

**Supplemental Digital Content, containing 4 tables and 2 figures. The Supplemental Digital Content was not copyedited by Archives of Pathology & Laboratory Medicine.**

**Supplemental Table 1. 4-hour Training of Participants – Structure and Content**

(1) 1-hour background lecture on the core principles of HER2 interpretation with a focus on findings frequently observed in low HER2 expressing breast cancers, approved by the steering-committee members.

Main references: ASCO/CAP 2018<sup>1</sup> and current literature on novel anti-HER2 therapies<sup>2,3,4</sup>

Step-wise reading of IHC stained slides followed 3 important precepts (acc. to Figure 2):

- Application of the magnification rule<sup>5,6</sup> as an aid for standardized intensity scoring
- Demonstration of staining patterns that are frequently observed in weak stained HER2 tumors. Typical patterns include incomplete patchy staining which must be distinguished from nonspecific stains such as basal membrane-like as well as cytoplasmic granular or dotted staining as a potential risk of false-positive scoring.
- Finally, the training focused on interpretation techniques to score cases bordering at the 10% cut-off that separates HER2-0 and IHC 1+ (HER2-low).

(2) Digital didactic microscope session with 6 (5 paired) demonstration cases

(3) Thereafter participants completed a “pre-test” of 5 different cases followed by a detailed case-by-case discussion.

(4) Main test with another 15 cases. Results were discussed included scanned images of both assays.

(5) Finally, participants were asked to complete a training questionnaire with feedback on issues such as length of training time, factors causing most concern for HER2-low interpretation, and adequacy of curriculum. Survey data not presented in this manuscript.

HER2, Human Epidermal Growth Factor Receptor 2; ASCO, American Society of Clinical Oncology; CAP, College of American Pathologists; IHC, Immunohistochemistry;

**Supplemental Table 2.** Human HER2-low study group: participants (list extract)

<b>First Name</b>	<b>Last Name</b>	<b>Company</b>
Robert	Babkowski	Stamford Health System
Sunil	Badve	Emory University
Alberto	Berjón García	Hospital Universitario La Paz - Servicio de Anatomía Patológica
Kenneth	Bloom	Invicro
Fouad	Boulos	Washington University School of Medicine (St. Louis)
Clinton	Boyd	Belfast Health & Social Care Trust - Royal Victoria Hospital
Elena	Brachtel	Maastricht UMC
Simonetta	Buglioni	Istituto Nazionale Tumori Regina Elena
Laura	Comerma Blesa	Hospital del Mar - Parc de Salut Mar
Chiara	Costanza Volpi	Fondazione IRCCS Istituto Nazionale dei Tumori
Elisa	De Camilli	IEO – Milano
Alexander	de Haas	Martini Hospital Groningen
Carsten	Denkert	Universitätsklinikum Giessen-Marburg
Erinn	Downs-Kelly	Cleveland Clinic
Ramona	Erber	Universitätsklinikum Erlangen, Institut für Pathologie
Sara	Fernández Ferrer	Hospital de Basurto
Guido	Ficarra	AOU Policlinico di Modena
Almuth	Forberger	Universitätsklinikum Carl Gustav Carus Dresden - Institut für Pathologie
Camille	Franchet	IUCT Oncopole Toulouse
Juliana	Freitas	Federal University of Bahia Medical School
Nicolas	Gallegos	Affiliated Pathologists Medical Group
Christian	Garbar	Institut Jean Godinot
Alessandro	Ginori	Centro Polispecialistico Monterosso Carrara
Gyungyub	Gong	Asan Medical Center
Paolo	Graziano	Ospedale Casa Sollievo della Sofferenza-IRCCS
Oi	Harada	Kameda Hospital
Malini	Harigopal	Yale University School of Medicine
Astrid	Herrera	Hospital Universitario y Politécnico La Fe
David	Hicks	Rochester University Medical Center
Akira	Hida	Matsuyama City Hospital
Zsolt	Hodi	Nottingham University Hospital NHS Trust
Miles	Howe	Royal Victoria Infirmary, Newcastle
Hee	Jin Lee	Asan Medical Center
Fatima	Kebir-Seladji	CHU Tenon
Jeffrey	Killeen	Kapi'olani Medical Center for Women and Children
Beena	Kumar	Omnipath
Magali	Lacroix	Gustave Roussy
Amy	Ly	Massachusetts General Hospital
Gaetan	MacGrogan	Institut Bergonié
Anthony	Magliocco	Protean BioDiagnostics
Aurelie	Maran-Gonzalez	ICM Montpellier
Shinobu	Masuda	Nihon University School of Medicine
Bernadette	McLaren	Delta Pathology Group

