IMPAKT BREAST CANCER CONFERENCE

IMProving cAre and Knowledge through Translational research

PRE-IMPAKT
EARLY CAREER TRAINING COURSE

6-7 MAY 2015

Validated assays in pathology

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Disclosure slide

- I have the following potential conflict of interest to disclose:
  - Participation in a company sponsored speaker's bureau from Genomic Health

OUTLINE

- Definitions
- Validation process
- Routinely evaluated biomarkers
- Controversial issues

DEFINITIONS

- Biomarker
  - A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention*
- Biomarkers in breast pathology
  - Prognostic biomarkers (to provide information on the natural course of disease)
    - Is further treatment needed?
  - Predictive biomarkers (to identify patients most likely to benefit from a specific drug)
    - Which treatment?

*AACR-FDA-NCI Cancer Biomarkers Collaborative Consensus Report

PROGNOSTIC AND PREDICTIVE BIOMARKERS IN BREAST CANCER

Prognostic markers

- Tumor size
- Histologic type
- Histologic grade
- Lymph-nodes status
- Lymphatic/Vascular invasion
- Extensive DCIS
- ER/PR status
- HER2 neu status

Predictive markers

- ER/PR status
- HER2 neu status

VALIDATION PROCESS FOR PROGNOSTIC AND PREDICTIVE BIOMARKERS

- **analytic validity:** the test has been shown to be accurate, reliable, and reproducible in the specimen type to be used in the clinical situation; test characteristics such as sensitivity, specificity, and reliability are confirmed
- **clinical validity:** the test separates a group into 2 or more distinct populations with different biological characteristics or clinical outcomes
- **clinical utility:** the use of the test has been shown with high levels of evidence to improve clinical outcomes compared with not using the test

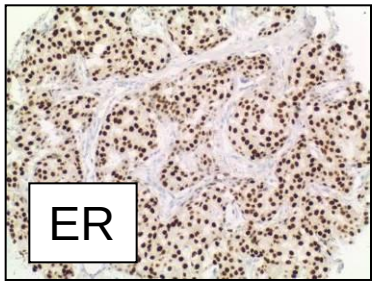
Evaluation of Genomic Applications in Practice and Prevention definitions, the Institute of Medicine

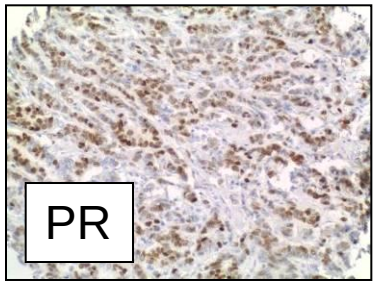
Table 1. Elements of tumor marker studies that constitute Levels of Evidence determination*

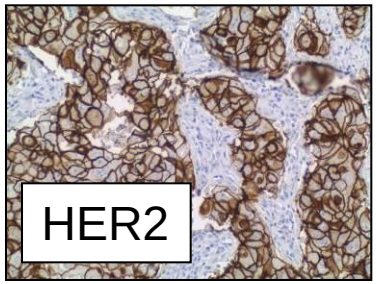
Category Element	A Prospective	B Prospective using archived samples	C Prospective/ observational	D Retrospective/ observational
Clinical trial	PCT designed to address tumor marker	Prospective trial not designed to address tumor marker, but design accommodates tumor marker utility Accommodation of predictive marker requires PRCT	Prospective observational registry, treatment and follow-up not dictated	No prospective aspect to study
Patients and patient data	Prospectively enrolled, treated, and followed in PCT	Prospectively enrolled, treated, and followed in clinical trial and, especially if a predictive utility is considered, a PRCT addressing the treatment of interest	Prospectively enrolled in registry, but treatment and follow-up standard of care	No prospective stipulation of treatment or follow-up; patient data collected by retrospective chart review
Specimen collection, processing, and archival	Specimens collected, processed, and assayed for specific marker in real time	Specimens collected, processed, and archived prospectively using generic SOPs. Assayed after trial completion	Specimens collected, processed, and archived prospectively using generic SOPs. Assayed after trial completion	Specimens collected, processed and archived with no prospective SOPs
Statistical design and analysis	Study powered to address tumor marker question	Study powered to address therapeutic question and underpowered to address tumor marker question	Study not prospectively powered at all. Retrospective study design confounded by selection of specimens for study	Study not prospectively powered at all. Retrospective study design confounded by selection of specimens for study
		Focused analysis plan for marker question developed before doing assays	Focused analysis plan for marker question developed before doing assays	No focused analysis plan for marker question developed before doing assays
Validation	Result unlikely to be play of chance	Result more likely to be play of chance that A but less likely than C	Result very likely to be play of chance	Result very likely to be play of chance
	Although preferred, validation not required	Requires one or more validation studies	Requires subsequent validation studies	Requires subsequent validation

* PCT = prospective controlled trial; PRCT = prospective randomized controlled trial; SOPs = standard operating practices.

