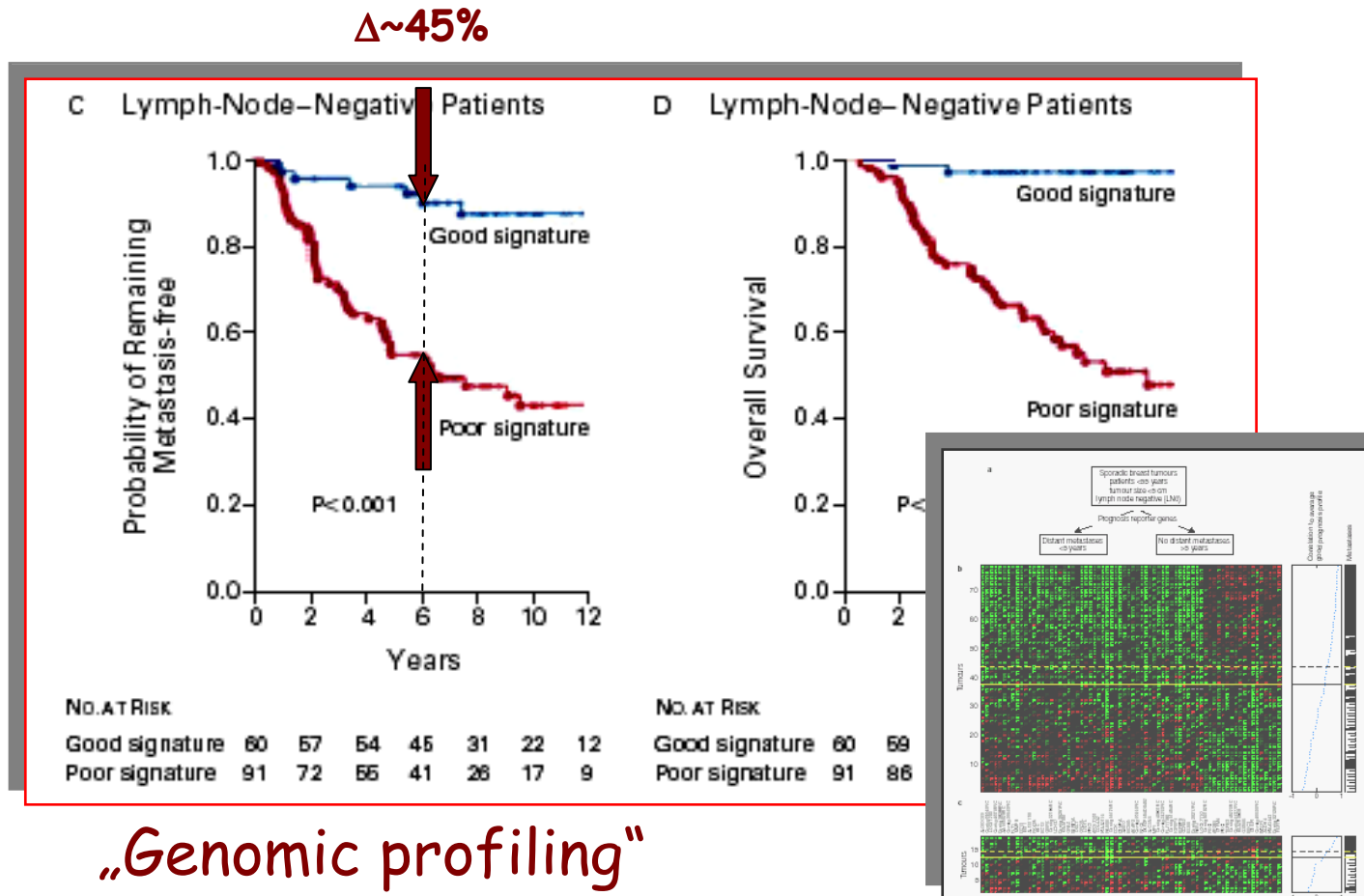


Liedtke C et al. JCO 2008; 26:1275-1281.