Dylan	Miller	Intermountain Central Laboratory
Aurelia	Noske	Pathologie-Zentrum Zürich
Cristiana	Nunes	Faculdade de Medicina da UFMG
Tomo	Osako	Japanese Foundation for Cancer Research
Belén	Pérez Mies	Hospital Universitario Ramón y Cajal - Servicio de Anatomía Patológica
Letizia	Perracchio	Istituto Nazionale Tumori Regina Elena
Elke	Peters	Haaglanden MC, location: Westeinde, The Hague
Bruno	Poulet	Institut de Pathologie de Paris
Paula	Rodriguez Martinez	Hospital Universitari Germans Trias i Pujol
Alejandro	Rojo Sebastián	MD Anderson Cancer Center
Luis	Samayoa	Norton Healthcare
Daman	Samrao	LMC Pathology Services- UNLV School of Medicine
Mary Ann	Sanders	Norton Healthcare
Alfredo	Santinelli	Ospedali Riuniti Marche Nord
Abeer	Shaaban	Univeristy Hospital Birmingham QE
Jian	Shen	Norton Health-CPA Labs
Kamaljeet	Singh	Brown University
Peter	Sinn	Universitätsklinikum Heidelberg
Stacey	Smith	ProPath Services
Olivia	Snir	Providence Hospital- Portland
Rinda	Soong	University of Pittsburgh Medical Center, Magee-Womens Hospital
Uffe	Stolborg	Sjællands Universitetshospital, Department of Pathology Roskilde
Sameer	Talwalkar	Norton Health-CPA Labs
Bruce	Tanchel	Birmingham Heartlands Hospital
Lucie	Tixier Deves	Centre Jean Perrin
Isabelle	Treilleux	Centre Léon Berard
Hitoshi	Tsuda	University of Nat. Diffence
Bradley	Turner	University of Rochester Medical Center, Strong Memorial Hospital/Highland Hospital
Andreas	Turzynski	Dr. med. Andreas Turzynski, PathoPlan GbR
Varone	Valeria	Istituto Nazionale Tumori IRCCS Fondazione "G. Pascale"
Zsuzsanna	Varga	UniversitätsSpitalZürich, Institut für Pathologie und Molekularpathologie
Reinhard	von Wasielewski	MVZ Pathologie Hildesheim Hannover-Zentrum GmbH
Shi	Wei	University of Alabama at Birmingham
Huina	Zhang	University of Rochester Medical Center, Strong Memorial Hospital/Highland Hospital

**Supplemental Table 3.** Cohen's  $\kappa$ ; per binary category

HER2-Testing:	4B5 $\pm$ ISH		HcT $\pm$ ISH	
Classification System:	<b>ASCO/CAP</b> Negative: [IHC 0, 1+, 2+/ISH-] Positive: [IHC 2+/ISH+, 3+]	<b>New Classification</b> 0: [IHC 0] Low: [IHC 1+, 2+/ISH-] Positive: [IHC 2+/ISH+, 3+]	<b>ASCO/CAP</b> Negative: [IHC 0, 1+, 2+/ISH-] Positive: [IHC 2+/ISH+, 3+]	<b>New Classification</b> 0: [IHC 0] Low: [IHC 1+, 2+/ISH-] Positive: [IHC 2+/ISH+, 3+]
Concordance, 1 <sup>st</sup> round, prior training:	0.955 [0.897-0.998]	0: 0.641 [0.378 - 0.833] Low: 0.658 [0.415 - 0.836] Positive: 0.956 [0.92 - 0.998] 0,Low,Positive: 0.747 [0.609 - 0.915]	0.837 [0.505 - 0.971]	0: 0.782 [0.537 - 0.918] L: 0.657 [0.381 - 0.853] P: 0.837 [0.515 - 0.975] 0,Low,Positive: 0.805 [0.701 - 0.945]
Concordance, 2 <sup>nd</sup> round, post-training:	0.97 [0.943 - 1] ( $P=0.26$ )	0: 0.699 [0.434 - 0.86] ( $P=0.037^a$ ) L: 0.701 [0.472 - 0.863] ( $P=0.125$ ) P: 0.97 [NA] ( $P=0.26$ ) 0,Low,Positive: 0.785 [0.643 - 0.933] ( $P=0.06$ )	0.849 [0.565 - 0.971] ( $P=0.67$ )	0: 0.803 [0.557 - 0.939] ( $P=0.46$ ) L: 0.677 [0.398 - 0.865] ( $P=0.66$ ) P: 0.849 [0.569 - 0.971] ( $P=0.67$ ) 0,Low,Positive: 0.823 [0.74 - 0.95] ( $P=0.47$ )