Simon et al JNCI 2009 101 (21) 1446-1452







- Prognostic biomarkers
- Help determine likelihood of patients responding to endocrine therapy
- Absence of benefit from endocrine treatments for women with ER-negative invasive breast cancers has been confirmed in large overviews of randomized clinical trials.
- Routinely assessed by IHC

- Prognostic biomarker
- Help determine likelihood of patients responding to anti-HER2 therapies
- Randomized clinical trials confirmed that patients with HER2-positive breast cancer benefit from effective HER2-targeted therapies
- Routinely assessed by IHC/ISH

To standardize and improve accuracy in testing and reporting

American Society of Clinical Oncology/College of American Pathologists Guideline Recommendations for Immunohistochemical Testing of Estrogen and Progesterone Receptors in Breast Cancer (Unabridged Version)

M. Elizabeth H. Hammond; Daniel F. Hayes; Mitch Dowsett; D. Craig Allred; Karen L. Hagerty; Sunil Badve; Patrick L. Fitzgibbons; Glenn Francis; Neil S. Goldstein; Malcolm Hayes; David G. Hicks; Susan Lester; Richard Love; Pamela B. Mangu; Lisa McShane; Keith Miller; C. Kent Osborne; Soonmyung Paik; Jane Perlmutter; Anthony Rhodes; Hironobu Sasano; Jared N. Schwartz; Fred C. G. Sweep; Sheila Taube; Emina Emilia Torlakovic; Paul Valenstein; Giuseppe Viale; Daniel Visscher; Thomas Wheeler; R. Bruce Williams; James L. Wittliff; Antonio C. Wolff

Recommendations for Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer

American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Update

Antonio C. Wolff, M. Elizabeth H. Hammond*, David G. Hicks*, Mitch Dowsett*, Lisa M. McShane*, Kimberly H. Allison, Donald C. Allred, John M.S. Bartlett, Michael Bilous, Patrick Fitzgibbons, Wedad Hanna, Robert B. Jenkins, Pamela B. Mangu, Soonmyung Paik, Edith A. Perez, Michael F. Press, Patricia A. Spears, Gail H. Vance, Giuseppe Viale, and Daniel F. Hayes**

Hammond et al. Arch Pathol Lab Med 2010; 134: e48-e72
Wolff et al. Arch Pathol Lab Med 2013; 138: 241-256

