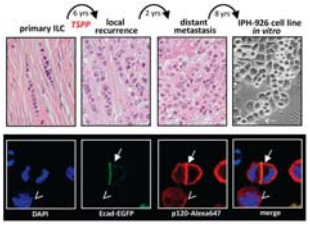
Molecular Markers in Breast Cancer:

Research and diagnostic testing with special reference to **HER2**, MDR1 and Ki67

Medizinische Hochschule Hannover

Disclaimer

- my primary research focus is...not HER2

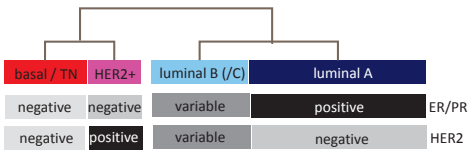


Christgen (2015) Breast Cancer Res 17:16

Disclaimer

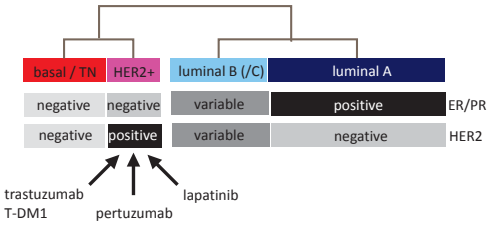
- my primary research focus is...not HER2
- perspective from routine diagnostics and reference laboratory service for clinical trials

Therapeutic decision-making depends on prognostic and predictive biomarkers



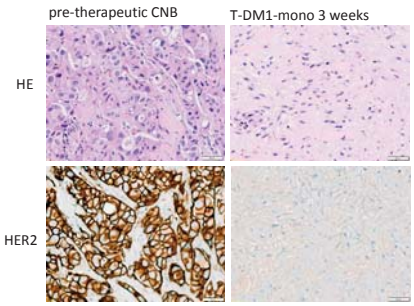
Campbel *et al.* (1981) Lancet 2:1317
Perou *et al.* (1999) PNAS 96:9212

Therapeutic decision-making depends on prognostic and predictive biomarkers



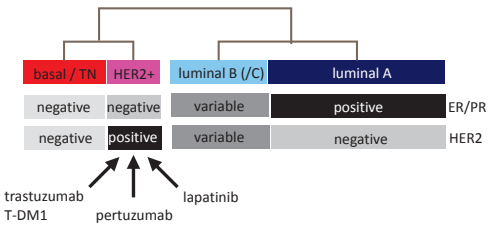
Baselga *et al.* (2009) Nat Rev Cancer 9:463
Verma *et al.* (2013) Oncologist 18:1153

Therapeutic decision-making depends on prognostic and predictive biomarkers



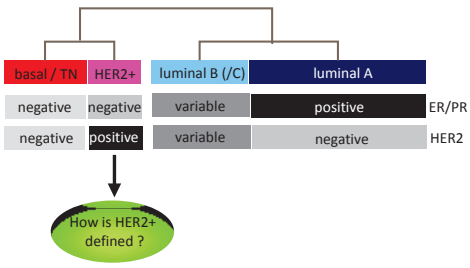
Mueller *et al.* (2015) Sci Translat Med: submitted

Therapeutic decision-making depends on prognostic and predictive biomarkers

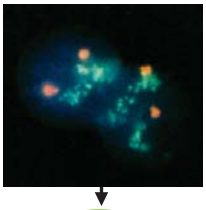


Baselga *et al.* (2009) Nat Rev Cancer 9:463
Verma *et al.* (2013) Oncologist 18:1153

Therapeutic decision-making depends on prognostic and predictive biomarkers



Therapeutic decision-making depends on prognostic and predictive biomarkers

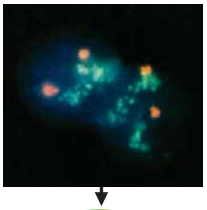


How is HER2+ defined ?

„straightforward“:
heavily amplified tumor cells with 25 to 100 copies per cell („cluster“) in FISH

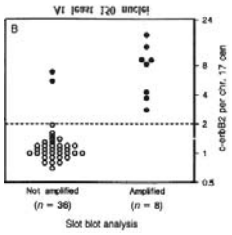
Kallioniemi *et al.* (1992) Proc Natl Acad Sci 89:5321
discussion section line 18-ff

Therapeutic decision-making depends on prognostic and predictive biomarkers



How is HER2+ defined ?

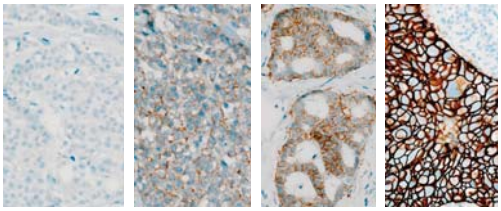
„straightforward“:
heavily amplified tumor cells with 25 to 100 copies per cell („cluster“) in FISH



- 17q12:CEP17 ratio ≥2,
- signals per nucleus ≥7

Kallioniemi *et al.* (1992) Proc Natl Acad Sci 89:5321
discussion section line 18-ff

