

Emerging breast cancer biomarkers as disease forecast markers and targets for therapy



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**Cancer
biomarkers**

Diagnosis

**Detection of
disease**

**Cancer
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Diagnosis

**Detection of
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**Disease
monitoring**

**Detection of
disease recurrence**

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**Localizing tumors
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**Pharmaco-
dynamics**

What dose ?

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What dose ?

**Predicting drug-
associated
toxicity**

Molecular alterations in tumors can be uncovered using technologies that assess changes in the content or sequence of

- ❖ **DNA,**
- ❖ **its transcription into messenger RNA or microRNA.**
- ❖ **The production of proteins or**
- ❖ **the synthesis of various metabolic products.**
- ❖ **Carbohydrates**
- ❖ **Lipids**
- ❖ **Other constituents in tissue or bodily fluids.**

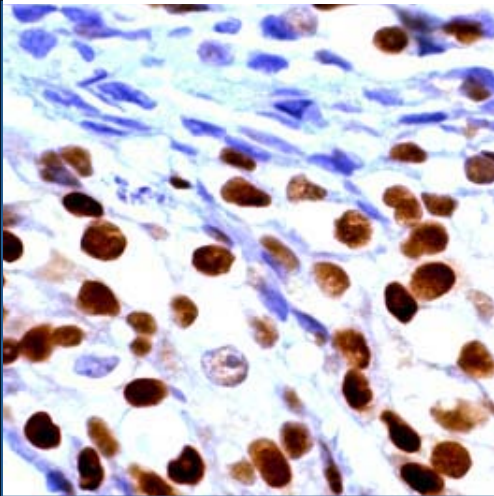
Sources of cancer biomarkers in breast cancer

- **Blood (plasma, serum, cells)**
- Nipple aspirate fluid
- Pleura effusion
- Urine
- **Breast cancer tissue**
- Lymph node tissue
- Metastatic tissue

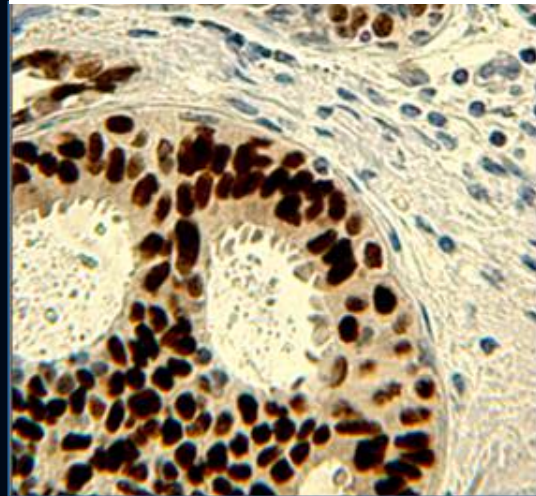


Target protein	Prognostic value	Predictive value	Cancer drug
ER; PR	Yes	Yes	Tamoxifen
HER2	Weak	Yes	Herceptin, Tyverb

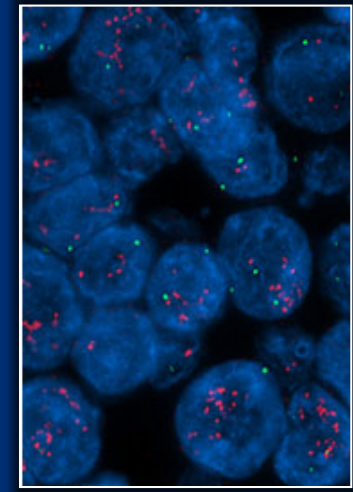
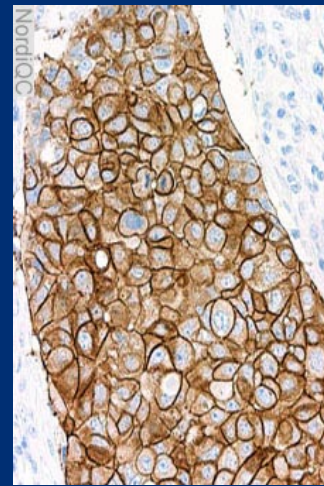
**Estrogen receptor,
breast cancer**



**Progesterone receptor,
breast cancer**



Her2, breast cancer



Tumor Marker Utility Grading System: a Framework to Evaluate Clinical Utility of Tumor Markers

*Daniel F. Hayes, Robert C. Bast, Christopher E. Desch, Herbert Fritsche, Jr., Nancy E. Kemeny, J. Milburn Jessup, Gershon Y. Locker, John S. Macdonald, Robert G. Mennel, Larry Norton, Peter Ravdin, Sheila Taube, Rodger J. Winn**

Level-of-evidence (LOE) 1 - 5

Level of evidence	Classification
1	Evidence derived from prospective high-powered clinical trial specifically addressing tumor marker utility OR overview or meta (pooled) analysis of lower LOE studies.
2	Evidence from tumor specimens which were collected prospectively and tumor marker utility determined as secondary aim of study in order to get ready for a large clinical trial.
3	Evidence from larger retrospective trials.
4	Evidence from small retrospective studies.
5	Evidence from small pilot studies designed to determine or estimate distribution of tumor biomarker levels in sample population.

Tumor marker utility grading system: Hayes J Natl Cancer Inst. 1996; 88:1456

American Society of Clinical Oncology 2007 Update of Recommendations for the Use of Tumor Markers in Breast Cancer

Lyndsay Harris, Herbert Fritsche, Robert Mennel, Larry Norton, Peter Ravdin, Sheila Taube, Mark R. Somerfield, Daniel F. Hayes, and Robert C. Bast Jr

Thirteen categories of breast tumor markers were considered, six of which were new for the guideline.

**The following categories demonstrated
insufficient evidence to support routine
use in clinical practice**

- ❖ **DNA/ploidy by flow cytometry**
- ❖ **detection of bone marrow micrometastases**
- ❖ **circulating tumor cells**
- ❖ **p53**
- ❖ **Cathepsin D**
- ❖ **cyclin E**
- ❖ **certain multiparameter gene assays**
- ❖ **proteomics**

The following categories showed evidence of clinical utility and were recommended for use in practice

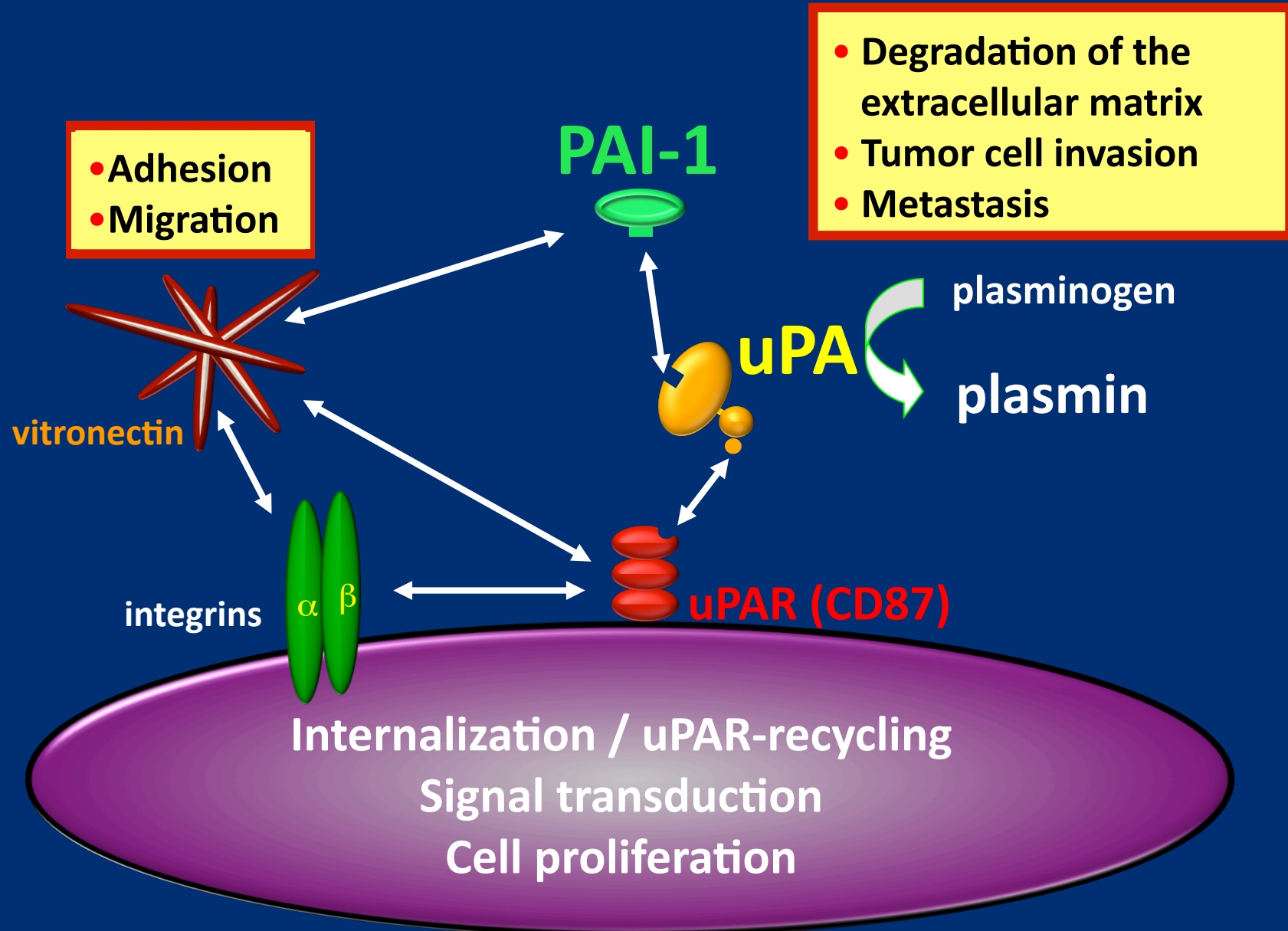
- ❖ CA 15-3 (blood)
- ❖ CA 27.29 (blood)
- ❖ Carcinoembryonic antigen (blood)

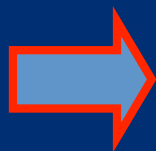
- ❖ Estrogen receptor, progesterone receptor (ER, PR)
- ❖ Human epidermal growth factor receptor 2 (HER2)
- ❖ Multiparameter gene expression assay (Oncotype DX)

- Urokinase plasminogen activator (uPA)
- Plasminogen activator inhibitor type-1 (PAI-1)

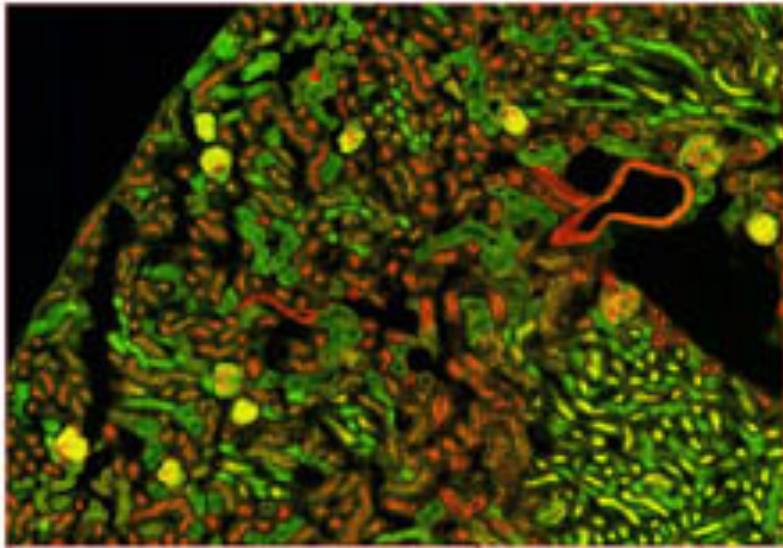
uPA and PAI-1

Tumor invasion and metastasis





Summary	Not Recommended	Recommended
P53	Screening, diagnosis, staging, prognosis, surveillance, or monitoring.	
Cathepsin D	Screening, diagnosis, staging, prognosis, surveillance, or monitoring.	
uPA and PAI-1	Screening, diagnosis, staging, surveillance, or monitoring.	To determine prognosis. For treatment planning. To guide use of CMF-based adjuvant chemotherapy.
Cyclin E Fragments	Screening, diagnosis, staging, prognosis, surveillance, or monitoring.	
Proteomic Analysis	Screening, diagnosis, staging, prognosis, surveillance, or monitoring.	
Multiparameter Gene Expression Analysis	Screening, diagnosis, staging, surveillance, or monitoring. Not for prediction of hormonal therapies other than tamoxifen or other chemotherapy regimens.	Oncotype [™] for prognosis for patients with node-negative, ER positive breast cancer who will receive tamoxifen. Guiding use of adjuvant tamoxifen and adjuvant chemotherapy (specifically CMF).
Multiparameter Gene Expression Analysis, other	Screening, diagnosis, staging, prognosis, surveillance, or monitoring.	
Bone Marrow Micrometastases	Screening, diagnosis, staging, prognosis, surveillance, or monitoring.	
Circulating tumor cell assays	Screening, diagnosis, staging, prognosis, surveillance, predicting or monitoring.	