ER/PR/HER2 testing

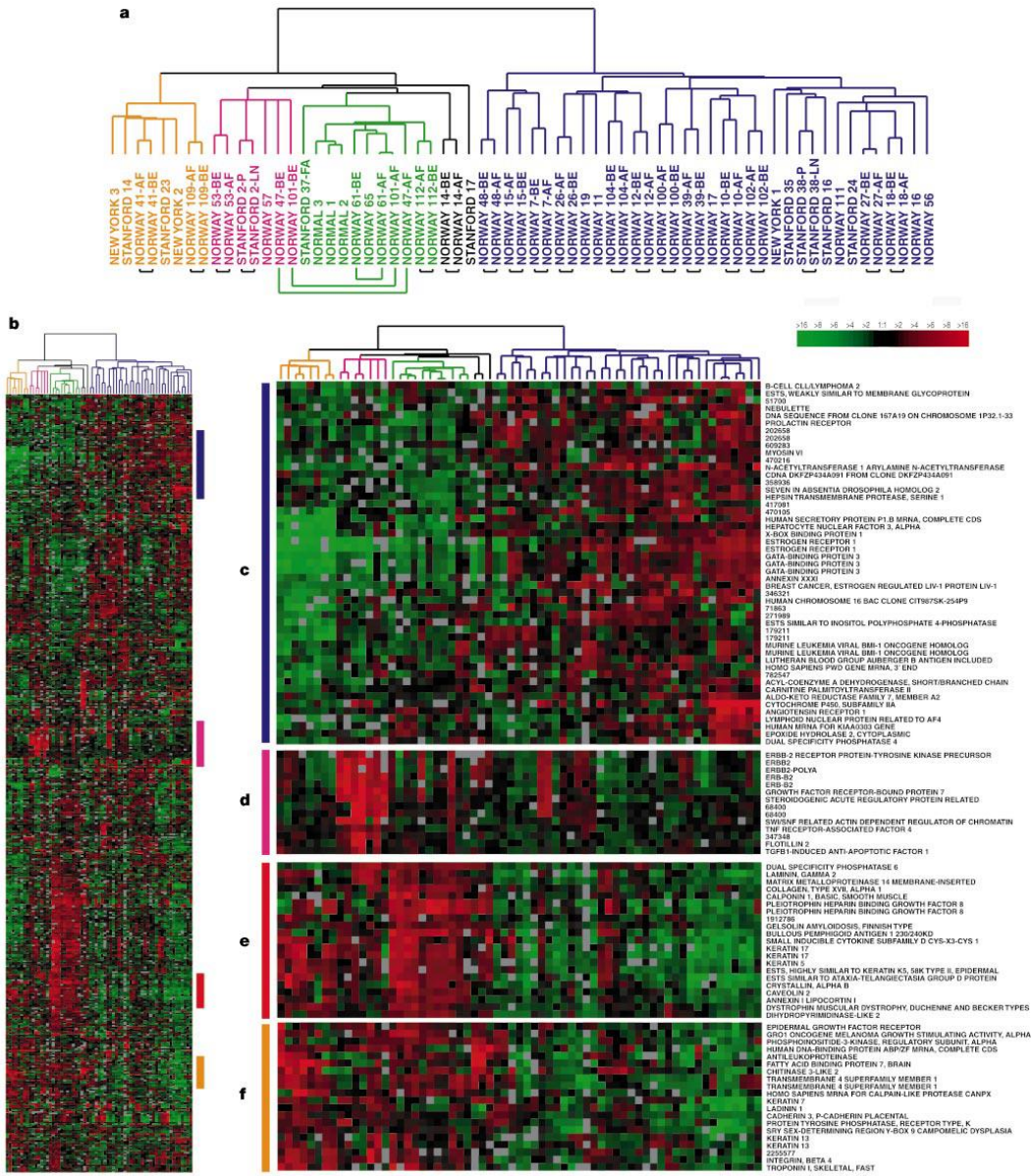
		ER/PR guidelines	HER2 guidelines
Pre-analytical	**Time to fixation**	≤ 1 hour	≤ 1 hour
	Fixation time	> 6 and < 72 hours	> 6 and < 72 hours
	Fixative	10% NBF	10% NBF
	Samples to be tested	Newly diagnosed BC and breast recurrences	Newly diagnosed BC and breast recurrences
Analytical	**Validated Assays**	IHC	IHC and ISH
	Methods	Validated against patients outcomes	Validated against patients outcomes
	Controls	Positive and negative controls; Positive internal control	Positive and negative controls Negative internal control
Post-analytical	**Evaluation**	% positive cells and intensity	IHC: %, intensity and pattern ISH: average HER2 copy number (c), HER2/CEP17 (r)
	Interpretation	Positive if ≥ 1% tumor cells stained	IHC: negative (0, 1+), equivocal (2+), positive (3+) ISH: negative, equivocal or positive

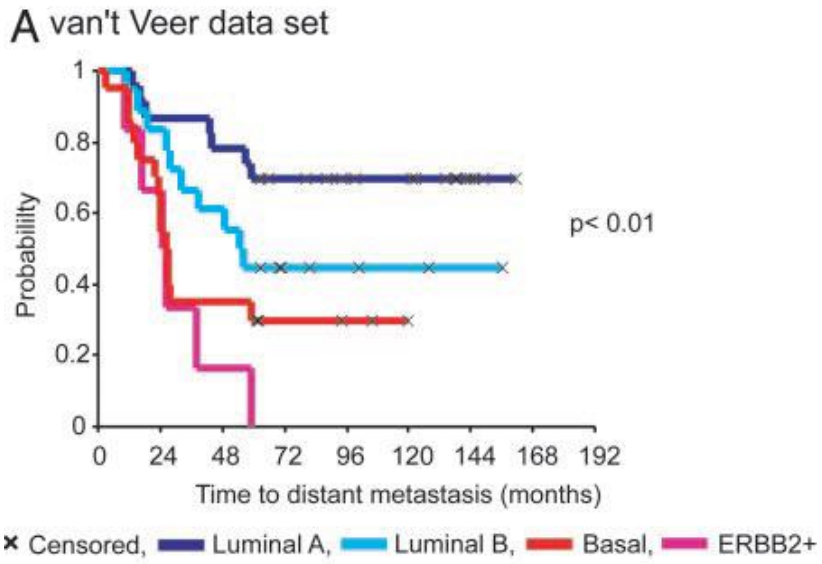
Hammond et al. Arch Pathol Lab Med 2010; 134: e48-e72
Wolff et al. Arch Pathol Lab Med 2013; 138: 241-256

