

Genexpressionsprofile in der Praxis

Michael Knauer

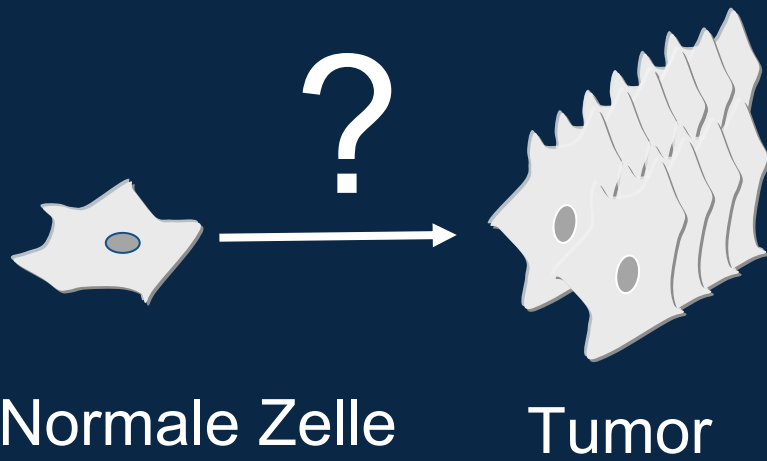
Zertifiziertes Brustzentrum Vorarlberg
LKH Feldkirch

Netherlands Cancer Institute
Amsterdam

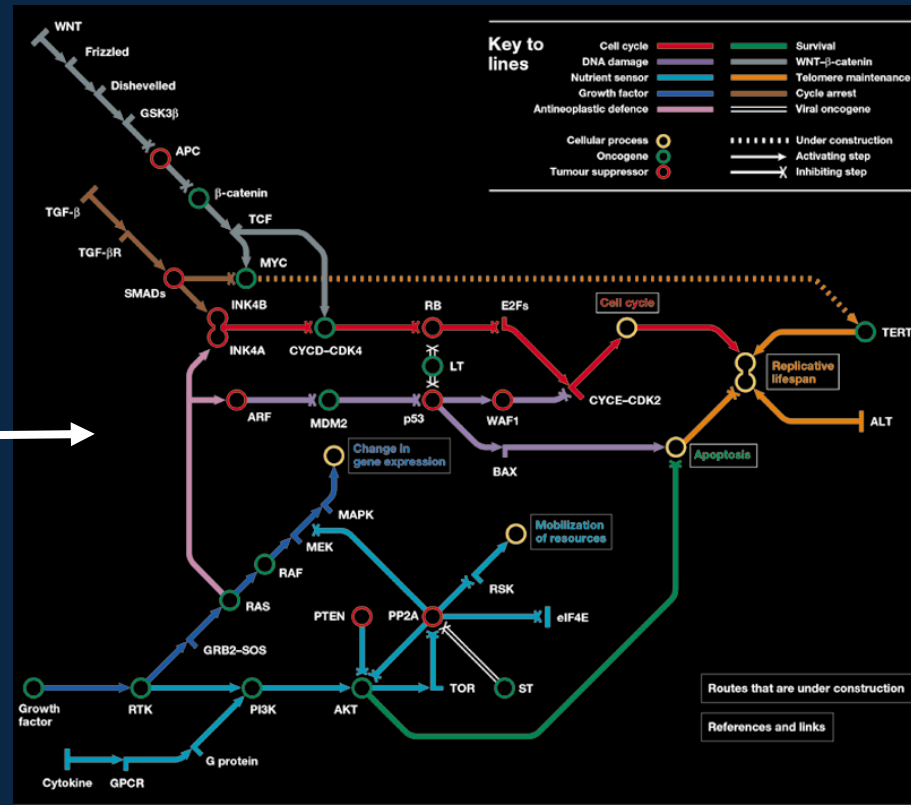
Jahrestagung Österr. Gesellschaft für Senologie
14.10.2010



30 Jahre Fortschritt in der Krebsforschung

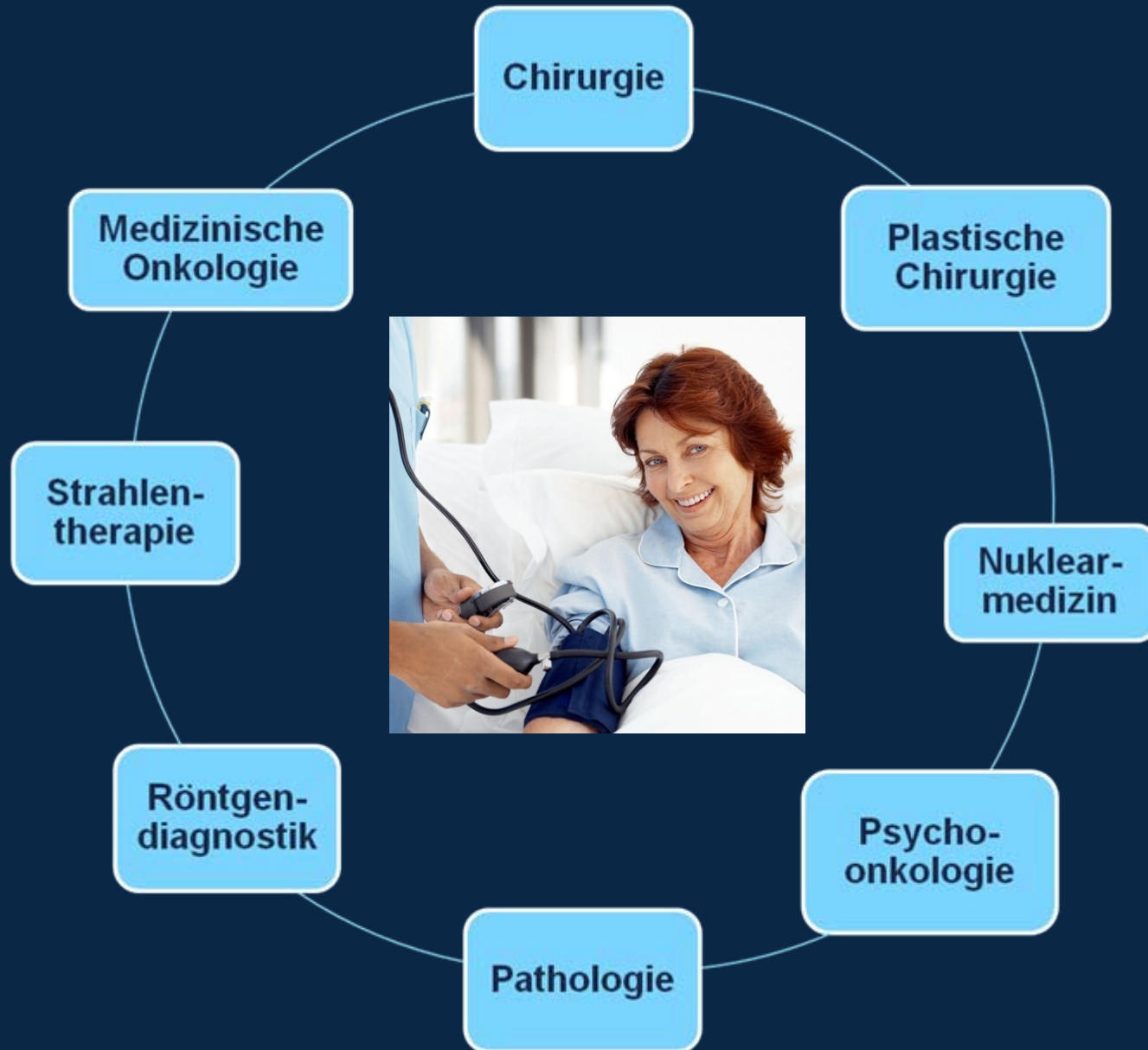


1980



2010

Interdisziplinäre Zusammenarbeit



Zwei wichtige Fragen

Wie schlimm ist mein Krebs?

Hilft mir das Medikament?



Individuelle Therapie

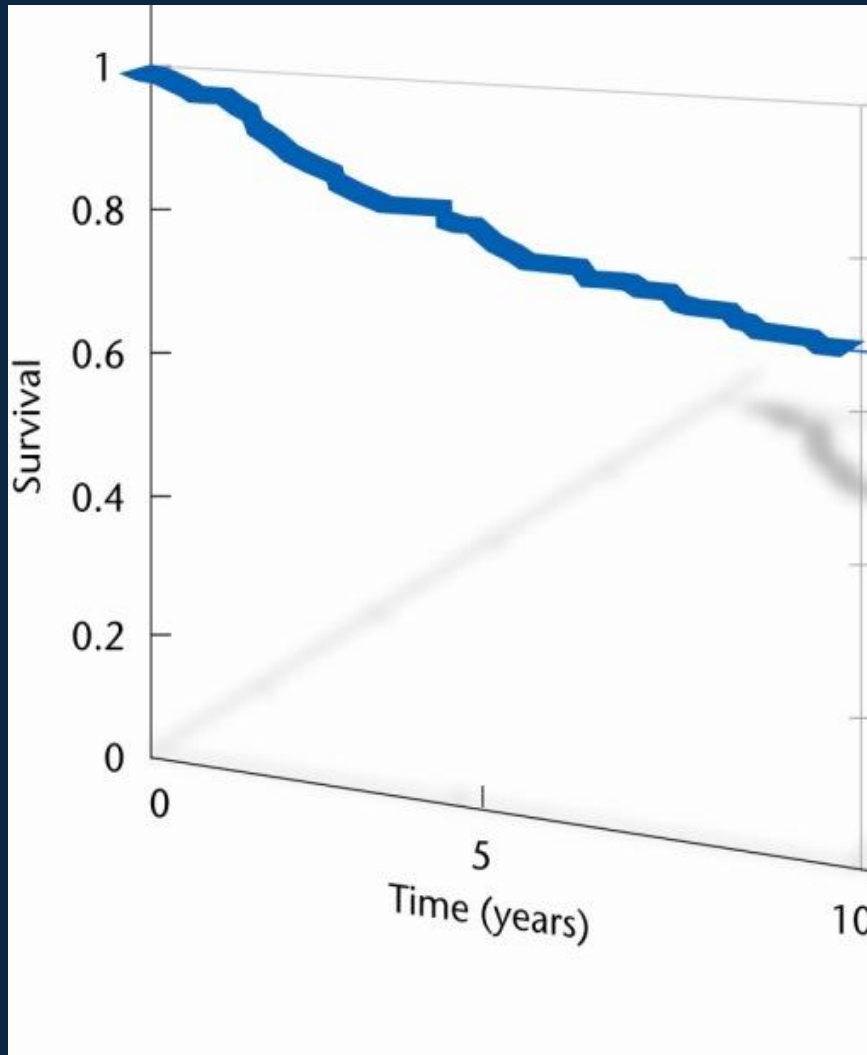


Vermeiden von
Über- und Unterbehandlung

Individualisierte Therapie

Einschätzung des Rezidivrisikos

Kaplan-Meier Survival Curve



Einschätzung des Rezidivrisikos

- Ähnliche klinisch-pathologische Eigenschaften
- Unterschiedlicher Verlauf
- Adjuvante Therapie auf Basis von Richtlinien

- Alter
- Tumorgroße
- Grad
- Lymphknoten-Status
- Hormonrezeptoren
- Her2-Status



St. Gallen 2007

- Low risk:
T1, N0, Grad 1, Alter > 35, ER/PR +, Her2 -

<u>St. Gallen</u>	All Patients: n (%)	Highly + Incompletely Responsive, Her2-negative
Low risk	145 (9%)	141 (16%)
Intermediate risk	1287 (76%)	714 (79%)
High risk	190 (11%)	42 (5%)
Not available	74 (4%)	6 (1%)
Total	1696	903/1696 (53)%

Adjuvant! for Breast Cancer (Version 8.0)

Patient Information

Age:

Comorbidity:

ER Status:

Tumor Grade:

Tumor Size:

Positive Nodes:

Calculate For:

10 Year Risk:

Adjuvant Therapy Effectiveness

Horm:

Chemo:

No additional therapy:



79.7 alive in 10 years.

16.7 die of cancer.

3.6 die of other causes.

With hormonal therapy: Benefit = 4.9 alive.



With chemotherapy: Benefit = 5.1 alive.



With combined therapy: Benefit = 8.4 alive.



- 3.5% profitieren von Chemotherapie
- 100% haben Risiko von Nebenwirkungen

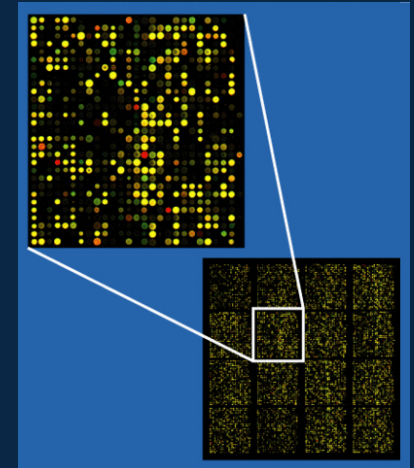
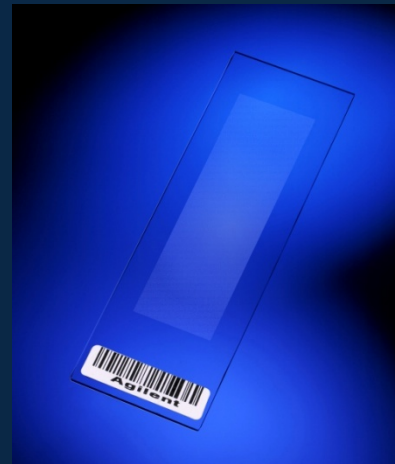
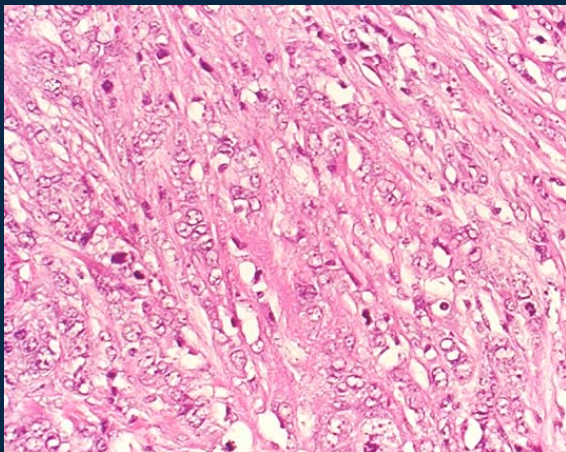
Neue diagnostische Möglichkeiten *vom Mikro-skop zum Micro-array*