Glass tissue slide

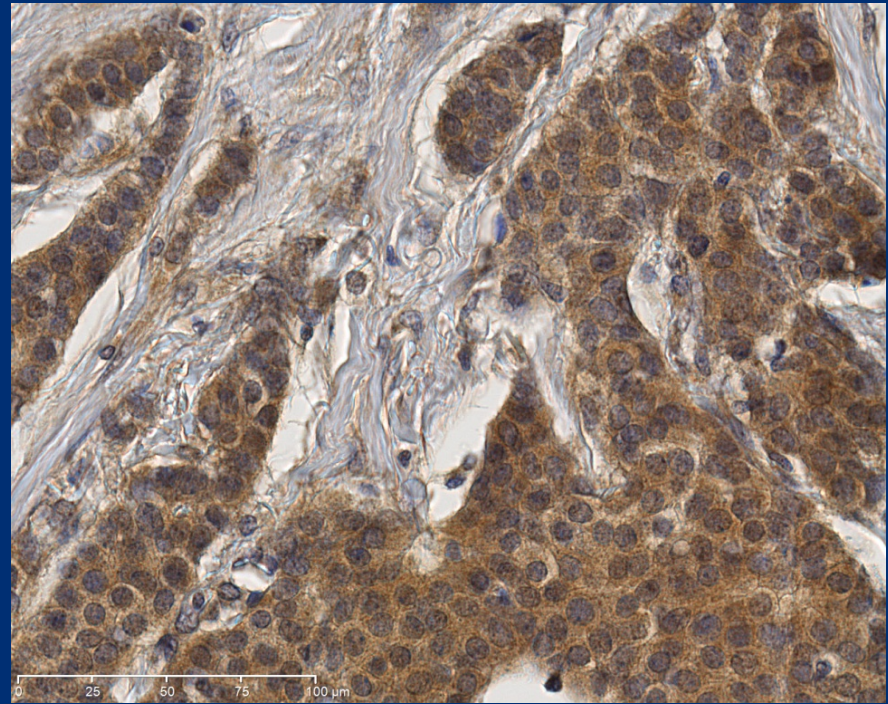
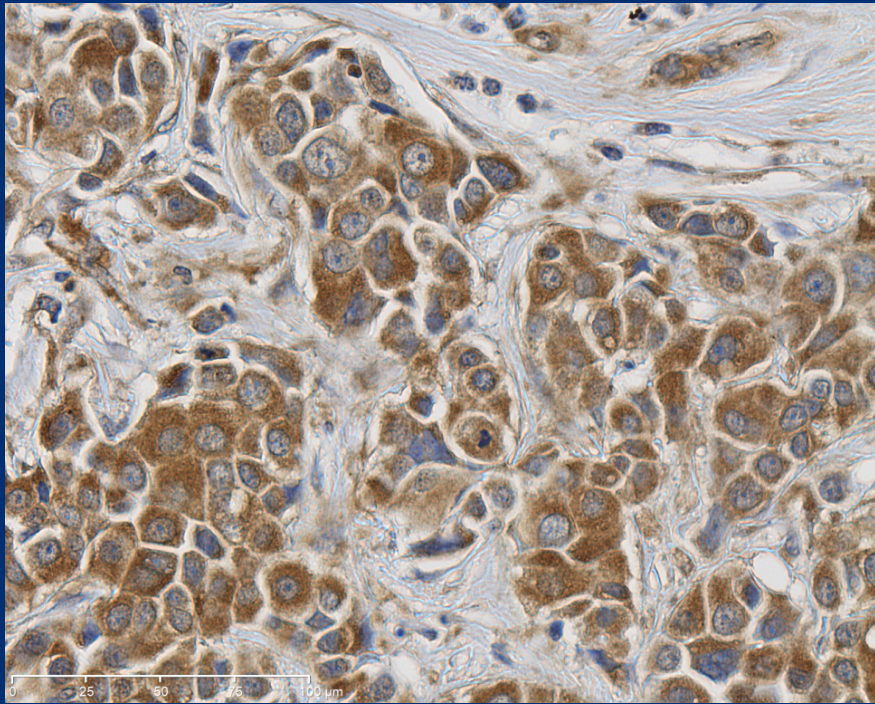


HAMAMATSU

Photon is our business



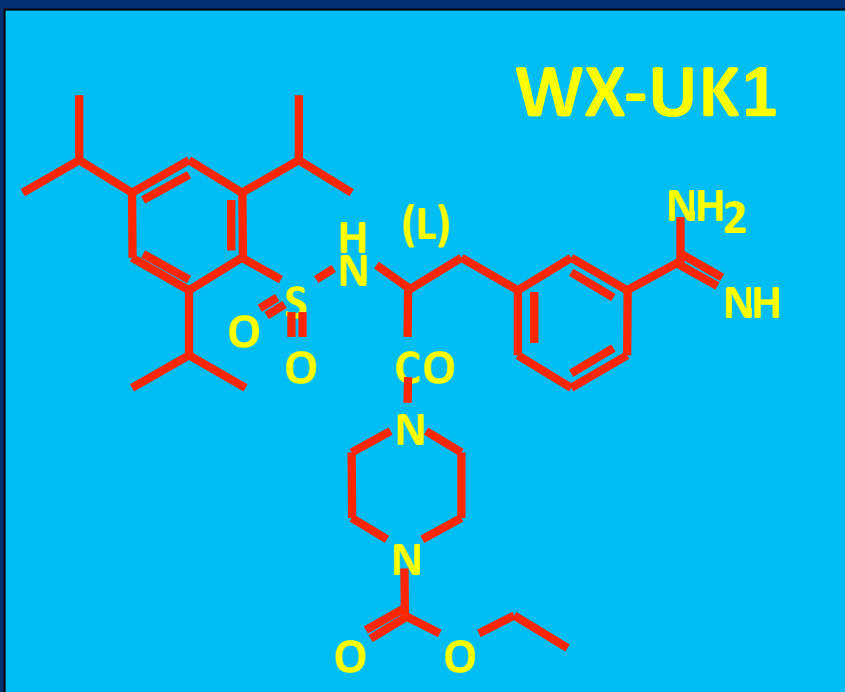
Fluorescence Illumination Optics
L10387



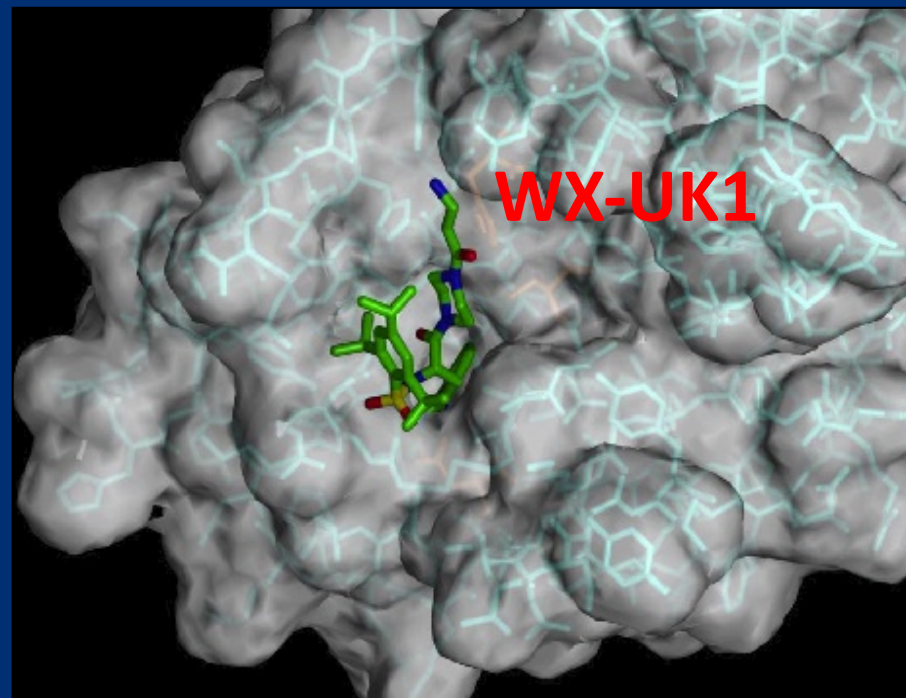
uPA (left) and PAI-1 (right) protein expression in ductal invasive breast cancer

brown: uPA, PAI-1 blue: hematoxylin (nuclei). Scanned with Hamamatsu NanoZoomer virtual microscope at 40 x. Monoclonal antibody #3689 (uPA), #3785 (PAI-1) American Diagnostica, CT, USA. No antigen retrieval. LSAB-method.

WX-UK1: N-Triisopropyl-phenylsulfonyl-amidinophenylalanin



WX-UK1 inhibits uPA, plasmin, thrombin



30. 09. 09: WILEX AG:

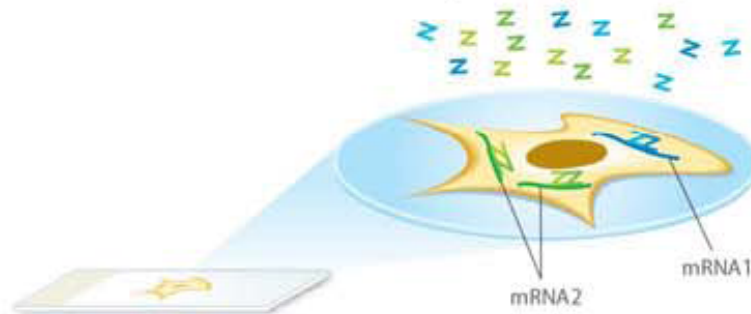
Preliminary data of MESUPRON (phase II study) show impressive increase in overall survival of patients with pancreatic cancer.

MESUPRON is an orally active pro-drug based on WX-UK1.