4B5, Ventana PATHWAY 4B5 Assay; ASCO/CAP, American Society of Clinical Oncology/College of American Pathologists;  
 HER2, human epidermal growth factor receptor 2; HcT, Dako HercepTest; IHC, immunohistochemistry; ISH, in situ hybridization;  
 Low, HER2-Low; NA, not applicable; 0, HER2-0; P, HER2-positive

- Cohen's weighted  $\kappa$  coefficient, 95% confidence intervall (if applicable)
- Concordance per category (0, Low, Positive)
- Training effect in each category
- <sup>a</sup>Statistically significant for 4B5 HER2-0 (almost significant for 4B5; entire new 3-step score)

**Supplemental Table 4.** Concordance and efficacy of training per classification category, as per Cohen’s weighted kappa ( $\kappa$ ) coefficient

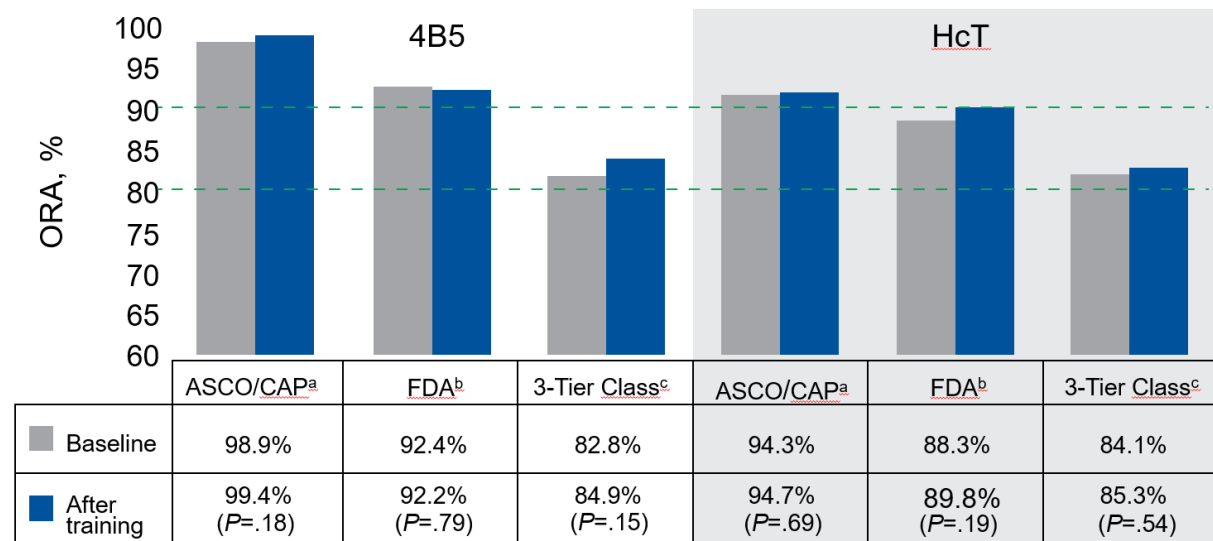
HER2-Testing Concordance	4B5 IHC ISH+/- [95% CI] ( <i>P</i> -value)		HcT IHC ISH+/- [95% CI] ( <i>P</i> -value)	
	ASCO/CAP <sup>a</sup> Negative: [IHC 0,1+,2+/ISH-] Positive: [IHC 2+/ISH+, 3+]	New 3-Tier Class <sup>b</sup> O: [IHC 0] L: [IHC 1+, 2+/ISH-] P: [IHC 2+/ISH+, 3+]	ASCO/CAP <sup>a</sup> Negative: [IHC 0, 1+, 2+/ISH-] Positive: [IHC 2+/ISH+, 3+]	New 3-Tier Class <sup>b</sup> O: [IHC 0] L: [IHC 1+, 2+/ISH-] P: [IHC 2+/ISH+, 3+]
<b>Baseline</b>	0.955 [0.897-0.998]	O: 0.641 [0.378-0.833] L: 0.658 [0.415-0.836] P: 0.956 [0.92-0.998] O,L,P: 0.747 [0.609-0.915]	0.837 [0.505-0.971]	O: 0.782 [0.537-0.918] L: 0.657 [0.381-0.853] P: 0.837 [0.515-0.975] O,L,P: 0.805 [0.701-0.945]
<b>After training</b>	0.97 [0.943-1.000] ( <i>P</i> =.26)	O: 0.699 [0.434-0.86] ( <i>P</i> =.037*) L: 0.701 [0.472-0.863] ( <i>P</i> =.125) P: 0.97 [NA] ( <i>P</i> =.26) O,L,P: 0.785 [0.643-0.933] ( <i>P</i> =.06)	0.849 [0.565-0.971] ( <i>P</i> =.67)	O: 0.803 [0.557-0.939] ( <i>P</i> =.46) L: 0.677 [0.398-0.865] ( <i>P</i> =.66) P: 0.849 [0.569-0.971] ( <i>P</i> =.67) O,L,P: 0.823 [0.74-0.95] ( <i>P</i> =.47)