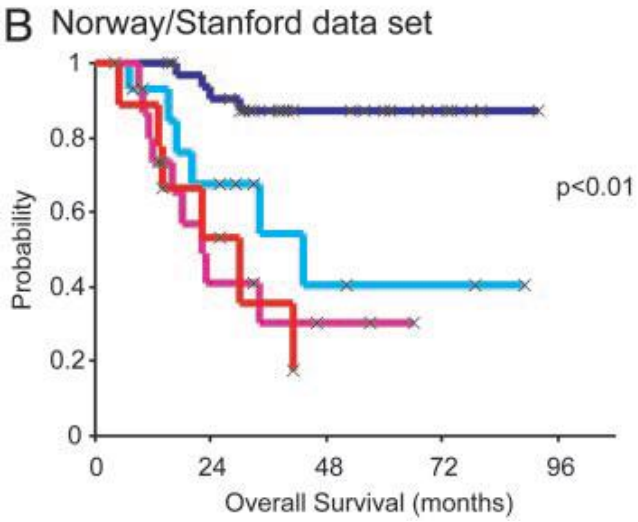
(Some) controversial issues

- ER/PR
  - ER low (<10%)
  - PR and response to endocrine therapy
  - Alternative methods (RT-PCR, microarrays)
- HER2
  - Polysomy 17
  - Intratumoral heterogeneity
  - Alternative methods (RT-PCR, microarrays)

Beyond ER/PR/HER2







Perou et al. Nature 406, 747-752 (2000)
Sorlie et al. PNAS 100, 8418-8422 (2003)

Ki67

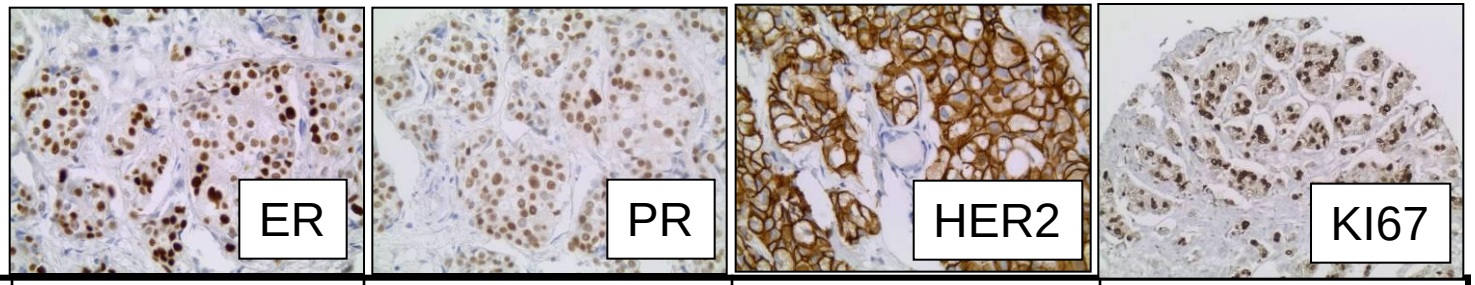
- Proliferation marker expressed by cells in G1, S, G2, M phase
- Ki67 is a prognostic factor
- High ki67 is associated with a high rate of pathological complete response (pCR) in the neo-adjuvant setting
- Ki67 might help distinguish between luminal A and luminal B HER2 negative tumors

de Azambuja et al. Br J Cancer. 2007; 96(10):1504-13
Stuart-Harris et al., Breast 2008; 17: 323–34
Cheang et al. JNCI 2009; 101:736–750
Luporsi et al BCRT 2012 132:895-915

Annals of Oncology 24: 2206–2223, 2013
doi:10.1093/annonc/mdt303

Personalizing the treatment of women with early breast cancer: highlights of the St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2013