# Molecular typing

- Typisierung nach Genexpression  
alternativ zur histologischen Typisierung
- Ersatz durch IHC für ER/EgP/HER2 ??
  - Konkordanz << 100%
- Bisher nur retrospektive Analysen
  - Prädiktiv für Therapie-Effekte?
  - Selektion Anthrazykline / Taxane...

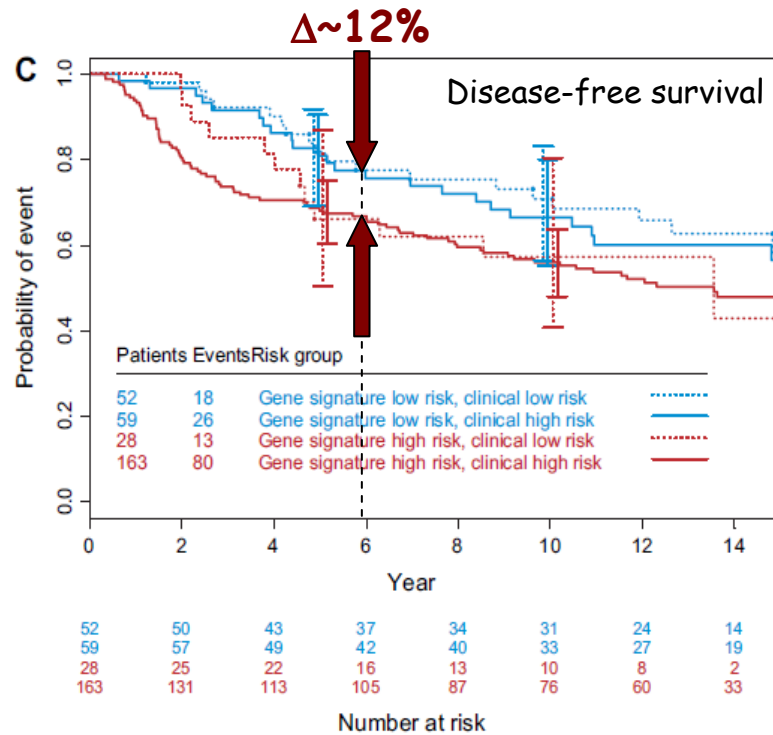
# Microarray of gene expression - a prognostic tool?



