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

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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.

Provided by the American Society for Clinical Pathology in partnership with Clinical Care Options, LLC.

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

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

Clinical Overview - CDK4/6 Inhibitors for Treatment

Manali Bhave, MD
Assistant Professor, Department of Hematology and Medical Oncology
Emory University School of Medicine
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
Immunohistochemistry developed for ER and PR analysis

1999
ER downregulator approved
AI approved as adjuvant therapy

2002
mTOR inhibitor + AI approved

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

2012
CDK4/6 inhibitor + AI approved

2015
PI3K Inhibitor approved

2019
PI3K Inhibitor approved

PI3K inhibit approved
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 cancer¹
- Short term inhibition of CDK4 & CDK6 leads to G1 arrest that rebounds upon withdrawal²
- Continuous inhibition leads to prolonged cell cycle arrest with initiation of apoptosis or senescence³
- This led to the hypothesis that continuous target inhibition could be an effective strategy

¹ Altucci L et al. 1996 Oncogene 12:2315-24
² Gelbert et al. 2014 Invest New Drugs 32: 825-37
³ Beckman et al. AACR Annual Meeting 2016
Approved CDK4/6 Inhibitors in Clinical Use

- **Palbociclib**
  - PD 0332991
  - FDA Approved (February 4, 2015)

- **Ribociclib**
  - LEE011
  - FDA Approved (March 13, 2017)

- **Abemaciclib**
  - LY2835219
  - FDA Approved (September 27, 2017)
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

<table>
<thead>
<tr>
<th></th>
<th>Palbociclib¹</th>
<th>Ribociclib²</th>
<th>Abemaciclib³</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dosage Form</strong></td>
<td>Capsule</td>
<td>Film-coated tablets</td>
<td>Tablets</td>
</tr>
<tr>
<td><strong>Recommended Dose</strong></td>
<td>125 mg</td>
<td>600 mg (3x200 mg)</td>
<td>150 mg (when used as combination therapy)</td>
</tr>
<tr>
<td><strong>Dosing Frequency</strong></td>
<td>Once daily for 21 consecutive days followed by 7 days off treatment (28-day cycle)</td>
<td>Once daily for 21 consecutive days followed by 7 days off treatment (28-day cycle)</td>
<td>Twice daily on a continuous dosing schedule</td>
</tr>
<tr>
<td><strong>Administration Considerations</strong></td>
<td>Should be taken with food</td>
<td>May be taken with or without food</td>
<td>May be taken with or without food</td>
</tr>
</tbody>
</table>
### CDK4/6 Inhibitors: First-Line Trials in Advanced Breast Cancer

<table>
<thead>
<tr>
<th></th>
<th>Palbociclib(^1)</th>
<th>Ribociclib(^2,3)</th>
<th>Abemaciclib(^4)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Endocrine Partner</strong></td>
<td>PALOMA-2</td>
<td>MONALEESA-2</td>
<td>MONARCH-3</td>
</tr>
<tr>
<td><strong>Eligibility</strong></td>
<td>Letrozole</td>
<td>Letrozole</td>
<td>Letrozole or Anastrozole</td>
</tr>
<tr>
<td><strong>Population</strong></td>
<td>N = 666</td>
<td>N = 668</td>
<td>N = 493</td>
</tr>
<tr>
<td><strong>ORR (%)</strong></td>
<td>55.3 vs 44.4</td>
<td>42.5 vs 28.7</td>
<td>49.7 vs 37.0</td>
</tr>
<tr>
<td><strong>CBR</strong></td>
<td>84.3 vs 71.0</td>
<td>79.6 vs 72.8</td>
<td>78 vs 71.5</td>
</tr>
<tr>
<td><strong>Median PFS (mo.)</strong></td>
<td>27.6 vs 14.5: HR, 0.56</td>
<td>25.3 vs 16.0; HR, 0.57</td>
<td>28.2 vs 14.8; HR, 0.53</td>
</tr>
</tbody>
</table>

