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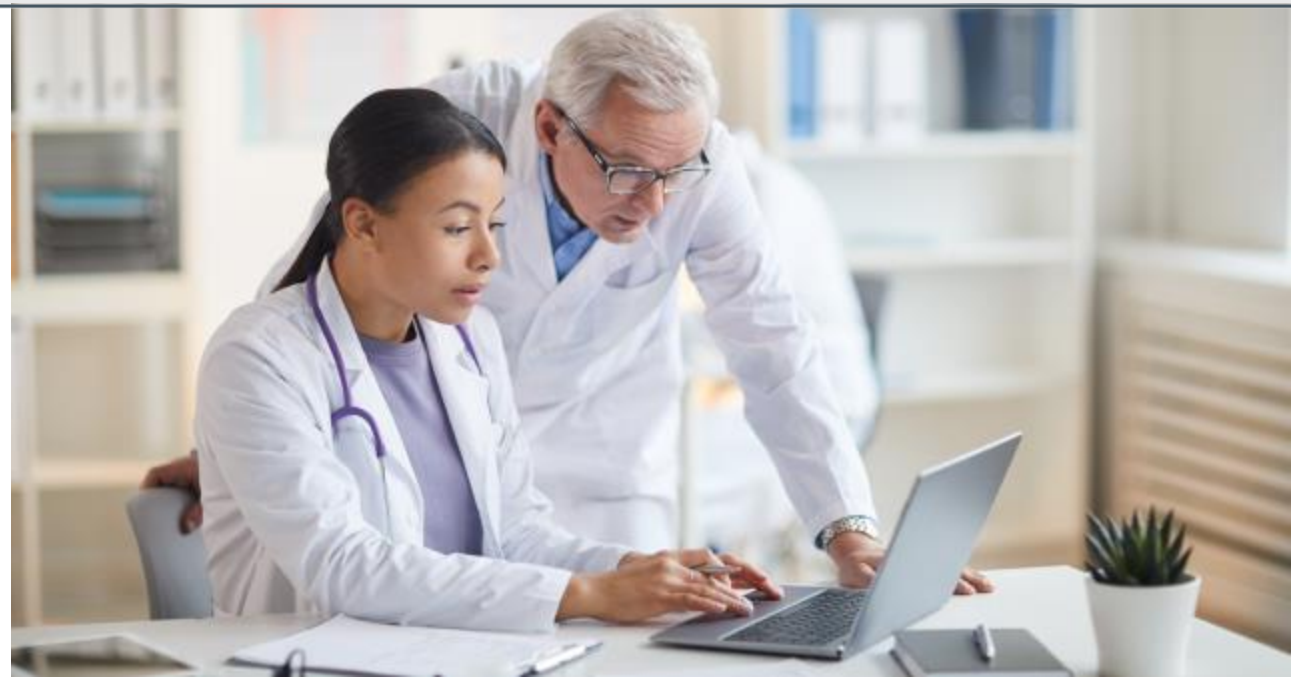
Optimal Utilization of Ki67 Testing in HR-Positive/HER2-Negative Early Breast Cancer: Education and Resources for the Oncology and Pathology Healthcare Teams

January 20, 2022



This activity is supported by an educational grant from Lilly.

Faculty Disclosures

- Manali Bhave, MD, faculty for this educational event, is a speaking consultant for Daiichi Sankyo, Inc. and on the advisory board for Merck. All of the relevant financial relationships listed for these individuals have been mitigated.
- Sunil S. Badve, MD, FRCPath, faculty for this educational event, is a consultant for Bristol Myers Squibb, on the speakers' bureau for Agilent and Targos/Discovery, and has received research support from Agilent. All of the relevant financial relationships listed for these individuals have been mitigated.

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Optimal Utilization of Ki67 Testing in HR-Positive/ HER2-Negative Early Breast Cancer

Manali Bhave, MD

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Emory University School of Medicine

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Emory University School of Medicine

Today's videoconference

Dr. Manali Bhave

Clinical Overview

CDK4/6 Inhibitors for Treatment

Dr. Sunil Badve

Ki67 Testing Methods,

Standardization & Interpretation

Dr. Manali Bhave

Application

with a Case

Interactive Polling & Brief Discussion

Optimal Utilization of Ki67 Testing in HR-Positive/ HER2-Negative Early Breast Cancer

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Optimal Utilization of Ki67 Testing in HR-Positive/ HER2-Negative Early Breast Cancer

Clinical Overview - CDK4/6 Inhibitors for Treatment

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ER+ Breast Cancer

- Account for ~70% of breast cancers
  - Even higher for older women
- Lower risk of ER+ breast cancer in women with first pregnancy <35 years and higher parity
- Recent use of OCPs associated with slight increase in ER+ breast cancer, particularly if use started before age 20 or prior to first pregnancy
- HRT use also associated with slight increase in ER+ breast cancer
- Overweight or obese women are at higher risk of ER+ breast cancer
- Tend to be more indolent -> caught at earlier stages compared to TNBC
- Genomic Assays can guide chemotherapy de-escalation

Treatment

- Treatment of stages I-III of disease are generally managed with surgical resections in combination with radiotherapy and/or systemic treatments (endocrine therapy +/- chemotherapy +/- CDK 4/6 inhibitor)

- Stages IV are managed by medical oncologists and usually consists of a combination endocrine therapy and targeted therapies

Source: Mayo Clinic, NCCN Guidelines 2020

Endocrine Therapy for Advanced Breast Cancer: Milestones

1896 — Oophorectomy and response to advanced disease (George Beatson)

1951 — Estrogen drives breast cancer

1977 — Estrogen receptor (ER) identified; Tamoxifen approved

1990s — Immuno-histochemistry developed for ER and PR analysis

1999 — First selective aromatase inhibitor (AI) approved for ABC

2002 — ER downregulator approved; AI approved as adjuvant therapy

2010 — Anti-HER2 + AI approved for ER/HER2+ ABC

2012 — mTOR inhibitor + AI approved

2015 — CDK4/6 inhibitor + AI approved

2019 — PI3K Inhibitor approved

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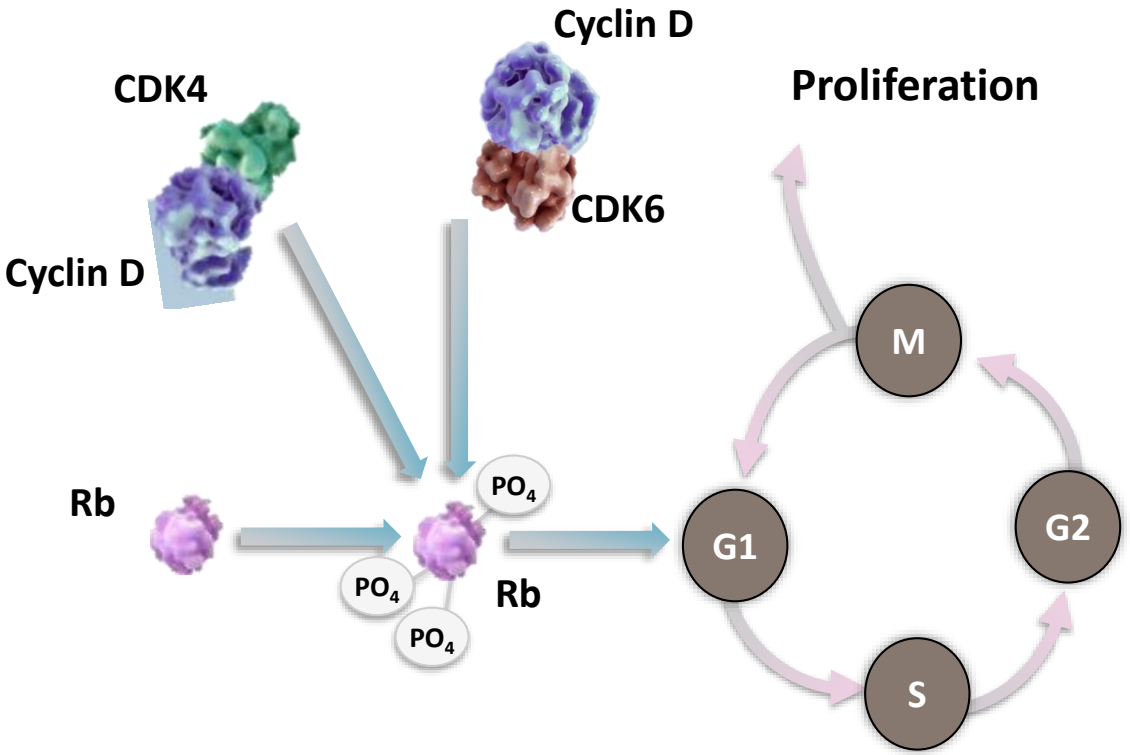
CDK4 & 6 in Breast Cancer

- D type cyclins activate CDK4 & 6 which phosphorylate Rb allowing G1 to S progression
- Estrogen stimulates cyclin D1 in HR+ breast cancer[1]
- Short term inhibition of CDK4 & CDK6 leads to G1 arrest that rebounds upon withdrawal[2]
- Continuous inhibition leads to prolonged cell cycle arrest with initiation of apoptosis or senescence[3]
- This led to the hypothesis that continuous target inhibition could be an effective strategy



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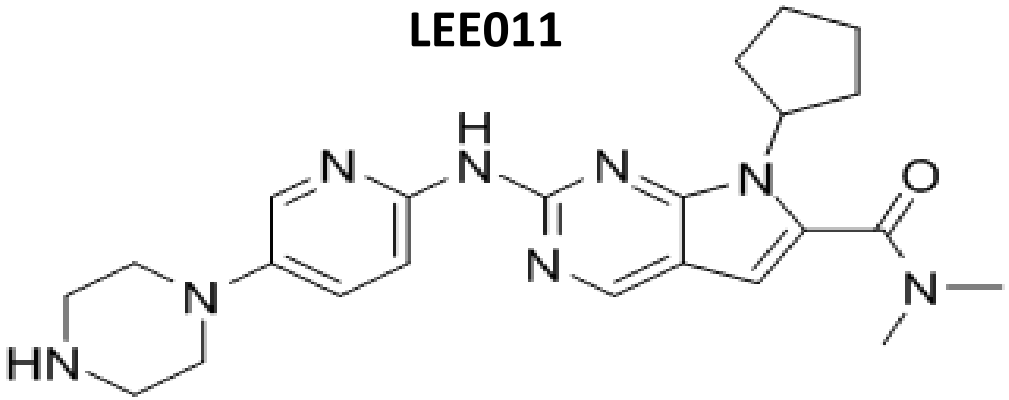
Altucci L et al. 1996 Oncogene 12:2315-24
Gelbert et al. 2014 Invest New Drugs 32: 825-37
Beckman et al. AACR Annual Meeting 2016