Therapeutic decision-making depends on prognostic and predictive biomarkers



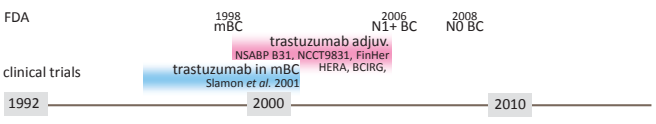
0
no staining

1+
weak,
incomplete
>10% of cells

2+
weak-moderate,
complete
>10% of cells

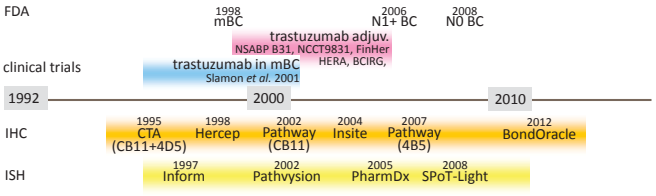
3+
intense,
complete
>10% of cells

Evolution of HER2-targeted therapy and HER2 testing



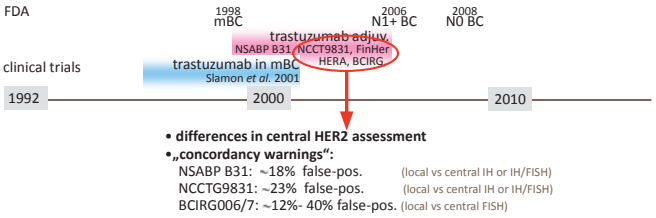
Cobleigh *et al.* (1999) J Clin Oncol 17:2639
Slamon *et al.* (2001) N Engl J Med 344:783
Ramond *et al.* (2005) N Engl J Med 353:1673
Piccart-Gebhart *et al.* (2005) N Engl J Med 353:1659
Joensuu *et al.* (2006) N Engl J Med 354:809

Evolution of HER2-targeted therapy and HER2 testing



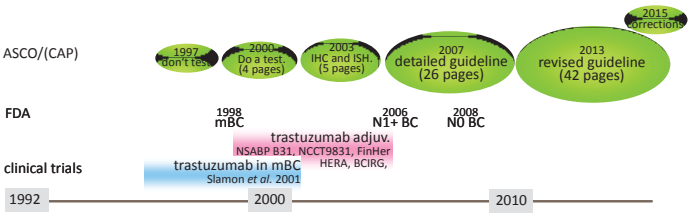
Ross *et al.* (2009) Oncologist 14:320

Evolution of HER2-targeted therapy and HER2 testing



Paik *et al.* (2002) J Natl Cancer Inst 94:852
Roche *et al.* (2002) J Natl Cancer Inst 94:855
Press *et al.* (2005) Clin Cancer Res 11:6598

Evolution of HER2-targeted therapy and HER2 testing



ASCO (1998) J Clin Oncol 16:793
Bast *et al.* (2001) J Clin Oncol 19:1865
Zarbo *et al.* (2003) Arch Pathol Lab Med 127:549
Wolff *et al.* (2007) J Clin Oncol 25:118
Wolff *et al.* (2013) J Clin Oncol 31:3997
Wolff *et al.* (2015) J Clin Oncol 33:1302

Background of the 2007 ASCO/CAP HER2 guideline

issues	provisions
IHC vs ISH	no gold standard - local validation (95% concordance) - swap methods on equivocals
high false-positive rates	IHC cutoff 10% → 30% ISH equivocal: ratio 1.8-2.2 (dp-ISH) 4 or 5 or 6 signals (sp-ISH)
quality control	test requirements (onslide controls etc) proficiency tests for accreditation

Wolff *et al.* (2007) J Clin Oncol 25:118
Appendix G, line 13: goals of the panel

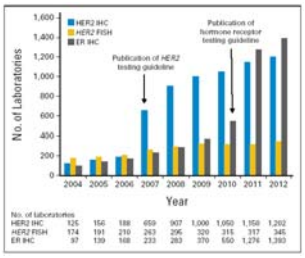
Background of the 2007 ASCO/CAP HER2 guideline

ASCO/(CAP)

issues	provisions	AGO 2008
IHC vs ISH	no gold standard - local validation (95% concordance) - swap methods on equivocals	yes
high false-positive rates	IHC cutoff 10% → 30%	yes
	ISH equivocal: ratio 1.8-2.2 (dp-ISH)	yes / no
	4 or 5 or 6 signals (sp-ISH)	yes
quality control	test requirements (onslide controls etc)	yes
	proficiency tests for accreditation	yes

Wolff *et al.* (2007) J Clin Oncol 25:118
Appendix G, line 13: goals of the panel

HER2 test consistency: trends in round robin tests and clinical trials



ALTTO trial (2007-2011)		central		
		neg./equi.	pos.	Σ
local	equi.	13	14	27
	pos.	58	971	1029

false-positive rate in locally HER2+ BC ~5.6%

Wolff *et al.* (2013) J Clin Oncol 31:3997
McCullough *et al.* (2014) Breast Cancer Res Treat 143:485

HER2 test consistency: trends in round robin tests and clinical trials

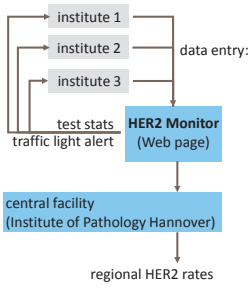
German HER2 round robin test 2012
- 220 participating institutes (IHC part)
- 90% success rate in the 1st run
- 100% sucess rate in the re-test

false-negative rate in locally HER2- BC <2%

PlanB trial final V076 (2009-2011) NCT01049425		central					
		IHC 0/1+	IHC 2+ FISH -	IHC 2+ FISH eq	IHC 2+ FISH +	IHC 3+ FISH +/n.a.	Σ
local	negative	3059 (97,3%)	44 (1,4%)	6 (0,2%)	12 (0,4%)	21 (0.7%)	3142 (100%)