Van't Veer L et al. , Nature 2002; 415: 530-6

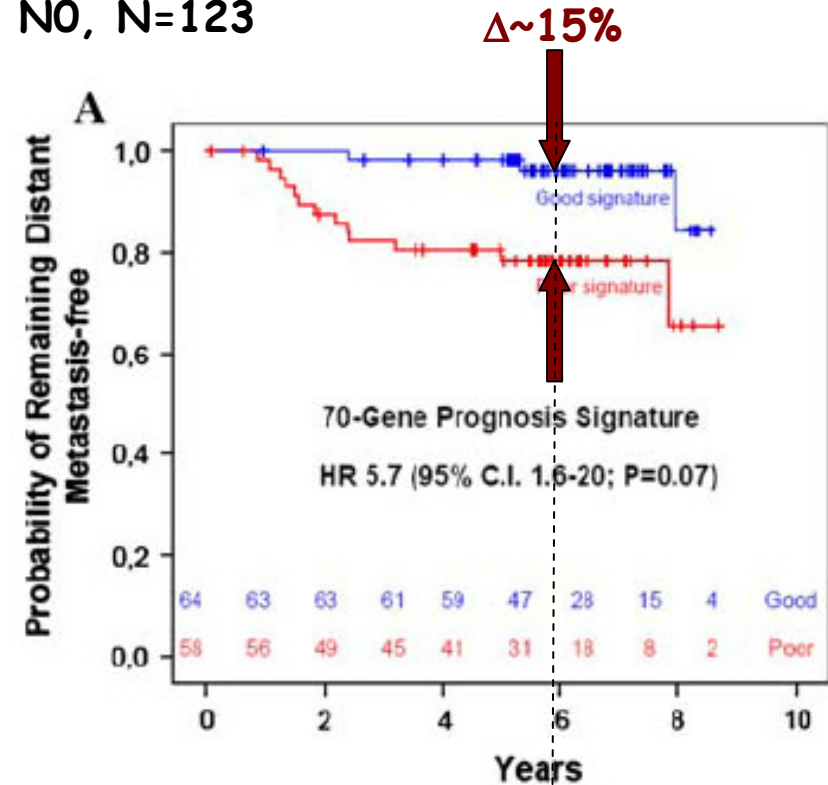
# 70-Gen-Profil: Validierung

N0, N=302



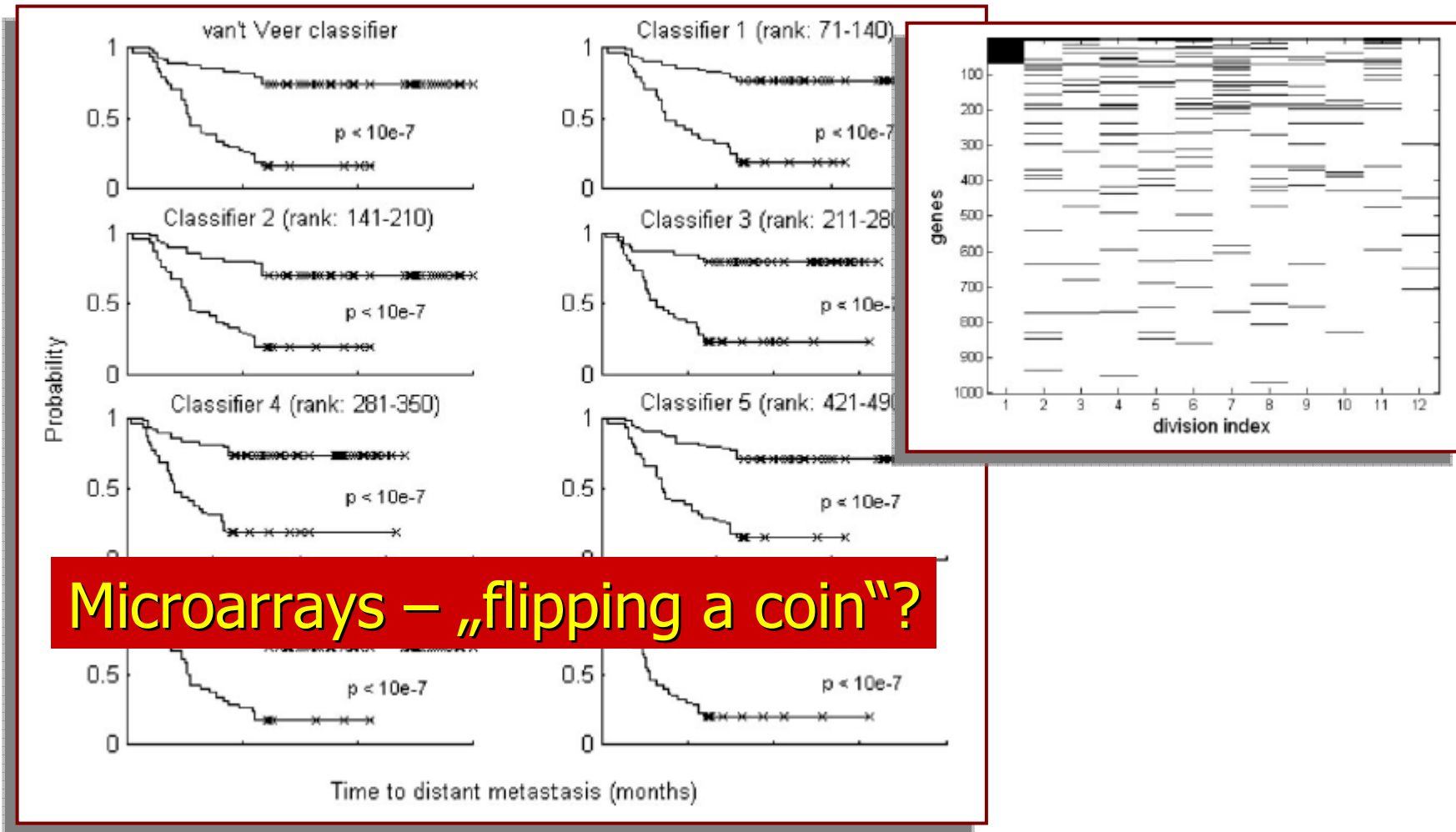
Buyse M et al. JNCI 2006

N0, N=123



Bueno de Mesquita et al. BCRT 2009