Approved CDK4/6 Inhibitors in Clinical Use

FDA Approved
(March 13, 2017)

Ribociclib

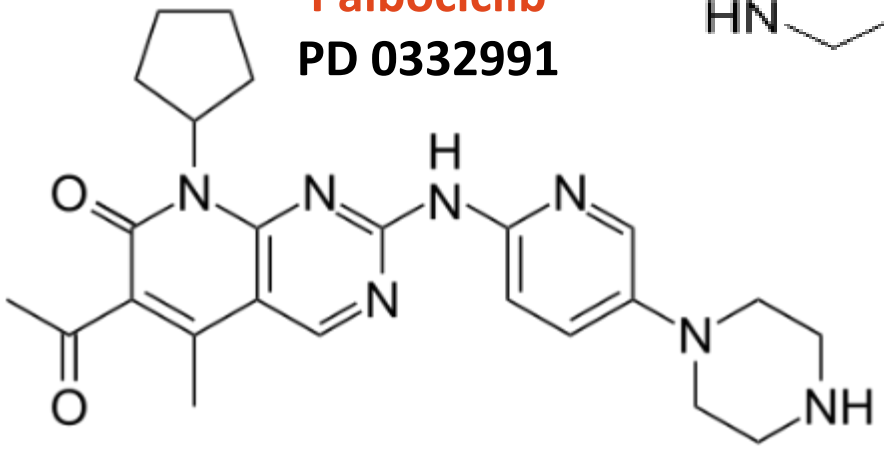
LEE011



FDA Approved
(February 4, 2015)

Palbociclib

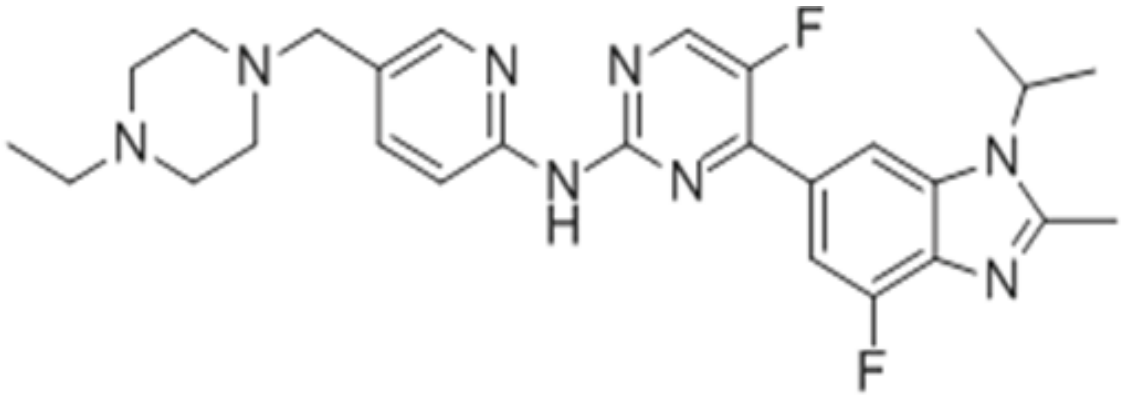
PD 0332991



FDA Approved
(September 27, 2017)

Abemaciclib

LY2835219



Polling Question

- Which two CDK4/6 inhibitors are given once daily for 21 consecutive days?
  A. Abemaciclib and Palbociclib
  B. Palbociclib and Ribociclib
  C. Abemaciclib and Ribociclib

CDK4/6 Inhibitors: Dosing Considerations

	Palbociclib[1]	Ribociclib[2]	Abemaciclib[3]	
Dosage Form	Capsule	Film-coated tablets	Tablets	
Recommended Dose	125 mg	600 mg (3x200 mg)	150 mg (when used as combination therapy)	200 mg (when used as monotherapy)
Dosing Frequency	Once daily for **21 consecutive days followed by 7 days off** treatment (28-day cycle)	Once daily for **21 consecutive days followed by 7 days off** treatment (28-day cycle)	Twice daily on a **continuous** dosing schedule	
Administration Considerations	Should be taken with food	May be taken with or without food	May be taken with or without food	

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Ibrance™ Product Monograph. Pfizer Canada Inc. June 5, 2018
Kisquali™ Product Monograph. Novartis Pharmaceuticals Canada Inc. March 19, 2018
Verzenio™ Product Monograph. Eli Lilly and Company. August 2018

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CDK4/6 Inhibitors: First-Line Trials in Advanced Breast Cancer

	Palbociclib[1]	RIbociclib[2,3]	Abemaciclib[4]
	PALOMA-2	MONALEESA-2	MONARCH-3
Endocrine Partner	Letrozole	Letrozole	Letrozole or Anastrozole
Eligibility	No prior met ET	No prior met ET No adj AI < 12 mo	No prior adv ET No adj AI < 12 mo
Population	N = 666	N = 668	N = 493
ORR (%)	55.3 vs 44.4	42.5 vs 28.7	49.7 vs 37.0
CBR	84.3 vs 71.0	79.6 vs 72.8	78 vs 71.5
Median PFS (mo.)	27.6 vs 14.5: HR, 0.56	25.3 vs 16.0; HR, 0.57	28.2 vs 14.8; HR, 0.53

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Finn RS, et al. N Engl J Med. 2016;375:1925-1936
Hortobagyi GN. Ann Oncol. 2018 Jul 1;29(7):1541-1547
Hortobagyi GN. N Engl J Med. 2016 Nov 3;375(18):1738-1748
Johnston S, et al. npj Breast Cancer 2019; 5:5

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CDK4/6 Inhibitors in Relapsed/Refractory HR+/HER2- MBC

Trial	Regimen	Phase	# patients	ORR*	PFS (months)	HR	95% CI
PALOMA-3[1,2]	Fulvestrant +/- palbociclib	III	521	11% vs 25%	4.6 vs 11.2[2]	0.50	0.36 to 0.59
MONARCH-2	Fulvestrant +/- abemaciclib	III	669	21% vs 48%	9.3 vs 16.4	0.55	0.45 to 0.68
MONALEESA-3	Fulvestrant +/- ribociclib	III	725	29% vs 41%	12.8 vs 20.5	0.59	0.48 to 0.73
MONARCH-1**	Abemaciclib monotherapy	II	132	20%	6.0	--	--

*in subset of pts with measurable dz at baseline

**progression on or after prior endo tx; 1-2 lines of chemo for MBC

Both PALOMA-3 and MONARCH-2: ~60% visceral disease; ~20% pre/perimenopausal (received LHRHa)

MONARCH-2: No prior met chemo; PALOMA-3: approx. 1/3 with 1 line prior met chemo

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Turner NC, et al. N Engl J Med. 2015;373:209-219
Cristofanilli M, et al. Lancet Oncol. 2016;17:425-439; Sledge GW Jr, et al. J Clin Oncol. 2017;35:2875-2884; Dickler MN et al. Clin Cancer Res. 2017;23:5218-5224; Slamon DJ. J Clin Oncol. 2018 Aug 20;36(24):2465-2472

CDK4/6 Inhibitor Safety Profiles

Palbociclib[1]	RIbociclib[2]	Abemaciclib[3]
PALOMA-2	MONALEESA-2	MONARCH-3
Neutropenia • Any grade (79.5%) • Grade 3/4 (57.5%)	**Neutropenia** • Any grade (74.3%) • Grade 3/4 (60.3%)	**Diarrhea** • Any grades (81.3%) • Grade 3 (9.5%)
	Liver abnormalities • Any grade, 30.6%) • Grade 3/4 (15%)	**Neutropenia** • Any grade (41.3%) • Grade 3/4 (21.1%)
	Prolonged QTcF interval (2.7%)	

Finn RS, et al. N Engl J Med. 2016;375:1925-1936
Hortobagyi GN. N Engl J Med. 2016 Nov 3;375(18):1738-1748
Goetz MP. J Clin Oncol. 2017 Nov 10;35(32):3638-3646.

Monitoring Requirements for CDK4/6 Inhibitors*

	Palbociclib[1]	Ribociclib[2]	Abemaciclib[3]
Complete blood count (CBC)	• Prior to starting therapy • At the beginning of each cycle • On Day 15 of the first two cycles • As clinically indicated	• Prior to starting therapy • Every 2 weeks for the first 2 cycles • At the beginning of each of the 4 subsequent cycles • As clinically indicated	• Prior to starting therapy • Every 2 weeks for the first 2 months • Monthly for the next 2 months • As clinically indicated
Liver function test (LFT)	N/A	• Prior to starting therapy • Every 2 weeks for the first 2 cycles • At the beginning of each of the 4 subsequent cycles • As clinically indicated	• Prior to starting therapy • Every 2 weeks for the first 2 months • Monthly for the next 2 months • As clinically indicated
Electrocardiography (ECG)	N/A	• Prior to starting therapy • During Cycle 1 at approximately Day 14 • At the beginning of Cycle 2 • At regular intervals thereafter during steady-state treatment (at approximately Day 14 of the cycle) • As clinically indicated	N/A
Serum electrolytes	N/A	• Prior to starting therapy • At regular intervals during steady-state treatment in later cycles • As clinically indicated	N/A

*** Individual practice may vary and additional tests beyond the Product Monograph requirements may be done.**

Ibrance™ Product Monograph. Pfizer Canada Inc. June 5, 2018

Kisquali™ Product Monograph. Novartis Pharmaceuticals Canada Inc. March 19, 2018

Verzenio™ Product Monograph. Eli Lilly and Company.

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Polling Question

- Which trials evaluated CDK4/6 inhibitors in early-stage HR+/HER2- breast cancer (select all that apply)?