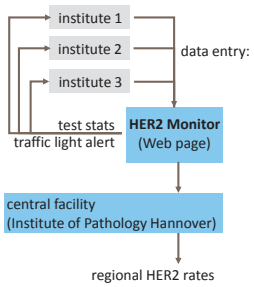
Liessem *et al.* (2014) Pathologe 35:61
Christgen *et al.* (2012) J Clin Oncol 30:3313

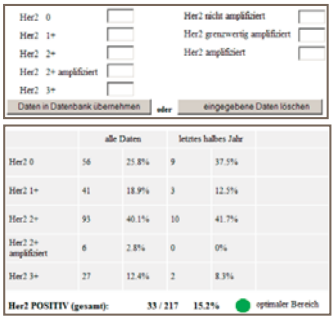
HER2 test consistency: trends in population-based registries



Chroritz *et al.* (2011) Virchows Arch 459:283

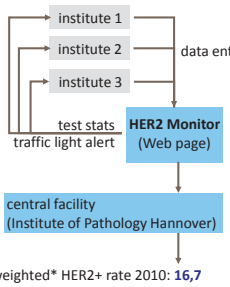
HER2 test consistency: trends in population-based registries





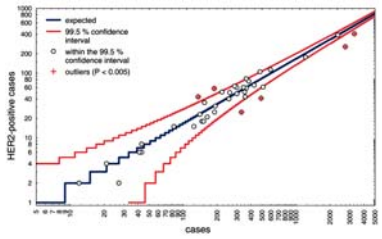
Chroritz *et al.* (2011) Virchows Arch 459:283

HER2 test consistency: trends in population-based registries



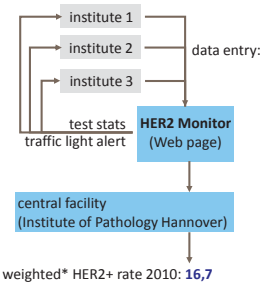
weighted* HER2+ rate 2010: **16,7**
weighted* HER2+ rate 2014: **14,6**

2010 Monitor data
- >18.000 cases
- 42 institutions (Germany, Austria, Switzerland)
- HER2+ rate per institute ranged 7,6% - 31,6%
- 6 institutions statistically „outliers" → traffic light red

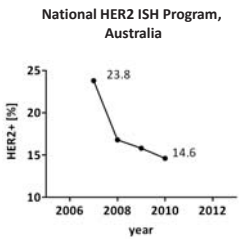


Chroritz *et al.* (2011) Virchows Arch 459:283

HER2 test consistency: trends in population-based registries

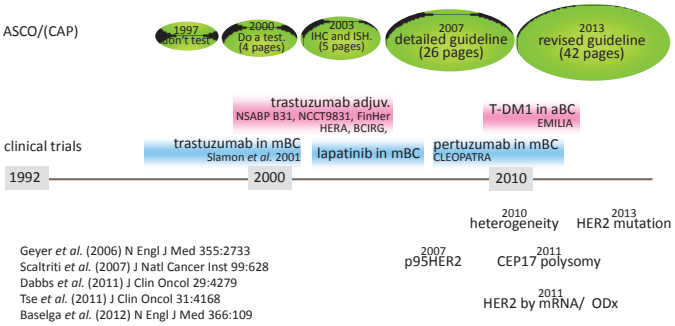


weighted* HER2+ rate 2010: **16,7**
weighted* HER2+ rate 2014: **14,6**



Bilous *et al.* (2012) Breast Cancer Res Treat 134:617
Chroritz *et al.* (2011) Virchows Arch 459:283

Background of the 2013 ASCO/CAP HER2 guideline



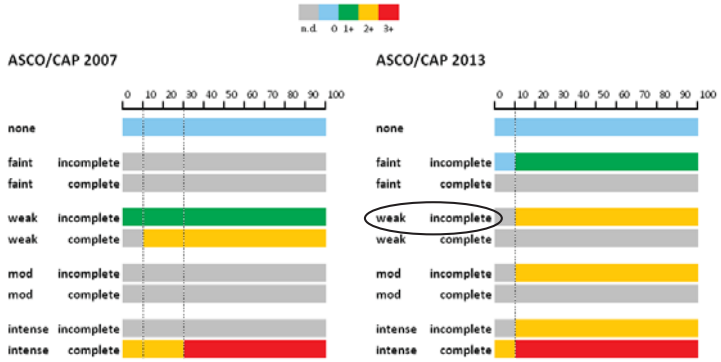
Geyer *et al.* (2006) N Engl J Med 355:2733
Scaltriti *et al.* (2007) J Natl Cancer Inst 99:628
Dabbs *et al.* (2011) J Clin Oncol 29:4279
Tse *et al.* (2011) J Clin Oncol 31:4168
Baselga *et al.* (2012) N Engl J Med 366:109
Verma *et al.* (2012) N Engl J Med 367:1783
Ross *et al.* (2013) Clin Cancer Res 19: 2668
Lien *et al.* (2015) Breast Cancer Res Treatment 150:447