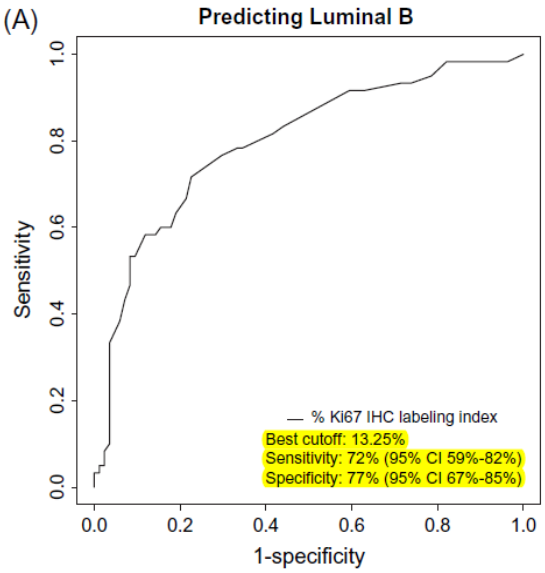
A. Goldhirsch[1*], E. P. Winer[2], A. S. Coates[3], R. D. Gelber[4], M. Piccart-Gebhart[5], B. Thürlimann[6] & H.-J. Senn[7] Panel members[†]

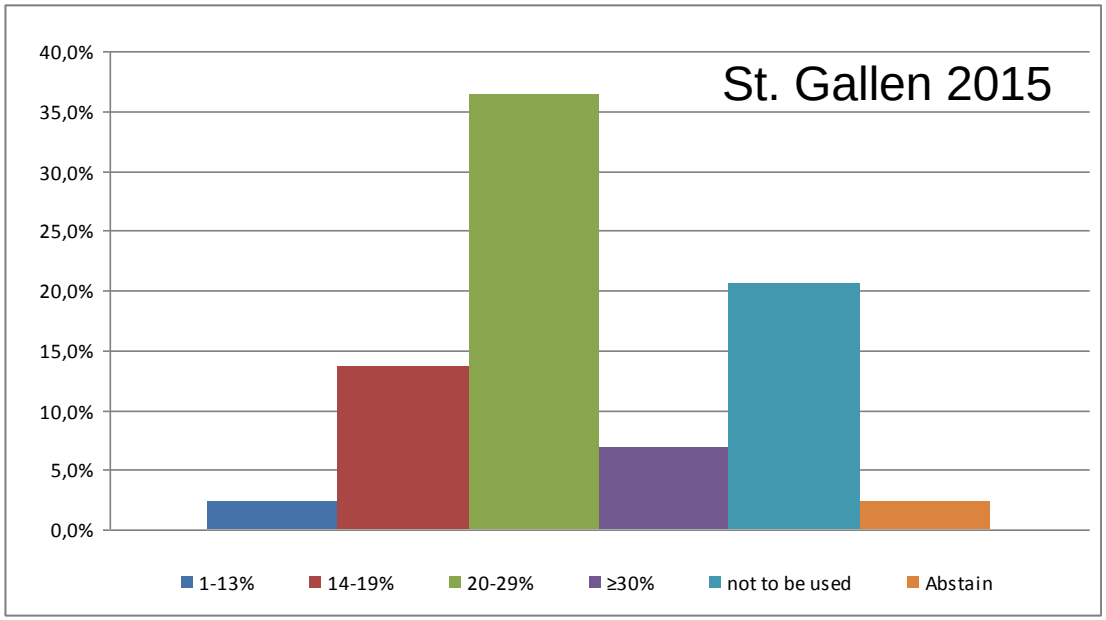


Luminal A-like	+	+	-	low
Luminal B-like (HER2 negative)	+	low	-	high
Luminal B-like (HER2 positive)	+	any	+	any
HER2 positive (non-luminal)	-	-	+	any
Triple negative (ductal)	-	-	-	any

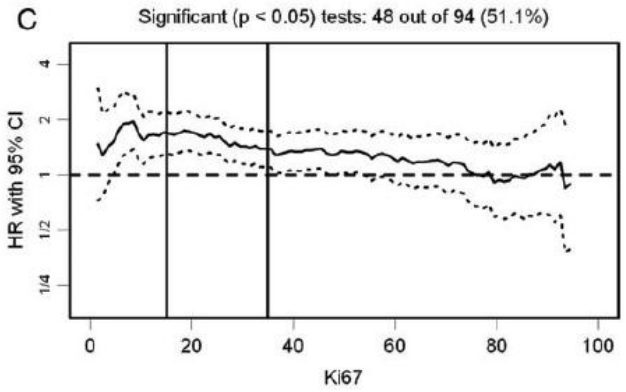
Which cut-point?