# Gene-Expressionsarrays Multiple Cluster-Analysen

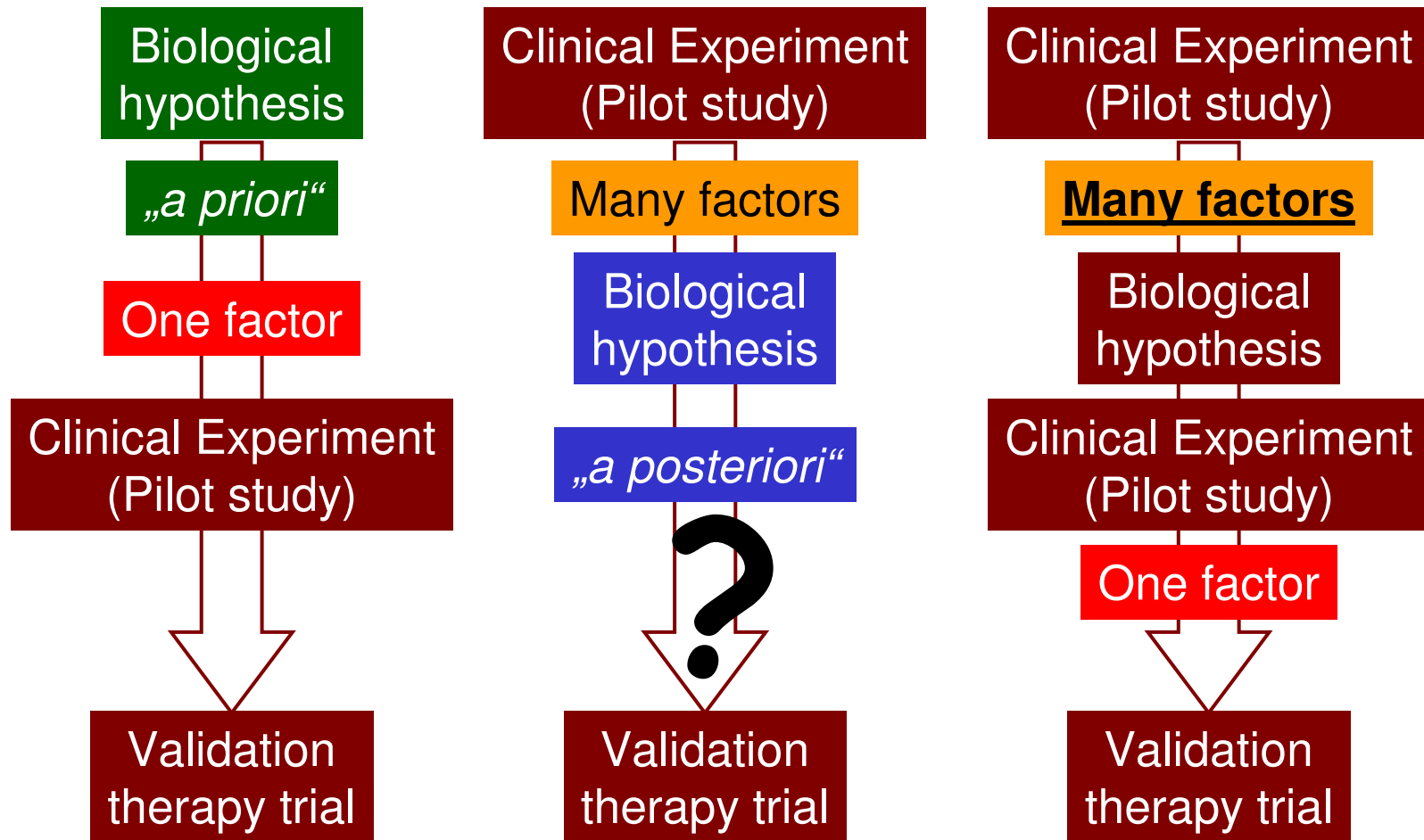


# Development of biological factors with clinical impact (e.g. progn./pred.)

„Classical research“

„gene expression profiles“

„gene expression profiles“



# Onco type DX (quantitative RT-PCR „TaqMan“)

- PCR-based assay:

Proliferation

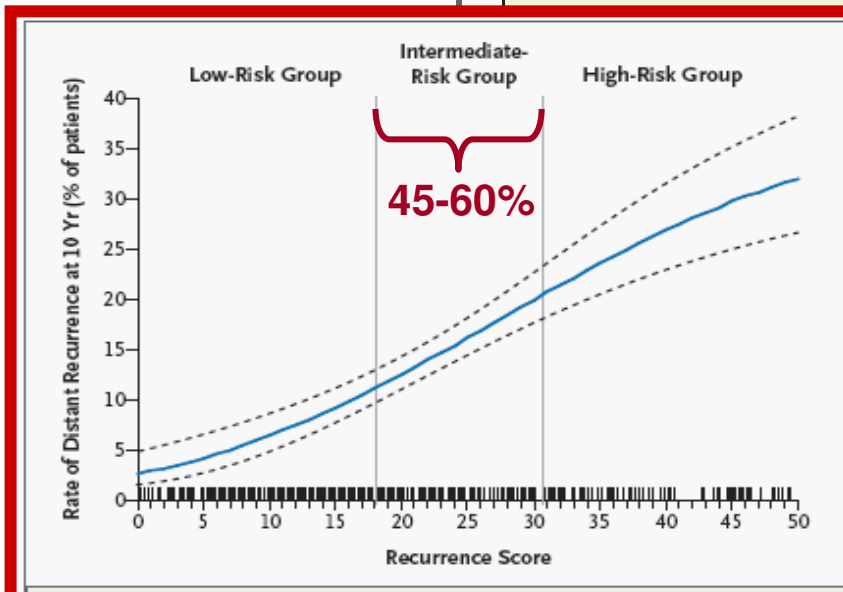
Ki67  
STK15  
Survivin

HER2

GRB7  
HER2

Estrogen

ER  
PGR  
BCL 2

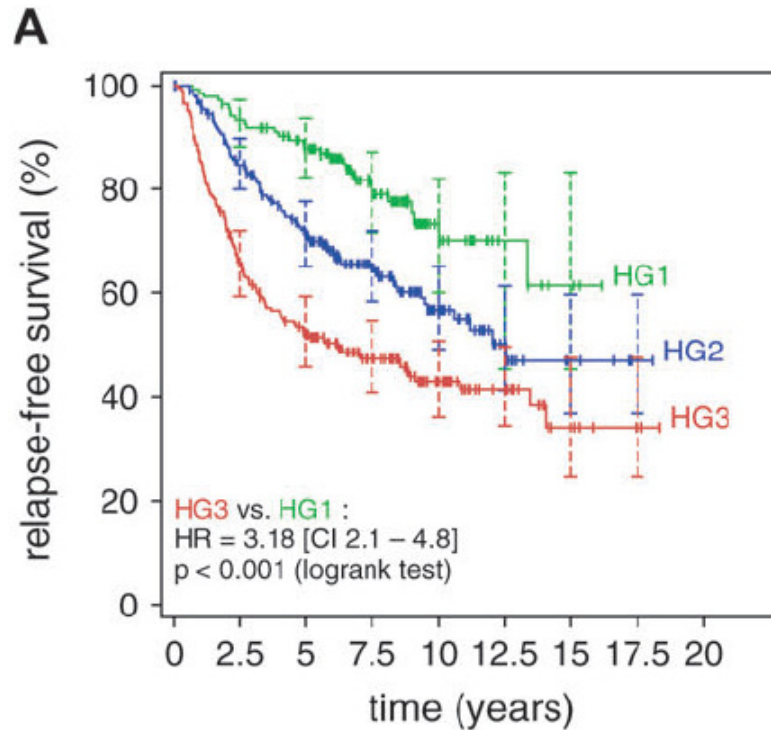


**Table 2.** Multivariate Cox Proportional Analysis of Age, Tumor Size, and Recurrence Score in Relation to the Likelihood of Distant Recurrence.\*