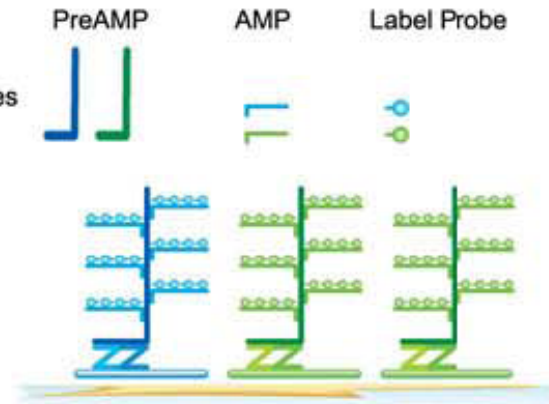
ADVANCED CELL DIAGNOSTICS, INC

ZZ Target RNA-Specific Oligo Probes



Step 1

Step 2



Step 3



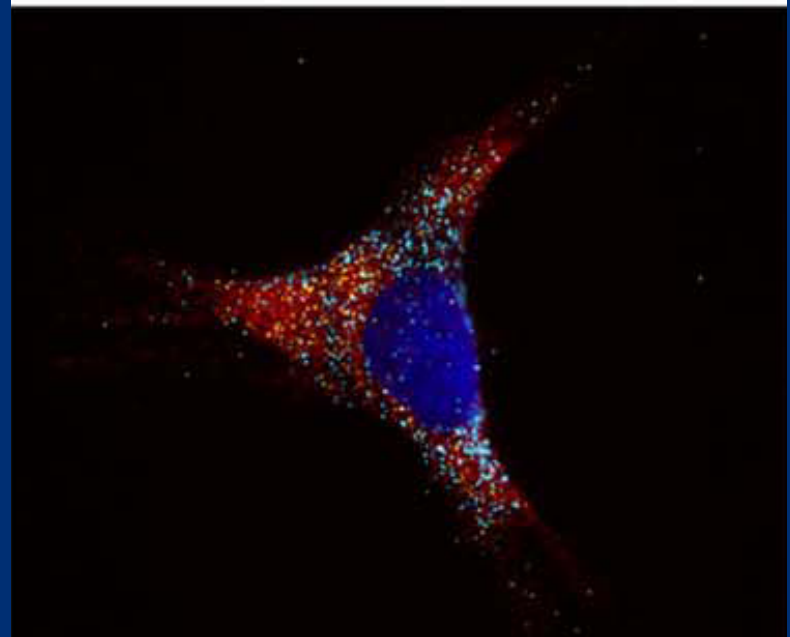
Step 4

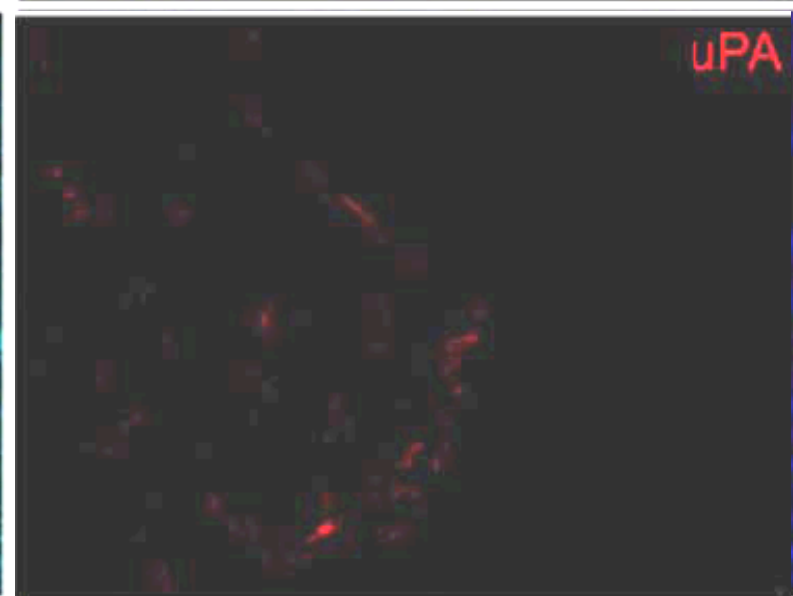
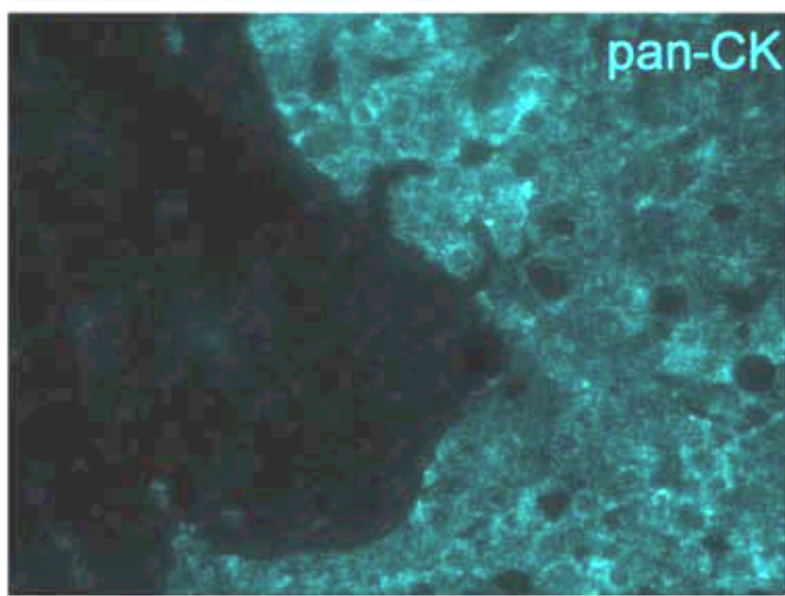
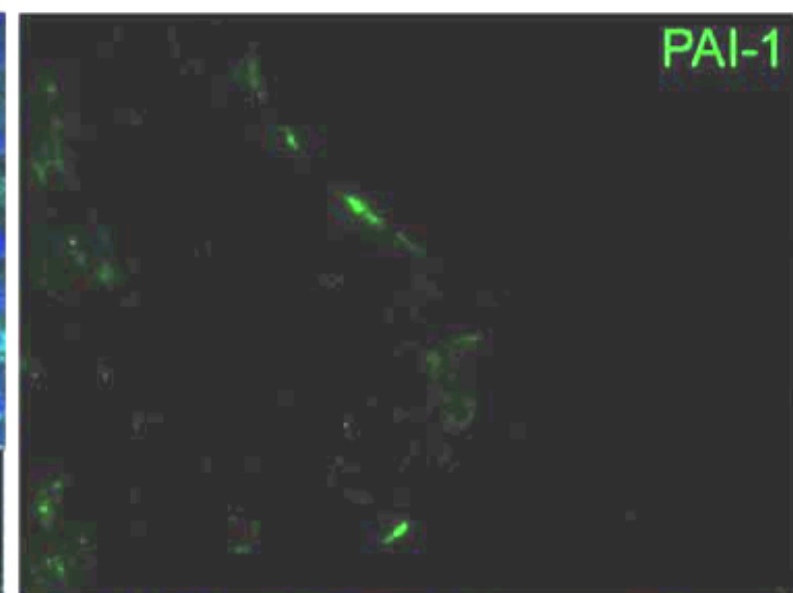
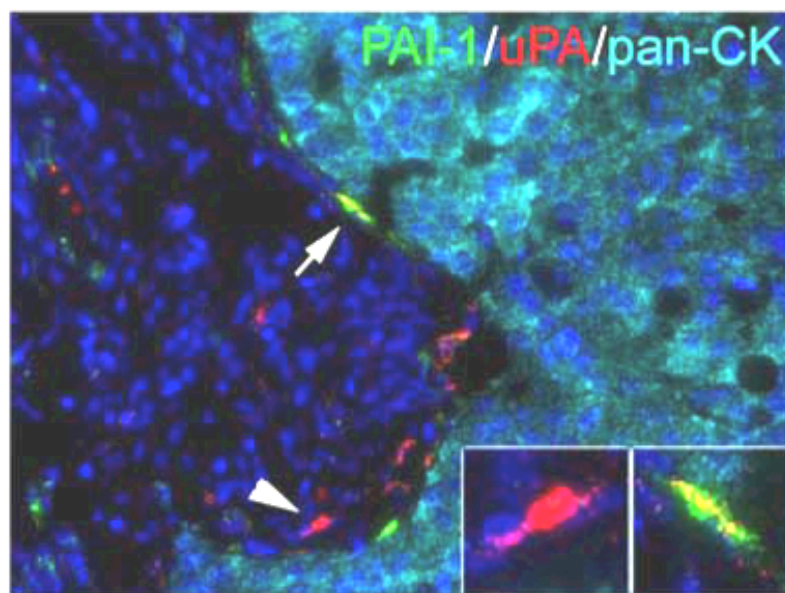
PPIB (peptidylprolyl isomerase B)

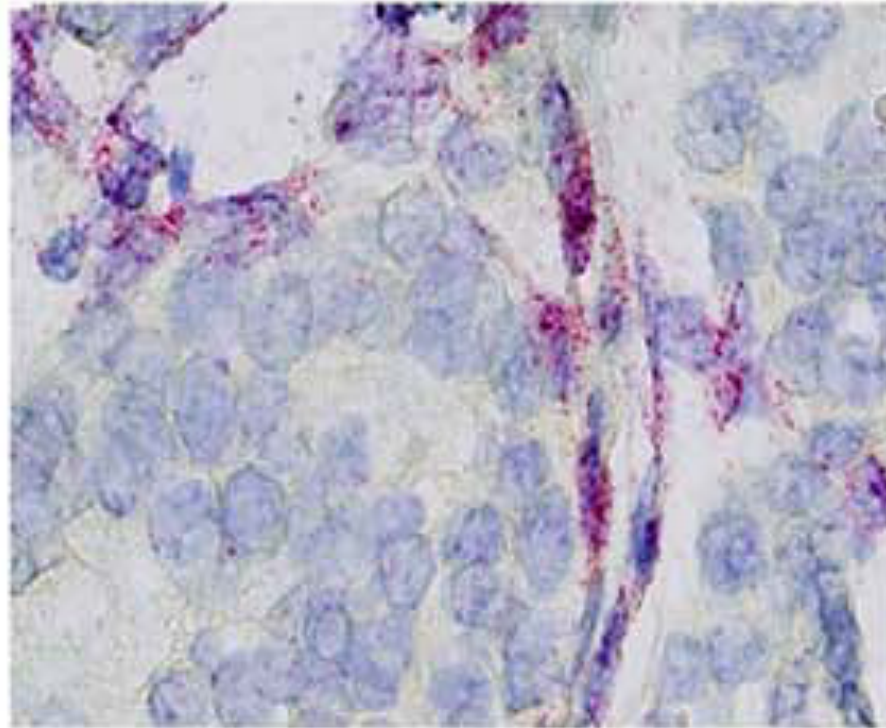
RPLP0 (60S acidic ribosomal protein P0)

HPRT-1 (hypoxanthine phosphoribosyl-transferase 1)