the minimum value of Ki-67 required for ‘Luminal B-like’ is:

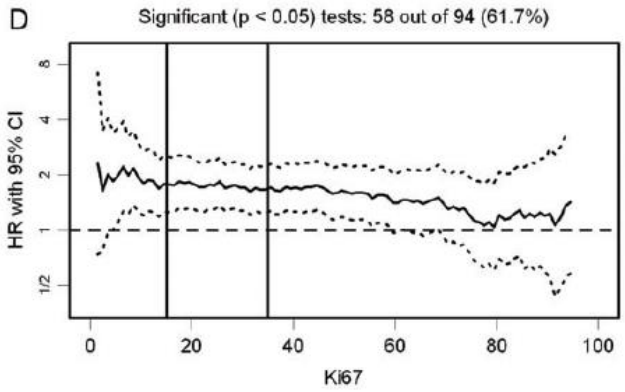




DFS



OS



Cheang et al. JNCI 2009; 101 (10): 736-750
Denkert et al. Ann Oncol 2013; 24: 2786-2793
http://www.oncoconferences.ch/Consensus2015

Analytical validity?

Assessment of Ki67 in Breast Cancer: Recommendations from the International Ki67 in Breast Cancer Working Group

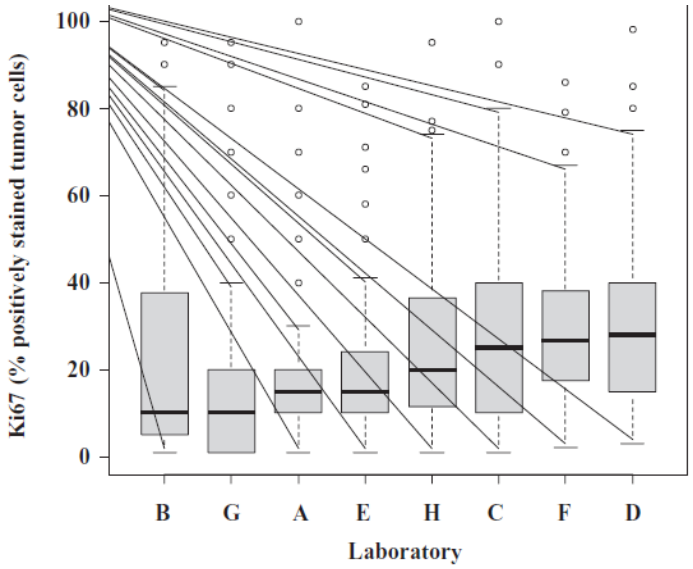
Mitch Dowsett, Torsten O. Nielsen, Roger A'Hern, John Bartlett, R. Charles Coombes, Jack Cuzick, Matthew Ellis, N. Lynn Henry, Judith C. Hugh, Tracy Lively, Lisa McShane, Soon Paik, Frederique Penault-Llorca, Ljudmila Prudkin, Meredith Regan, Janine Salter, Christos Sotiriou, Ian E. Smith, Giuseppe Viale, Jo Anne Zujewski, Daniel F. Hayes

- Tissue handling guidelines for ER/HER2 are more than adequate for Ki67
- IHC for Ki67 (MIB1 antibody) is the gold standard
- Count 3 randomly selected HPF at least 500 cells, at the periphery, including hot spots
- Unable to come to consensus regarding the ideal cut point(s)