Variable	P Value	Hazard Ratio (95% CI)†
<b>Analysis without recurrence score</b>		
Age at surgery	0.004	0.57 (0.39–0.83)
Clinical tumor size	0.06	1.44 (0.99–2.11)
<b>Analysis with recurrence score‡</b>		
Age at surgery	0.08	0.71 (0.48–1.05)
Clinical tumor size	0.23	1.26 (0.86–1.86)
Recurrence score	<0.001	3.21 (2.23–4.61)

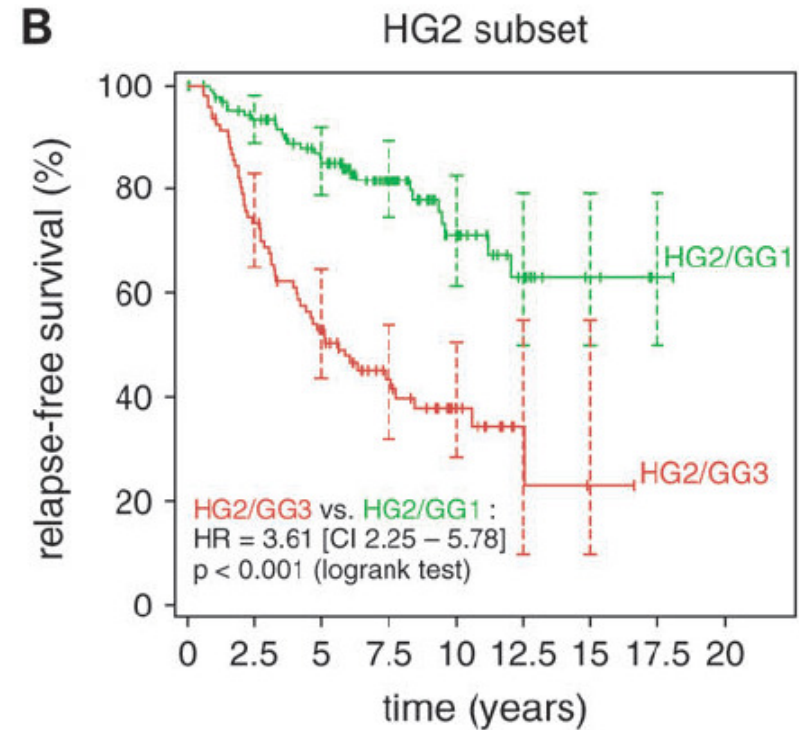
Tamoxifen adjuvant, med. F/U >10 Jahre

# Genomic Grading Index



number at risk

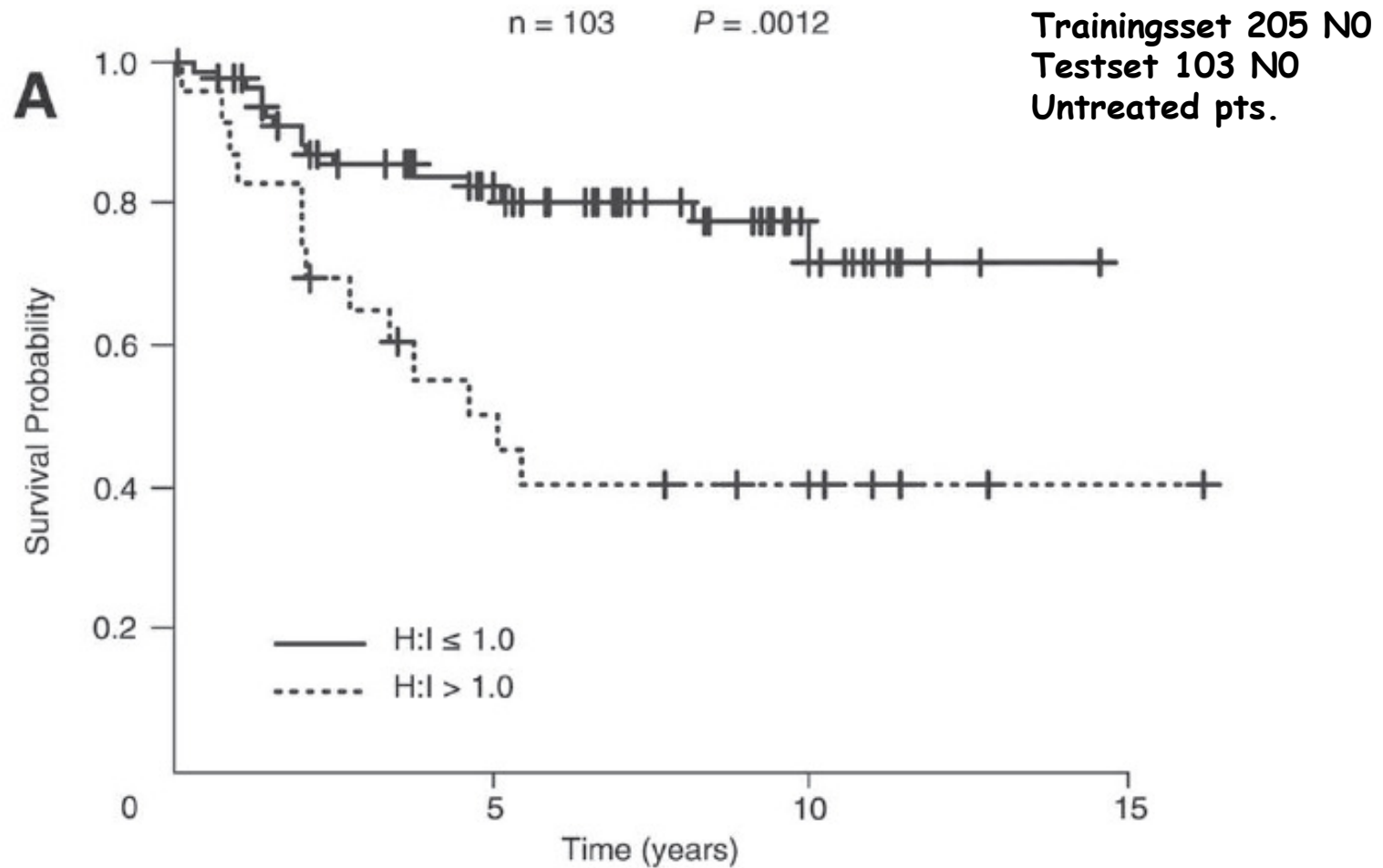
HG1	134	123	107	59	23	8	4	
HG2	216	174	136	80	40	16	6	1
HG3	220	137	102	67	35	20	6	2
total	570	434	345	206	98	44	16	3



number at risk

HG2/GG1	124	108	91	55	28	13	5	1
HG2/GG3	92	66	45	25	12	3	1	
total	216	174	136	80	40	16	6	1

# HOXB13:IL17BR Expression Index (H:I-Ratio)



# EGAPP-Recommendations

*(Evaluation of Genomic Applications in Practice and Prevention Working Group)*

- Mammaprint<sup>®</sup>, Oncotype DX<sup>®</sup>, H:I Ratio
- The evidence is insufficient to assess the balance of benefits and harms of the proposed uses of the tests. The EWG encourages further development and evaluation of these technologies.
- These technologies have potential for both benefit and harm.

*EGAPP, Genet Med 2009;11(1):66 -73.*

*Marchionni et al. Ann Intern Med. 2008;148:358-369*



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# Genexpressions-Profile

- Biologisches Modell *a posteriori*
  - Testverfahren unizentrisch, QS !
  - Pilot: retrospektiv
  - Validierung: retrospektiv, mehrere Studien
  - Keine prospektive Validierung
  - Therapiekonsequenz: Studien offen
  - Metaanalyse: vielversprechend, ausreichende Validierung fehlt
- => nicht reif für die Routine