Mikro-skop



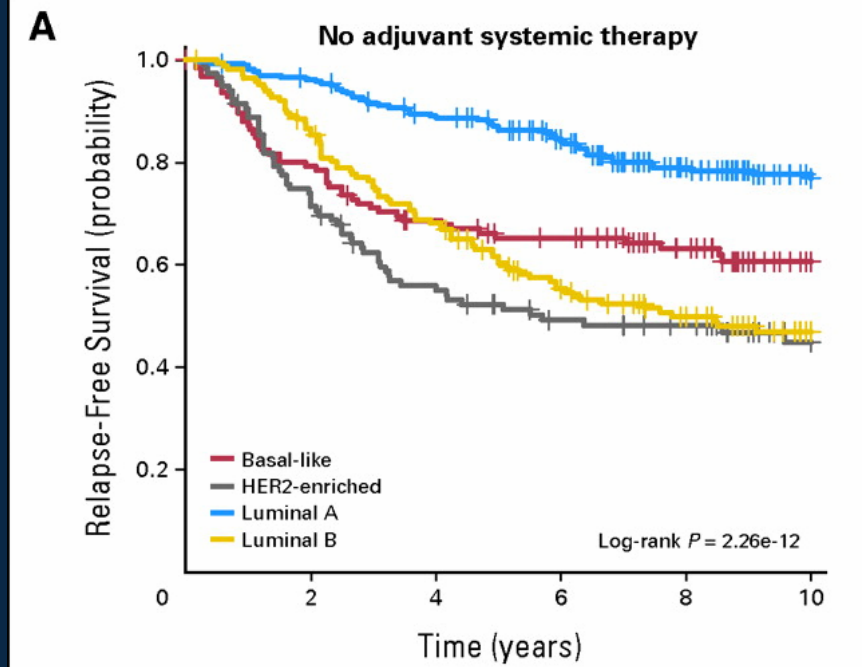
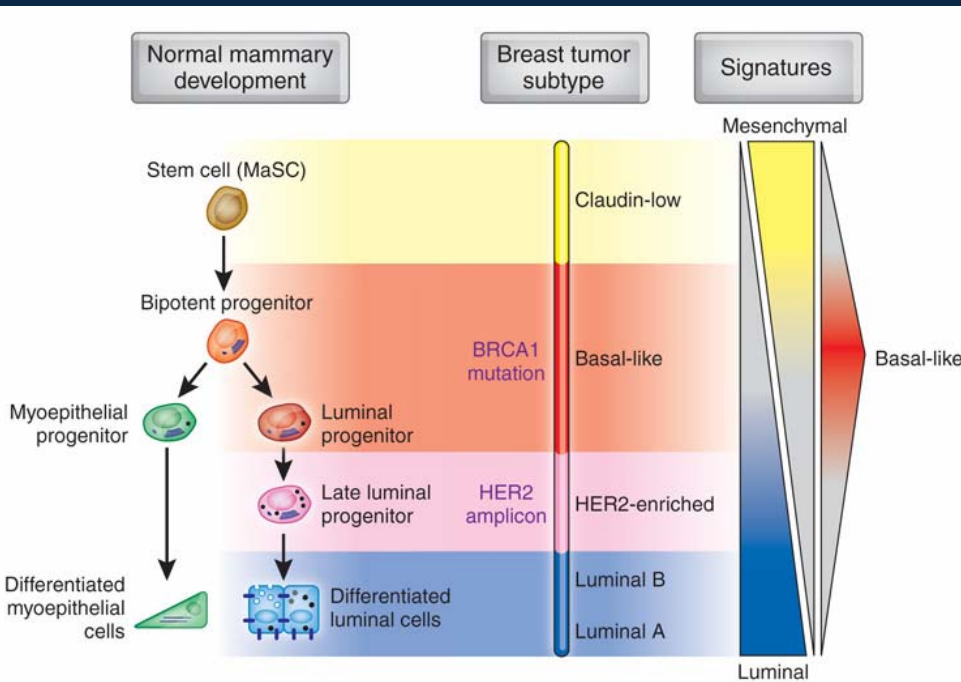
Micro-array



Entwicklung Multigen-Assays

Neue Klassifikation: intrinsic subtypes¹

Entwicklung von prognostischen Profilen²

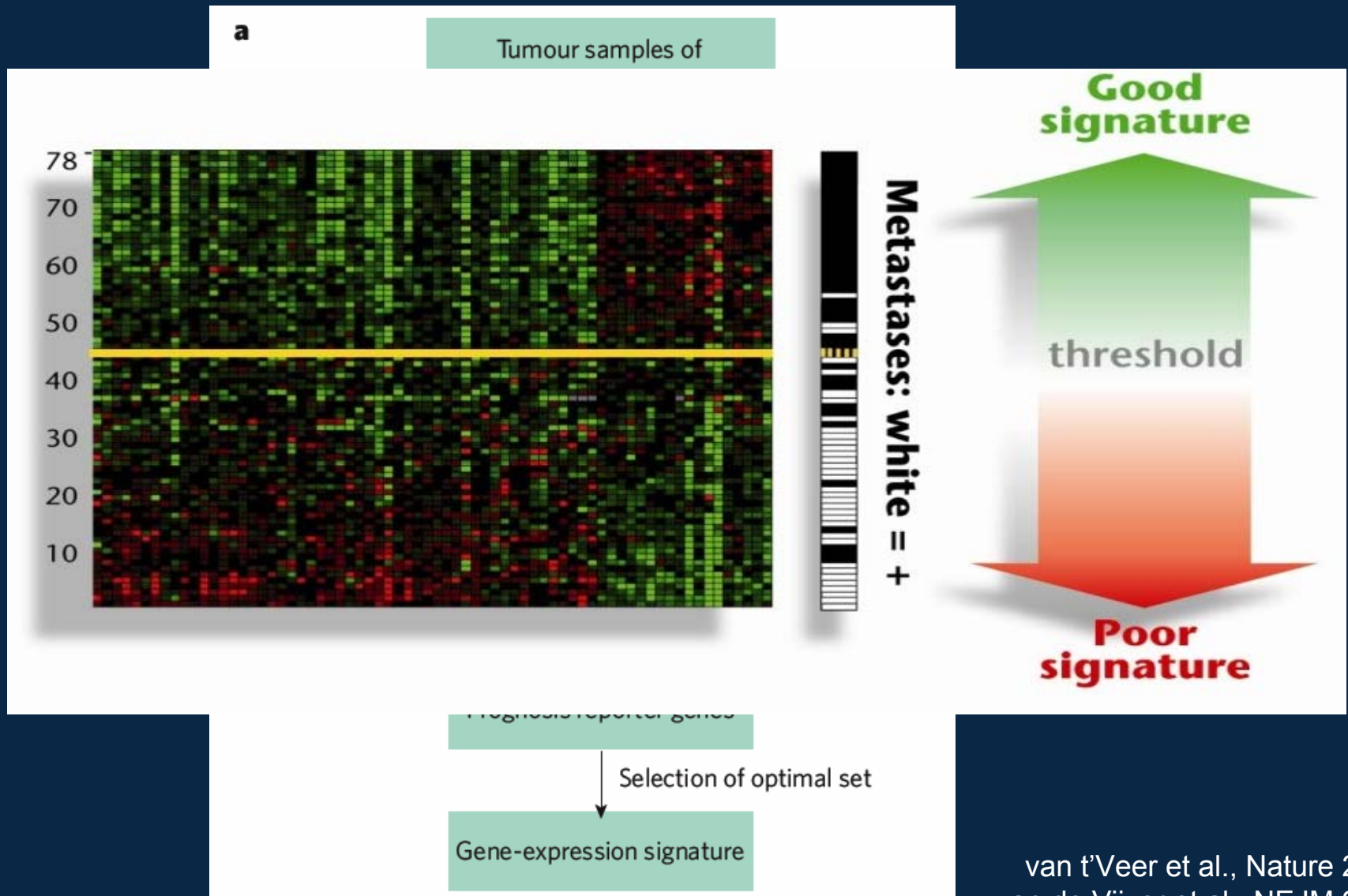


Paradigmenwechsel

Weg von vornehmlich
stadienbasierter
Risikoabschätzung...

...hin zur Integration von
individuellen, biologiebasierten
Risikoprofilen

Entwicklung Multigen-Assays



Wie funktioniert es?

Aktive Gene → mRNA

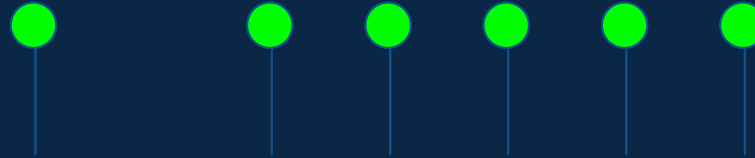
Tumor RNA



Gene

1 2 3 4 5 6 7 8

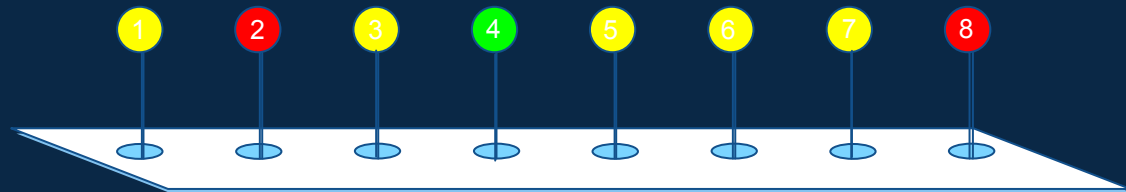
Referenz RNA



● Über-Expression

● Unter-Expression

● Gleiche Expression



Profile beim Mammakarzinom

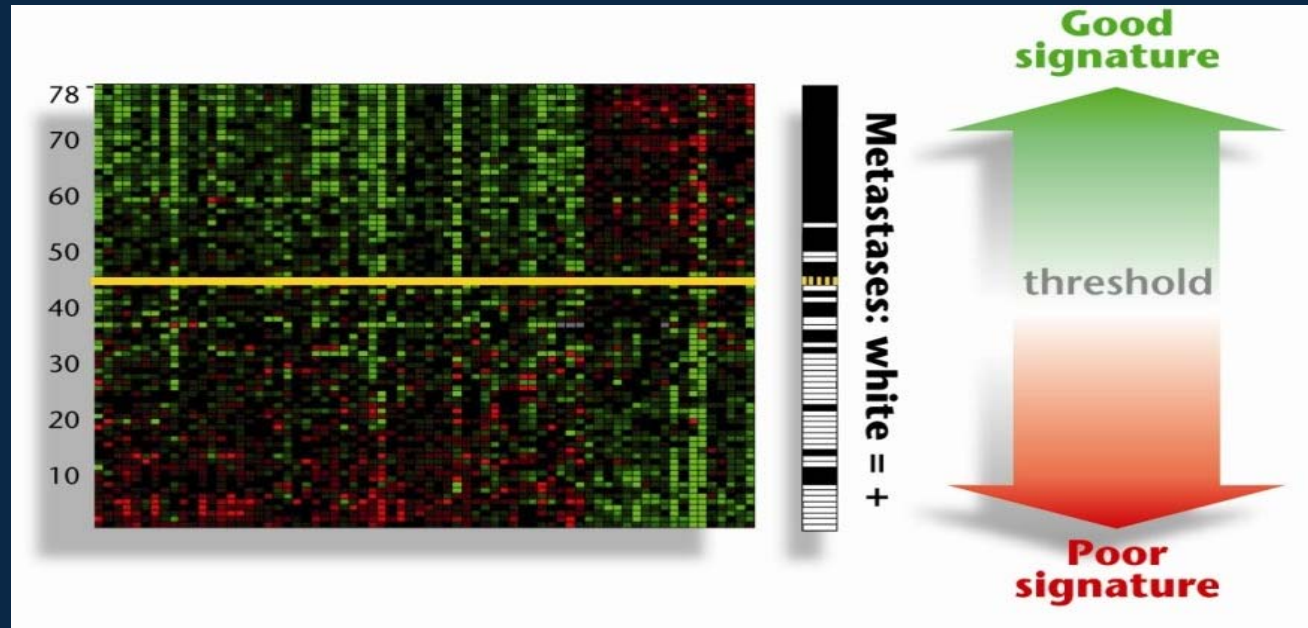
Profil	Gewebe	Technik	Evidenzlevel
70-Genprofil (MammaPrint)	fresh - frozen	DNA microarray	III
21-Gen Recurrence Score (OncotypeDX)	FFPE	rt-PCR	II
HOXB13/IL17BR ratio (Breast Cancer Index)	FFPE	rt-PCR	III
Genomic Grade Index (MapQuant Dx)	fresh - frozen	DNA microarray	III
Molecular subtypes (PAM50)	FFPE	rt-PCR	III
76-Genprofil (Rotterdam)	fresh - frozen	DNA microarray	III
Wound-response signature	fresh - frozen	DNA microarray	III
...			