  A. PALLAS

  B. PENELOPE-A

  C. monarchER

  D. monarchE

CDK4/6 INHIBITOR STUDIES IN EARLY-STAGE HR+/HER2- BREAST CANCER

Study	Description	Population*	IDFS (%)	median f/u (mos)
PALLAS	PALBO x 2 years + ET	Stage II/III	3y: 88.2 vs 88.5	23.7
PENELOPE-B	PALBO x 13 cycles + ET	All received preop chemo CPS-EG score ≥ 3 or CPS-EG score 2 with ypN+	4y: 73 vs 72.4	42.8
monarchE	ABEMA x 2 years + ET	1-3LN + high-risk (T≥ 5cm, Gr 3, or Ki67 ≥ 20%) or ≥4 LN	3y: 88.8 vs 83.4[#]	27.0
NATALEE[‡]	RIBO x 3 years + ET	Stage II (N1 or T2-T3N0 + Gr 2-3, or Ki67 ≥ 20%) or Stage III	Not reported	Not reported

*All studies included pre & post menopausal; [#]Statistically significant; [‡]amended to include more high-risk patients after PALLAS & monarchE

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monarchE Updates

monarchE
Adjuvant Abemaciclib + ET in High-Risk, Node+, HR+/HER2- EBC
ESMO 2021 Update:
27 mos follow-up

monarchE Study Design

- International, randomized, open-label phase III trial

Women or men with high-risk, node-positive HR+/HER2- EBC; prior (neo)adjuvant CT permitted; pre- or postmenopausal; no distant metastasis; ≤16 mo from surgery to randomization; ≤12 wk of ET after last non-ET
(ITT: N = 5637;
NAC subgroup: n = 2056)

ITT Population (Cohorts 1 + 2)

Cohort 1
≥4 positive ALN *or* 1-3 positive ALN plus histologic grade 3 and/or tumor ≥5 cm

Cohort 2
1-3 positive ALN, Ki67 ≥20% per central testing, not grade 3, tumor size <5 cm

Stratified by prior CT (NAC vs adjuvant CT vs none), menopausal status, region

Abemaciclib 150 mg BID up to 2 yr + **ET** per standard of care of physician's choice for 5-10 yr as clinically indicated
(ITT: n = 2808; NAC subgroup: n = 1025)

ET per standard of care of physician's choice for 5-10 yr as clinically indicated
(ITT: n = 2829; NAC subgroup: n = 1031)

- Primary endpoint: iDFS (primary outcome analysis occurred after 395 iDFS events in ITT population)
- Key secondary endpoints: distant RFS, iDFS in Ki67-high (≥20%) population, OS, safety, PROs, PK

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Slide credit: clinicaloptions.com

Martin. ASCO 2021. Abstr 517. Johnston. JCO. 2020;38:3987. Rastogi. SABCS 2020. Abstr GS1-01.

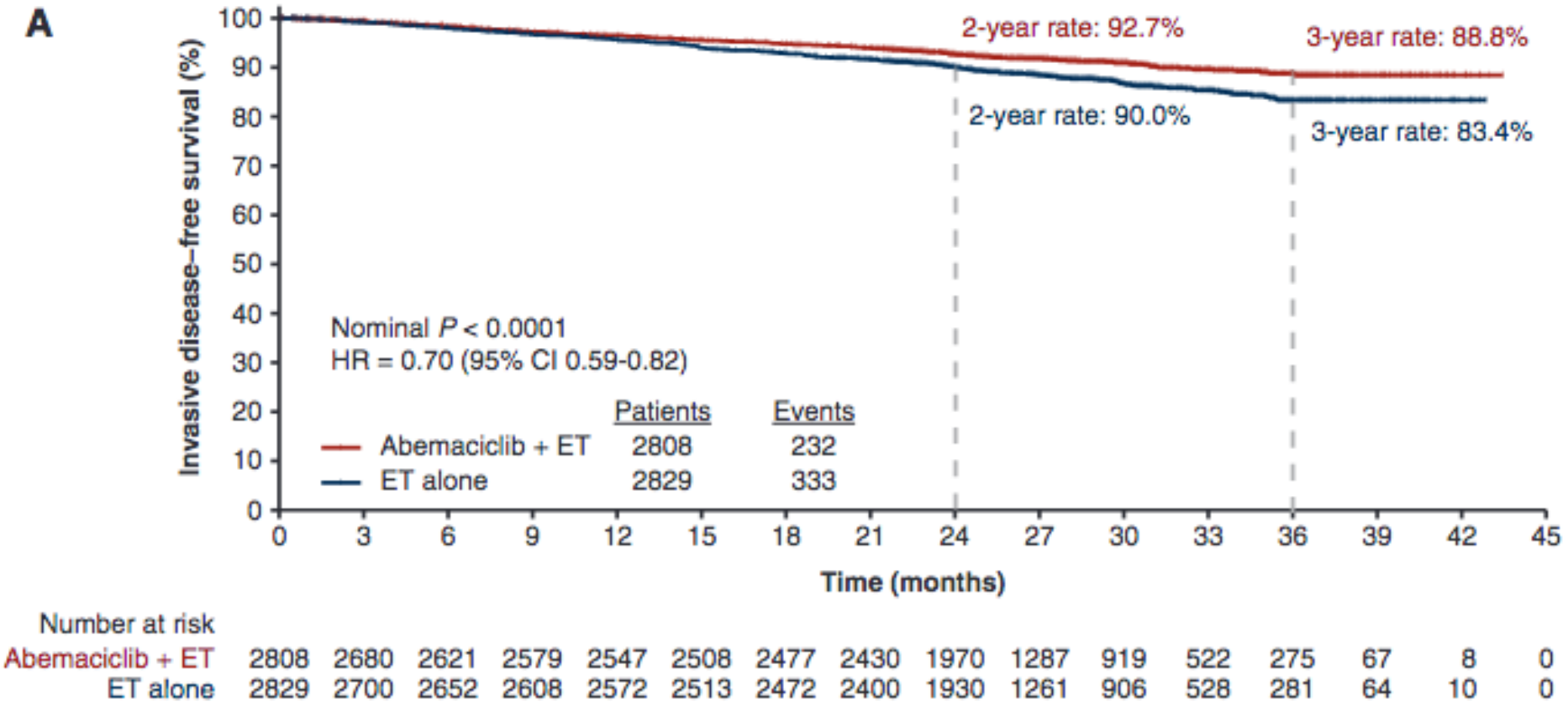
monarchE: Baseline Characteristics

Characteristic	Abemaciclib + ET (n = 2808)	ET Alone (n = 2829)
Median age, yrs (range) ▪ < 65 ▪ ≥ 65	51 (23-89) 84.4 15.6	51 (22-86) 85.4 14.6
North America and Europe/Asia/other, %	52.4/20.4/27.2	52.3/20.6/27.1
Pre/postmenopausal, %	43.5/56.5	43.5/56.5
Prior CT, % ▪ Neoadjuvant ▪ Adjuvant ▪ None	 37.0 58.5 4.5	 37.0 58.2 4.7
Prior neoadjuvant/ adjuvant RT, %	2.5/93.3	2.9/92.9
Positive axillary LN, % ▪ 0 ▪ 1-3 ▪ ≥ 4	 0.2 39.9 59.8	 0.2 40.4 59.3
ER/PgR positive, %	99.1/86.2	99.2/86.7

Characteristic, %	Abemaciclib + ET (n = 2808)	ET Alone (n = 2829)
Pathologic tumor size ▪ < 2 cm ▪ 2-5 cm ▪ ≥ 5 cm	 27.8 48.8 21.7	 27.0 50.2 21.6
Histologic grade at diagnosis ▪ 1 ▪ 2 ▪ 3 ▪ Not assessed	 7.4 48.9 38.8 4.5	 7.6 49.3 37.7 4.9
Ki-67 index < 20/≥ 20	33.9/44.9	34.4/43.6
TNM stage (derived) ▪ IA ▪ IIA ▪ IIB ▪ IIIA ▪ IIIB ▪ IIIC	 0.1 11.5 13.9 36.6 3.7 33.8	 0 12.5 13.7 36.2 3.2 34.0

Johnston JCO 2020

monarchE: IDFS



O'Shaughnessy Annals of Oncology 2021

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monarchE: Ki-67 and Prognosis

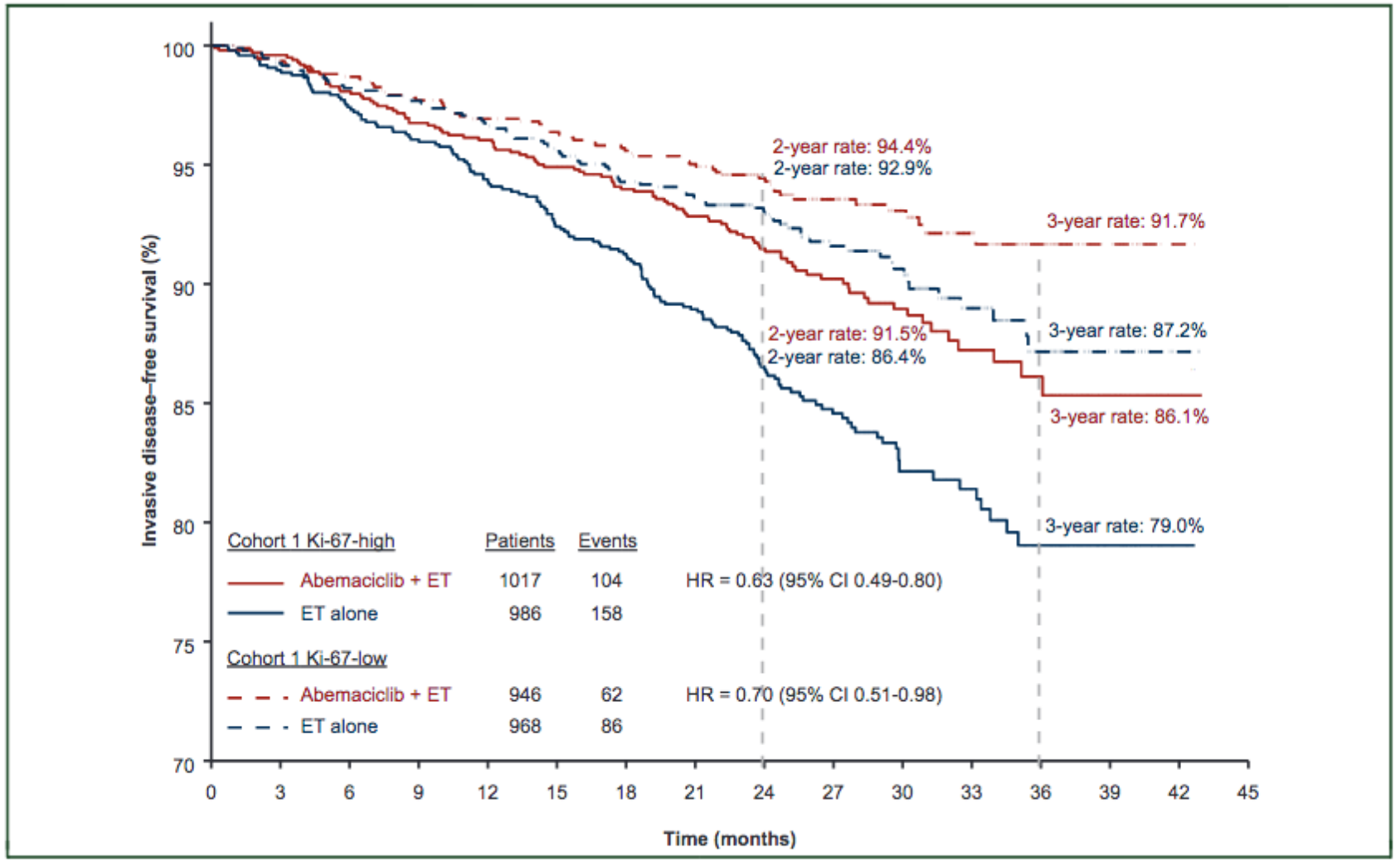

Figure 3. Kaplan-Meier curves of invasive disease-free survival in Cohort 1 Ki-67 high versus Ki-67 low at additional follow-up 1 (AFU1).
CI, confidence interval; ET, endocrine therapy; HR, hazard ratio.