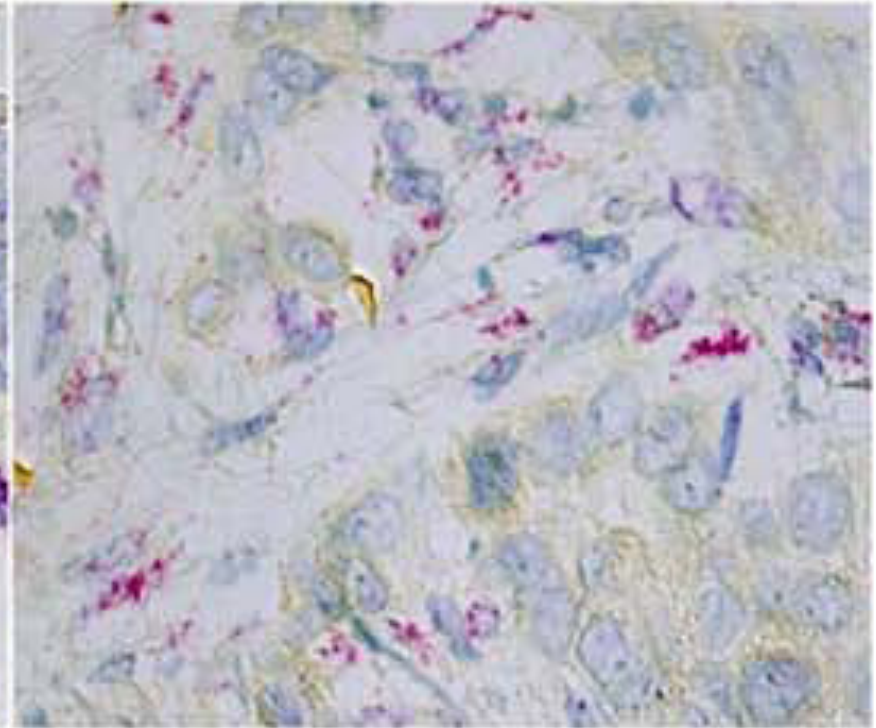
DAPI + B-Actin + HPRT-1 + RPLP0 + PPIB





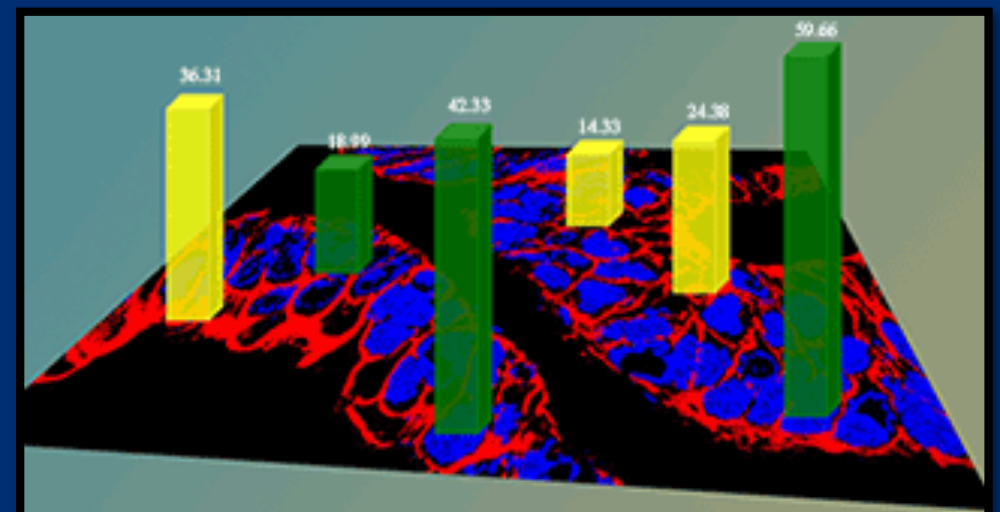
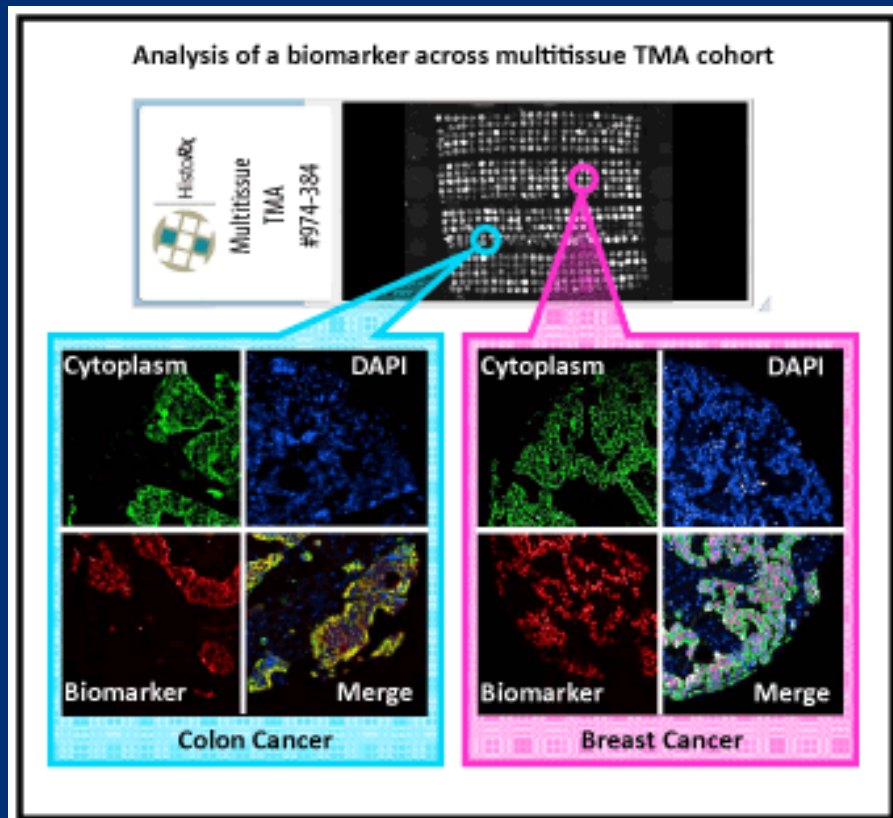
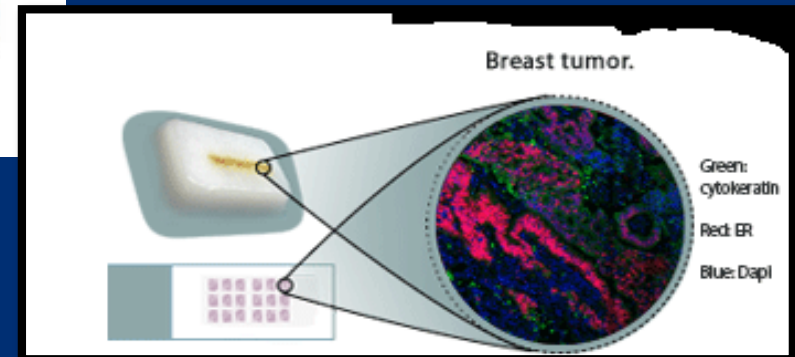
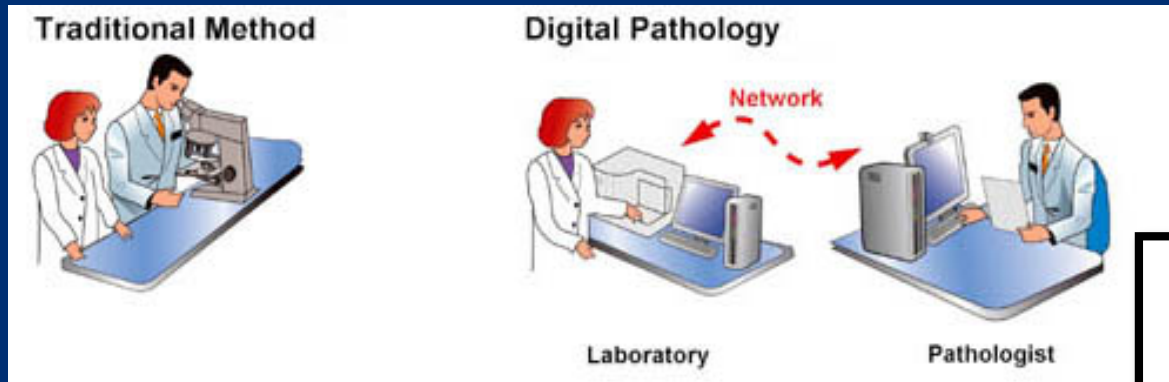


uPA/Hematoxylin



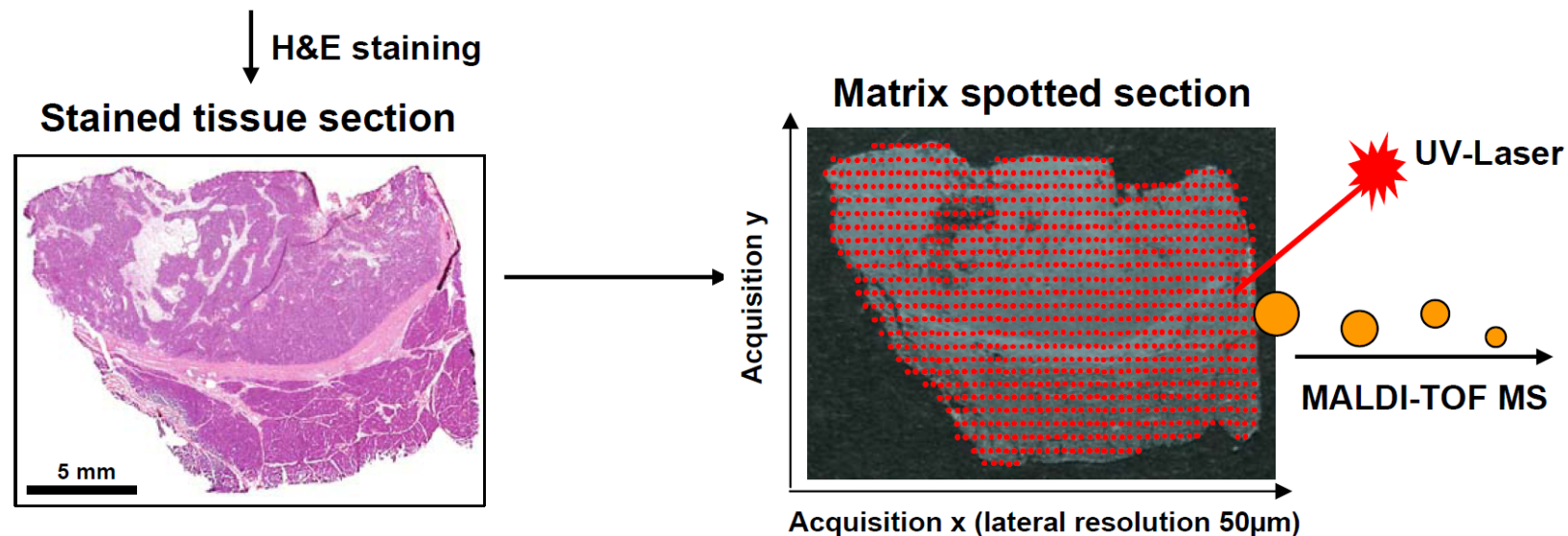
PAI-1/Hematoxylin

Improving the automatic biomarker IHC analysis further by employing the AQUA system

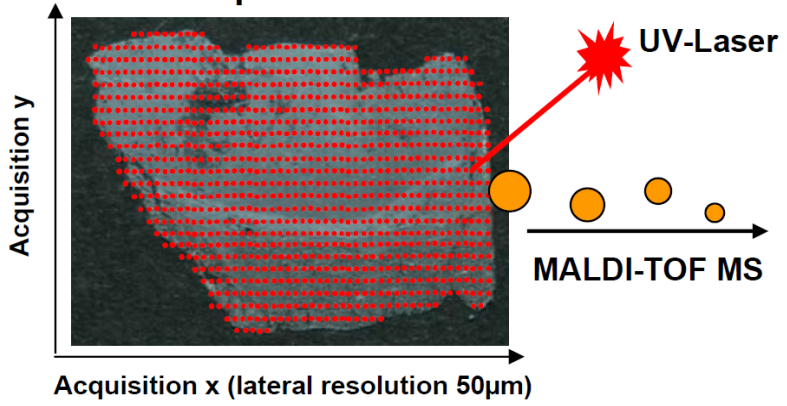


MALDI imaging mass spectrometry for direct tissue analysis: a new frontier for molecular histology

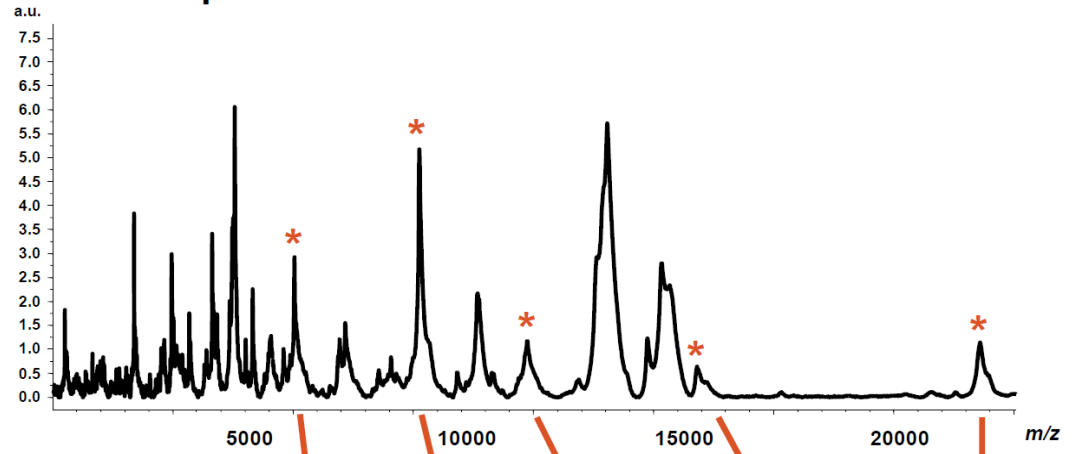
Axel Walch · Sandra Rauser · Sören-Oliver Deininger ·
Heinz Höfler



Matrix spotted section

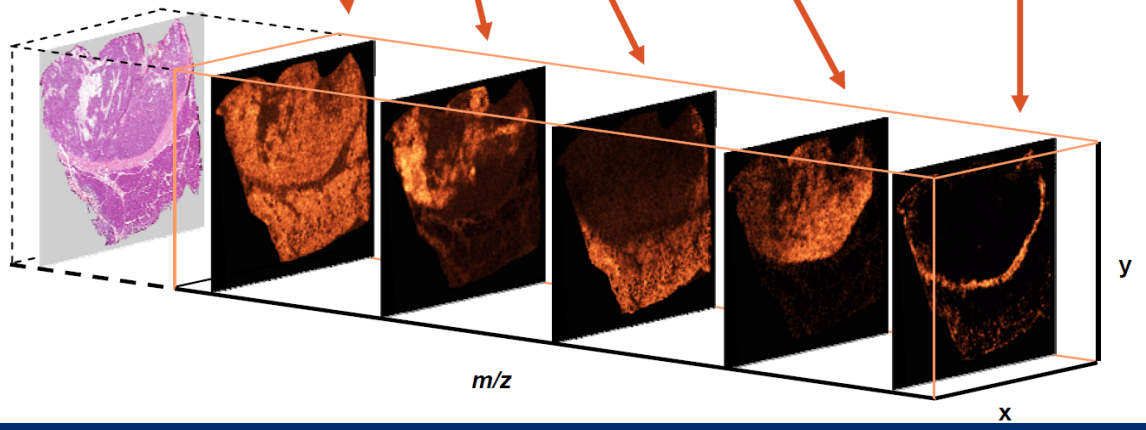
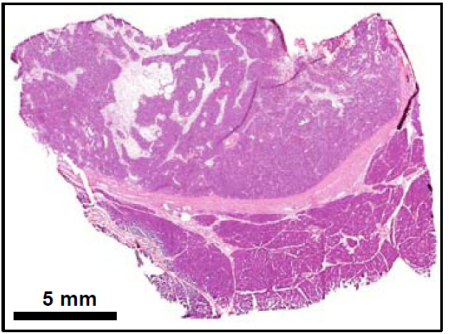