70-Genprofil MammaPrint™

Entwickelt aus 78 eingefrorenen Tumorproben¹

Validiert in 3 unabhängigen Studien²⁻⁴

- Stadium I/II
- ER+ und ER-
- N0 und N1
- FDA- und ISO-zertifiziert



- 1: van 't Veer et al, Nature 2002
- 2: van de Vijver et al, NEJM 2002
- 3: Buyse et al, JNCI 2006
- 4: Bueno-de-Mesquita, BCRT 2008

21-gene recurrence score – Oncotype Dx™

16 Cancer and 5 Reference Genes From 3 Studies

PROLIFERATION

Ki-67
STK15
Survivin
Cyclin B1
MYBL2

ESTROGEN

ER
PR
Bcl2
SCUBE2

GSTM1

INVASION

Stromolysin 3
Cathepsin L2

CD68

BAG1

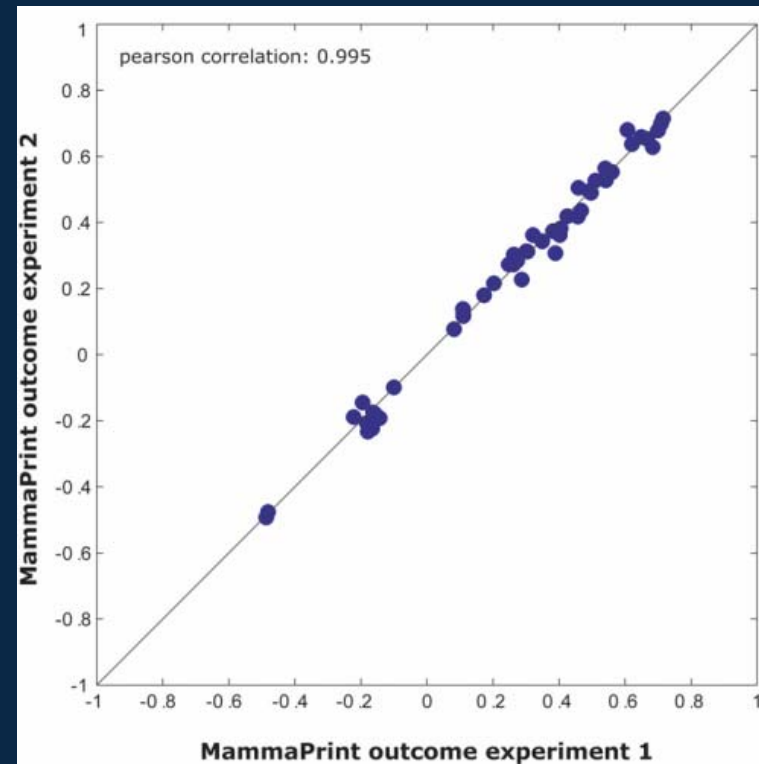
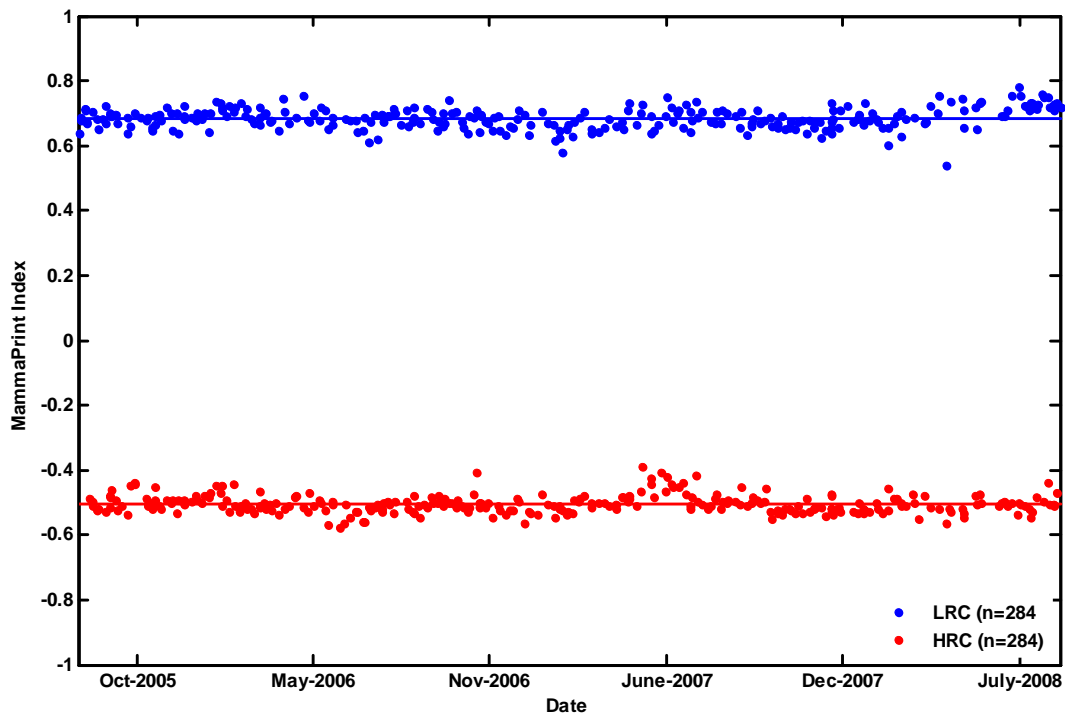
HER2
GRB7
HER2

REFERENCE

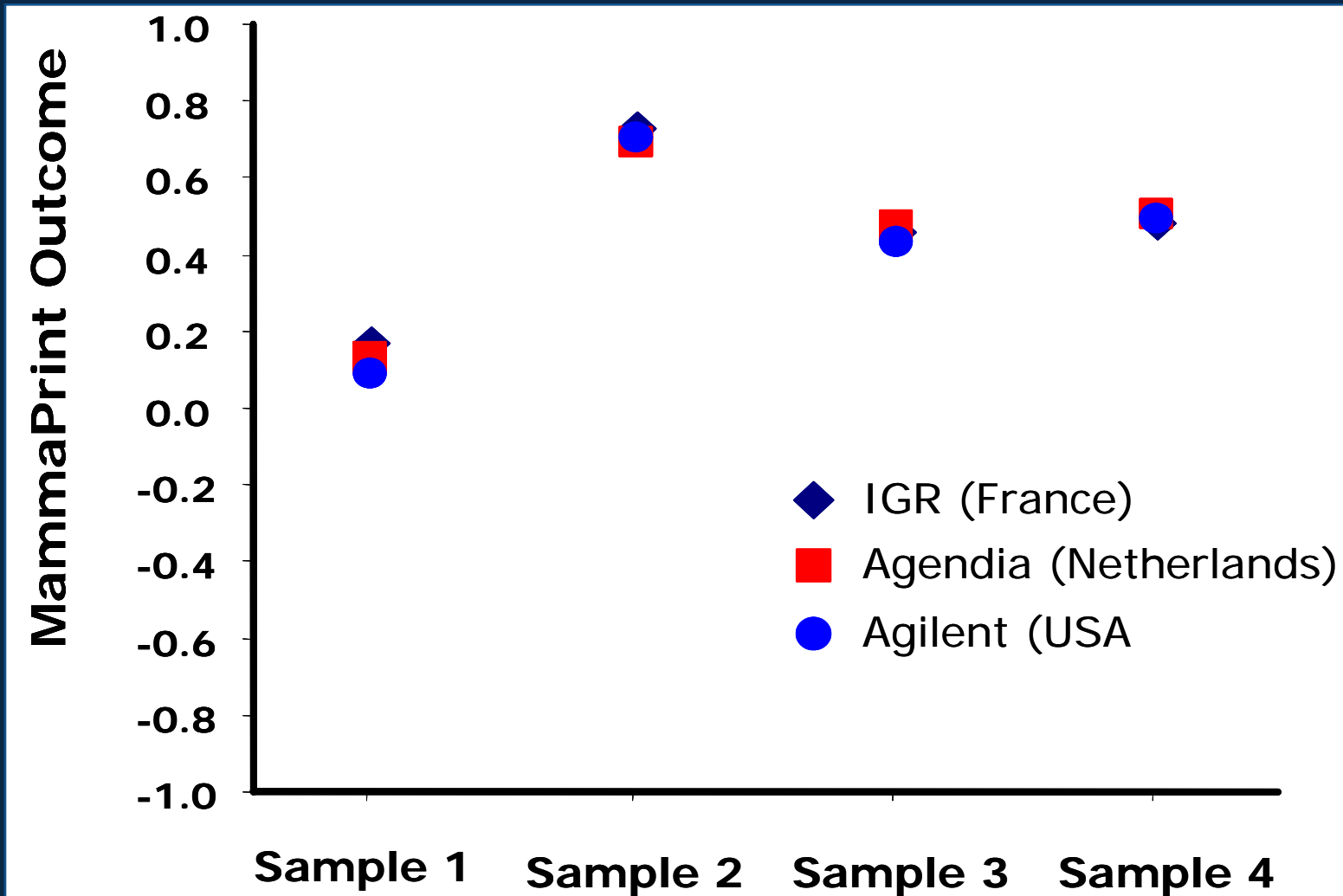
Beta-actin
GAPDH
RPLPO
GUS
TFRC

Category	RS (0 – 100)
Low risk	RS < 18
Intermediate risk	RS 18 - 30
High risk	RS ≥ 31

Stabilität und Reproduzierbarkeit



Stabilität und Reproduzierbarkeit



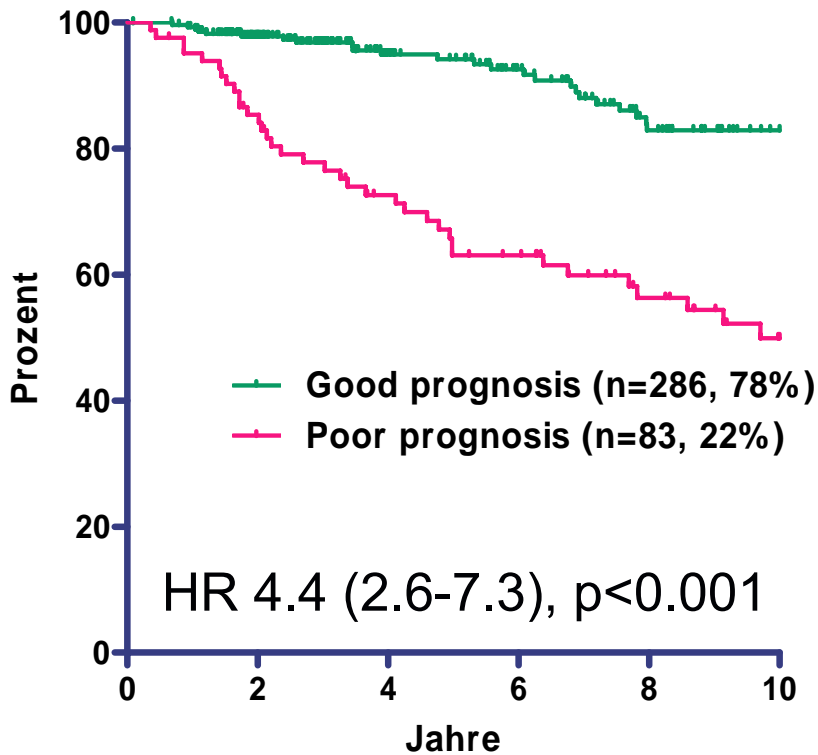
21-Genscore: NSABP-B14

Category	NCCN		St Gallen		OncotypeDx	
	% of pts	DRFS ₁₀	% of pts	DRFS ₁₀	% of pts	DRFS ₁₀
Low	7.9	0.93	7.9	0.95	50.6	0.93
Intermediate	-	-	33.2	0.91	22.3	0.86
High	92.1	0.85	58.8	0.81	27.1	0.69

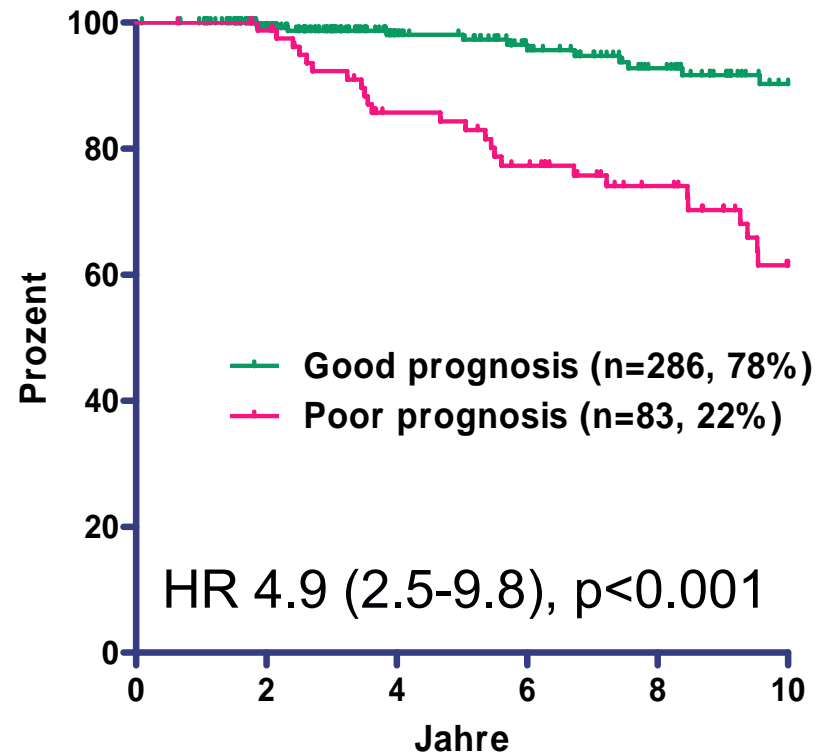
70-Genprofil

Rezeptor-positives Karzinom, keine adj. Therapie

Metastasenfreies Überleben (n=369)



Krebsspezifisches Überleben (n=369)



Raster Studie

- Machbarkeitsstudie Implementierung MammaPrint 2004 – 2006
- 16 öffentliche Krankenhäuser in den Niederlanden
- 427 Patienten
- 51% low risk, 49% high risk
- 4 Jahre Nachbeobachtung
- Erste prospektive Ergebnisse 2011



The MINDACT logo, consisting of the word "MINDACT" in a bold, black, uppercase, sans-serif font. The letters are set against a bright green rectangular background that has a slight gradient.