O'Shaughnessy Annals of Oncology 2021

FDA Approval – Abemaciclib in Early-Stage HR+ HER2- Node-positive BC

- Approval is for patients with **high-risk clinical and pathological factors** and a **Ki-67 score ≥20%.**
  - ≥4 positive axillary lymph nodes (ALN) and Ki-67 score of ≥20% OR
  - 3 positive ALN with either Grade 3 disease and/or tumor size ≥5 cm and Ki-67 score of ≥20%.

How Do We Explain the Different Outcomes?

Study	Risk	Adherence	Drug and/or Schedule	Duration of Follow-up
PALLAS	Lower risk relative to monarchE	42% drop-out rate; 32% completed 2y	21d on, 7d off for 2 years	Median 2 years
PENELOPE-B	Different definition	80% completed 13 cycles	21d on, 7d off for 13 cycles	Median 4 years
monarchE	28% greater rate of patients with ≥4 LN relative to PALLAS	16.6% drop-out rate	Continuous dosing for 2 years More potent CDK4 inhibition	Median 27 months

Mayer ESMO 2020; Loibl SABCS 2020; Johnston ESMO 2020

Ki-67: Integration of a new prognostic marker in Early Stage HR+ HER- breast cancer

- monarchE was the first phase III registration trial to analyze the utility of centrally confirmed Ki-67
- Not predictive of abemaciclib treatment benefit, but prognostic of recurrence
- Supports the use of Ki-67 along with clinical and pathologic features of high-risk disease to identify those who may benefit from adjuvant abemaciclib x 2 years

Optimal Utilization of Ki67 Testing in HR-Positive/ HER2-Negative Early Breast Cancer

Ki67 Testing Methods, Standardization & Interpretation

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Emory University School of Medicine

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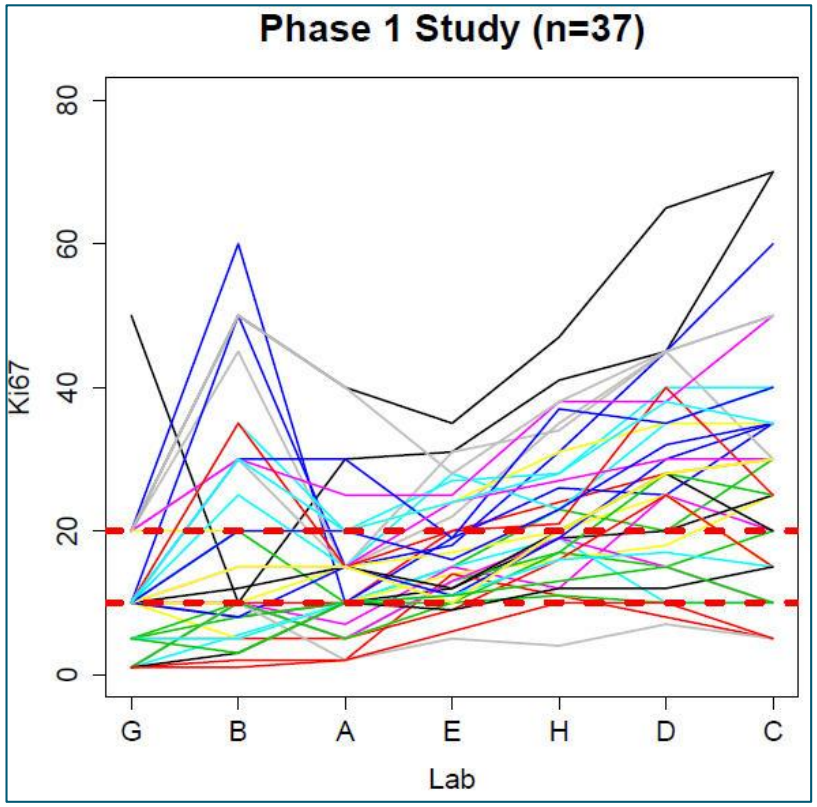
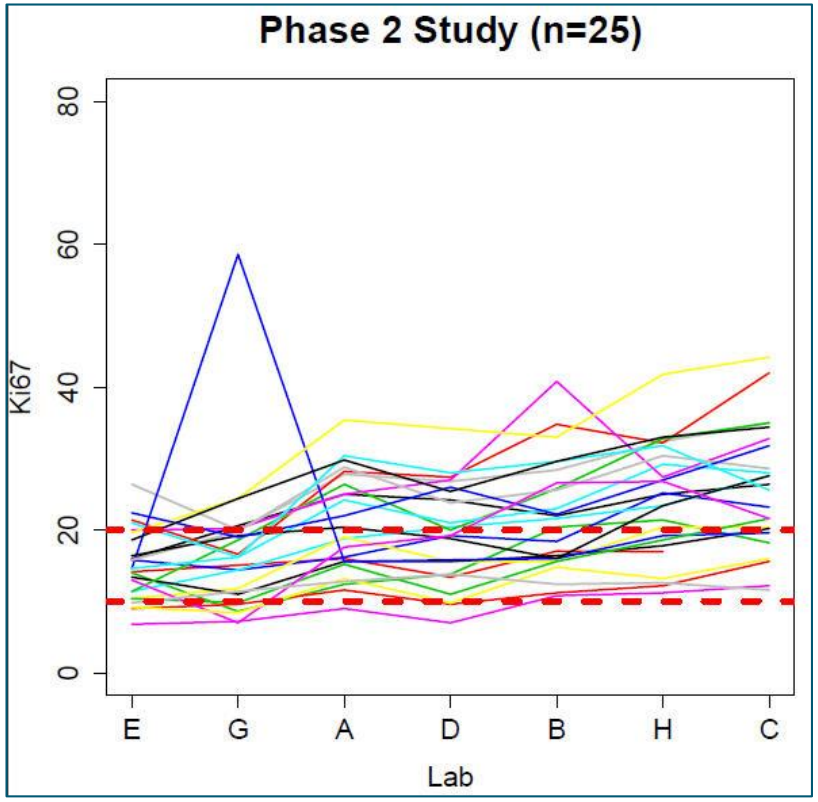
Current methodologies for Ki67 quantification

- International Ki67 Working Group recommendations
- Preanalytic considerations in tissue handling/processing for hormone receptor and HER2 testing
- Challenges in adopting standardized methodologies
- Best practices for quality assurance and quality control in Ki67 testing
- Optimal reporting

International Ki67 Working Group Spaghetti plots: Ki67 of 10-20% (7 labs common to both phases)



37 cases scored by ≥ 1 lab as 10-20%.
0 of the 37 scored by all labs as 10-20%.



25 cases scored by ≥ 1 lab as 10-20%.

0 of the 25 scored by all 7 labs as 10-20%.

1 case, scored by 5 of the 7 labs, was scored by all 5 labs as 10-20%.

Nielsen TO et al SABCS 2013

Current methodologies for Ki67 quantification

International Ki67 Working Group recommendations

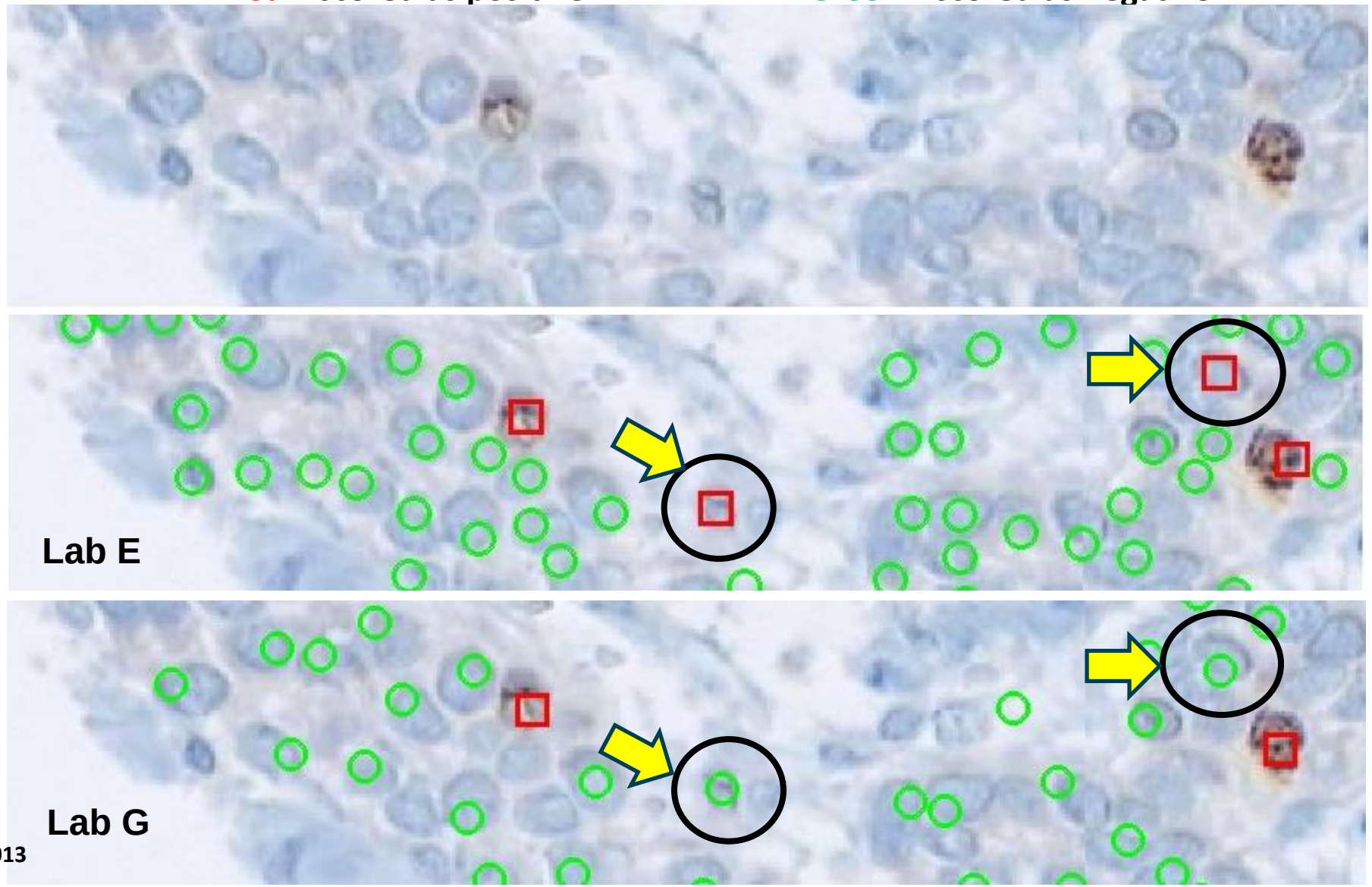
- Reagents – minor impact on variability
- Pathologists are the major cause of variability
  - What is a positive?
    - Any brown is positive (note this different from CDx definition)
  - Method of analysis
    - Global analysis is more consistent
  - Meticulous analysis is required