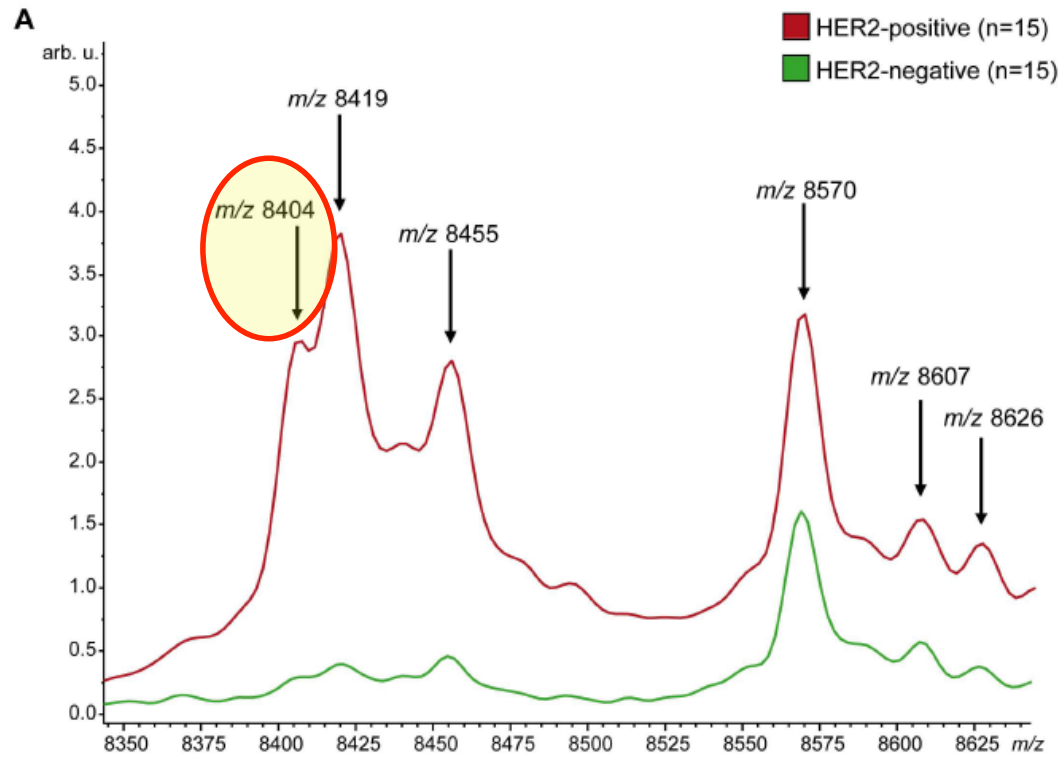
Mass spectrum



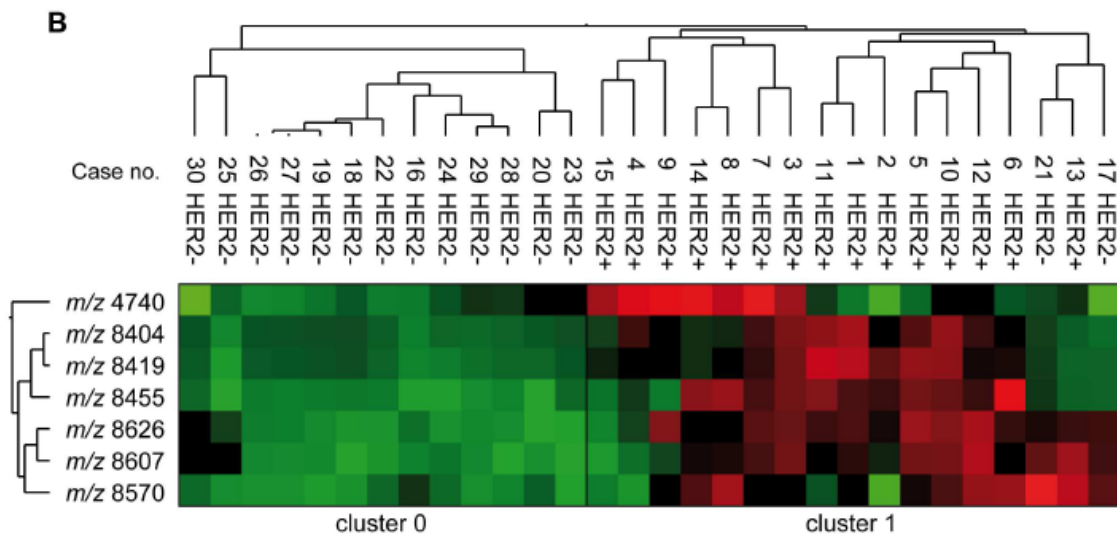
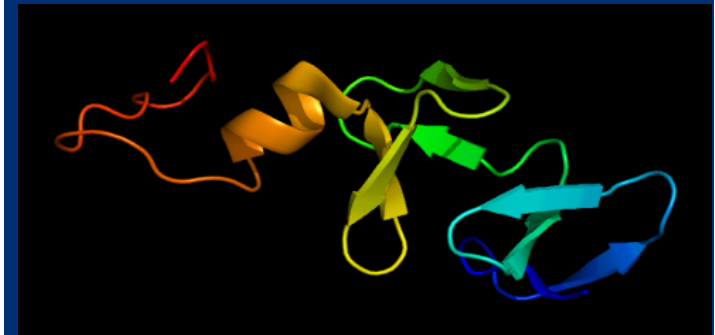
H&E staining

Stained tissue section





m/z 8404 =
Cysteine-rich protein 1
(CRIP1_HUMAN)
Zn-binding protein



Journal of Proteome Research, 2010, Feb. 14, published ahead

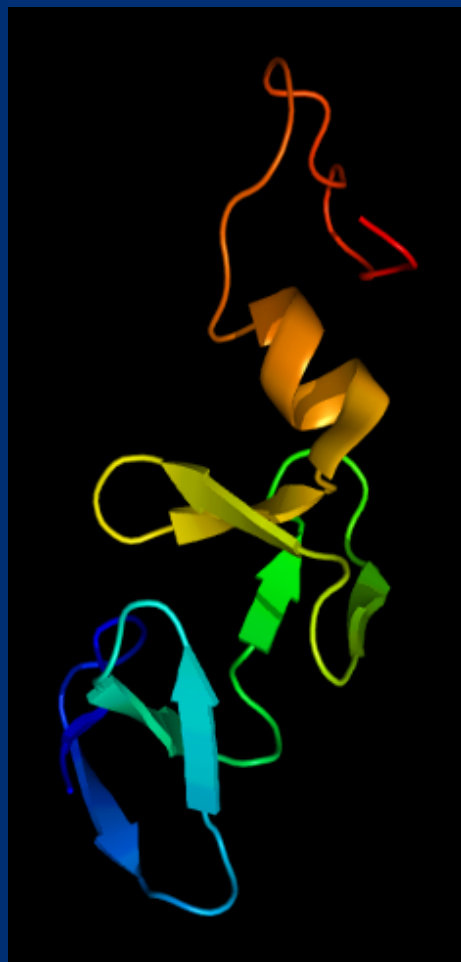
Classification of HER2 Receptor Status in Breast Cancer Tissues by MALDI Imaging Mass Spectrometry

Sandra Rauser, Claudio Marquardt, Benjamin Balluff, Sören-Oliver Deininger, Christian Albers, Eckhard Belau, Ralf Hartmer, Detlev Suckau, Katja Specht, Matthias P. Ebert, Manfred Schmitt, Michaela Aubele, Heinz Höfler, Axel Walch

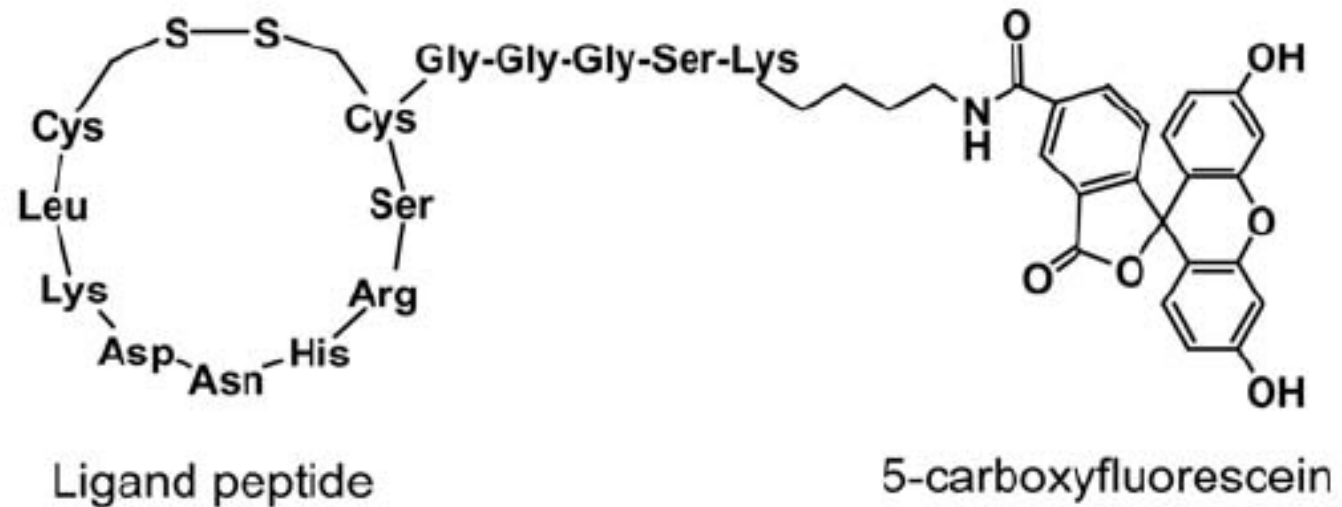
Institute of Pathology, Helmholtz Zentrum München - German Research Center for Environmental Health, 85764 Neuherberg; Department of Medicine II, Klinikum rechts der Isar, Technische Universität München, 81675 Munich; Bruker Daltonik GmbH, 28359 Bremen; Institute of Pathology, Technische Universität München, 81675 Munich; Department of Obstetrics and Gynecology, Klinikum rechts der Isar, Technische Universität München, 81675 Munich, Germany

Identification and Rational Redesign of Peptide Ligands to CRIP1, A Novel Biomarker for Cancers

Jihua Hao^{1,9}, Adrian W. R. Serohijos^{2,3,9}, Gail Newton^{4,10a}, Gina Tassone^{4,10b}, Zuncai Wang⁴, Dennis C. Sgroi⁴, Nikolay V. Dokholyan^{2,5*}, James P. Basilion^{1,6,7*}



Fluorescent Probe

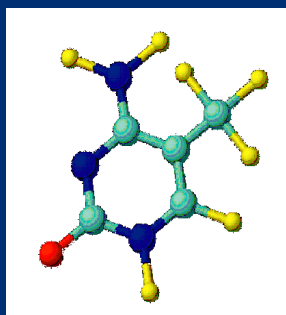


Factor	Number of patients	Assay	Association with	Reference
PITX2, PITX2, BMP4, FGF4, C20orf55, PSAT-1, STMN1, S100A2, SFN, PRKCD, SYK, VTN, GRIN2D, TGFBR2, COX7AL2	1,708	Microarray/ QM-PCR	Distant metastasis, OS, DFS, clinical outcome	Hartmann; 2009. Golouh; 2008. Nimmrich; 2008. Harbeck; 2008. Maier; 2007. Martens; 2005.
STMN1	246	Microarray/ IHC	DFS, DSS, clinical outcome	Golouh; 2008. Oishi; 2007
PRKCD	208	QRT-PCR, IB, ELISA	OS	McKiernan; 2008
DNMT3B	228	QRT-PCR	RFS	Girault; 2003
EZH2	966	QRT-PCR	PFS	Reijm; 2010
H3K27me3	142	IHC	OS	Wei; 2008
HDAC1	200	IHC	DFS	Krusche; 2005
BRCA1	140	MSP	Clinical response	Yuan; 2009
EFEMP1	234	Microarray, QRT-PCR, BisSeq, IHC	DFS, OS	Sadr-Nabavi; 2009
ESR1, ARH1, CYP1B1	148	Microarray/ QMS-PCR	DSF, OS	Widschwendter; 2004
LZTS1	150	QRT-PCR, BisSeq, IHC	Lymph node metastasis	Chen; 2009
RASSF1A, APC, ESR1, HSD17B4, HIC1	270	QMS-PCR	Prognosis-associated with pre-treatment serum levels, RFS, OS	Muller; 2004 Fiegl; 2005
NEUROD1	95	QMS-PCR	RFS, OS	Fiegl; 2008
p16	41	MSP	Persistent plasma levels after mastectomy predicts micrometastatic disease	Silva; 2002