- 2 prospektiv randomisierte Studien laufen:

TAILORx (USA): 21-Genescore: >10.000 Frauen

MINDACT (Europa): 70-Genprofil: > 6.000 Frauen,

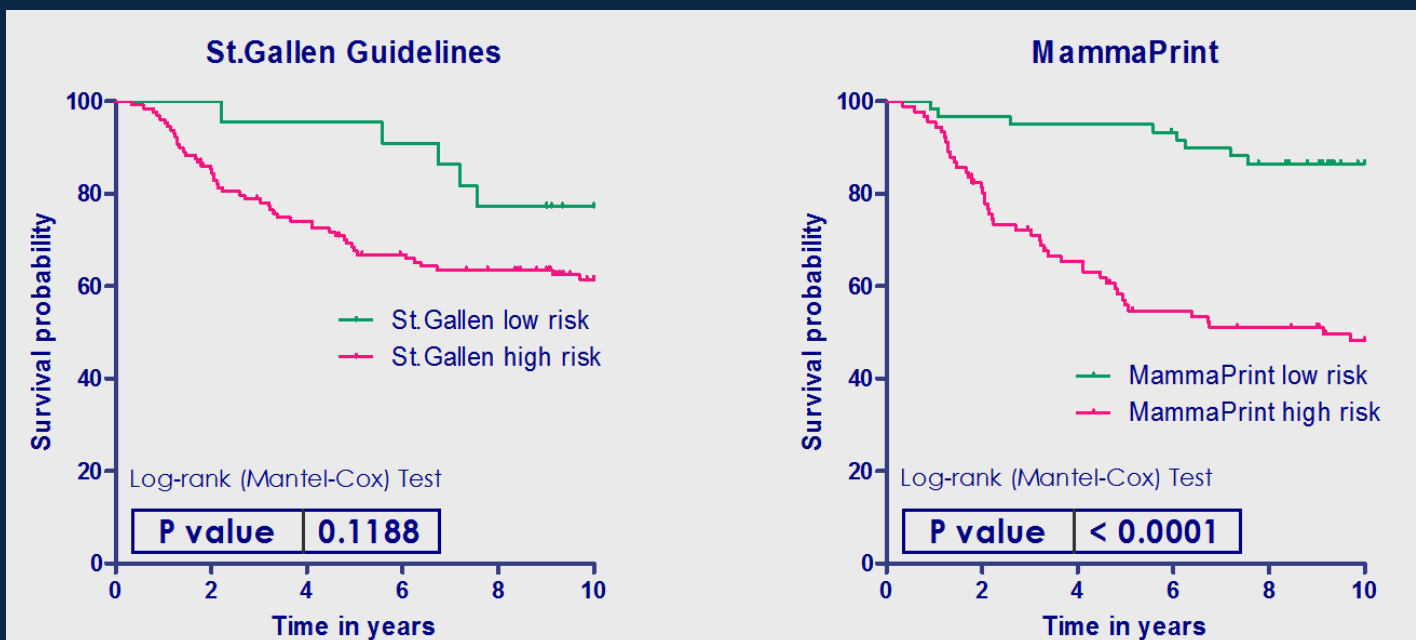
24.9.2010: 4.335 eingeschlossen

- Rekrutierung bis 2012, Ergebnisse noch später

- seit 2004: >100.000 OncotypeDx Tests, jede 8. Patientin

St. Gallen vs. 70-Genprofil

<u>Mammaprint</u>	All Patients: n (%)	Highly + Incompletely Responsive, Her2-negative
Low risk	792 (47%)	564 (63%)
High risk	904 (53%)	339 (37%)
Total	1696	903



Prognostischer Wert

- Ergebnisse sind logisch und nachvollziehbar
- Unabhängige Information über Prognose einer einzelnen Patientin
- Überprüft in mehreren unabhängigen Studien

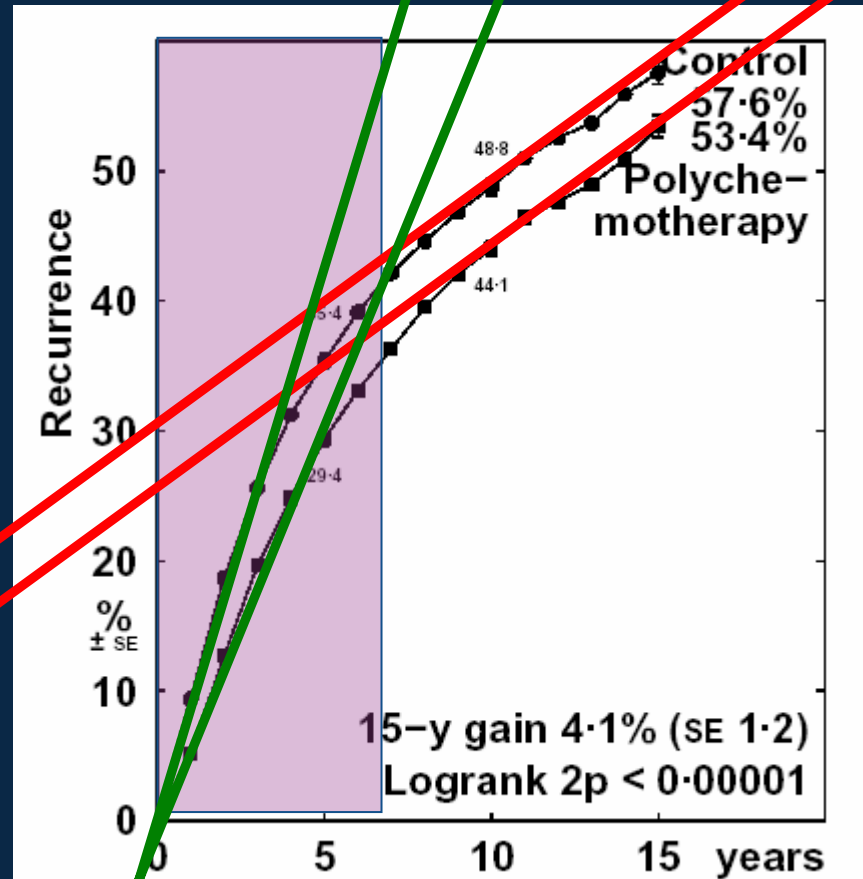
PROGNOSE

- Interessant zu wissen
- Veränderung dieser (schlechten) Prognose

PRÄDIKTION

- Vorhersage des Ansprechens auf die Behandlung
- Wer profitiert von welcher Therapie?

Chemotherapie hat wenig Effekt auf späte Rezidive

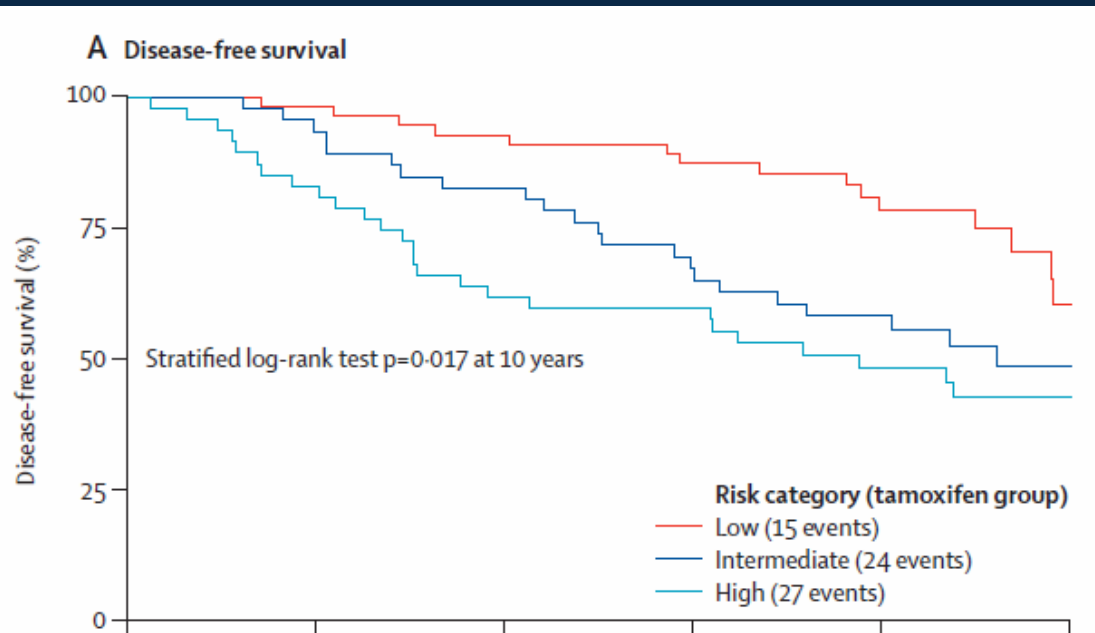


Kein Effekt von Chemo

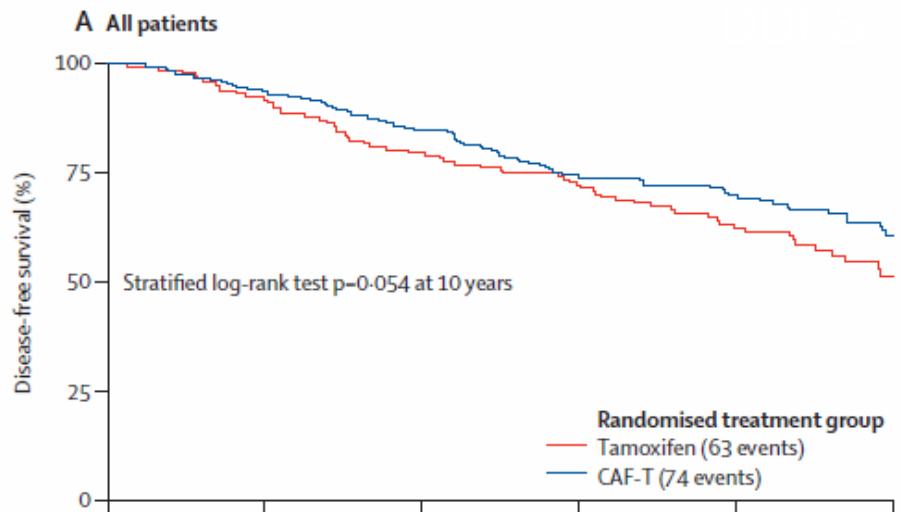
Wirkung von Chemotherapie

Chemotherapie: 21-Genscore

- Retrospektive Analyse der randomisierten SWOG-8814 Studie
- 367 Patientinnen mit nodal-positivem Karzinom

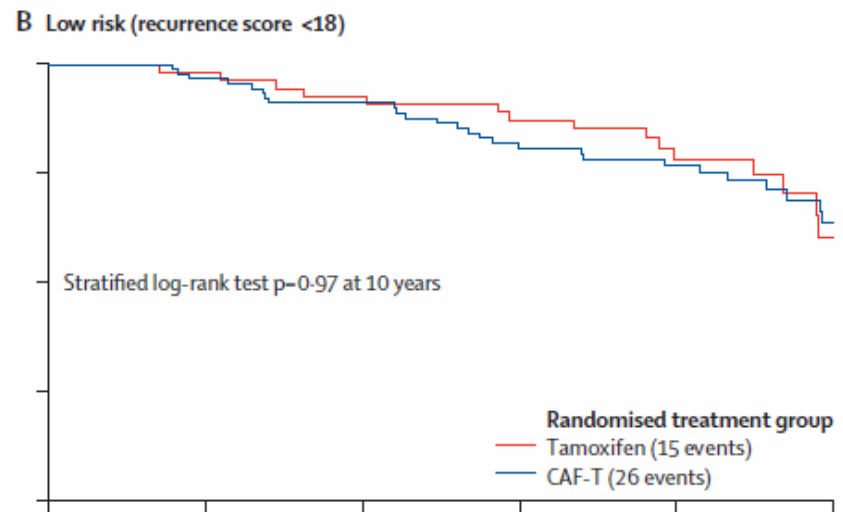


Number at risk							
Low	55	54	51	48	32	10	
Intermediate	46	43	38	30	23	8	
High	47	39	29	28	20	8	



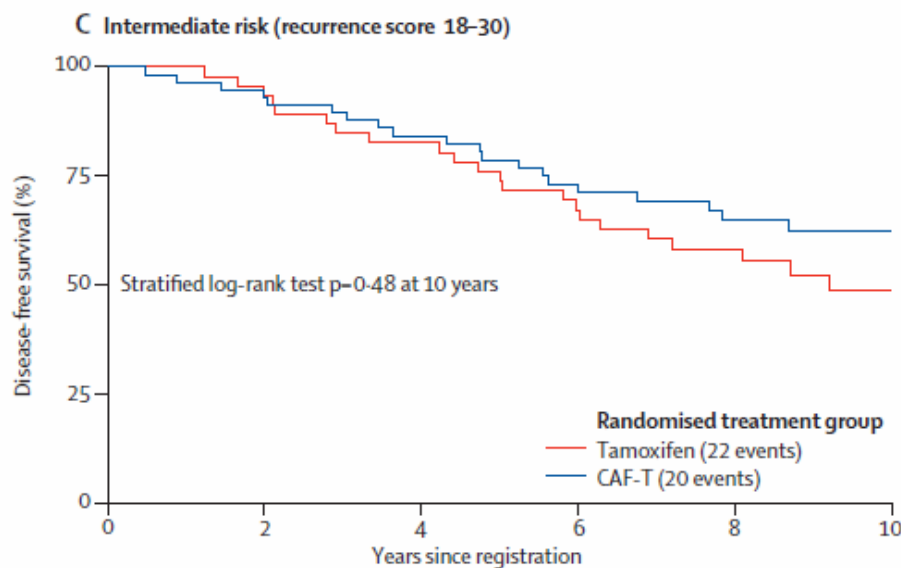
Number at risk

Tamoxifen	148	136	118	106	75	26
CAF-T	219	205	182	152	114	55



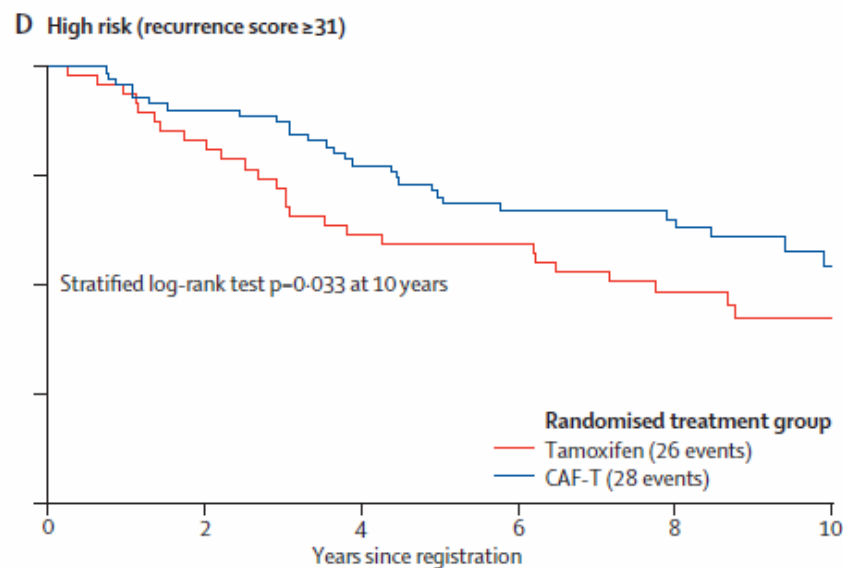
Number at risk

Tamoxifen	55	54	51	48	32	10
CAF-T	91	88	81	71	53	21



Number at risk

Tamoxifen	46	43	38	30	23	8
CAF-T	57	53	47	37	29	18

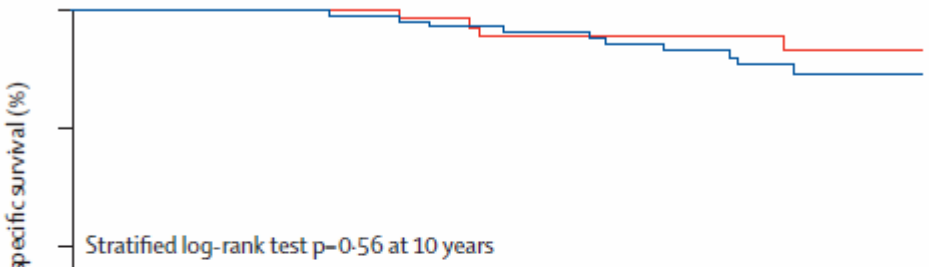


Number at risk

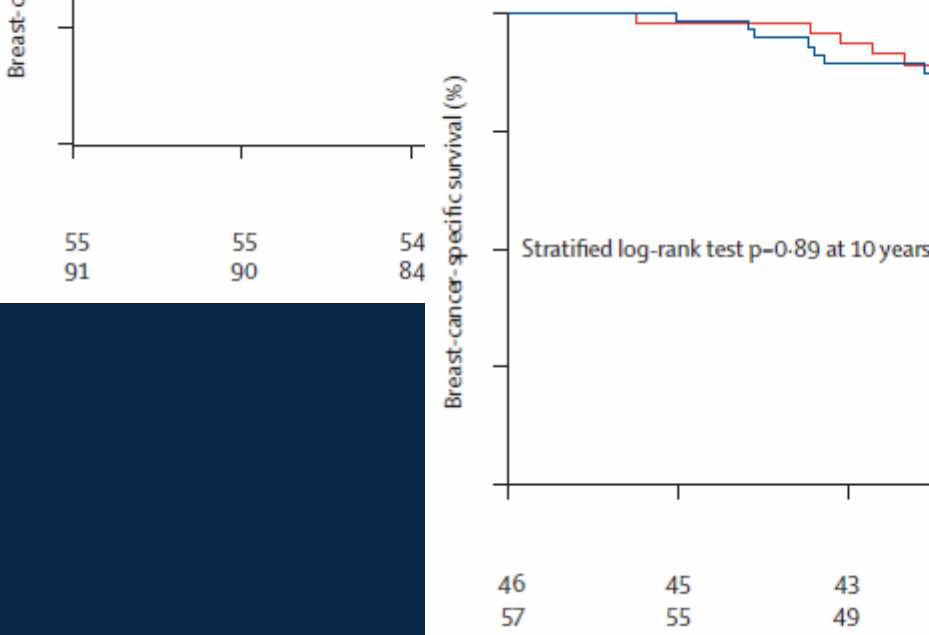
Tamoxifen	47	39	29	28	20	8
CAF-T	71	64	54	44	32	16