Background of the 2013 ASCO/CAP HER2 guideline

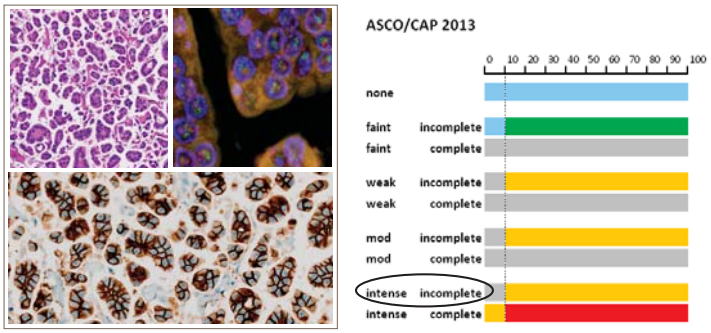
issues	provisions
↑ test consistency , ↓ HER2+ rates	IHC weak incomplete: 1+ → 2+
	IHC cutoff 30% → 10%
Definition of „equivocal“ (sp vs dp ISH)	Ratio 1.8-2.2 suspended
Specific constellations - IHC intense incomplete - CEP17 monosomy - fixation time „friday night specimen“ - CNB vs resections	- IHC intense incomplete → 2+ - CEP17 monosomy → ISH+ - fixation time 6-48 h → 6-72h -„histological concordance“ retest G3 TN

Wolff *et al.* (2013) J Clin Oncol 31:3997

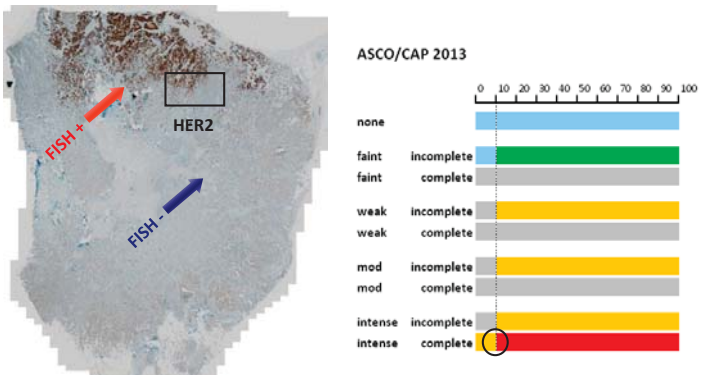
2013 ASCO/CAP guideline: changes in IHC scoring



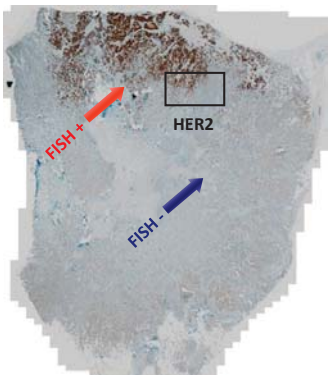
2013 ASCO/CAP guideline: changes in IHC scoring

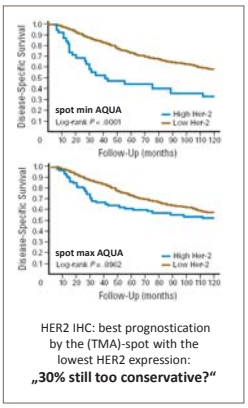


2013 ASCO/CAP guideline: changes in IHC scoring



2013 ASCO/CAP guideline: changes in IHC scoring

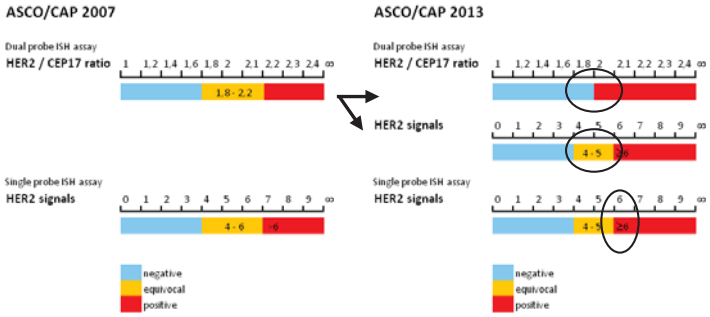




HER2 IHC: best prognostication by the (TMA)-spot with the lowest HER2 expression:
„30% still too conservative?“

Moeder *et al.* (2007) J Clin Oncol 25:5418

2013 ASCO/CAP guideline: changes in ISH scoring



2013 ASCO/CAP guideline: changes in ISH scoring impact on clinical trials

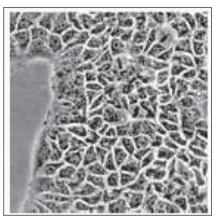
ADAPT HR+/HER2+ interimV2SBST1 (2012-...) NCT01745965		central					
		IHC 0/1+	IHC 2+ FISH - (r)	IHC 2+ FISH eq (r)	IHC 2+ FISH + (r)	IHC 3+	Σ
local	positive	26 (5,8%)	22 (4,9%)	13 (2,9%)	34 (7,6%)	353 (78,8%)	448