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\(^2\) Hortobagyi GN. *Ann Oncol.* 2018 Jul 1;29(7):1541-1547

\(^3\) Hortobagyi GN. *N Engl J Med.* 2016 Nov 3;375(18):1738-1748

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

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

*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

# CDK4/6 Inhibitor Safety Profiles

<table>
<thead>
<tr>
<th></th>
<th>Palbociclib&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Ribociclib&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Abemaciclib&lt;sup&gt;3&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Neutropenia</strong></td>
<td>PALOMA-2</td>
<td>MONALEESA-2</td>
<td>MONARCH-3</td>
</tr>
<tr>
<td>• Any grade</td>
<td>(79.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Grade 3/4</td>
<td>(57.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Neutropenia</strong></td>
<td>MONALEESA-2</td>
<td>MONARCH-3</td>
<td></td>
</tr>
<tr>
<td>• Any grade</td>
<td>(74.3%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Grade 3/4</td>
<td>(60.3%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Diarrhea</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Any grades</td>
<td>(81.3%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Grade 3</td>
<td>(9.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Liver abnormalities</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Any grade</td>
<td>(30.6%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Grade 3/4</td>
<td>(15%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Neutropenia</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Any grade</td>
<td>(41.3%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Grade 3/4</td>
<td>(21.1%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Prolonged QTcF interval (2.7%)

Hortobagyi GN. N Engl J Med. 2016 Nov 3;375(18):1738-1748
## Monitoring Requirements for CDK4/6 Inhibitors*

<table>
<thead>
<tr>
<th></th>
<th>Palbociclib&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Ribociclib&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Abemaciclib&lt;sup&gt;3&lt;/sup&gt;</th>
</tr>
</thead>
</table>
| **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<sup>TM</sup> Product Monograph. Pfizer Canada Inc. June 5, 2018  
Kisqali<sup>TM</sup> Product Monograph. Novartis Pharmaceuticals Canada Inc. March 19, 2018  
Verzenio<sup>TM</sup> Product Monograph. Eli Lilly and Company.
Polling Question

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

A. PALLAS
B. PENEOLOPE-A
C. monarchER
D. monarchE
### CDK4/6 INHIBITOR STUDIES IN EARLY-STAGE HR+/HER2- BREAST CANCER

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

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

- Primary endpoint: iDFS (primary outcome analysis occurred after 395 iDFS events in ITT population)

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)

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

- Key secondary endpoints: distant RFS, iDFS in Ki67-high (≥20%) population, OS, safety, PROs, PK

Slide credit: clinicaloptions.com
monarchE: Baseline Characteristics

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

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

O’Shaughnessy Annals of Oncology 2021
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.
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?

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

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

Sunil Badve, MD, FRCPath
Vice Chair, Pathology Cancer Programs, Department of Pathology and Lab Medicine
Emory University School of Medicine
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- monarchE was the first phase III registration trial to analyze the utility of centrally confirmed Ki-67

- 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
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)

Phase 1 Study (n=37)

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

Phase 2 Study (n=25)