BCSS

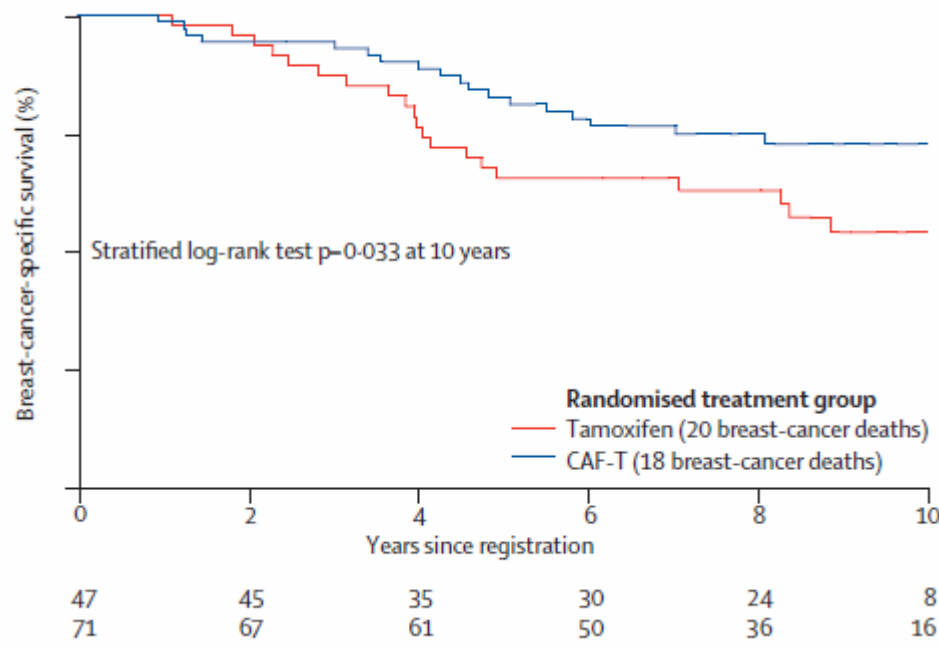
B Breast-cancer-specific survival, low risk (recurrence score <18)



D Breast-cancer-specific survival, intermediate risk (recurrence score 18-30)

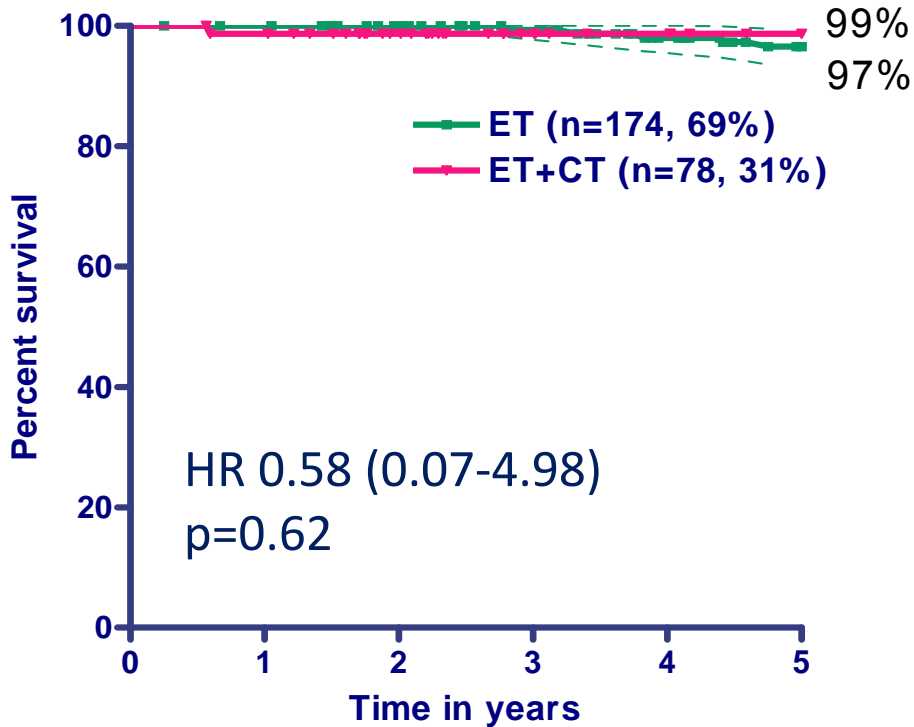


F Breast-cancer-specific survival, high risk (recurrence score ≥31)

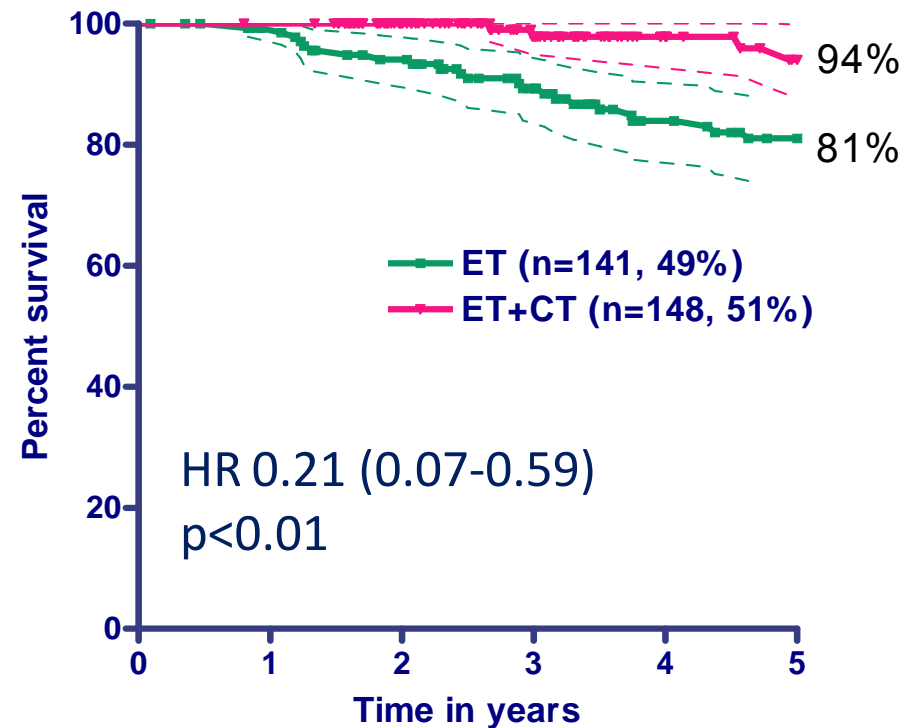


Chemotherapie: 70-Genprofil

BCSS: MammaPrint **LOW RISK**
(n=252)



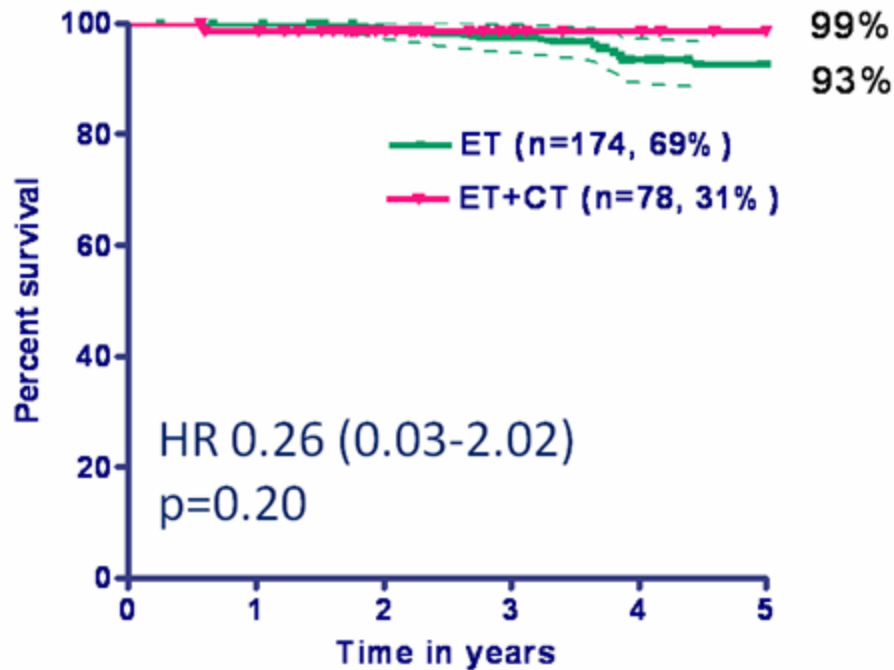
BCSS: MammaPrint **HIGH RISK**
(n=289)



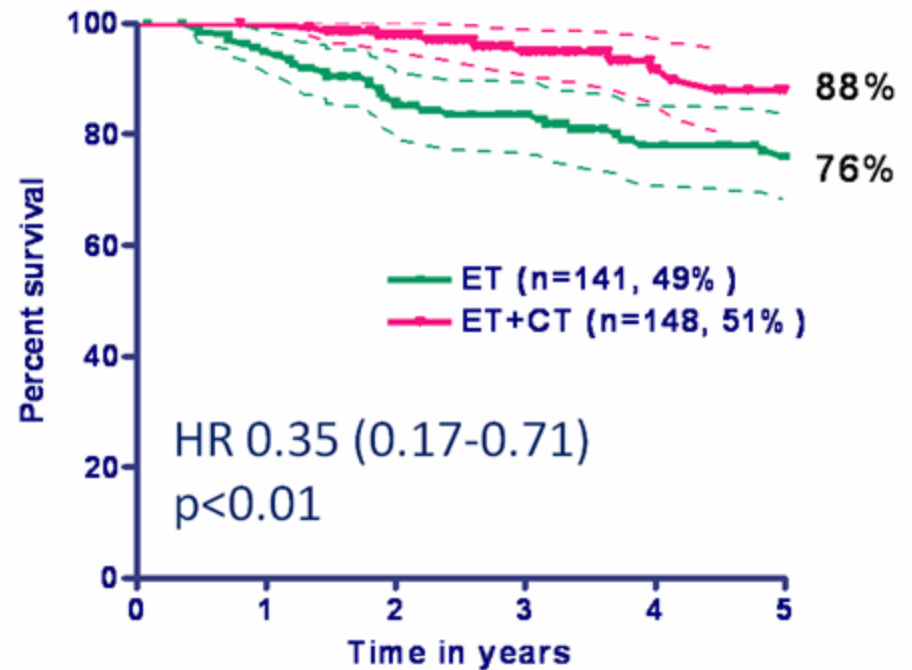
13% absolute benefit

Chemotherapie: 70-Genprofil

DDFS: MammaPrint **LOW RISK**
(n=252)

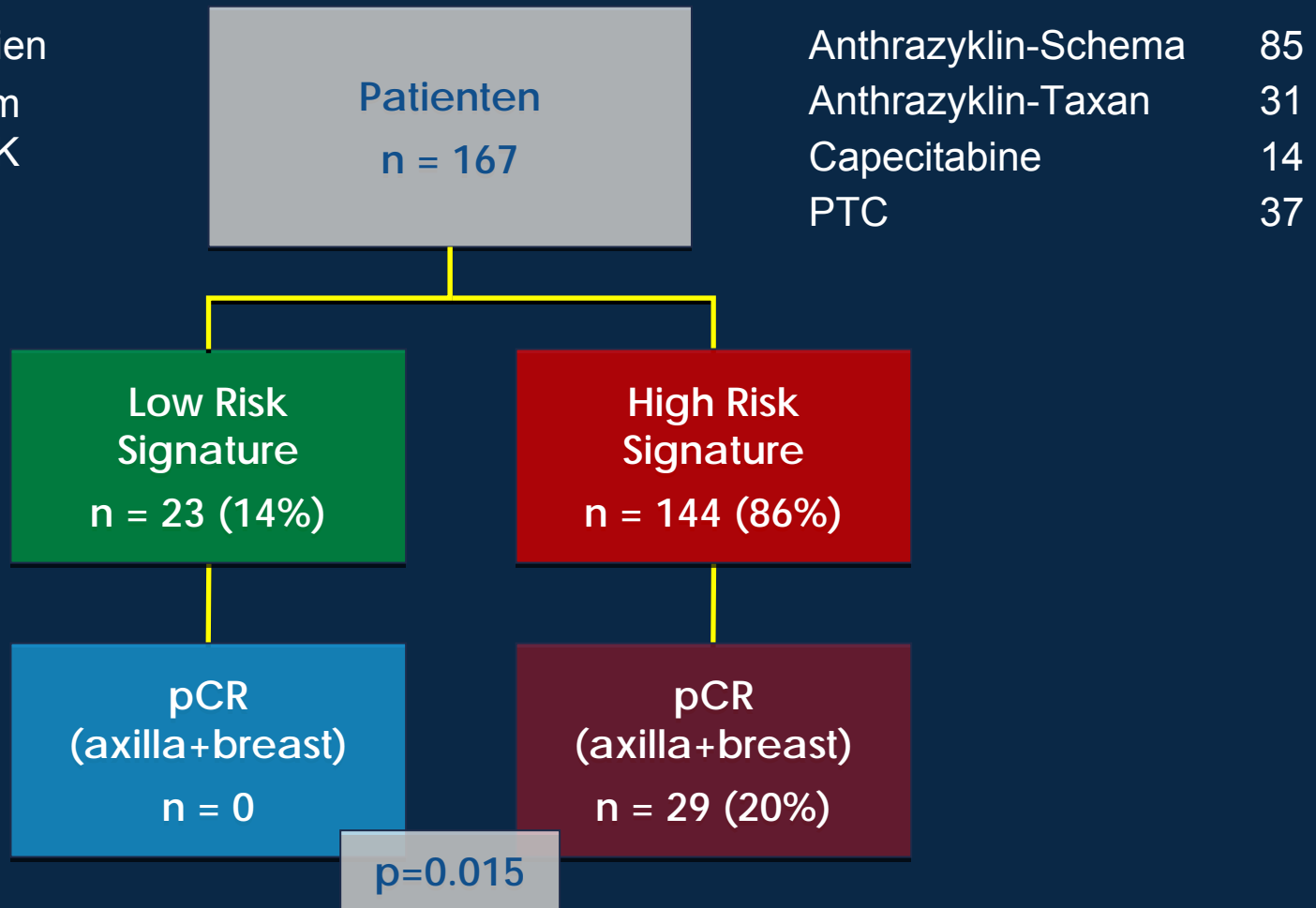


DDFS: MammaPrint **HIGH RISK**
(n=289)