Current methodologies for Ki67 quantification

- Challenges in adopting standardized methodologies
  - Different reagents and kits
  - Differences in definition of positivity
    - Groups
    - Labs
  - Differences in analysis methods
    - Hotspots versus Global
  - Differences in cutoffs

Ki67- “what is brown”

Red = scored as positive **Green = scored as negative**



Nielsen TO et al SABCS 2013

IKWG

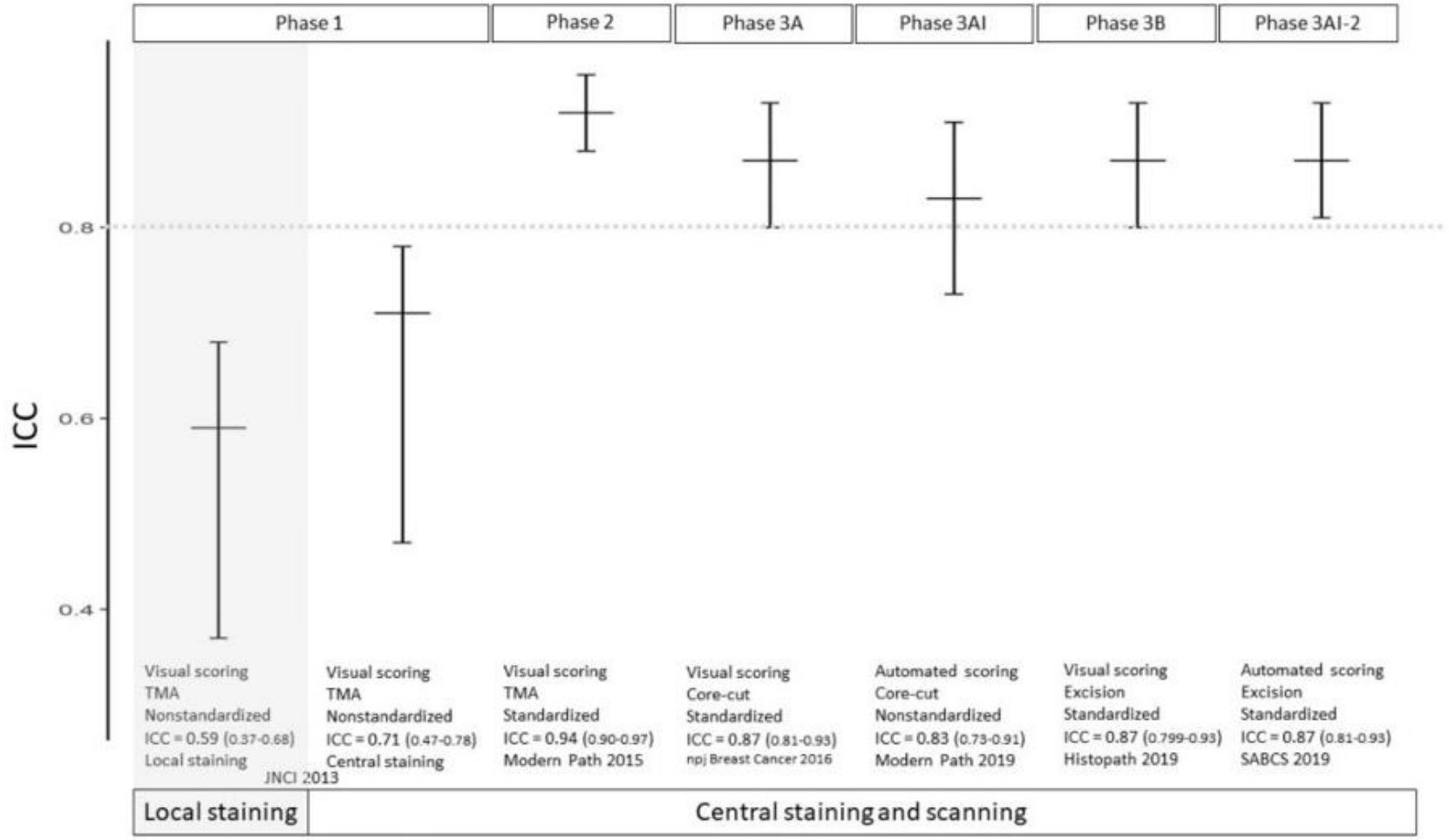


Figure 1. The series of International Ki67 Working Group (IKWG) studies to standardize methods for visual scoring of Ki67 index in breast cancer. Intraclass correlation coefficient (ICC) through the 3 study phases (1, 2, 3 A visual and automated [3AI], 3B visual and automated [3AI-2]) are shown with error bars representing the lower and upper 95% credible intervals. The numeric values of the various ICCs are shown at the x-axis labels with the 95% credible intervals in parentheses. The horizontal bar lines represent observed ICCs. The extent of the vertical lines indicates 95% credible interval. The dotted grey color line indicates ICC = 0.8. TMA = tissue microarray.

Nielsen et al JNCI 2021

Pre-analytical

Nielsen et al JNCI 2021

Table 1. Factors that may affect Ki67 IHC[a]

Setting	Factor	Variables	Comments
Preanalytical	Type of specimen	Core vs excision	Both are suitable, but core biopsies are preferred. Use case must be specimen type specific, eg, cutpoint for core cut may differ from excision; changes in Ki67 at multiple time points must be based on measurement on the same specimen type.
	Fixation	Prefixation delays (warm and cold ischemia time); tissue thickness; fixative type; time spent in fixative)	Affects morphologic nuclear integrity and intensity of nuclear IHC stain. Inadequate fixation decreases Ki67 (20). Ethanol-fixed or decalcified preparations should not be used. ASCO/CAP guidelines for breast tissue handling for ER/HER apply (19).
	Means of storage	Tissue in paraffin block vs unstained slides	Prolonged storage of formalin-fixed paraffin-embedded tissue block at room temperature has little effect on Ki67 (21). Avoid prolonged exposure to air of cut sections on glass slides.
Analytical	Antigen retrieval	Yes vs no	Required. High-temperature antigen retrieval mandatory.
	Specific antibody	MIB1 vs other antibodies against Ki67 antigen	MIB1 is the most widely validated antibody; 30-9, K2, MM1, and SP6 are also commonly used. Particular automated immunostainers have recommended antibodies (eg, MIB1 for Dako, 30-9 for Ventana, K2 for Leica). Some evidence indicates poor performance of MM1 (41), although this might be confined to its use on non-Leica platforms (42).
	Colorimetric detection system	Avidin-biotin immunoperoxidase vs polymer detection vs amplified systems	Avidin-biotin systems have substantially lower sensitivity and have largely been replaced by polymer detection (43) on automated platforms. Amplified systems such as OptiView+Amp (Ventana) produce powerful, open-ended amplification that is difficult to standardize (UK NEQAS internal observations).
	Counterstain	Completeness and intensity of stain	Important that all negative nuclei are counterstained (otherwise apparent Ki67 index can be falsely high).
	Quality assurance/ quality control	—	Should be established in each laboratory and systematically maintained. Quantitative external quality assessment should be established and participation should be mandatory.
Interpretation and scoring	Method of scoring	Cellular component, staining intensity	1) Count all positive invasive carcinoma cells within region in which all nuclei have been stained. 2) Scoring requires determination of percentage cells positive among total number of invasive cancer cells. 3) No interpretation of intensity.
	Area of slide read	Average value across slide vs value in hot spot	Controversial: global (average) scores across the section had higher reproducibility than hot spot methods in IKWG studies, although differences were not statistically significant.
	Digital imaging	Visual vs automated analysis	IKWG-standardized visual counting (Box 1) under light microscopy or from a digital image is validated. Automated scoring is still investigational, but evidence to date suggests that automated score is not worse than standardized visual scoring for core-cuts.
	Data format and cutpoints	Categorical or continuous	Capture Ki67 data as a continuous percentage variable rather than in bins relative to specific cutpoint(s). Log transformation is required for parametric statistical testing.

aASCO/CAP = American Society of Clinical Oncology and the College of American Pathologists; ER = estrogen receptor; IHC = immunohistochemistry; NEQAS = National External Quality Assessment Scheme.

IKWG scoring method

Box 1: IKWG Scoring Method for Ki67 in Breast Cancer

1) Before first use, access the IKWG website (https://www.ki67inbreastcancerwg.org/) and complete the Ki67 calibration exercise
2) From Tools, link to the Online scoring app (or download and install the Ki67 counting app) and use the global method
3) Using a regular light microscope, review the Ki67-stained breast cancer slide and input estimates of the percent area with negligible, low, medium, or high Ki67 index
4) Score 100 nuclei negative or positive in each field type (as directed by the app)
5) Record "Weighted global score" output as the Ki67 index for that slide

Neilsen et al JNCI 2021

Cutoffs for positivity

- Global counting vs “hot spot” counting

- Scoring thresholds
  - 10%
  - 13.25%
  - 20%

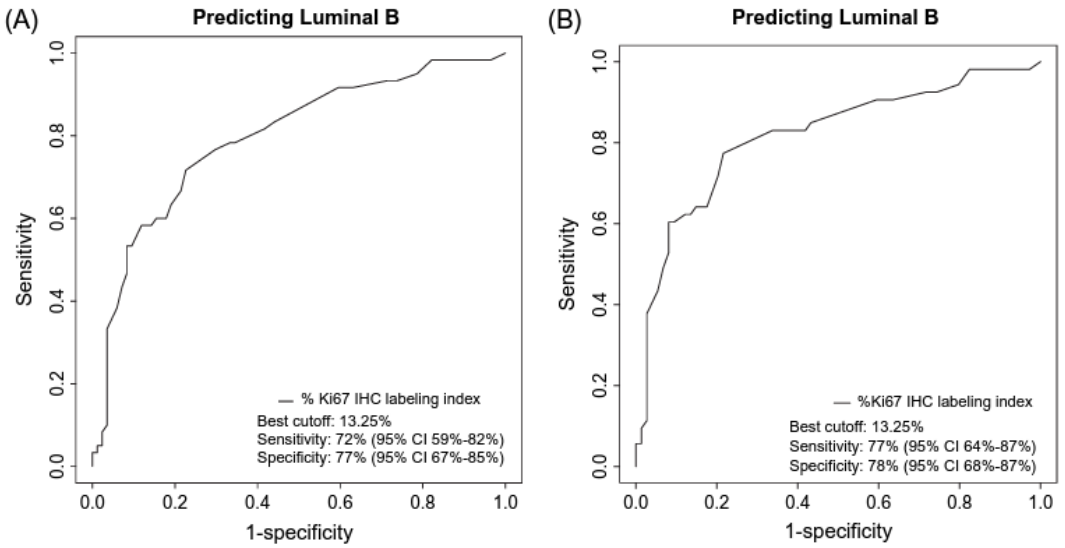


Figure 2. The x and y axes of ROC curve are true positive rate and false positive rate respectively. True positive rate equals to sensitivity and false positive rate is 1-specificity. Establishment of Ki67 cut point. True positive rate equals to sensitivity and false positive rate is 1-specificity. **A**) ROC analysis of 144 luminal A and B tumors with Ki67 IHC data to identify luminal B tumors as defined by a 50-gene classifier. Gene expression data for the classifier were obtained by quantitative reverse transcription–polymerase chain reaction. The selected best cut point for the Ki67 index was 13.25%. **B**) ROC analysis that was confined to 127 luminal A and B tumors with Spearman rank correlation coefficients of more than 0.1. CI = confidence interval; ROC = receiver operating characteristic; IHS = immunohistochemistry.