# Proliferation Markers

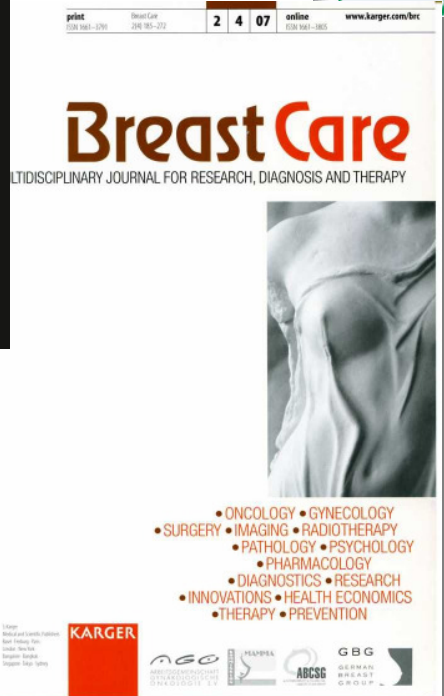
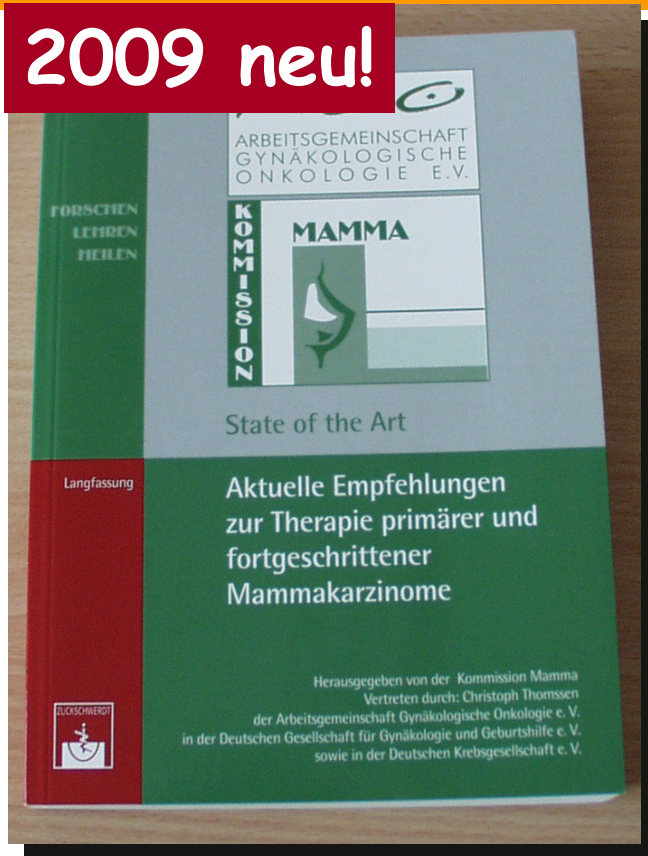
- De Azambuja et al. (**BJC** 2007;96(10):1504-13)
  - Our meta-analysis suggests that Ki-67/MIB-1 positivity confers a higher risk of relapse and a worse survival in patients with early BC. (n=12.155)  
Significant heterogeneity!
- Stuart-Harris R et al. (**Breast** 2008;17(4):323-34)
  - Ki-67, MI, PCNA and LI are associated with worse survival outcomes in early breast cancer. However, whether these proliferation markers provide additional prognostic information to commonly used prognostic indices remains unclear. (n=32.825)

# Das Rennen ist offen....

- Ähnliche Probleme
  - Fehlende prospektive Studien an unbehandelten Patientinnen
    - Prognose↓, Prädiktion↑
  - Gewebe-Asservierung (Frischgewebe od. FFPE)
    - Stanzmaterial
  - Tumorgewebe-Heterogenität
  - Effektstärke im Validierungskollektiv
  - Heterogenität der bisherigen Ergebnisse
  - Zentrale Testung vs dezentrale Testung
  - Qualitätssicherung
  - PROSPEKTIVE STUDIEN !! z.B. planB (AGO/WSG)

# AGO-Empfehlungen 2009 / 2010

2009 neu!



[www.ago-online.de](http://www.ago-online.de)

IF=0,52

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