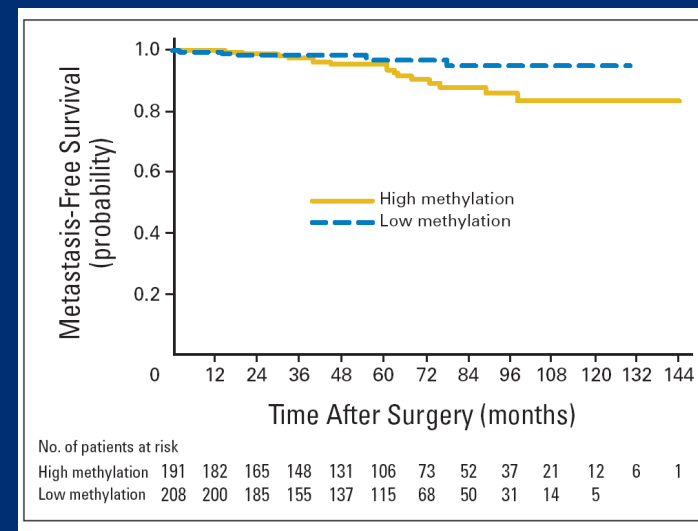
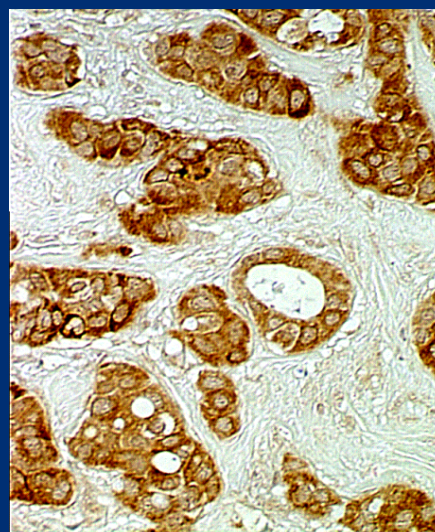
From the Departments of Obstetrics and Gynecology and Pathology, Technical University of Munich, Munich; Epigenomics AG; Charite Hospital, Humboldt University, Berlin; Institute of Pathology, University of Regensburg, Regensburg; Department of Visceral, Thoracic, and Vascular Surgery, University Hospital Carl Gustav Carus, Technical University Dresden, Dresden; Department of Gynecology, University Hospital Hamburg Eppendorf, Hamburg; Institute of Pathology, University of Erlangen, Erlangen, Germany; Institute of Clinical Pathology; University Hospital, Zurich, Switzerland; Institute of Oncology, Ljubljana, Slovenia; Clinical Experimental Oncology Laboratory, National Cancer Institute, Bari, Italy; Halitus Instituto Medico, Buenos Aires, Argentina; Department of Medical Oncology, Erasmus Medical Center, Rotterdam, the Netherlands; and Albany Medical College, Albany, New York.

Multicenter Study Using Paraffin-Embedded Tumor Tissue Testing *PITX2* DNA Methylation As a Marker for Outcome Prediction in Tamoxifen-Treated, Node-Negative Breast Cancer Patients

Nadia Harbeck, Inko Nimmrich, Arndt Hartmann, Jeffrey S. Ross, Tanja Cufer, Robert Grützmann, Glen Kristiansen, Angelo Paradiso, Oliver Hartmann, Astrid Margossian, John Martens, Ina Schwöpe, Antje Lukas, Volkmar Müller, Karin Milde-Langosch, Jörg Nährig, John Foekens, Sabine Maier, Manfred Schmitt, and Ralf Lesche



PITX2:
paired-like
homeodomain
transcription
factor 2



Factor	Number of patients	Assay	Association with	Reference
TOP2A	378	RT-PCR	mRNA levels predict recurrence in AC arm	Sparano; 2009
TOP2A, HER2	805	IHC, FISH	TOP2A amplification is associated with better RFS in the FEC treatment arm	Knoop; 2005
TOP2A, HER2	438	FISH	Better RFS in CEF arm compared to CMF arm with amplified TOP2A loci	O'Malley; 2009
HER2	3,487	IHC, FISH, PCR	HER2+ tumors show better DFS and DDFS in PAF arm vs PF arm. Increased RFS, OS, DFS in HER2+ treated with AC. Better response rates and RFS for T vs A in HER2+ cancers.	Paik; 1998, 2000 Di Leo; 2004 Pritchard; 2006
TOP2A, HER2, Ch17CEP	2,098	IHC/FISH	TOP2A/Ch17CEP ratio is predictive for response. Ch17CEP amplification is associated with prognosis. Better RFS with low HER2 status for ANT+CMF arm as compared to CMF therapy	Kawachi; 2010 Bartlett; 2008, 2010

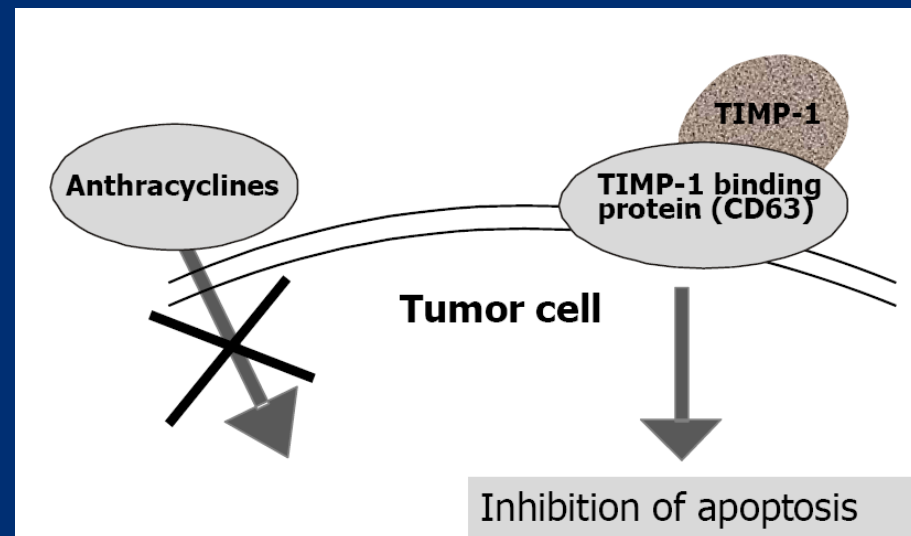
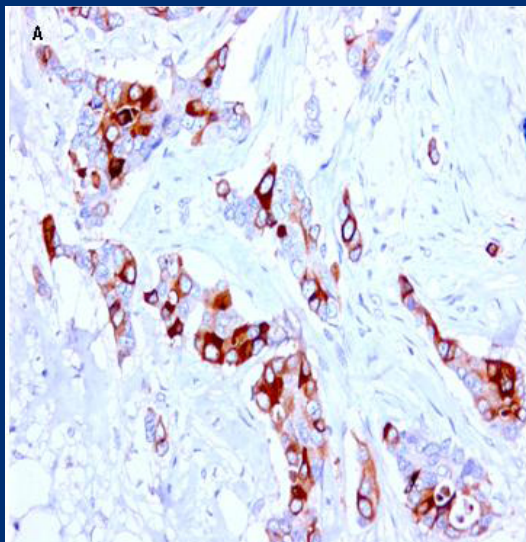
Factor	Number of patients	Assay	Association with	Reference
TIMP-1, HER2, TOP2A	1,270	IHC	Better IDFS in TIMP-1 negative patients treated with FEC as compared to CMF. TIMP-1 negativity combined with TOP2A overexpression is a significant predictor for better IDFS in the CEF arm as compared to CMF	Willemoes; 2009 Ejlertsen; 2010
Tau, HER2, ERCC1	2,200	Microarray/ IHC	Tau negativity is significantly associated with pathological complete response. Better DFS for Tau-expressing patients in AC+T arm compared to AC. Tau associated with OS and response despite association with nuclear grade and ER status	Rouzier; 2005 Pusztai; 2009 Shao; 2010



Lack of TIMP-1 tumour cell immunoreactivity predicts effect of adjuvant anthracycline-based chemotherapy in patients (n=647) with primary breast cancer. A Danish Breast Cancer Cooperative Group Study

Gro L. Willemoe, Pernille B. Hertel Annette Bartels, Maj-Britt Jensen, Eva Balslev, Birgitte B. Rasmussen, Henning Mouridsen, Bent Ejlersen, Nils Brüner

Department of Pathology, Rigshospitalet, Copenhagen, Section of Biomedicine, Department of Disease Biology, Faculty of Life Sciences, University of Copenhagen, Ridebanevej 3, 1870 Frederiksberg C, Copenhagen, Department of Oncology, Rigshospitalet, Copenhagen, Danish Breast Cancer Cooperative Group, Copenhagen, Department of Pathology, Herlev University Hospital, Herlev, Denmark



CD63:
a member of the tetraspanin family mediating signal transduction events

a	Number of patients	Assay	Association with	Reference
miR-10b, miR-21, miR-30a, miR-30e, miR-125b, miR-141, miR-200b, miR-200c, and miR-205	13	Microarray/ QRT-PCR	Associated with metastasis	Baffa; 2009
miR-7, miR-128a, miR-210, miR-516-3p	179	Microarray/ QRT-PCR	Aggressiveness for ER +/N0- cancers; miR-210 associated with metastasis in ER-, N0 and triple-negative cancers	Foekens; 2008
Let-7a, miR-195	192	QRT-PCR	Monitoring of therapy efficacy in blood samples	Heneghan; 2010
miR-21	457	QRT-PCR	LNM, OS independent of stage and nuclear grade . Aggressive disease, grade, ER/PR-, ductal carcinoma, TGFβ1; early stage patients showed association with poor DFS	Yan; 2008 Qian; 2009
miR-210	219	QRT-PCR	DFS, OS	Camps; 2008
Dicer		IHC/QRT-PCR	ER/PR status, luminal type carcinoma, metastatic disease	Grelier; 2009

Factor	Number of patients	Assay	Association with	Reference
HLA I, Treg	677	IHC	Better RFS and OS in treated patient cohort only	de Kruijf; 2010
CXCL9, ITSN2, GNAI2, H2AFX, INDO, MGC10986	70	Microarray/ QRT-PCR	CXCL9 overexpression is associated with prolonged DFS	Specht; 2009
CXCL9, CD3D, PTPRC, infiltrative lymphocytes	1,192	IHC/RT-PCR	Intratumoral lymphocytes and inflammatory markers are associated with pathological complete response in AC/T arm	Denkert; 2010
GPR30	373	IHC	HER2 status, tumor size, metastasis	Filardo; 2006

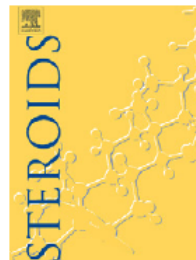


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Association of the membrane estrogen receptor, GPR30, with breast tumor metastasis and transactivation of the epidermal growth factor receptor