Cheang et al JNCI 2009

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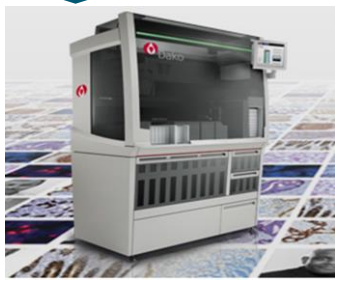
Pharm Dx assay

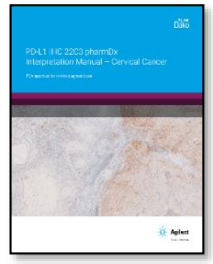
Dako Omnis



Agilent/DAKO Antibody

DAKO EnVision FLEX + DAB Enhancer

Scoring System



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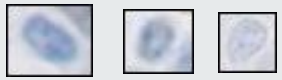
Ki-67 Score in breast carcinoma

Determined by estimating the percentage of viable invasive tumor cells with nuclear staining intensities 1+ and higher

Staining Intensity Scale and Assessment Parameters

Score		Intensity	Qualitative Description
3+		Strong Staining	Dark Chocolate Brown
2+		Moderate Staining	Dark Golden Brown *can see through*
1+		Weak Staining	Light Brown
0		No Staining	Blue or Gray

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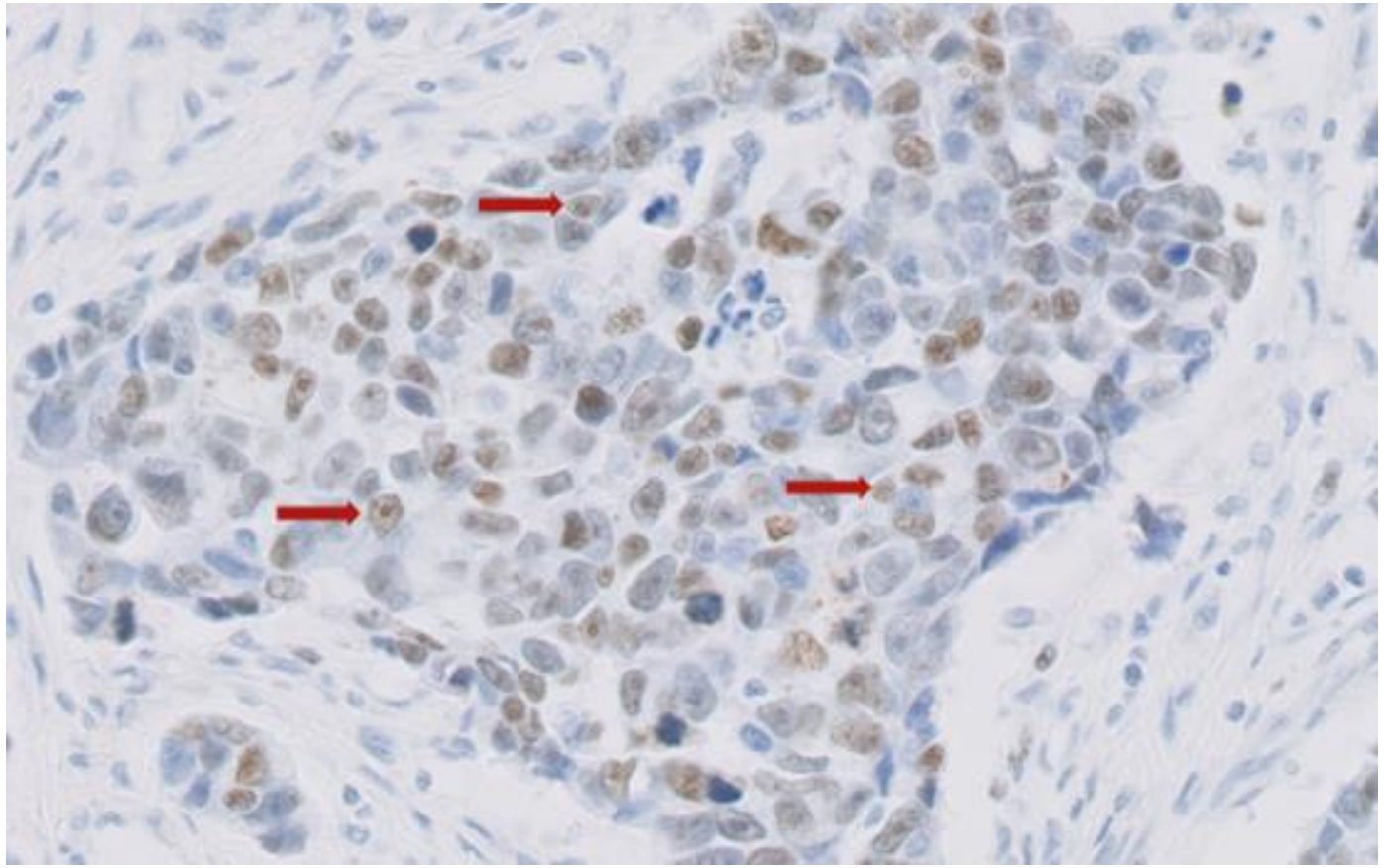
Tumor Cells

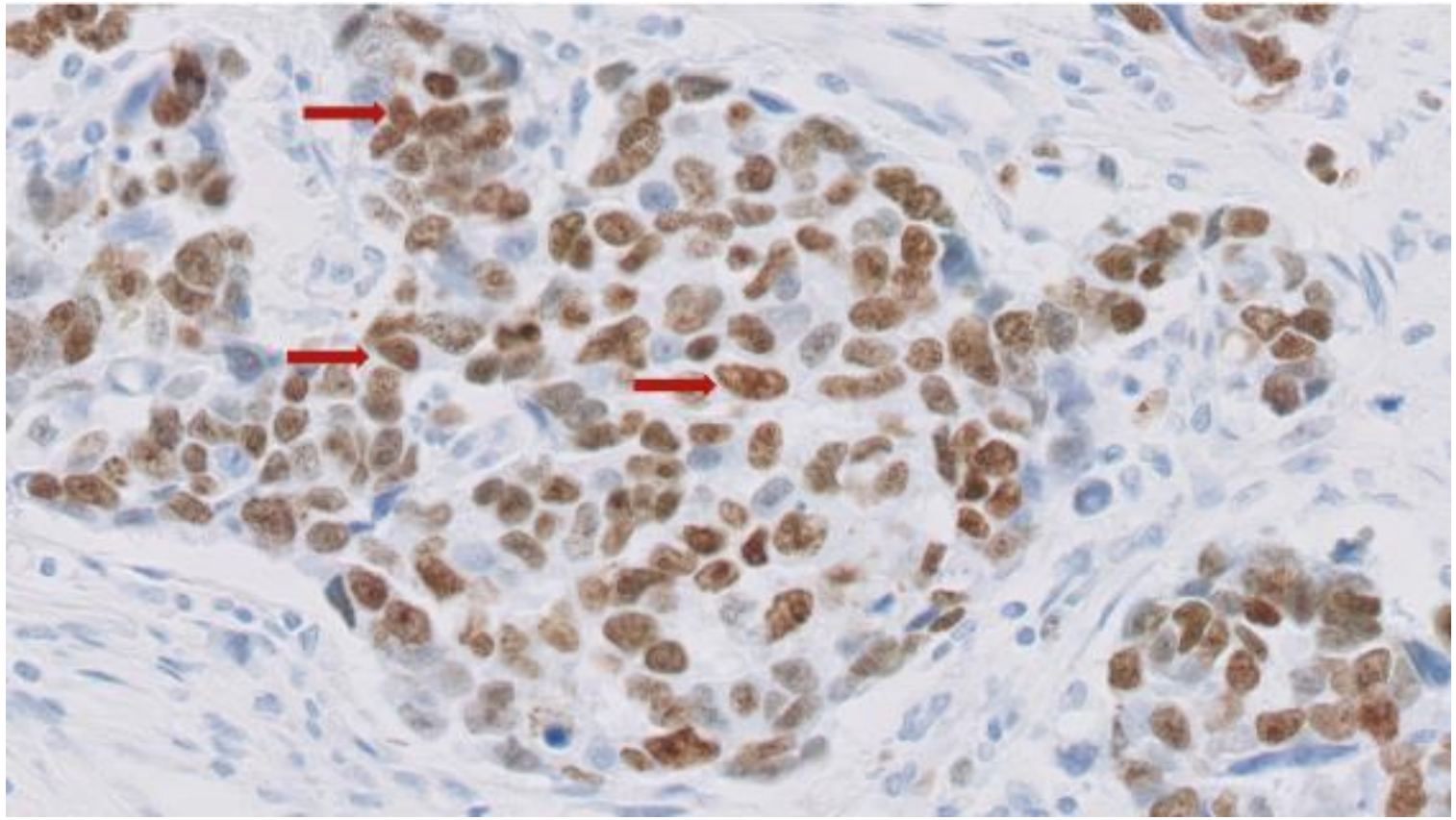
- **Nuclear Staining**
- Tumor cells exhibiting convincing nuclear staining at all intensities 1+ to 3+ should be considered Ki-67 positive.
- Convincing nuclear staining is determined by the following parameters:

1. The signal must be unequivocally brown
2. The staining must correspond to a nucleus
3. The staining must cover **the whole chromatin distribution** within the nucleus
4. The staining must correspond to non – apoptotic cells

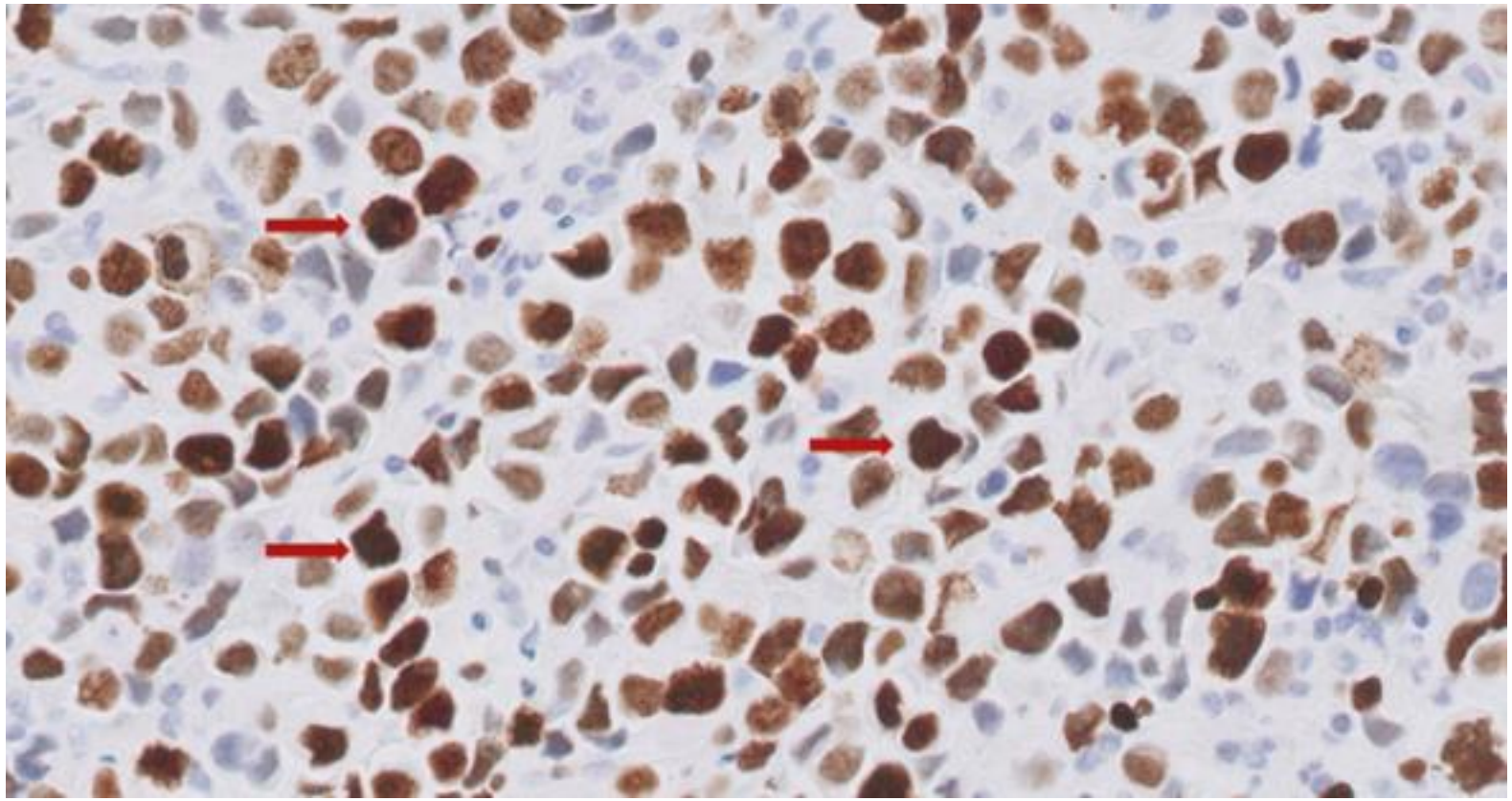
Nuclear Staining: 1+ Intensity



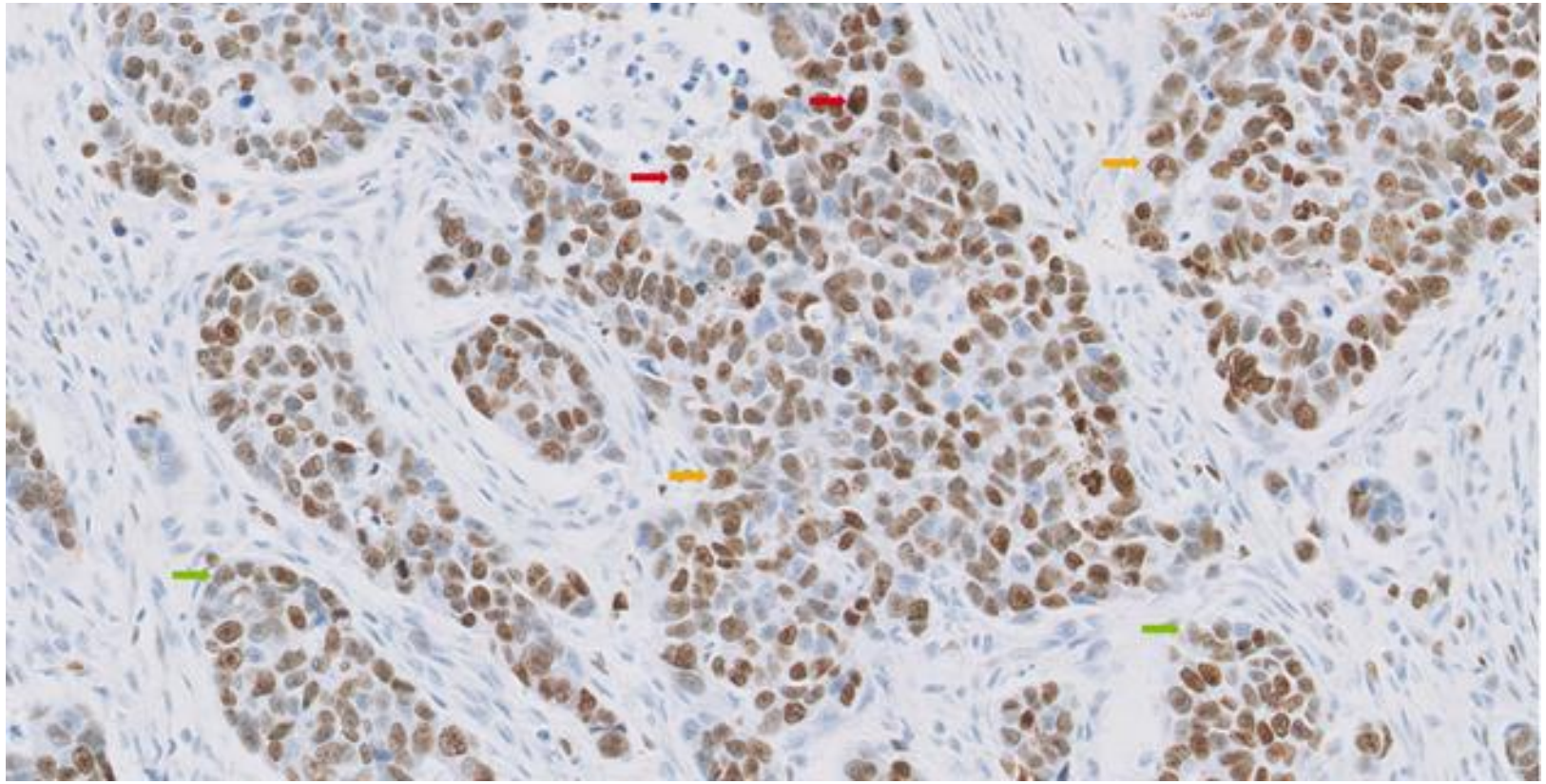
Nuclear Staining: 2+ Intensity



Nuclear Staining: 3+ Intensity



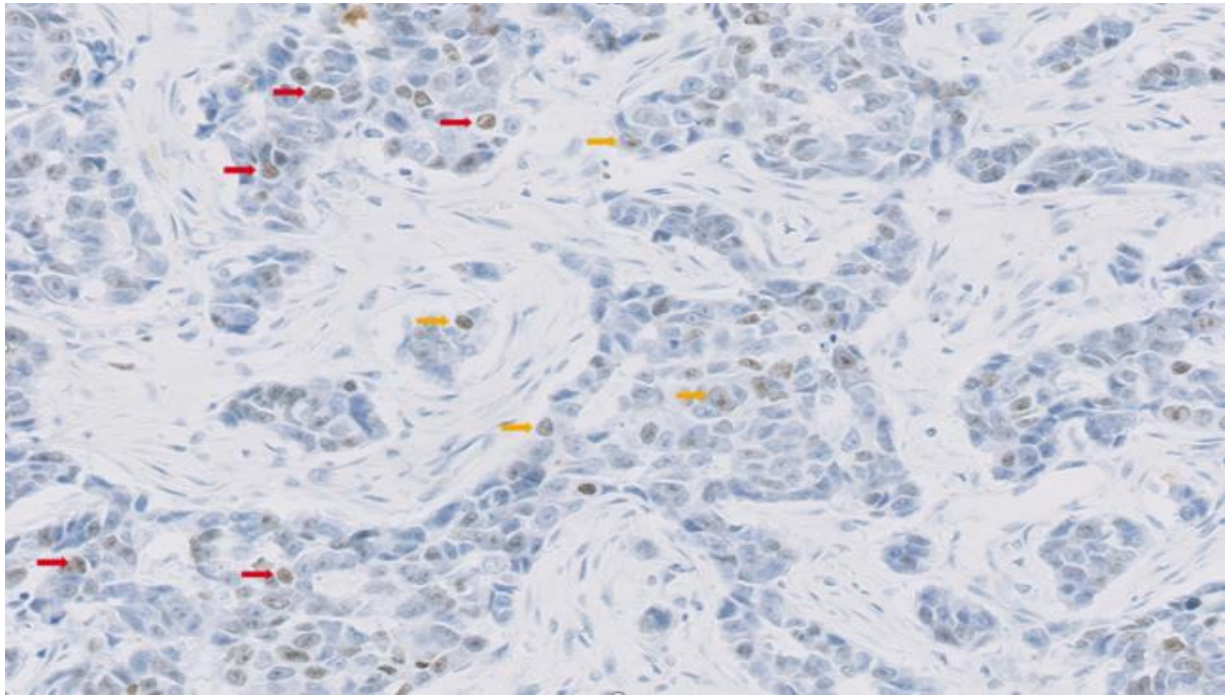
Convincing staining of tumor cells is often heterogeneous, with various staining intensities present



Red Arrows indicate 3+ staining intensities, yellow indicate 2+ staining intensities, and green indicate 1+ staining intensities. (20× magnification).