ADAPT HR+/HER2- interimV2SBOP (2012-...) NCT01779206		central					
		IHC 0/1+	IHC 2+ FISH - (r)	IHC 2+ FISH eq (r)	IHC 2+ FISH + (r)	IHC 3+ FISH +(r)/n.a.	Σ
local	negative	1985 (73,6%)	558 (20,7%)	86 (3.2%)	39 (1.4%)	30 (1.1%)	2698

local HER2 neagtive **+** central HER2 equivocal
the most common constellation
demanding clinicians to re-consider the therapy strategy in ADAPT HR+/HER2-

HER2 equivocal: a debated clinical category and its counterpart in cell biology

MDA-MB-453



HER2 (IHC): 2+
17q12/CEP ratio: 2.0-3.0

↓

limited 4D5 response

↓

LAR type expression profile
AR overexpression
AR mutation

↓

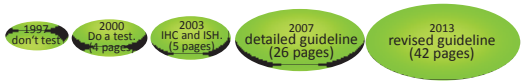
model of TN/AR+ BC in cell biology

control for HER2+ in diagnostic labs

Brinkley *et al.* (1980) Cancer Res 40:3118
Rhodes *et al.* (2002) Am J Clin Pathol 117:81
Lewis *et al.* (1996) Cancer Res 56:1996
Doane *et al.* (2006) Oncogene 25:3994
Lehmann *et al.* (2011) JNCI 121:2750
Robinson *et al.* (2011) EMBO J 30:3019

AGO statement on 2013 ASCO/CAP HER2 guideline

ASCO/(CAP)



issues	provisions	AGO 2014
↑ test consistency , ↓ HER2+ rates	IHC weak incomplete: 1+ → 2+	no
	IHC cutoff 30% → 10%	yes
Definition of „equivocal“ (sp vs dp ISH)	Ratio 1.8-2.2 suspended	yes
Specific constellations		
- IHC intense incomplete	- IHC intense incomplete → 2+	yes
- CEP17 monosomy	- CEP17 monosomy → ISH+	yes
- fixation time „friday night specimen“	- fixation time 6-48 h → 6-72h	yes
- CNB vs resections	-„histological concordance“ retest G3 TN	no

Wolff *et al.* (2013) J Clin Oncol 31:3997

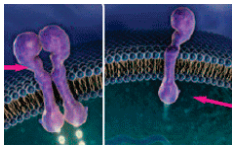
AGO statement on 2013 ASCO/CAP HER2 guideline

ASCO/(CAP)

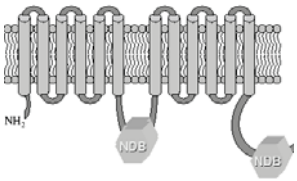
issues	provisions
↑ test consistency , ↓ HER2+ rates	~~IHC weak incomplete: 1+ → 2+~~
	IHC cutoff 30% → 10%
Definition of „equivocal“ (sp vs dp ISH)	Ratio 1.8-2.2 suspended
Specific constellations	
- IHC intense incomplete	- IHC intense incomplete → 2+
- CEP17 monosomy	- CEP17 monosomy → ISH+
- fixation time „friday night specimen“	- fixation time 6-48 h → 6-72h
- CNB vs resections	~~„histological concordance“ retest G3 TN~~

Wolff *et al.* (2015) J Clin Oncol 33:1302

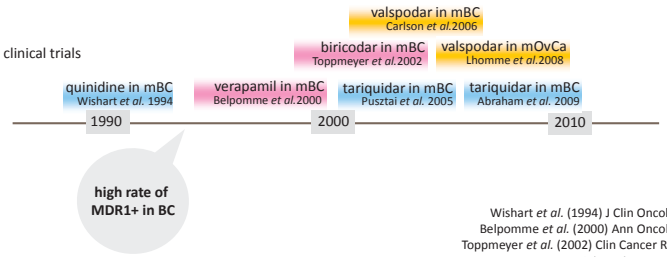
HER2 / ErbB2



P-gp / MDR1 /ABCB1

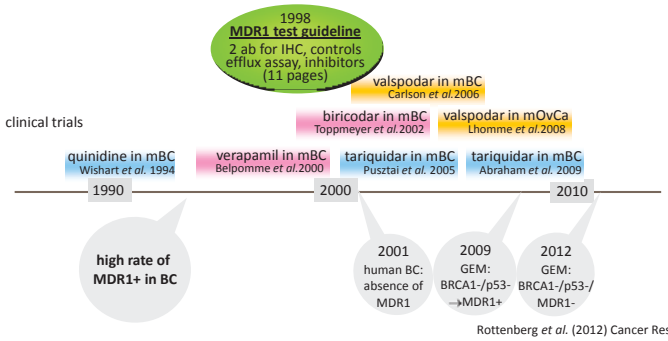


Evolution of MDR1-targeted therapy and MDR1 testing



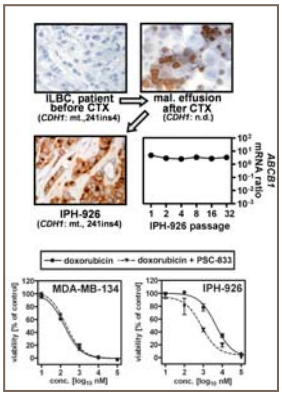
Wishart *et al.* (1994) J Clin Oncol 12:1771
Belpomme *et al.* (2000) Ann Oncol 11:1471
Toppmeyer *et al.* (2002) Clin Cancer Res 8:670
Pusztai *et al.* (2005) Cancer 104:682
Carlson *et al.* (2006) Cancer Invest 24:677
Lhomme *et al.* (2008) J Clin Oncol 26:2674
Abraham *et al.* (2009) Clin Cancer Res 15:3574