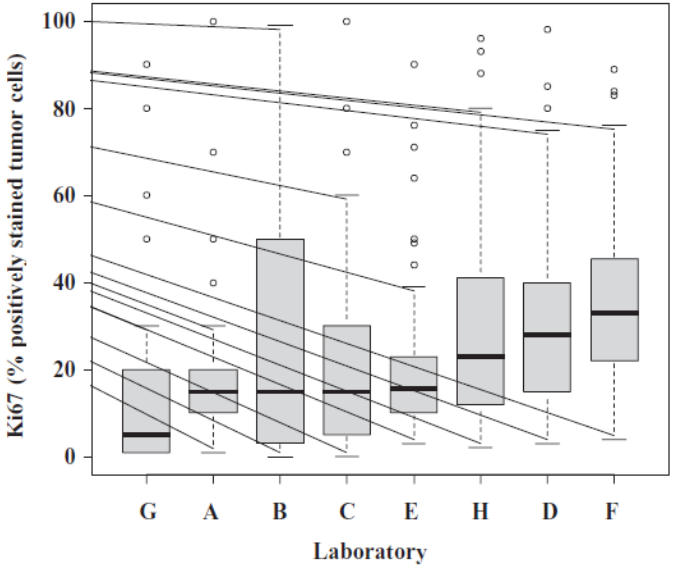
Dowsett et al. JNCI 2011; 103:1656–1664

An International Ki67 Reproducibility Study

Tissue Microarrays Centrally stained, locally scored



Locally stained, locally scored



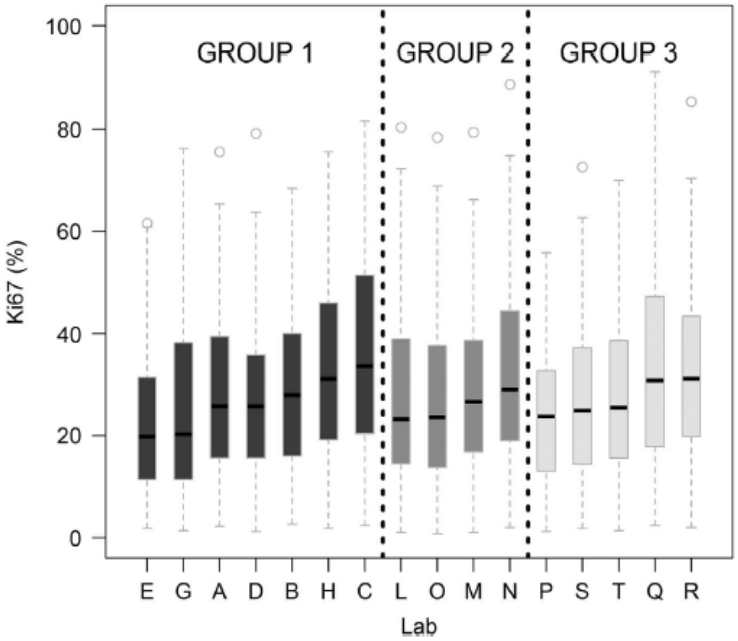
"the analytical validity for Ki67 assay is unacceptably poor".

"clinical utility of Ki67 in breast cancer remains elusive because of analytical concerns"

Polley et al. JNCI 2013;105:1897–1906

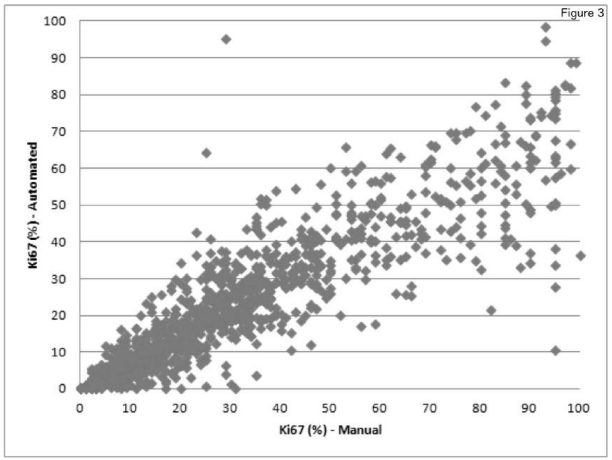
16 laboratories
50 centrally MIB-1 stained TMAs

“ After calibrating to a common scoring method via a web-based tool, laboratories can achieve high inter-laboratory reproducibility in Ki67 scoring on centrally stained tissue microarray slides”