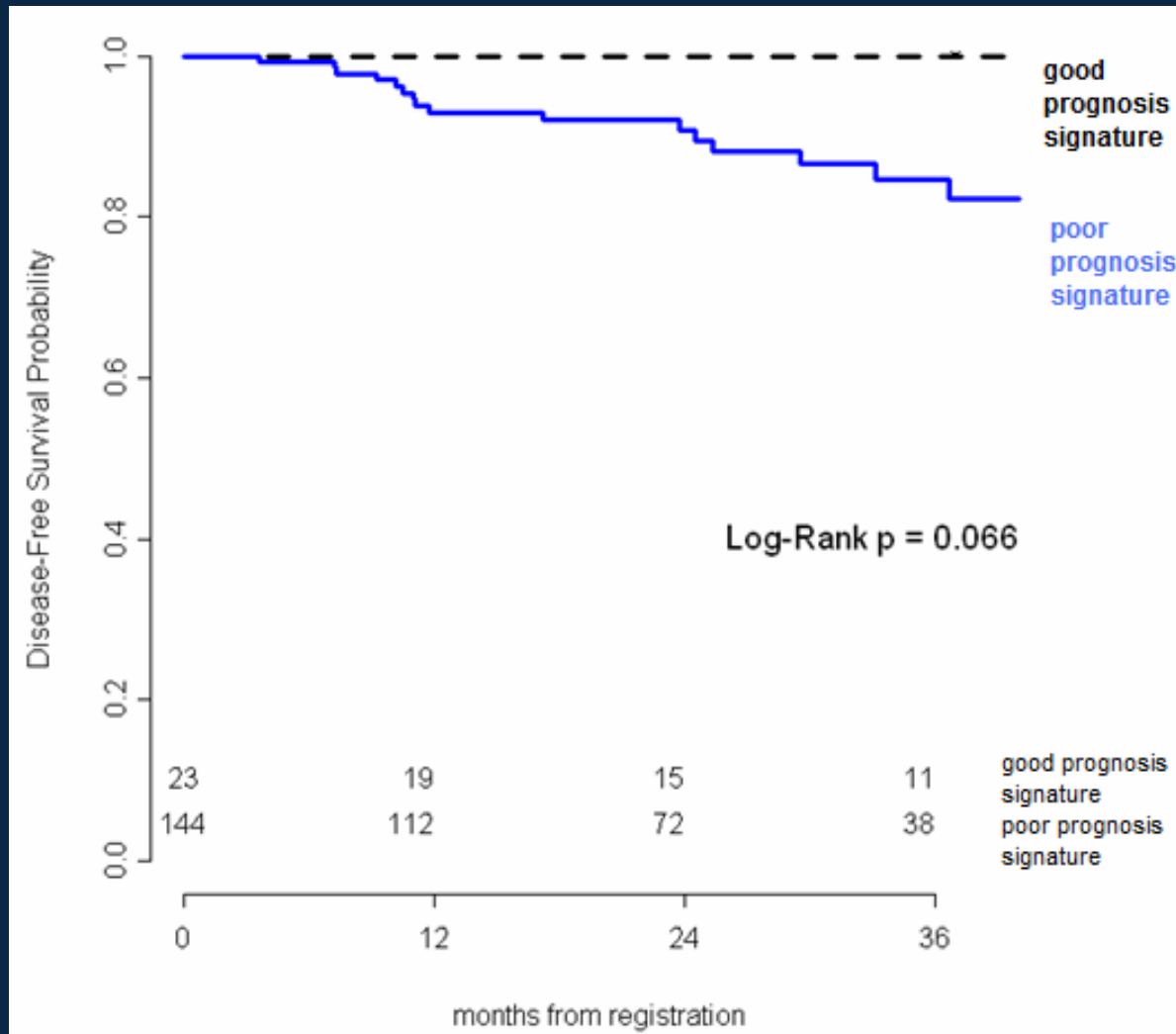


Neoadjuvante Chemotherapie

- 2 klinische Studien
- T-Stadium >3 cm und/oder pos. LK
- Ultraschall-Stanzbiopsien
- MRI
- Histologie



Prognose nach neoadjuvanter Chemotherapie



Zusammenfassung prädiktiver Wert

1. Patientinnen mit “Low Risk” 70-Genprofil haben eine sehr gute Chance, gesund zu bleiben.
Die adjuvante Chemotherapie hat nur einen geringen, nicht signifikanten Benefit gegenüber Hormontherapie alleine
2. Patientinnen mit “High Risk” 70-Genprofil haben ein hohes Risiko für Fernmetastasen und zeigen einen signifikanten Benefit von zusätzlicher Chemotherapie
3. MINDACT, die europaweite klinische Studie wird wahrscheinlich diese retrospektiven Analyse bestätigen

Individualisierung der Therapie beginnt vor der OP

- Präoperative Stanzbiopsie ist Routine:
ER, PR, Her2
- Möglichkeit der Genexpressionsanalyse
- Hohe Wahrscheinlichkeit für pCR:
genaue Markierung und Responsemonitoring
essentiell
- Lokalrezidivrate abhängig von molekularen
Subtypen: Optimale Lokaltherapie

Perioperative Veränderungen

- Operationsplanung und Logistik
- Stanzbiopsie aus dem frischen Tumor gleichzeitig mit Resektionsrandbeurteilung
- Verarbeitung innerhalb einer Stunde: RNA-Konservierung
- 7 Tage bis zur Verarbeitung: Lagerung bei -80°C



Agendia Laboratory Process

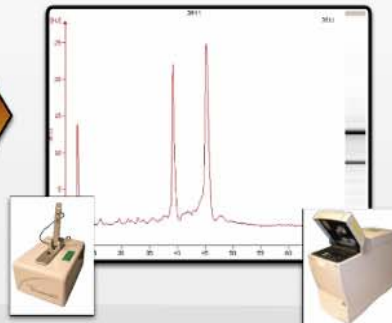
Tumor % determination



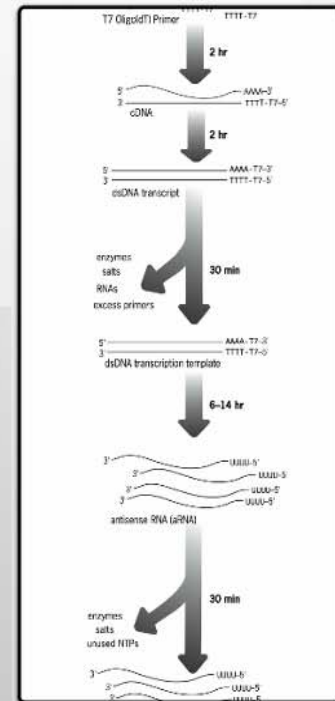
RNA Extraction



Quality Control



Amplification & Labeling



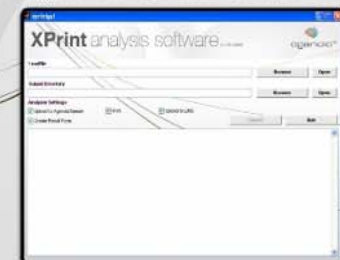
Sample Slicing



Sample Reception



Algorithm / Risk Calculation



Result

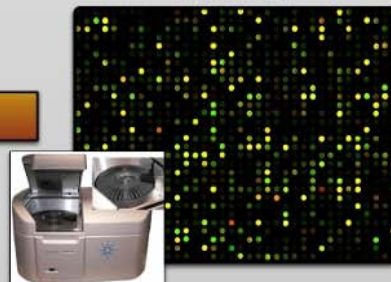


Spot Quantization

Sequence	Gene	Accession	Gene	Accession
CCGAGTTCACCG	7	UPO	UPO	UPO
AGAGAGAGAGAG	8	UPO	UPO	UPO
AGAGAGAGAGAG	9	UPO	UPO	UPO
AGAGAGAGAGAG	10	UPO	UPO	UPO
AGAGAGAGAGAG	11	UPO	UPO	UPO
AGAGAGAGAGAG	12	UPO	UPO	UPO
AGAGAGAGAGAG	13	UPO	UPO	UPO
AGAGAGAGAGAG	14	UPO	UPO	UPO
AGAGAGAGAGAG	15	UPO	UPO	UPO
AGAGAGAGAGAG	16	UPO	UPO	UPO
AGAGAGAGAGAG	17	UPO	UPO	UPO
AGAGAGAGAGAG	18	UPO	UPO	UPO
AGAGAGAGAGAG	19	UPO	UPO	UPO
AGAGAGAGAGAG	20	UPO	UPO	UPO
AGAGAGAGAGAG	21	UPO	UPO	UPO
AGAGAGAGAGAG	22	UPO	UPO	UPO
AGAGAGAGAGAG	23	UPO	UPO	UPO
AGAGAGAGAGAG	24	UPO	UPO	UPO
AGAGAGAGAGAG	25	UPO	UPO	UPO
AGAGAGAGAGAG	26	UPO	UPO	UPO
AGAGAGAGAGAG	27	UPO	UPO	UPO
AGAGAGAGAGAG	28	UPO	UPO	UPO
AGAGAGAGAGAG	29	UPO	UPO	UPO
AGAGAGAGAGAG	30	UPO	UPO	UPO
AGAGAGAGAGAG	31	UPO	UPO	UPO
AGAGAGAGAGAG	32	UPO	UPO	UPO
AGAGAGAGAGAG	33	UPO	UPO	UPO
AGAGAGAGAGAG	34	UPO	UPO	UPO
AGAGAGAGAGAG	35	UPO	UPO	UPO
AGAGAGAGAGAG	36	UPO	UPO	UPO
AGAGAGAGAGAG	37	UPO	UPO	UPO
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AGAGAGAGAGAG	67	UPO	UPO	UPO
AGAGAGAGAGAG	68	UPO	UPO	UPO
AGAGAGAGAGAG	69	UPO	UPO	UPO
AGAGAGAGAGAG	70	UPO	UPO	UPO



Scanning



Binding to MicroArray





mammaprint®
decoding breast cancer.

Page 1 of 2

CUSTOMER

Doctor: Anton Haid
Account: Landeskrankenhaus Feldkirch

Address: Carinagasse 47-49

City: Feldkirch-Tisis 6807
Country: Austria

SPECIMEN

Requisition #: 00008843
Collection Date: 28-Sep-2010
Test Request Date: 29-Sep-2010
Date Received: 30-Sep-2010
Report Date: 11-Oct-2010
Specimen Type: Surgical Specimen
Customer Ref.: BZ16071963

PATIENT

Patient:

DOB:
Patient #:
Gender: Female

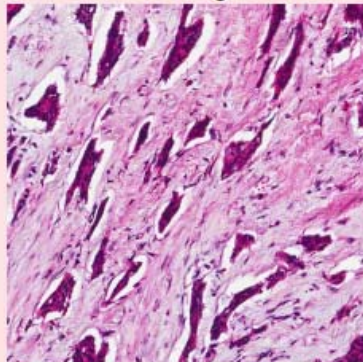
Gene Profile Result

LOW RISK

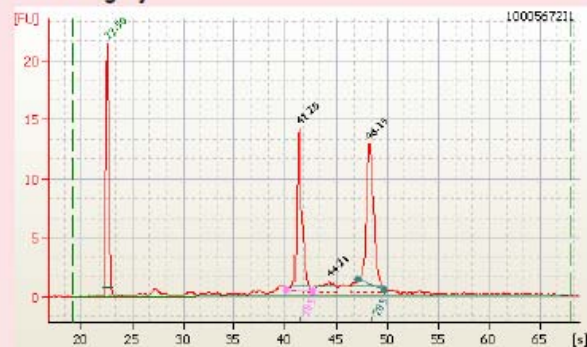
The breast cancer tissue sample submitted was analyzed by MammaPrint, an FDA Cleared IVDMA 70 - Gene Profile of Breast Cancer for Metastatic Risk that has been validated to correlate with high or low outcome risk for distant metastases in women with invasive breast cancer.¹ In the reference group as published, "Low Risk" means that a lymph node negative breast cancer patient has a 10% chance (95% CI 4-15) that her cancer will recur within 10 years without any additional adjuvant treatment, either hormonal therapy or chemotherapy.²

Clinicopathologic Findings

Tumor Cell Percentage: 65%



RNA Integrity Score: 9.6





targetprint™

Page 1 of 1

CUSTOMER

Doctor: Anton Haid
Account: Landeskrankenhaus Feldkirch

Address: Carinagasse 47-49

City: Feldkirch-Tisis 6807
Country: Austria

SPECIMEN

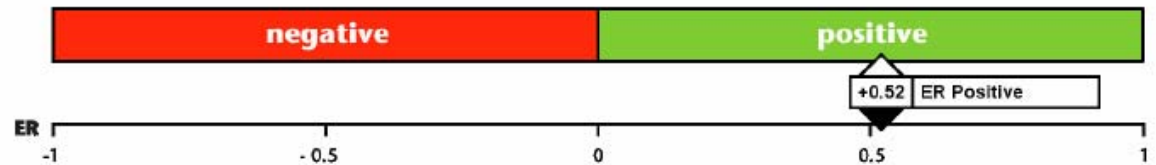
Requisition #: 00008843
Collection Date: 28-Sep-2010
Test Request Date: 29-Sep-2010
Date Received: 30-Sep-2010
Report Date: 11-Oct-2010
Specimen Type: Surgical Specimen
Customer Ref.: BZ16071963