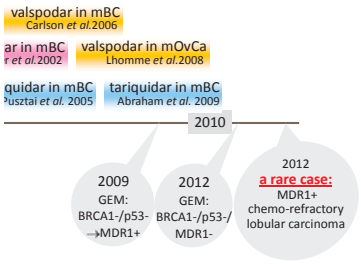
Evolution of MDR1-targeted therapy and MDR1 testing



Rottenberg *et al.* (2012) Cancer Res 72:2350
Pajic *et al.* (2009) Cancer Res 69:6396
Faneyete *et al.* (2001) Int J Cancer 93:114
Beck *et al.* (1998) Cancer Res 56:310

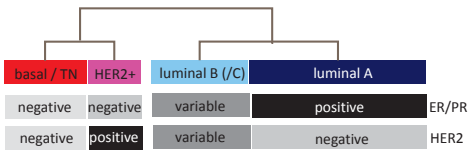
Our experience: MDR1-mediated drug resistance - - *does* exist - -



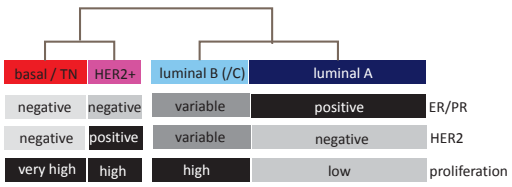


Krech *et al.* (2012) Cancer Lett 315:153

Therapeutic decision-making depends on prognostic and predictive biomarkers

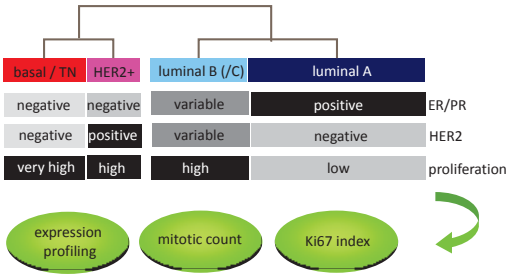


Therapeutic decision-making depends on prognostic and predictive biomarkers



Desmedt *et al.* (2008) Clin Cancer Res 14:5158

Therapeutic decision-making depends on prognostic and predictive biomarkers



Cheang *et al.* (2009) J Nat Cancer Inst 101:736
Allison *et al.* (2012) Breast Cancer Res Treat 131:413
Sahebjam *et al.* (2011) Br J Cancer 105:1342

The value of Ki67 is controversial

pros

prognostic
predictive
low-cost assay
available

St. Gallen 2013 and 2015:
Ki67 recommended, cutoff <20%

cons

insufficient standardization
antibodies (SP6, Mib1, 30-9)
interpretation (intensity, nucleoli)
scoring (eyeballing, counting)

ASCO/CAP „Molecular Markers“:
Ki67 not recommended

Harris *et al.* (2007) J Clin Oncol 33:5287
Varga *et al.* (2012) PlosOne 7:e137379
Polley *et al.* (2013) J Nat Cancer Inst 105:1897
Harbeck *et al.* (2013) Breast Care 8:102

The value of Ki67 is controversial

Ki67 Working Group : Reproducibility study

n= 100 breast cancers,
TMAs, 1 mm cores
Mib1 staining, central

↓

n= 6 expert laboratories

↓

moderate
reproducibility (ICC ~0.71)

cons

insufficient standardization
antibodies (SP6, Mib1, 30-9)
interpretation (intensity, nucleoli)
scoring (eyeballing, counting)

ASCO/CAP „Molecular Markers“:
Ki67 not recommended

Polley *et al.* (2013) J Nat Cancer Inst 105:1897

The value of Ki67 is controversial

Ki67 Working Group : Reproducibility study

G1 = 32%
G2 = 44%
G3 = 24%

n= 100 breast cancers,
TMAs, 1 mm cores
Mib1 staining, central

limiting hetero-geneity

↓

n= 6 expert laboratories

ICC vs 2-categorial IRR

↓

moderate
reproducibility (ICC ~0.71)

→ **possibly even too optimistic?**

cons

insufficient standardization
antibodies (SP6, Mib1, 30-9)
interpretation (intensity, nucleoli)
scoring (eyeballing, counting)

ASCO/CAP „Molecular Markers“:
Ki67 not recommended

Polley *et al.* (2013) J Nat Cancer Inst 105:1897

The value of Ki67 is controversial

Ki67 Working Group Scoring-Training, round robin tests,

Computer-assisted Image Analysis (CAIA)

cons

insufficient standardization
antibodies (SP6, Mib1, 30-9)
interpretation (intensity, nucleoli)
scoring (eyeballing, counting)

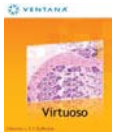
ASCO/CAP „Molecular Markers“: Ki67 not recommended

Polley *et al.* (2015) Mod Pathol 28:778
Klauschen *et al.* (2015) Clin Cancer Res: in press