PharmDx - Negative vs. Weakly Positive Cells

Cells that exhibit a “grey” color in the nucleus are excluded. If the nucleus is not unequivocally brown, then the cell is considered to negative.



Negative cells show grey hematoxylin counterstaining and are indicated with yellow arrows, and weak 1+ staining indicated with red arrows. (arrows) (20× magnification).

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Steps to Determine Ki-67 Score

1. **Confirm diagnosis of invasive breast carcinoma.**

2. **A minimum of 200 viable invasive tumor cells must be present to be considered adequate for evaluation.**
   - For specimens with less than 200 viable tumor cells, use sections from a deeper level or another block.

3. **At lower magnification**
   - Examine all well-preserved tumor areas
   - Evaluate overall areas of Ki-67 staining and non-staining tumor cells
   - Keep in mind that 1+ nuclear staining may be difficult to see at low magnifications.

4. **At higher magnification**
   - Estimate the total number of viable invasive tumor cells, both Ki-67 staining and non-staining (Ki-67 Score denominator)
   - Estimate the number of Ki-67 staining viable invasive tumor cells (Ki-67 Score numerator)
   - Determine Ki-67 Score

Ki-67 Inclusion and Exclusion for PharmDx

- Any convincing nuclear staining (≥ 1+) of viable invasive tumor cells that is perceived
  - **included** in the Ki-67 Score
- Any nuclear staining of lymphocytes and stromal cells (mononuclear inflammatory cells, MICs) within tumor nests and/or adjacent supporting stroma is not considered Ki-67 staining
  - **excluded** from the Ki-67 Score
- Staining of in-situ breast carcinoma and tumor cell membrane/cytoplasmic staining
  - **excluded** from the Ki-67 Score
- Staining of non-neoplastic breast epithelium and necrosis/apoptosis
  - **excluded** from the Ki-67 Score
- Edge effect, processing artifacts and non-specific background
  - **excluded** from the Ki-67 Score

Optimal Utilization of Ki67 Testing in HR-Positive/ HER2-Negative Early Breast Cancer

Application with a Case

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Emory University School of Medicine

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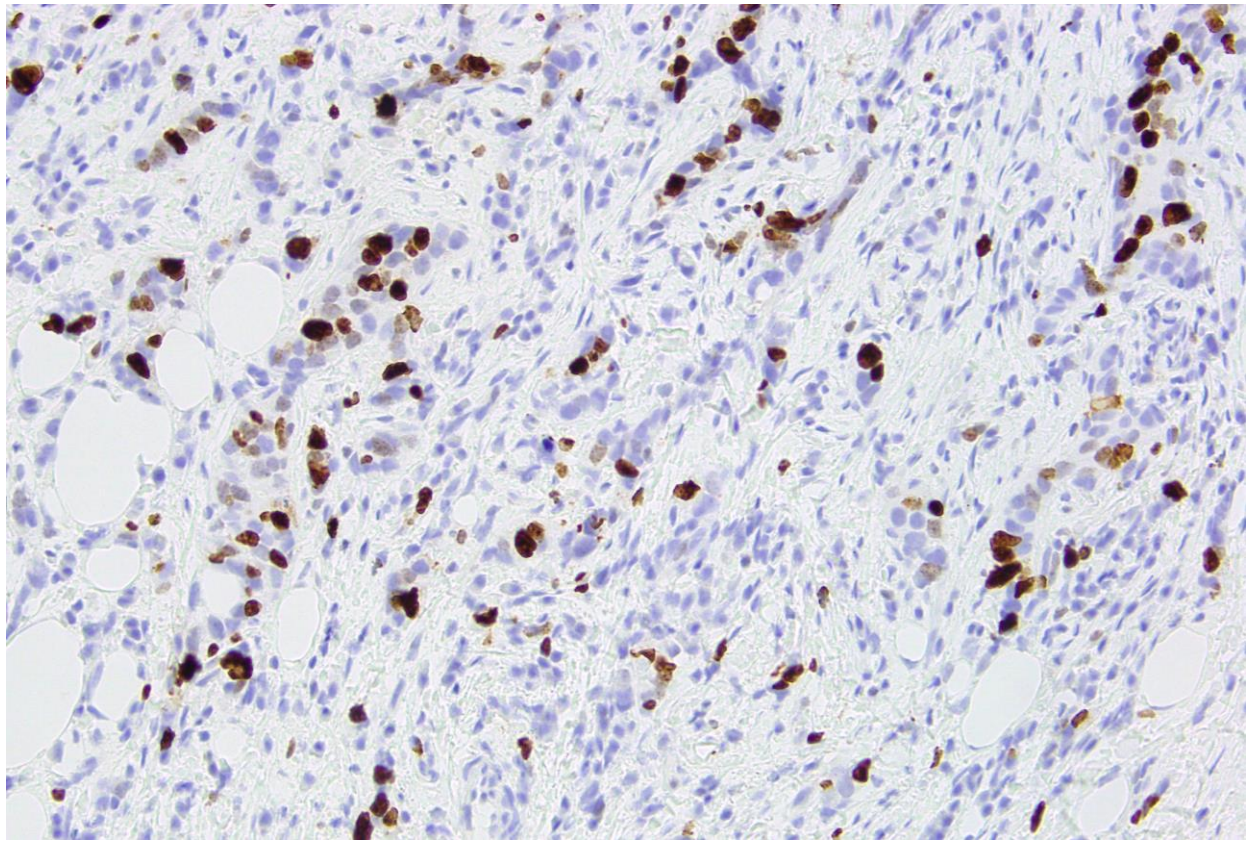
Clinical Case

- 62 year old post-menopausal female found to have a left breast abnormality on screening MMG
- Left breast diagnostic MMG showed pleomorphic calcifications in the upper outer quadrant of the left breast with ultrasound showing an irregular hypoechoic solid mass measuring 35mm corresponding to abnormality seen on the screening MMG
- Left axillary ultrasound showed one abnormal appearing lymph node with cortical thickening

Clinical Case Continued

- Left breast core needle biopsy confirmed invasive ductal carcinoma, grade 3, ER 85%, PR 40%, HER2 1+, Ki-67 30%
- Left axillary lymph node biopsy confirmed metastatic mammary carcinoma

Clinical Case Continued – Ki67



Ki67 score 30 (picture taken at 20x magnification)

Clinical Case Continued

- Patient underwent left breast segmental mastectomy with SLNB that showed IDC, nottingham histologic grade 3, 43mm in greatest dimension, lymphovascular invasion focally present
  - DCIS, intermediate nuclear grade, 8mm
  - Margins negative
- Left axilla sentinel lymph nodes showed one of three lymph nodes positive for metastatic carcinoma
  - Metastatic deposit measuring 11mm in greatest dimension
  - Negative for extranodal extension

Clinical Case Continued

- Oncotype Dx score was sent and returned at 23
- Met with medical oncology to discuss systemic therapy
- ???

Polling Question

- What adjuvant systemic therapy would you recommend?

  A. Chemotherapy with TC x 4 cycles + Endocrine therapy x 5 years

  B. Endocrine therapy x 5 years + Abemaciclib x 2 years

  C. Chemotherapy with TC x 4 cycles, Endocrine therapy x 5 years + Abemaciclib x 2 years

Clinical Case Continued

- No adjuvant chemotherapy recommended based on RxPONDER
- Discussed adjuvant endocrine therapy with an aromatase inhibitor + abemaclicib 150mg BID x 2 years

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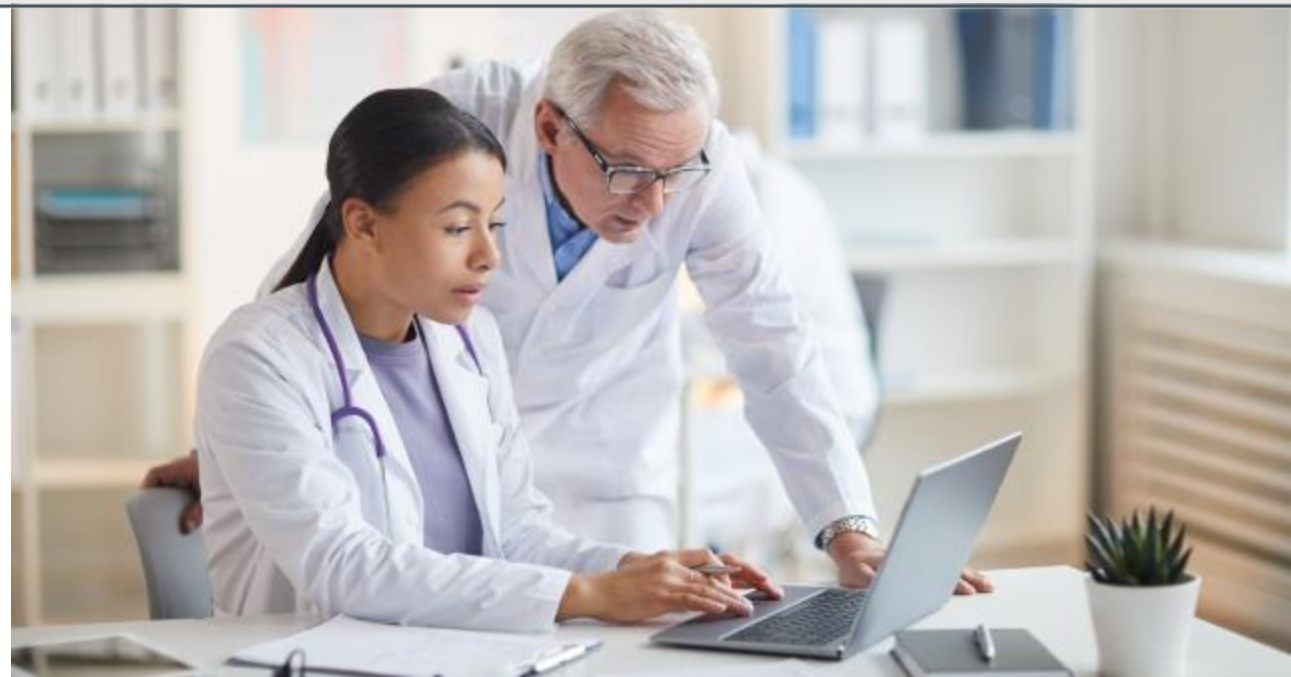
Optimal Utilization of Ki67 Testing in HR-Positive/HER2-Negative Early Breast Cancer: Education and Resources for the Oncology and Pathology Healthcare Teams

Access additional resources on breast cancer

https://www.ascp.org/content/learning/breast-cancer



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