4B5, Ventana PATHWAY 4B5 Assay; ASCO/CAP, American Society of Clinical Oncology/College of American Pathologists; HER2, human epidermal growth factor receptor 2; HcT, Dako HercepTest; IHC, immunohistochemistry; ISH, in situ hybridization; L, HER2-Low; NA, not applicable; O, HER2-0; P, HER2-positive

<sup>a</sup>Current ASCO/CAP 2018 guideline classification; HER2-positive (IHC 3+ or IHC 2+/ISH+) vs HER2-negative (IHC 2+/ISH-, IHC 1+, IHC 0).

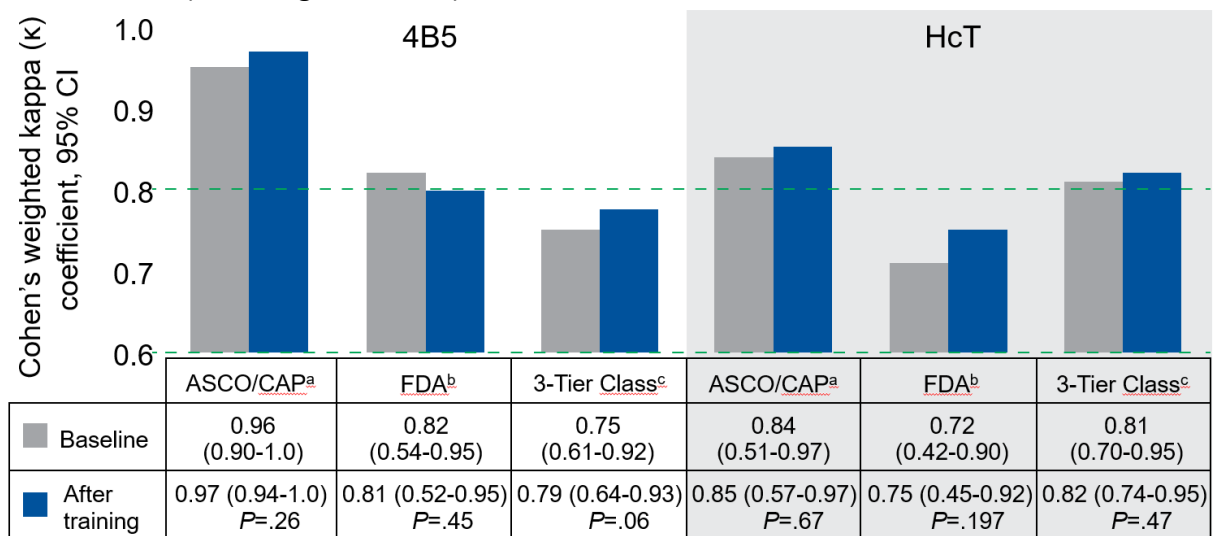
<sup>b</sup>New 3-tier classification with a category for HER2-low; HER2-positive (IHC 3+ or IHC 2+/ISH+) vs HER2-low (IHC 2+/ISH- or IHC 1+) vs HER2-0 (IHC 0).

**Supplement Supplemental Figure 1.** Overall Rater Agreement Between Reference Group and Participating Pathologists for HER2 Binary (<sup>a</sup>ASCO/CAP and <sup>b</sup>FDA) and <sup>c</sup>New 3-Tier Classification (Including HER2-Low)



4B5, Ventana PATHWAY 4B5 Assay; HcT, Dako HercepTest; ASCO/CAP, American Society of Clinical Oncology/College of American Pathologists; FDA, U.S. Food and Drug Administration; ORA, Overall Rate of Agreement

**Supplement Figure 2.** Kappa Analysis for the Concordance Between Reference Group and Participating Pathologists for HER2 Binary (<sup>a</sup>ASCO/CAP and <sup>b</sup>FDA) and <sup>c</sup>New 3-Tier Classification (Including HER2-Low)



4B5, Ventana PATHWAY 4B5 Assay; HcT, Dako HercepTest; ASCO/CAP, American Society of Clinical Oncology/College of American Pathologists; FDA, U.S. Food and Drug Administration;

## REFERENCES

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6. Scheel AH, Penault-Llorca F, Hanna W, et al. Physical basis of the 'magnification rule' for standardized Immunohistochemical scoring of HER2 in breast and gastric cancer. *Diagn Pathol*. 2018;13(1):19.