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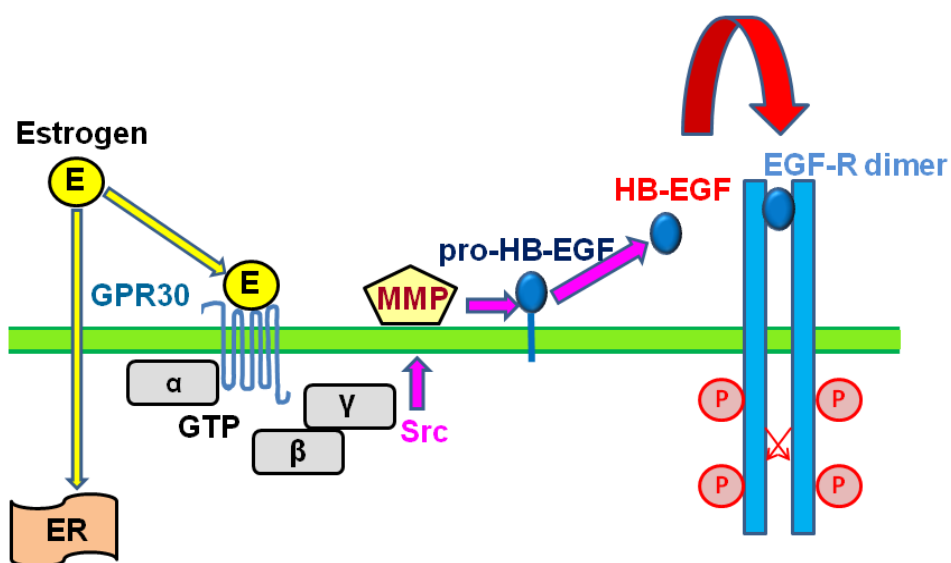
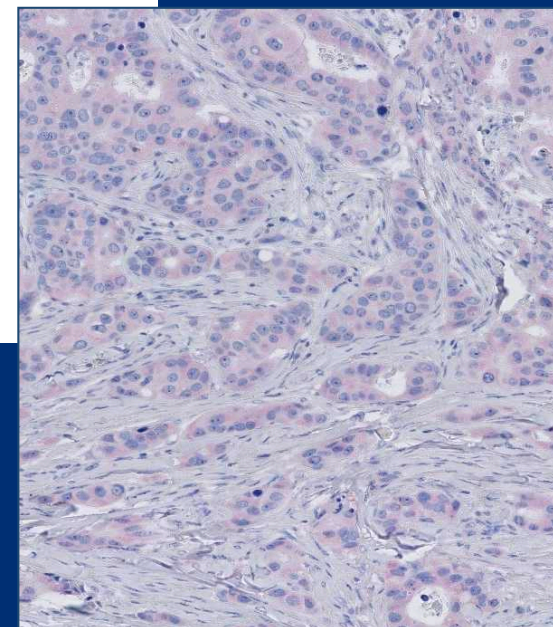
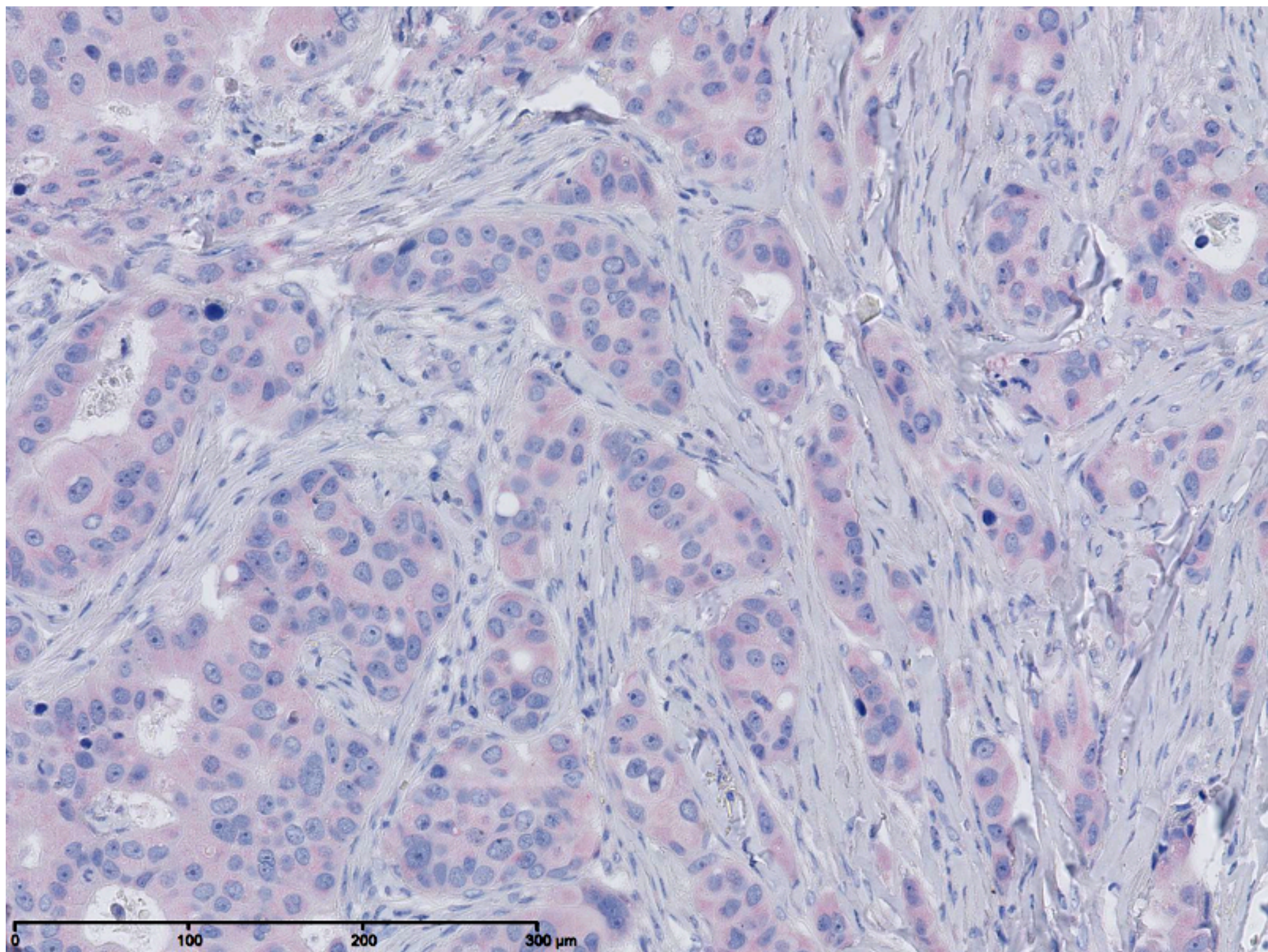


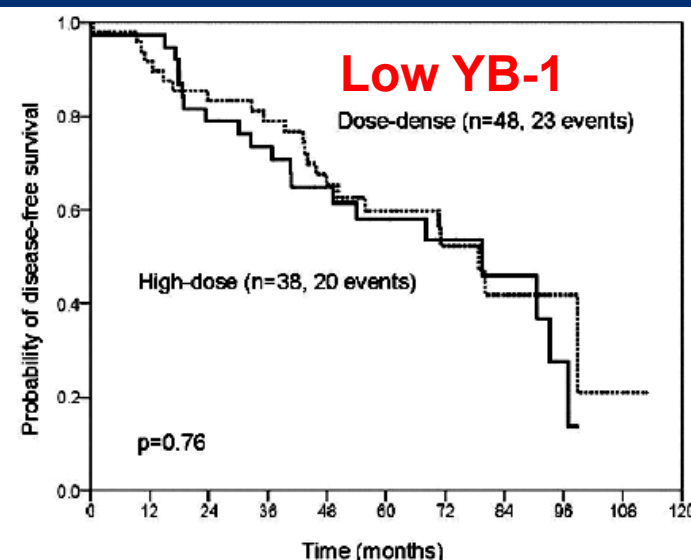
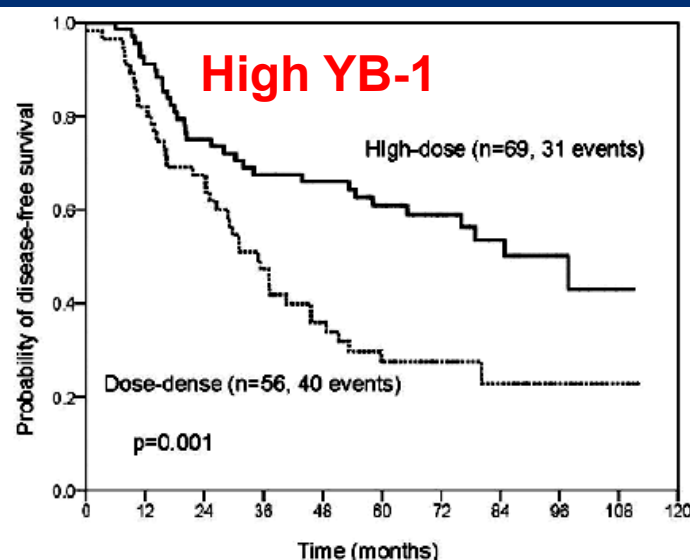
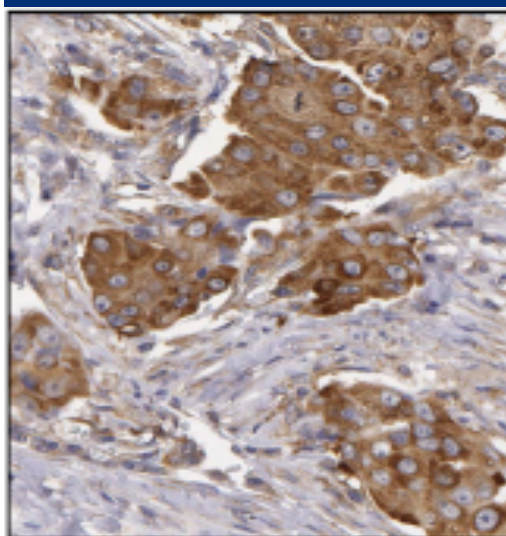
Fig. 1 – Mechanism by which GPR30 triggers release of EGF-related polypeptides. This cartoon illuminates the cellular signaling events that promote GPR30-mediated release of HB-EGF. GPR30 action occurs independently of the known estrogen receptors, ER α and ER β and is also promoted by ER antagonists.