Computer-assissted image analysis (CAIA) promises improved Ki67 quantification

anti-Ki67 antibody 30-9
Benchmark Autostainer

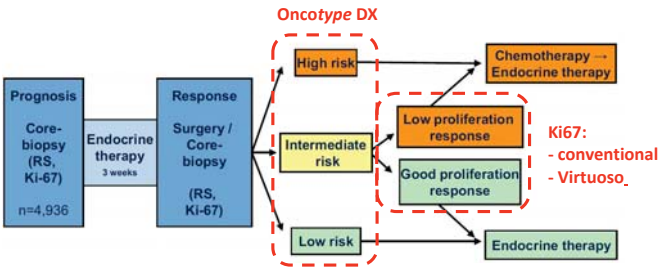
iScan Coreo Scanner



Virtuoso Software

FDA 510(k)-clearance in 2013

Computer-assissted image analysis for Ki67 in prospective clinical trials (e.g. ADAPT)

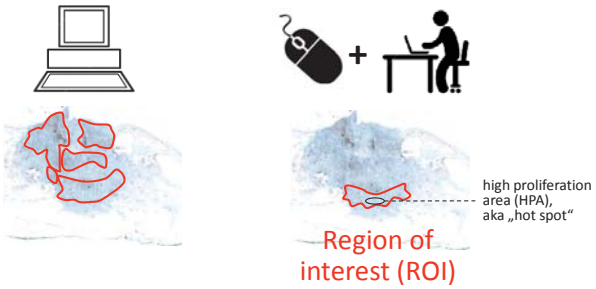


Harbeck *et al.* (2014) Cancer Treatment Rev 40:434
Hofmann *et al.* (2013) Trials 14:261

Ki67 quantification: automated *versus* semi-automated

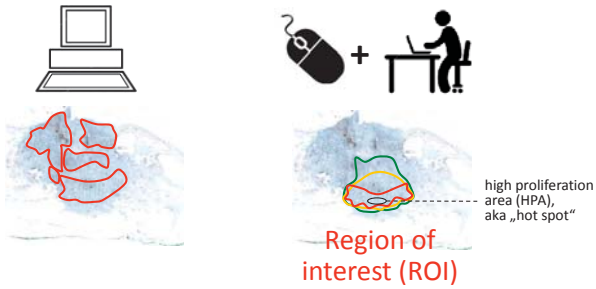
Region of interest (ROI)

Ki67 quantification: automated *versus* semi-automated



Dowsett *et al.* (2011) J Natl Cancer Inst 103:1656

Ki67 quantification: automated *versus* semi-automated



Dowsett *et al.* (2011) J Natl Cancer Inst 103:1656

PD Dr.med. PhD M.Christgen
Institute of Pathology
Hannover Medical School

2nd NordiQC Conference
on Applied Immunohistochemistry
Aalborg, 9th-12th June 2015

MHH Medizinische Hochschule Hannover

Ki67 quantification: Does the ROI size impact on Virtuoso readouts?

- **n=100 breast cancers (resection specimens)**
- Ki67 staining
- full slide scan on iScan Coreo

- **Virtuoso**
- systematic variantion of the ROI size (50 – 20.000 cells)

- **descriptive statistics:**
- non-linear regression: Ki67 index vs ROI size
- re-classification rate (Ki67-low vs high, cutoff <20%) in relation to the ROI

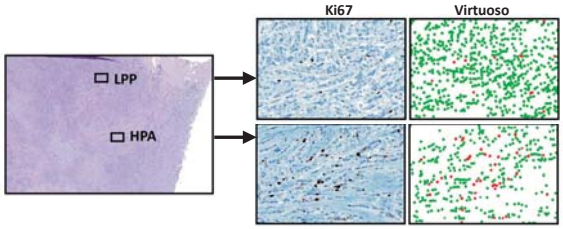
Christgen *et al.* (2015) Hum Pathol: in press

PD Dr.med. PhD M.Christgen
Institute of Pathology
Hannover Medical School

2nd NordiQC Conference
on Applied Immunohistochemistry
Aalborg, 9th-12th June 2015

MHH Medizinische Hochschule Hannover

Case expamle: Virtuoso with multiple ROI sizes



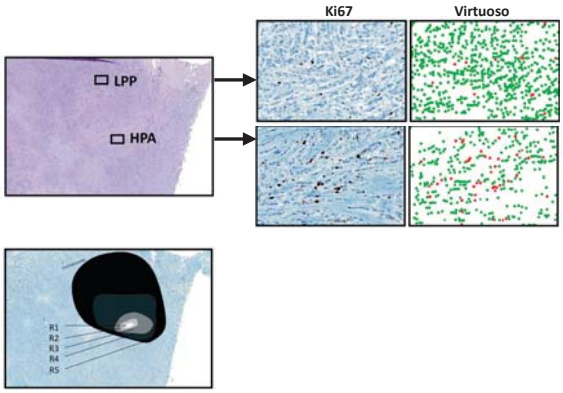
Christgen *et al.* (2015) Hum Pathol: in press

PD Dr.med. PhD M.Christgen
Institute of Pathology
Hannover Medical School

2nd NordiQC Conference
on Applied Immunohistochemistry
Aalborg, 9th-12th June 2015