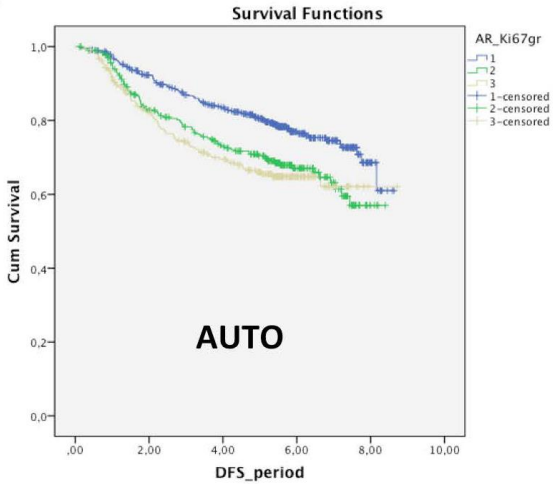


Polley et al. Mod Pathol 2015

Automated scoring systems



A



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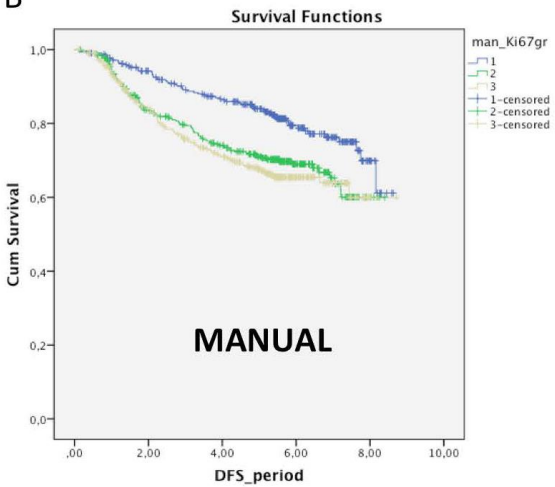

Klauschen et al Clin Cancer Res 2014

Ki67 as predictive biomarker

- No prospective studies
- Results from retrospective studies on randomized trials assessing the role of Ki67 as predictive marker for chemotherapy response in the adjuvant setting are contradictory
- Need to further validation
- Need to standardize pre-analytical analytical and post-analytical phases

Dowsett et al. JNCI 2011; 103:1656–1664
Luporsi et al BCRT 2012 132:895-915
Andre *et al*. *Nat. Rev. Clin. Oncol*. 17 March 2015

Multiparameters molecular markers

	Oncotype Dx™	MammaPrint®	GGI	PAM50 (ROR-S)	Breast Cancer Index	EndoPredict
Provider	Genomic Health	Agendia	Ipsogen	–	Biotheranostics	Sividon Diagnostics
Type of assay	21-Gene recurrence score	70-Gene assay	97-Gene assay	50-Gene assay	2-Gene ratio HOXB13 to IL17R and molecular grade index	11-Gene assay
Tissue sample	FFPE	Fresh or frozen	Fresh or frozen	FFPE	FFPE	FFPE
Technique	qRT–PCR	DNA microarray	DNA microarray	qRT–PCR	qRT–PCR	qRT–PCR

GGI, Genomic Grade Index; FFPE, formalin-fixed paraffin-embedded; qRT–PCR, quantitative reverse transcriptase–polymerase chain reaction.

Table 5. Recommendations/guidelines from other groups including the 2012 IMPAKT Working Group

	Year	Signatures	Statement
ASCO	2007	Oncotype Dx™ MammaPrint® Breast Cancer Gene Expression Ratio	• Oncotype Dx® can be used for prognosis in ER+, pN0, tamoxifen-treated • Oncotype Dx® may be used to assign chemotherapy
Institut National du Cancer, France	2009	Oncotype Dx™ MammaPrint® uPA-PAI-1	• Level II 'Oncotype Dx®': prognosis in ER+, pN0 • Level II 'Oncotype Dx®': prediction in ER+, CMF-treated
EGAPP		Oncotype Dx™ MammaPrint® Breast Cancer Gene Expression Ratio	• Inadequate analytical validity 'both' • Adequate clinical validity 'Oncotype Dx®' • Inadequate clinical utility 'both'
St Gallen	2011	Oncotype Dx™ MammaPrint®	• Oncotype Dx® may be used to assign chemotherapy • MammaPrint®: insufficient data
IMPAKT Working Group	2012	Oncotype Dx™ MammaPrint® Genomic Grade Index PAM50 (ROR-S) Breast Cancer Index EndoPredict	• Analytical validity: convincing 'Oncotype DX™, MammaPrint®' • Clinical validity: convincing 'Oncotype DX ™, MammaPrint®' • Clinical utility: convincing 'none'

ASCO, American Society of Clinical Oncology; ER+, estrogen receptor-positive; pN0, pathologic node negative; CMF, cyclophosphamide, methotrexate, 5-flouruoracil; EGAPP, Evaluation of Genomic Applications in Practice and Prevention.

Azim et al. Annals of Oncology 24, 647–654 (2013)

Multiparameters molecular markers

- Multiparameter molecular markers including Mammaprint, Breast Cancer Index, Oncotype Dx, Prosigna, Endopredict demonstrated clinical validity as prognostic markers in patients with ER+ breast cancer
- Analytical validity studies have been published for some of these tests
- Definitive evidence of clinical utility for Mammaprint Oncotype Dx and Prosigna will derive from three ongoing prospective randomized trials
- Need to integrate prognostic/predictive information deriving from these tests with traditional clinico-pathological parameters

Thanks!