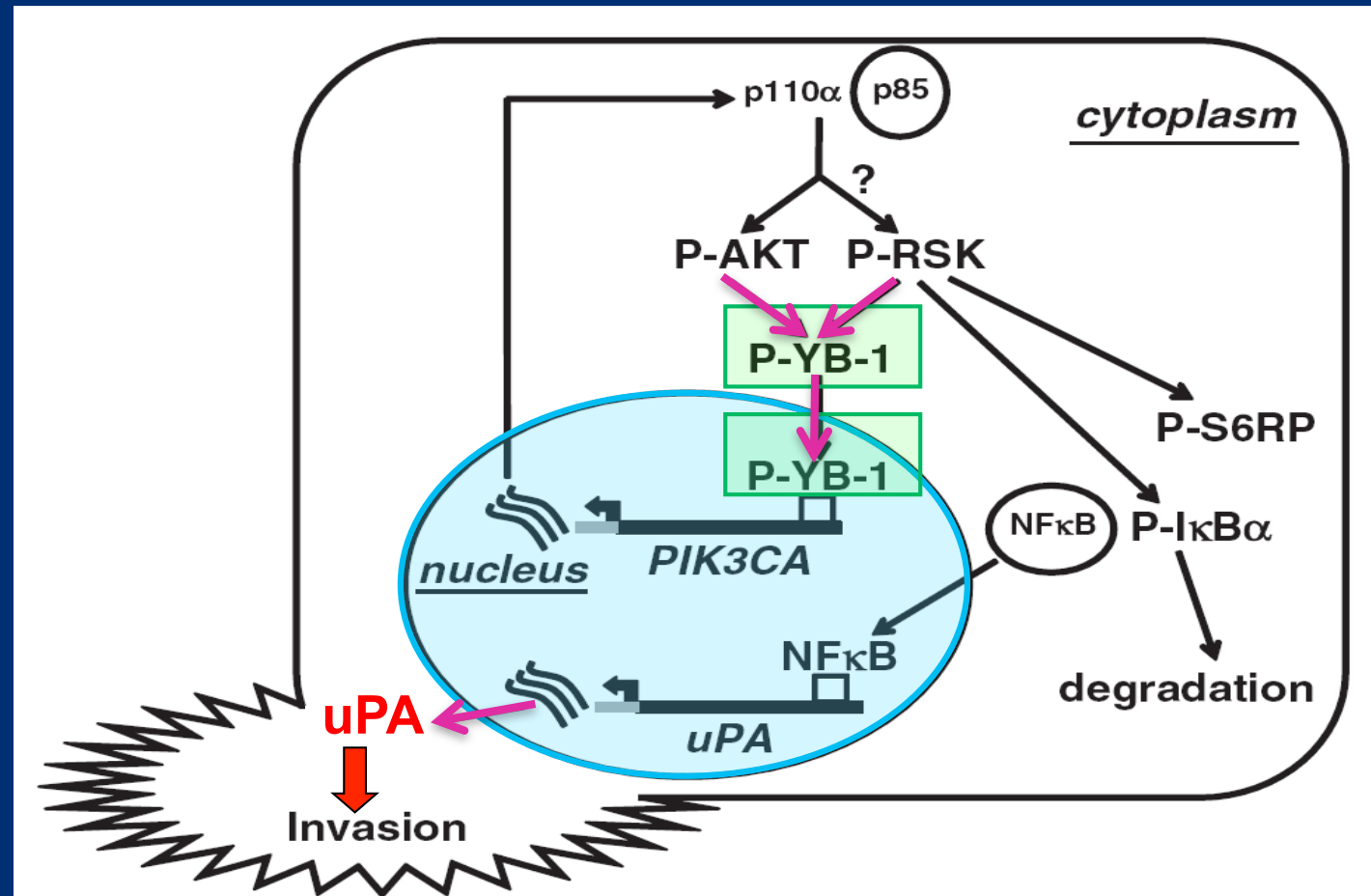


Factor	Number of patients	Assay	Association with	Reference
YB-1	83	IHC	ER/PR-; DFS and poor response to adjuvant chemotherapy; not with HER2/uPA/PAI-1	Janz; 2002
YB-1	63	IHC	Nuclear expression with PR+; p-glycoprotein expression; CD68, nuclear grade; not with RFS	Saji; 2003
YB-1	135	IHC	N+/ER-; RFS in patients without adjuvant CT; response to CMF and relapse under anthracycline-containing adjuvant CTX; not with nuclear grade/p-glycoprotein expression	Huang; 2005
YB-1	27	IHC	Paclitaxel treatment increased YB-1 and p-glycoprotein expression and induces YB-1 transfer to the nucleus; nuclear translocation of YB-1 correlates with p-glycoprotein expression; translocation to the nucleus in response to paclitaxel	Fujita; 2005
YB-1	389	IHC	ER-; high proliferation, HER2+, EGFR+; independent prognostic significance: decreased patient survival and early death in multivariate analysis; not correlated with tumor size or lymph node status	Wu; 2006
YB-1	285	IHC	Triple-negative tumors	Stratford; 2007
YB-1	4,049	IHC	Young age; ER-; high nuclear grade; HER2 amplification; independent marker for RFS and BCSS; independent of cancer subtype and N-/+ , as well as +/- adjuvant CTX; superior to tumor grade, ER, and HER2 status	Habibi; 2008
YB-1	211	IHC	ER/PR-, poor nuclear grade, high proliferation, HER2+; p53 staining; independent prognostic significance: OS and benefit of rapidly cycled tandem high-dose CTX	Gluz; 2009
YB-1	106	IHC	HER2+; not with response prediction in triple-negative subgroup	Darb-Esfahani; 2009
YB-1	369	IHC	Nuclear YB-1 with OS, but not independent; high tumor stage; poor nuclear grade; PR-	Dahl; 2009

Y-Box–Binding Protein YB-1 Identifies High-Risk Patients With Primary Breast Cancer Benefiting From Rapidly Cycled Tandem High-Dose Adjuvant Chemotherapy

Oleg Gluz, Karin Mengele, Manfred Schmitt, Ronald Kates, Raihana Diallo-Danebrock, Frauke Neff, Hans-Dieter Royer, Niels Eckstein, Svjetlana Mohrmann, Evelyn Ting, Marion Kiechle, Christopher Poremba, Ulrike Nitz, and Nadia Harbeck





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ORIGINAL ARTICLE

The transcriptional induction of *PIK3CA* in tumor cells is dependent on the oncoprotein Y-box binding protein-1

A Astanehe¹, MR Finkbeiner¹, P Hojabrpour², K To¹, A Fotovati¹, A Shadeo³, AL Stratford¹, WL Lam³, IM Berquin⁴, V Duronio² and SE Dunn¹

21-gene signature (mRNA) FFPE-tissue, breast cancer

The Oncotype DX assay is intended to be used by women with early-stage (Stage I or II), node-negative, estrogen receptor-positive (ER+) invasive breast cancer who will be treated with hormone therapy.

70-gene signature (mRNA) Fresh tissue, breast cancer

By measuring the activity of 70 genes in surgically-removed tumors, MammaPrint determines whether the patient is at a low or high risk of recurrence of the disease.

MOLECULAR ORIGINS OF CANCER

Gene-Expression Signatures in Breast Cancer

Christos Sotiriou, M.D., D.Phil., and Lajos Pusztai, M.D., D.Phil.

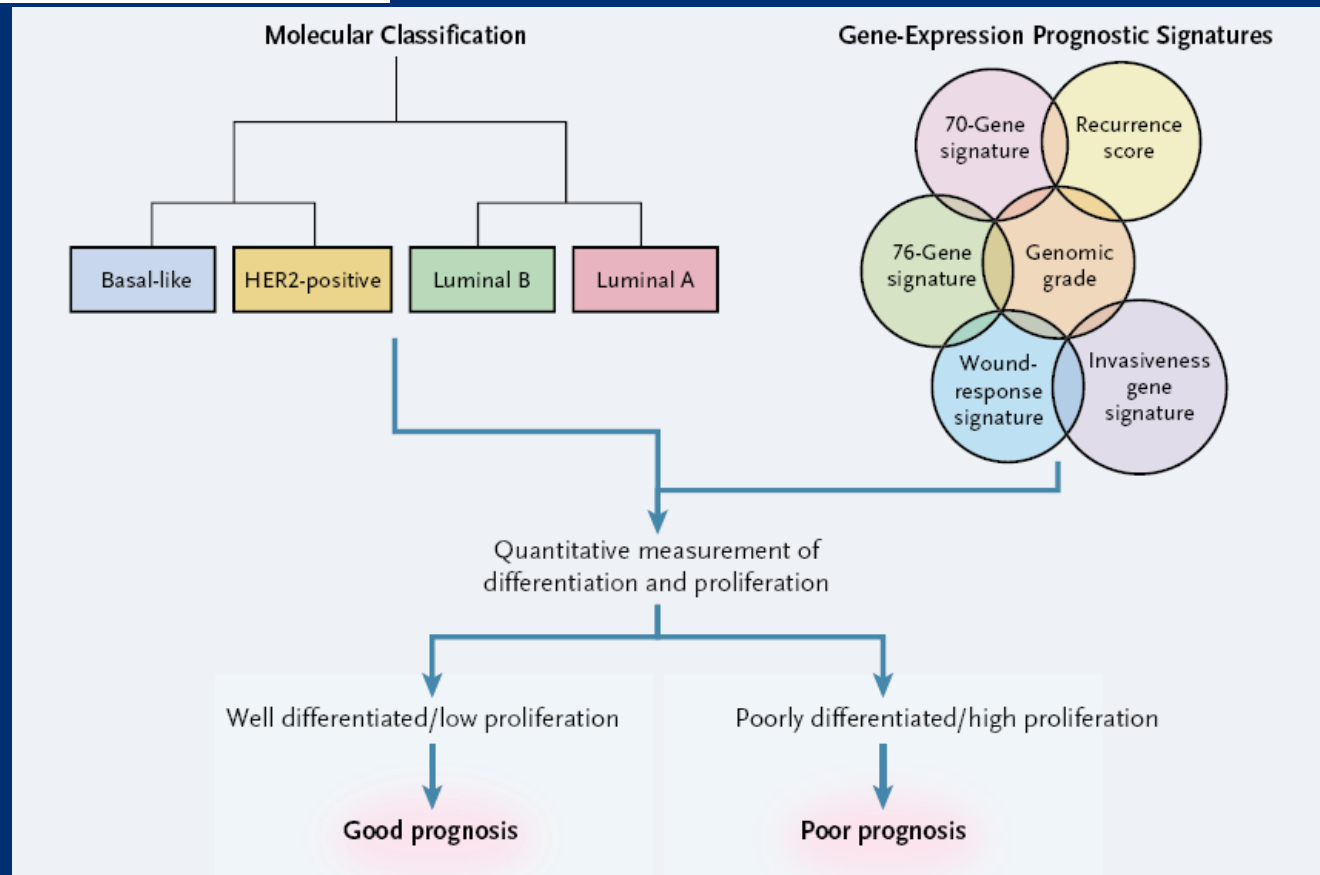
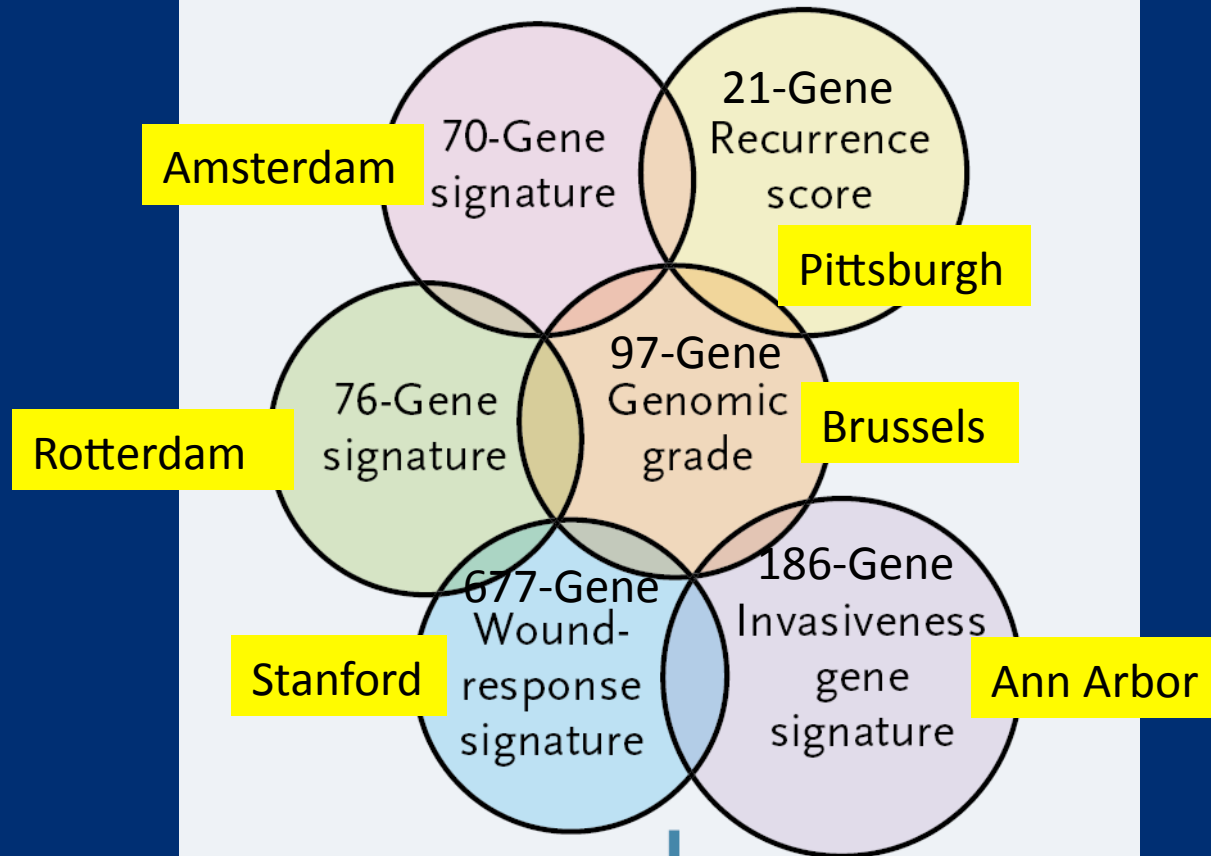


Figure 3. Molecular Classification, Gene-Expression Signatures, and Clinical Outcome.

Genes that are associated with tumor differentiation and cell cycle drive the prognostic power of the intrinsic molecular classification and several gene-expression signatures.

Gene-Expression Prognostic Signatures



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Multicenter Validation of a Gene Expression–Based Prognostic Signature in Lymph Node–Negative Primary Breast Cancer

John A. Foekens, David Atkins, Yi Zhang, Fred C.G.J. Sweep, Nadia Harbeck, Angelo Paradiso, Tanja Cufer, Anieta M. Sieuwerts, Dmitri Talantov, Paul N. Span, Vivianne C.G. Tjan-Heijnen, Alfredo F. Zito, Katja Specht, Heinz Hoefler, Rastko Golouh, Francesco Schittulli, Manfred Schmitt, Louk V.A.M. Beex, Jan G.M. Klijn, and Yixin Wang