PATIENT

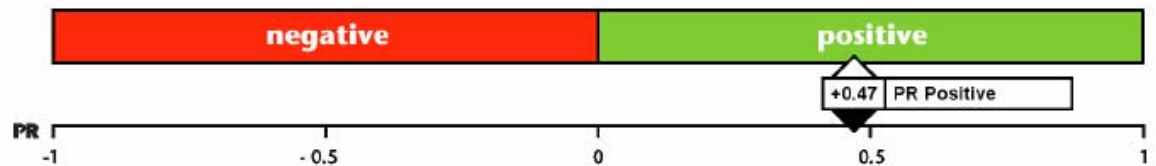
Patient:
DOB:
Patient #:
Gender: Female

Quantitative Gene Expression Results

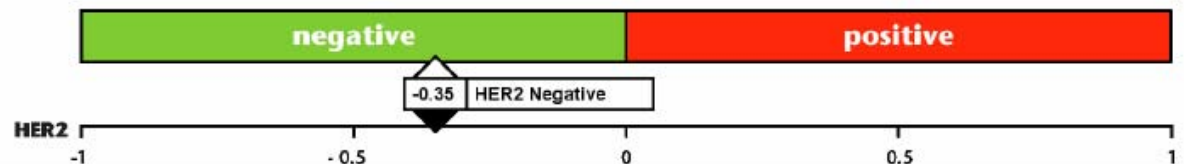
Estrogen Receptor
ER Positive



Progesterone Receptor
PR Positive



HER2/neu
HER2 Negative





CUSTOMER

Doctor: Anton Haid
Account: Landeskrankenhaus Feldkirch

Address: Carinagasse 47-49

City: Feldkirch-Tisis 6807
Country: Austria

SPECIMEN

Requisition #: 00008843
Collection Date: 28-Sep-2010
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PATIENT

Patient:

DOB:
Patient #:
Gender: Female

Molecular Subtyping Result

Luminal-type

Luminal-type breast cancers are characterized by gene expression of luminal epithelial cells that line the breast ducts and glands. The Luminal-type cancers are typically hormone receptor positive tumors and therefore responsive to hormonal therapy. A Luminal-type molecular subtyping result means that the tumor phenotype most closely resembles the Luminal-type intrinsic subtype. Patients classified as MammaPrint® 70-gene signature “low risk” and Luminal-type can be expected to have a clinical course similar to luminal A, usually treated with hormonal therapy, whereas those with a MammaPrint “high risk” and Luminal-type, a clinical course similar to luminal B patients who usually benefit from more aggressive treatment which may include chemotherapy.

Assay Description

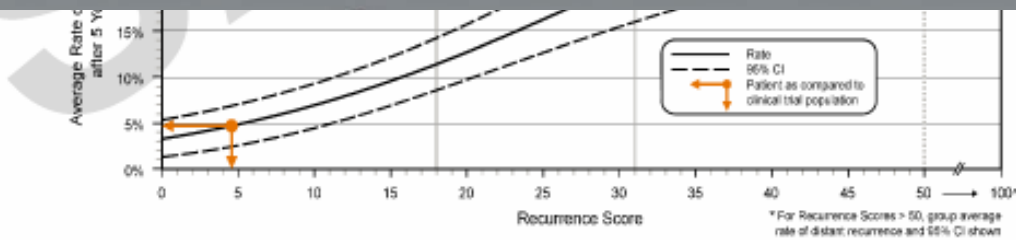
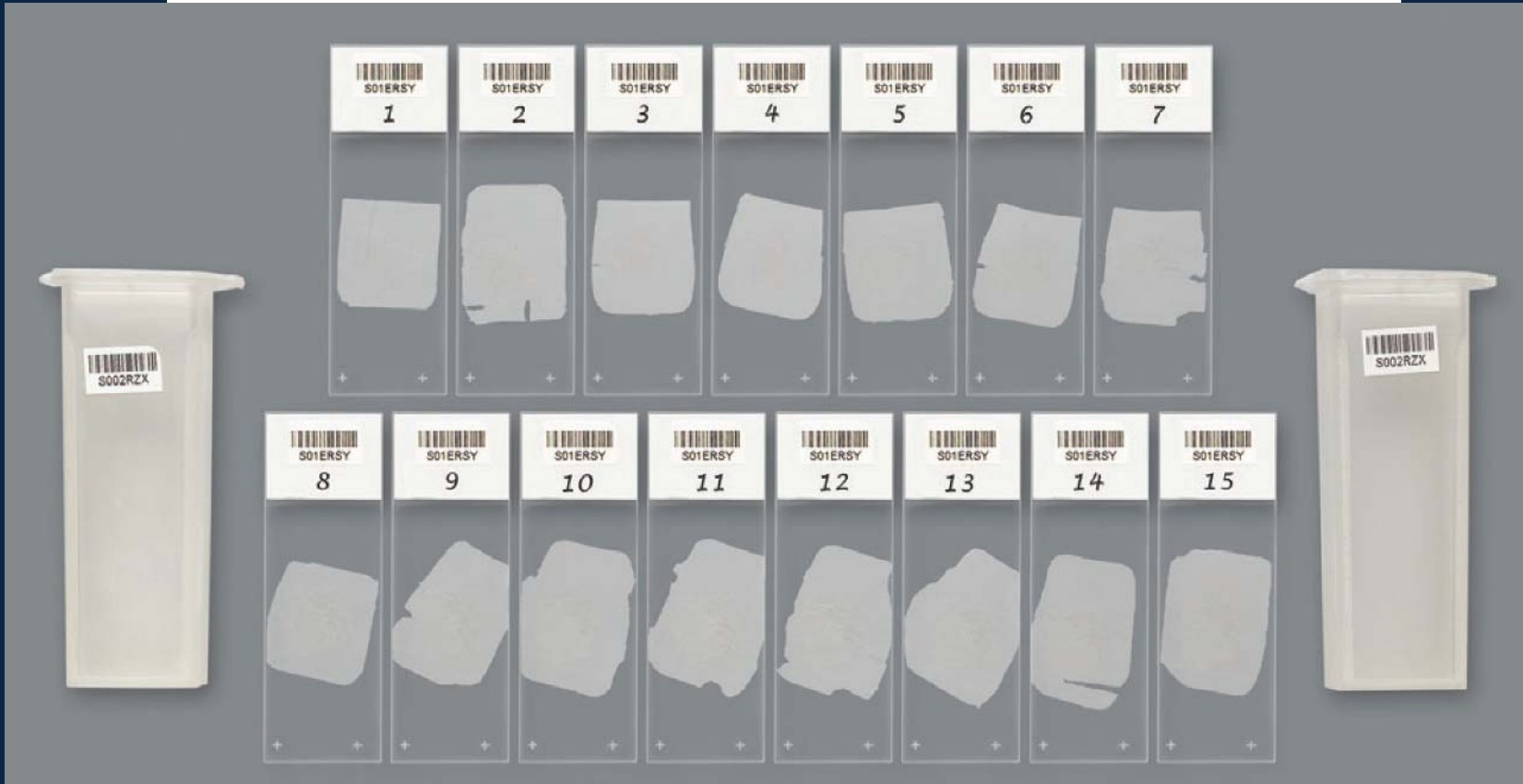
Gene expression analysis has confirmed the heterogeneity of breast cancer, revealing it to be a disease with intrinsic subgroups that can be uncovered by genomic profiling.¹ The BluePrint molecular subtyping profile was designed to distinguish the Basal-type, Luminal-type and ERBB2-type (HER2/neu positive) intrinsic subgroups of tumors.² The BluePrint signature determines the RNA levels of 80 genes that best discriminate among these three distinctive subtypes. Tumors from a cohort of 295 patients were used for the development of gene expression profiles specific for the Basal-type, Luminal-type and ERBB2-type breast cancers. Using state of the art bioinformatics tools, Agendia identified genes whose expression ratios best discriminate between the three subgroups. Subtype specific gene expression profiles were identified in a 3-fold cross-validation procedure. Optimal classification of the training samples in the corresponding Basal-type, Luminal-type and ERBB2-type subgroups was reached with a set of 80 genes. Next, a nearest-centroid classification procedure utilizing the 80-gene profile was developed that most accurately classified the breast cancer molecular subtypes on all samples. The BluePrint molecular subtyping profile was subsequently validated on 374 independent samples and demonstrated high concordance with the subgroups (excluding normal-like) described by Perou et al.^{1,2}



PATIENT REPORT

Patient: Doe, Jane
Sex: Female
DOB: 01/01/1950
Medical Record/Patient #: 556677771

Requisition: R00003G
Order Received: 2/01/2008
Date Reported: 2/13/2008
Client: Community Medical Center



Einsatz MammaPrint am LKH Feldkirch

JA:

Invasiv duktale Karzinome

Grad 1-3

0-3 positive Lymphknoten

Hormonrezeptoren positiv

Familiäre Belastung: BRCA1,2

NEIN:

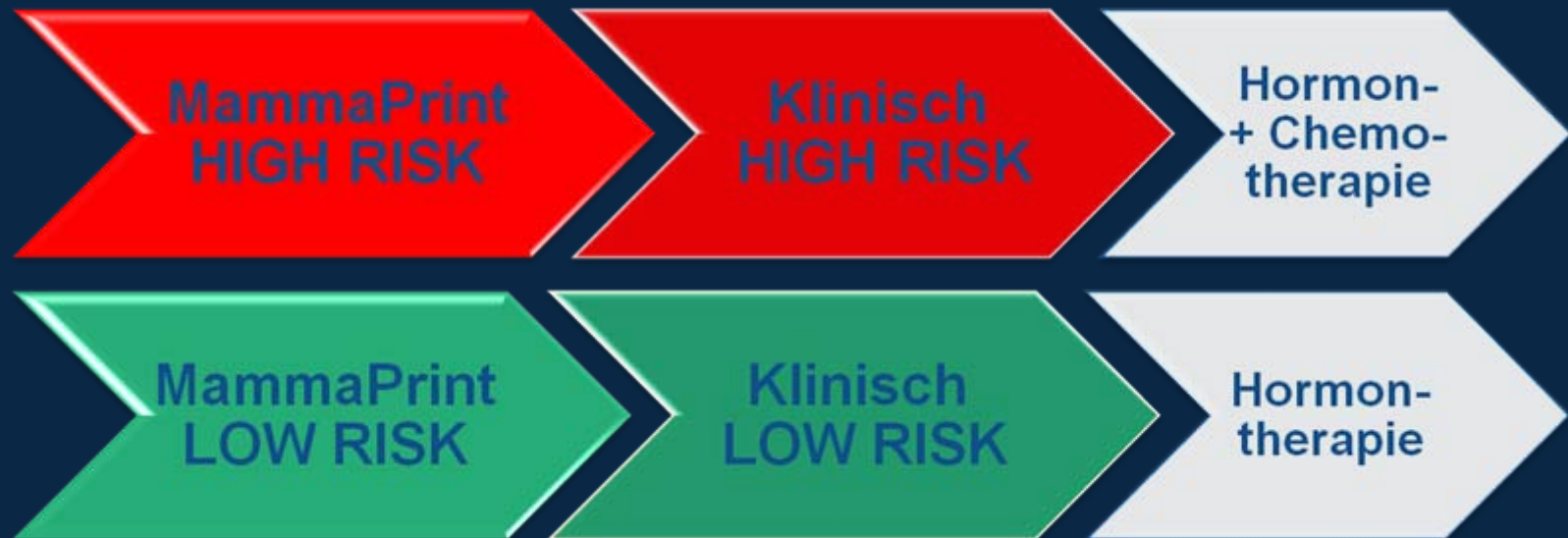
Invasiv lobuläre Karzinome

Triple negative Tumoren

Her2 positive Tumore

DCIS

MÖGLICHER PRAKTISCHER EINSATZ BEIM REZEPTOR-POSITIVEN MAMMAKARZINOM



MÖGLICHER PRAKTISCHER EINSATZ BEIM REZEPTOR-POSITIVEN MAMMAKARZINOM



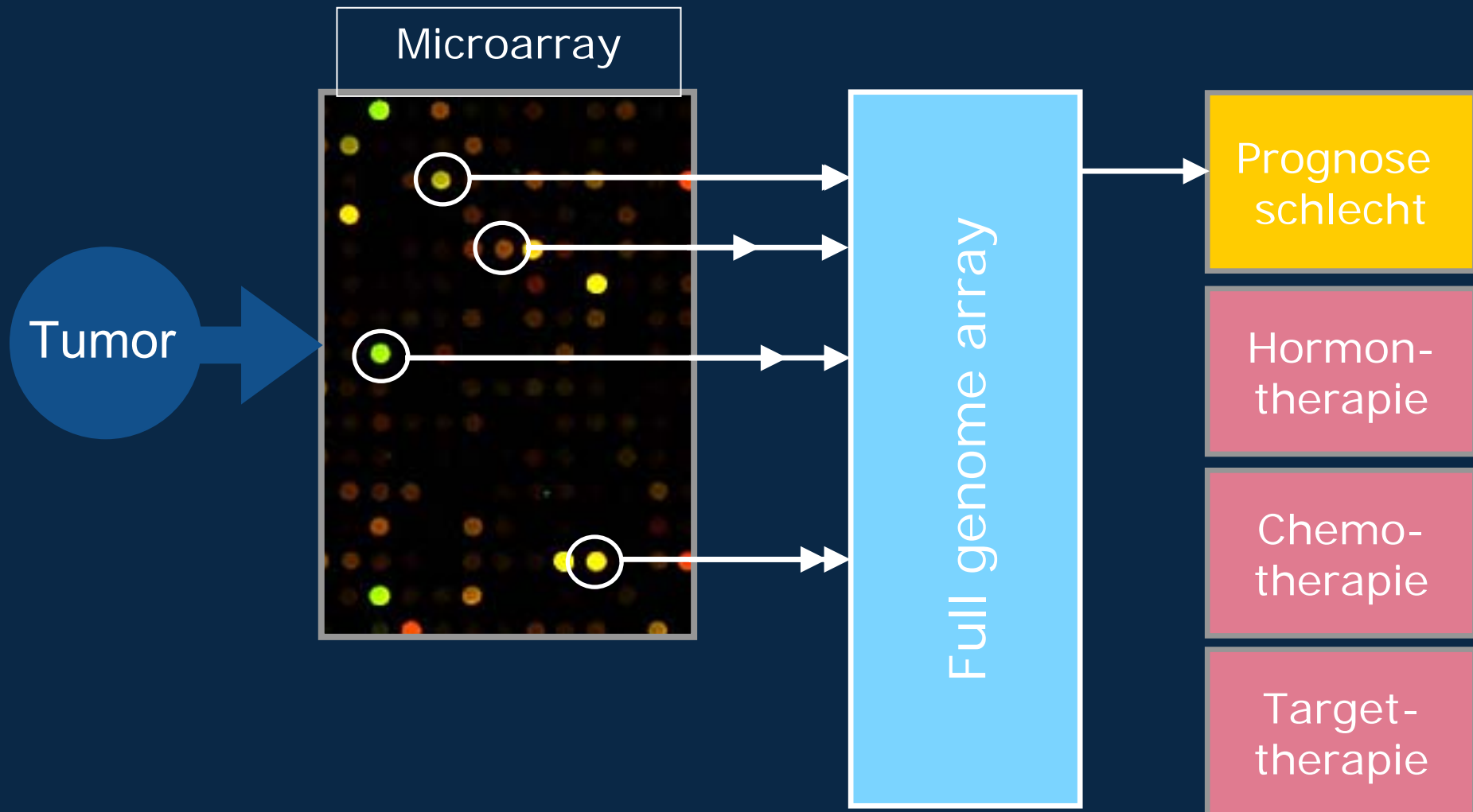
(1) If the patient is highly endocrine-responsive, otherwise consider CT