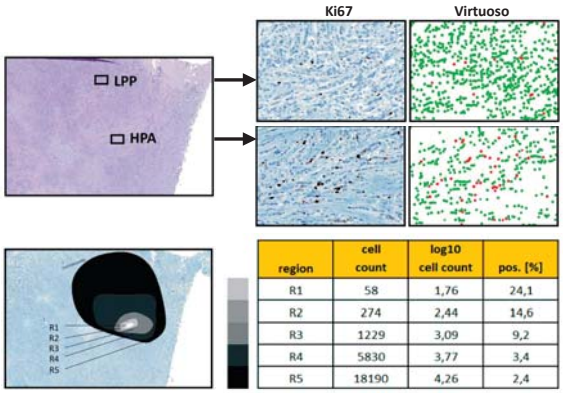
MHH Medizinische Hochschule Hannover

Case expamle: Virtuoso with multiple ROI sizes



Christgen *et al.* (2015) Hum Pathol: in press

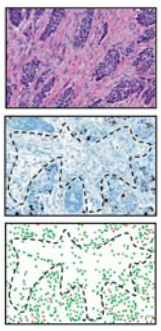
Case expamle: Virtuoso with multiple ROI sizes

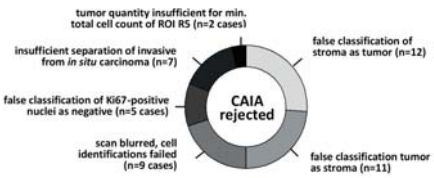


region	cell count	log10 cell count	pos. [%]
R1	58	1,76	24,1
R2	274	2,44	14,6
R3	1229	3,09	9,2
R4	5830	3,77	3,4
R5	18190	4,26	2,4

Christgen *et al.* (2015) Hum Pathol: in press

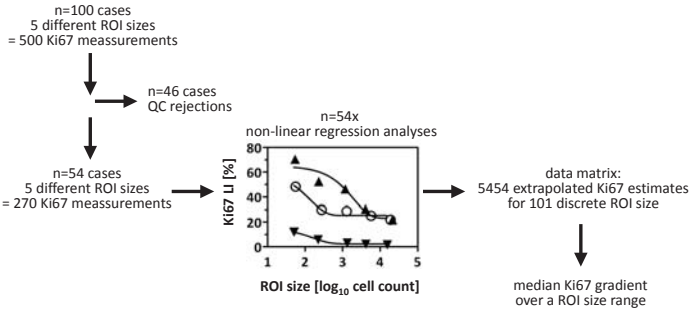
Rigorous morphological QC: Virtuoso rejected in 46% of cases





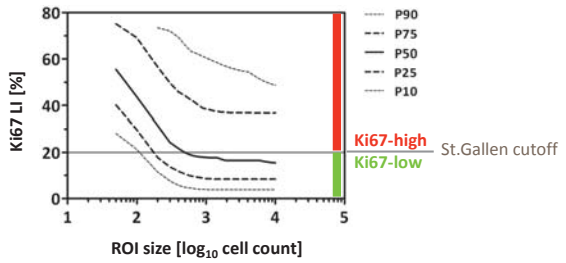
Christgen *et al.* (2015) Hum Pathol: in press

Modelling the gradient of the median Ki67 index



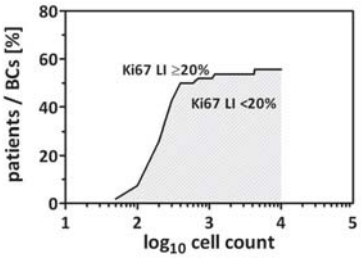
Christgen *et al.* (2015) Hum Pathol: in press

The median Ki67 index varies between 55% and 15% depending on the ROI size



Christgen *et al.* (2015) Hum Pathol: in press

Ki67-low *versus* Ki67-high: 50% of cases are re-classifiably by re-shaping the ROI



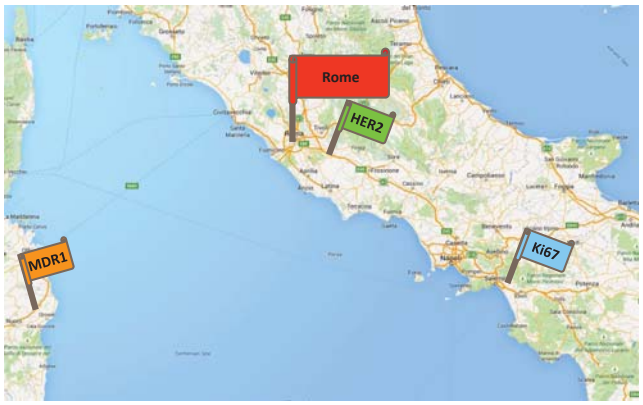
Christgen *et al.* (2015) Hum Pathol: in press

Semi-automated CAIA for Ki67 may require an adjustment for the ROI size

	Ki67 test sample		Ki67 reference cohort		
(A)	Ki67 LI	13	median	19,8	(D)
(B)	ROI size	500	P90	65,9	
			P75	44,2	
			P25	10,5	
			P10	4,9	
			Cohen's kappa[1]	0,67	(E)
			Cohen's kappa[2]	0,11	
			estimated ROI size-specific quantile of test sample[3]	29	(F)

Christgen *et al.* (2015) Hum Pathol: in press

Summary



Institute of Pathology
Hannover Medical School, Prof. Dr. H. Kreipe

H. Christgen | K. Buckmeier | S. von Ahsen | D. Schmidt | Dr. M. Raap





Womens Healthcare Study Group
Prof. N. Harbeck
Prof. N. Nitz
Dr. O. Gluz
Dr. R. Kates
Dr. H. Hofmann

Thank you so much for your attention

MHH Medizinische Hochschule Hannover