- 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

Lab E

Lab G

Nielsen TO et al SABCS 2013
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 9 study phases (1, 2, 3A visual and automated [3A], 3B visual and automated [3A-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.
<table>
<thead>
<tr>
<th>Setting</th>
<th>Factor</th>
<th>Variables</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preanalytical</td>
<td>Type of specimen</td>
<td>Core vs excision</td>
<td>Both are suitable, but core biopsies are preferred. Use case must be specimen type specific, eg, culprit for core cut may differ from excision; changes in Ki67 at multiple time points must be based on measurement on the same specimen type.</td>
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<td></td>
<td>Fixation</td>
<td>Prefixation delays (warm and cold ischemia time); tissue thickness; fixative type; time spent in fixative</td>
<td>Affects morphologic nuclear integrity and intensity of nuclear IHC stain. Inadequate fixation decreases Ki67. Ethanol-fixed or desiccated preparations should not be used. ASCO/CAP guidelines for breast tissue handling for ER/HER apply.</td>
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<tr>
<td></td>
<td>Means of storage</td>
<td>Tissue in paraffin block vs unstained slides</td>
<td>Prolonged storage of formalin-fixed paraffin-embedded tissue block at room temperature has little effect on Ki67. Avoid prolonged exposure to air of cut sections on glass slides.</td>
</tr>
<tr>
<td>Analytical</td>
<td>Antigen retrieval</td>
<td>Yes vs no</td>
<td>Required. High-temperature antigen retrieval mandatory. 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, although this might be confined to its use on non-Leica platforms.</td>
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<tr>
<td></td>
<td>Specific antibody</td>
<td>MIB1 vs other antibodies against Ki67 antigen</td>
<td>Avidin-biotin systems have substantially lower sensitivity and have largely been replaced by polymer detection on automated platforms. Amplified systems such as OptiView -Amp (Ventana) produce powerful, open-ended amplification that is difficult to standardize (UK NEQAS internal observations).</td>
</tr>
<tr>
<td></td>
<td>Colorimetric detection system</td>
<td>Avidin-biotin immunoperoxidase vs polymer detection vs amplified systems</td>
<td>Avidin-biotin systems have substantially lower sensitivity and have largely been replaced by polymer detection on automated platforms. Amplified systems such as OptiView -Amp (Ventana) produce powerful, open-ended amplification that is difficult to standardize.</td>
</tr>
<tr>
<td></td>
<td>Counterstain</td>
<td>Completeness and intensity of stain</td>
<td>Important that all negative nuclei are counterstained (otherwise apparent Ki67 index can be falsely high).</td>
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<tr>
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<td>Quality assurance/quality control</td>
<td>---</td>
<td>Should be established in each laboratory and systematically maintained. Quantitative external quality assessment should be established and participation should be mandatory.</td>
</tr>
<tr>
<td>Interpretation and scoring</td>
<td>Method of scoring</td>
<td>Cellular component, staining intensity</td>
<td>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.</td>
</tr>
<tr>
<td></td>
<td>Area of slide read</td>
<td>Average value across slide vs value in hot spot</td>
<td>Controversial: global (average) scores across the section had higher reproducibility than hot spot methods in iKNG studies; although differences were not statistically significant.</td>
</tr>
<tr>
<td>Digital Imaging</td>
<td>Visual vs automated analysis</td>
<td>iKNG-standardized visual scoring (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.</td>
<td></td>
</tr>
<tr>
<td>Data format and output</td>
<td>Categorical or continuous</td>
<td>Capture Ki67 data as a continuous percentage variable rather than in bins relative to specific cutoffs(4). Log transformation is required for parametric statistical testing.</td>
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</table>

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

Neilson 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; IHC = Immunohistochemistry.

Cheang et al JNCI 2009
Pharm Dx assay

- Dako Omnis
- Agilent/DAKO Antibody
- DAKO EnVision FLEX + DAB Enhancer
- Scoring System
**Ki-67 Score in breast carcinoma**

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

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### Staining Intensity Scale and Assessment Parameters

<table>
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<tr>
<th>Score</th>
<th>Intensity</th>
<th>Qualitative Description</th>
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<tbody>
<tr>
<td>3+</td>
<td>Strong Staining</td>
<td>Dark Chocolate Brown</td>
</tr>
<tr>
<td>2+</td>
<td>Moderate Staining</td>
<td>Dark Golden Brown <em>can see through</em></td>
</tr>
<tr>
<td>1+</td>
<td>Weak Staining</td>
<td>Light Brown</td>
</tr>
<tr>
<td>0</td>
<td>No Staining</td>
<td>Blue or Gray</td>
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</tbody>
</table>
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).
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).
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
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
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
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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