(2) If the patient is highly endocrine-responsive:
ET only is an option, no adjuvant therapy is not advised

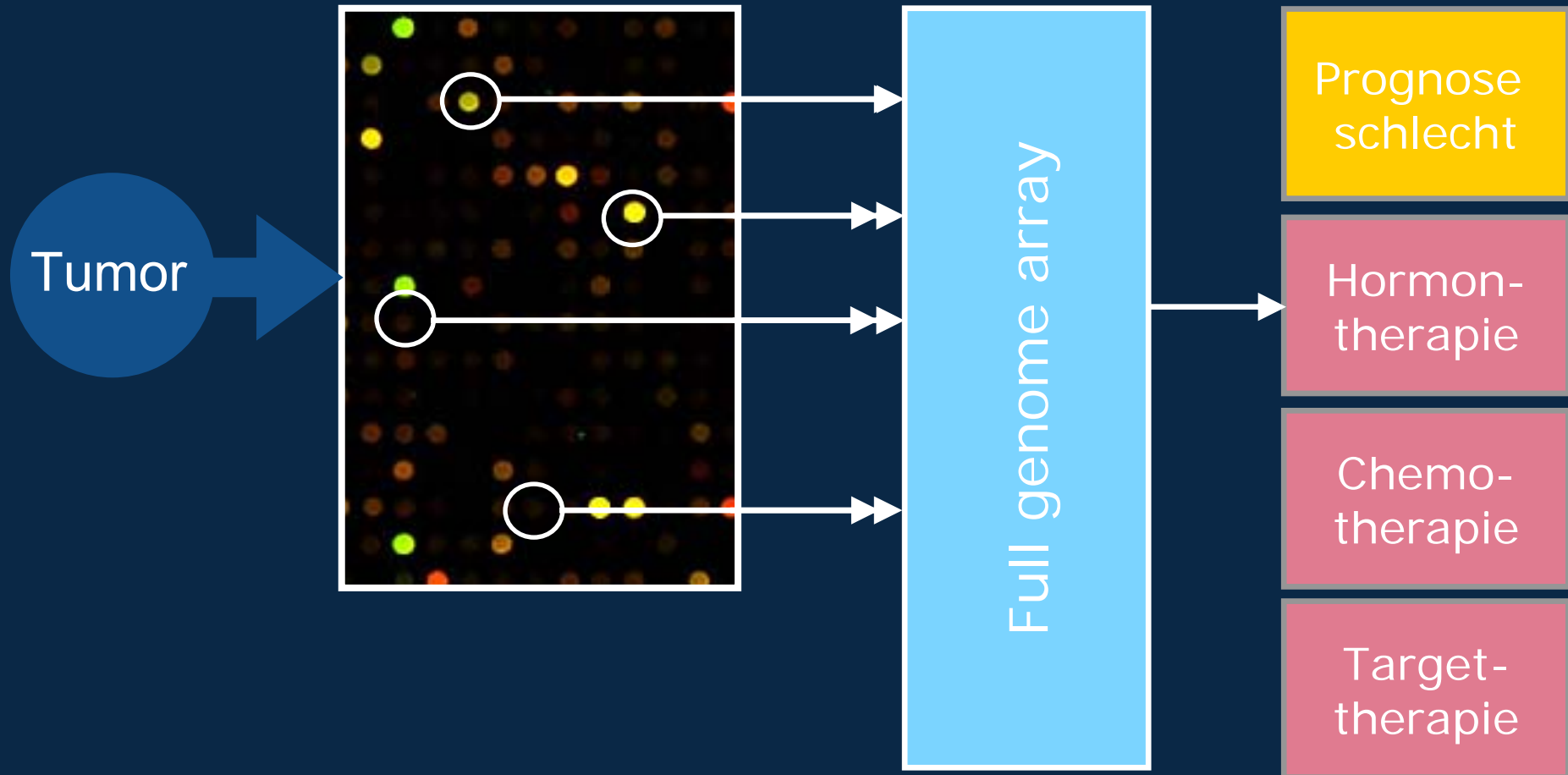
St. Gallen 2009

	Chemo- Endokrin	Nicht zielführend	Endokrin
Rezeptoren	ER und PR niedrig		ER und PR hoch
Grad	Grad 3	Grad 2	Grad 1
Proliferation	Hoch	Mittel	Niedrig
Lymphknoten	≥ 4	1 – 3	0
PVI	Extensive PVI		
Tumorgröße	$> 5\text{cm}$	2,1 – 5 cm	$\leq 2\text{cm}$
Patientenwunsch	Alle Therapie- optionen		Vermeidung von NW
Multigensignatur	High risk	Intermediate	Low risk

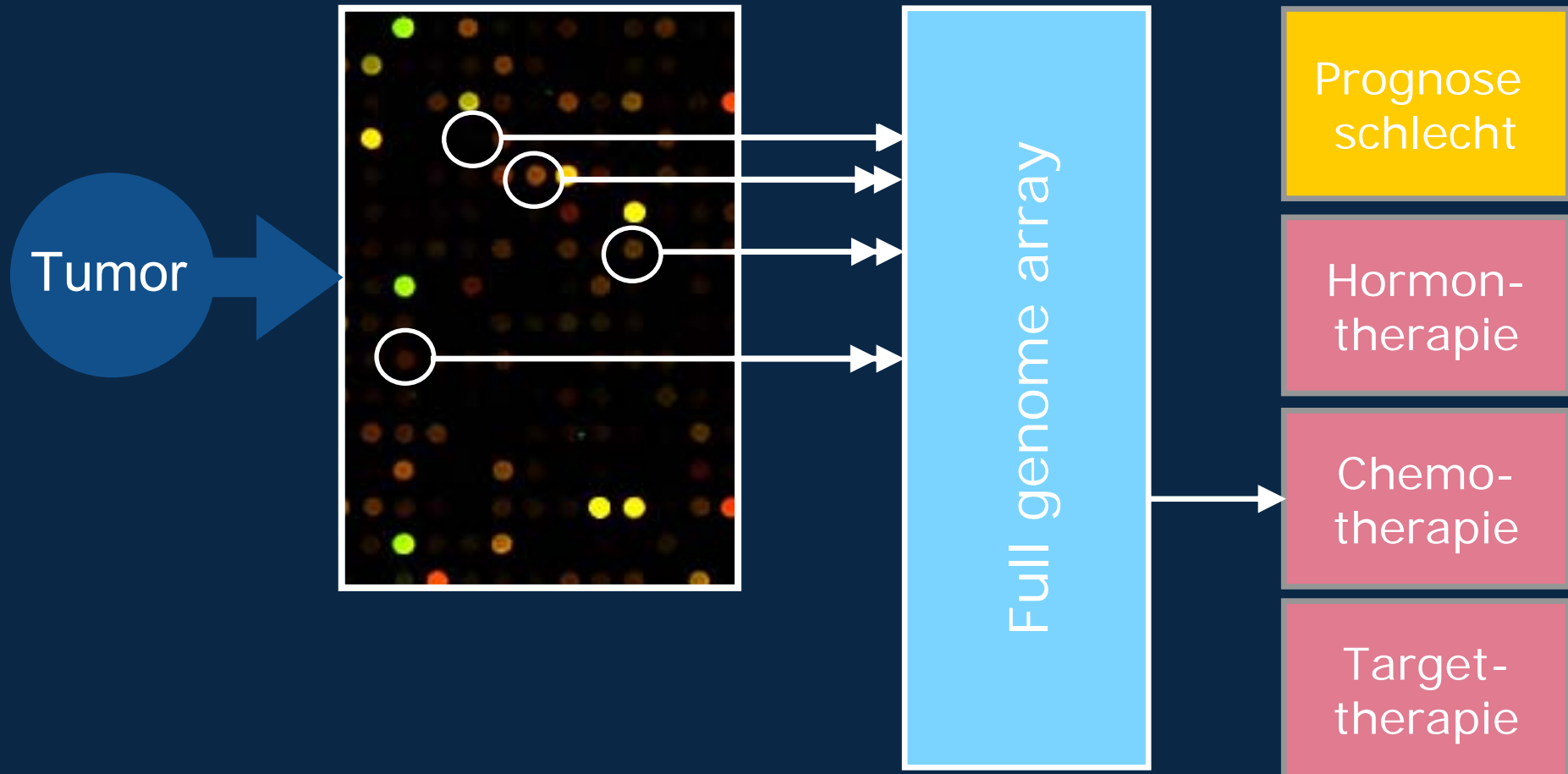
WAS KOMMT ALS NÄCHSTES?



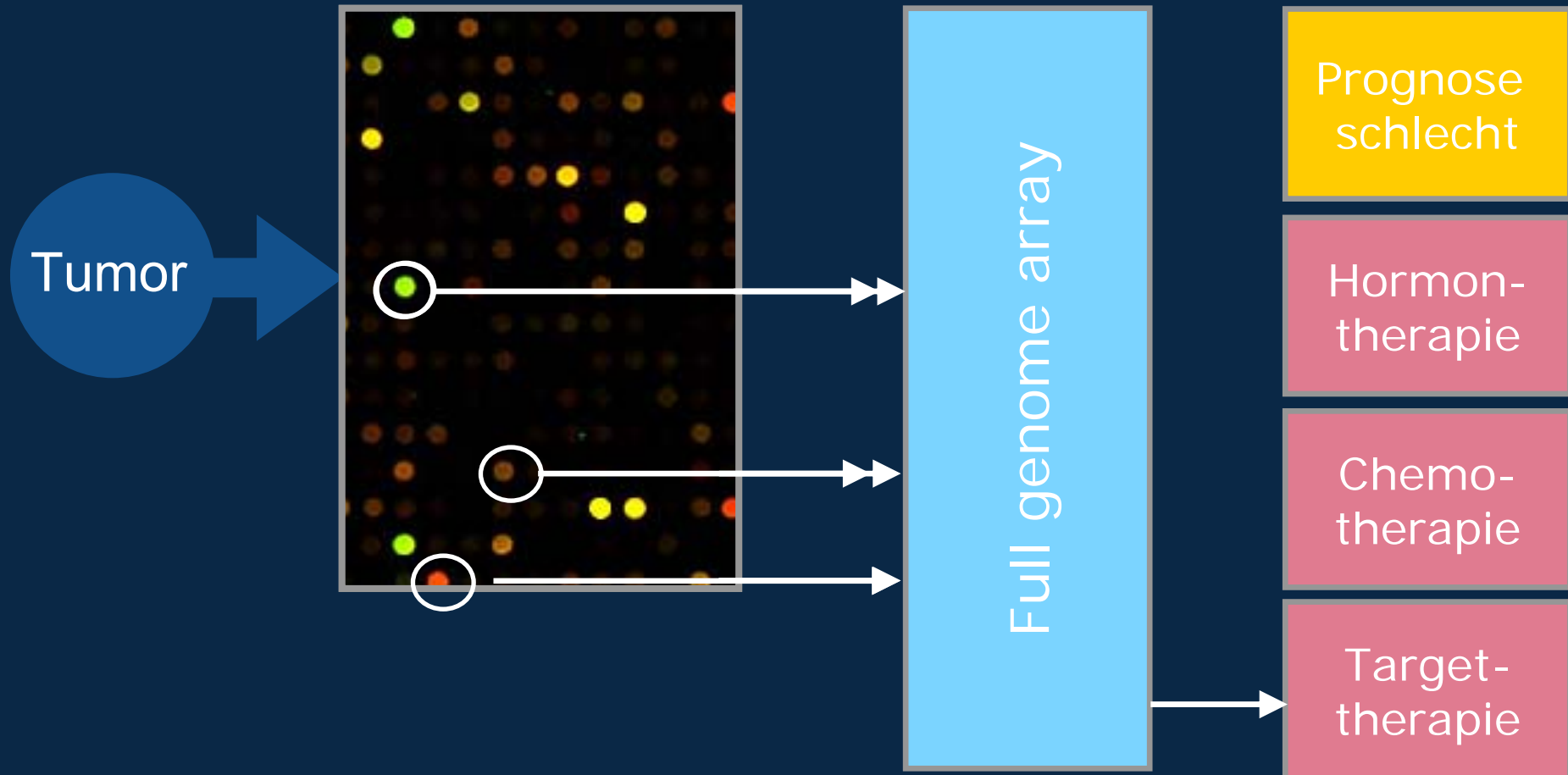
1 TEST → VIELE INDIVIDUELLE PROFILE



1 TEST → VIELE INDIVIDUELLE PROFILE



1 TEST → VIELE INDIVIDUELLE PROFILE



Vielen Dank

Netherlands Cancer Institute:

E. Rutgers, S. Linn, L. van 't Veer, S. Mook, V. Retel, J. Bueno-de-Mesquita, M. Kok, W. van Harten, S. Rodenhuis, M. van de Vijver

Österreich:

A. Haid, E. Wenzl, Z. Jasarevic, R. Bartsch, M. Gnant

vorarlberg

