Standardisation of Ki67 in breast cancer prognostication

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Overview

• What is Ki67 protein – historical perspectives and modern knowledge
• Clinical use in breast cancer – prognostication and prediction
• How should we assess it?
  • Historical perspectives – Ki67 reproducibility group Phase 1 and 2 studies
  • Recent advances – Phase 3 study and the Global method
• Using Ki67 Global method scores
  • Standalone measurements
  • Multi-parameter indices – IHC4+C
Ki67 protein in breast cancer – historical perspectives

- Antibody against Ki67 protein first described by Harald Stein’s group in 1984\(^1\)
  - Present in cells at all stages of the cell cycle, except G0 i.e. identifies all proliferating cells
  - Functional in frozen tissues only

What is Ki67 protein – historical perspectives

- First described use in breast cancer, 1986

Growth fractions in breast cancers determined in situ with monoclonal antibody Ki-67

J Gerdes, R J Lelle, H Pickart, W Heidenreich, R Schwarting, L Kurtsiefer, G Stauch, H Stein

From the *Institute of Pathology, Klinikum Steglitz, Free University Berlin; the †Department of Obstetrics and Gynaecology, Medical School, University of Hannover; and the ‡Institute of Pathology, Hannover, Federal Republic of Germany

SUMMARY The growth fractions of 160 mammary carcinomas and 30 benign mammary lesions were determined in situ by immunostaining with the monoclonal antibody Ki-67. Benign lesions had a mean value of 3% Ki-67 positive cells, whereas the mean value of mammary carcinomas was 16-6%. A comparison of the mean values of Ki-67 positive cells with the histological grade of the tumours showed a correlation between these two variables—that is, histological grade 1 showed 9%, grade 2 16%, and grade 3 26% proliferating cells.

Considering the individual Ki-67 values in the different histological grades, it was evident that there was considerable scatter in the number of proliferating cells, so that the proliferation rates of grades 1, 2, and 3 overlapped each other. This indicates a dissociation between histological grade of malignancy and size of the growth fraction in most breast cancers. Follow up studies are needed to establish which of the two variables—that is, morphological degree of malignancy, or the proportion of Ki-67 positive cells—correlates better with response to treatment and survival in individual cases.

What is Ki67 protein – historical perspectives

• Equivalent antibody that works in FFPE - MIB $^1$

What is Ki67 protein – historical perspectives

Ki 67 Pubmed citation count

0 200 400 600 800 1000 1200 1400 1600 1800

Standardisation of Ki67 in breast cancer prognostication
What is Ki67 protein – modern knowledge

• What is the cellular function of Ki67?


*Given the vast amount of information we have on the structure, localization and regulation of pKi67, it is extraordinary that so little is known about the function of this protein...*¹
What is Ki67 protein – modern knowledge

- What is the cellular function of Ki67?
What is Ki67 protein – modern knowledge

- Used siRNA technology to knock-out Ki67 expression in HeLa cells
What is Ki67 protein – modern knowledge

- Structure of Ki-67 protein
  - The N terminus contains a phosphopeptide-binding Forkhead-associated (FHA) domain
  - The central region consists of 16 tandem-repeats
  - The C-terminal part is enriched in leucine and arginine (LR) residue pairs
What is Ki67 protein – modern knowledge

• Structure of Ki-67 protein
  • Ki-67 has an amphiphilic structure
  • The short C-terminal LR domain of Ki-67 has high attraction to chromatin (RED)
  • The long N-terminal domain has high attraction to the cytoplasm – hydrophilic, and is excluded from chromatin (GREEN)
What is Ki67 protein – modern knowledge

- Structure of Ki-67 protein
  - Ki-67 forms a repulsive barrier surrounding the entire surface of the chromosome
Ki67 protein in breast cancer – why do we assess it?

• There is strong evidence that proliferation as defined by Ki67 levels is prognostic of a tumour’s behavior and patient outcome

• Recurrence-free survival according to tertiles of tumor Ki67 expression at baseline (top panel) and after 2 weeks of treatment (bottom panel) from patients included in the IMPACT trial. (Marion and Dowsett. Endocr Relat Cancer 2010;17:R245-R262)
Ki67 protein in breast cancer – why do we assess it?

- In early as well as locally advanced breast cancer, baseline Ki67 has been found to predict for response to chemotherapy (but not response to endocrine treatment).

Ki67 protein in breast cancer – why do we assess it?

Review

Clinical use of biomarkers in breast cancer: Updated guidelines from the European Group on Tumor Markers (EGTM)

M.J. Duffy a,⁎, N. Harbeck b, M. Nap c, R. Molina d, A. Nicolini e, E. Senkus f, F. Cardoso g

European Journal of Cancer 75 (2017) 284–298
Available online at www.sciencedirect.com
ScienceDirect
journal homepage: www.ejcancer.com

CrossMark
Ki67 protein in breast cancer – why do we assess it?

Abstract Biomarkers play an essential role in the management of patients with invasive breast cancer. For selecting patients likely to respond to endocrine therapy, both oestrogen receptors (ERs) and progesterone receptors (PRs) should be measured on all newly diagnosed invasive breast cancers. On the other hand, for selecting likely response to all forms of anti-HER2 therapy (trastuzumab, pertuzumab, lapatinib or ado-trastuzumab emtansine), determination of HER2 expression or gene copy number is mandatory. Where feasible, measurement of ER, PR and HER2 should be performed on recurrent lesions and the primary invasive tumour. Although methodological problems exist in the determination of Ki67, because of its clearly established clinical value, wide availability and low costs relative to the available multianalyte signatures, Ki67 may be used for determining prognosis, especially if values are low or high. In oestrogen receptor (ER)-positive, HER2-negative, lymph node-negative patients, multianalyte tests such as urokinase plasminogen activator (uPA)-PAI-1, Oncotype DX, MammaPrint, EndoPredict, Breast Cancer Index (BCI) and Prosigna (PAM50) may be used to predict outcome and aid adjunct therapy decision-making. Oncotype DX, MammaPrint, EndoPredict and Prosigna may be similarly used in patients with 1–3 metastatic lymph nodes. All laboratories measuring biomarkers for patient management should use analytically...
Ki67 protein in breast cancer – **how** do we assess it?

11. **Ki67: recommendations for further research**

- Improve interlaboratory variation with assay standardisation.
- Establish an optimum cut-off point or evaluate the use of Ki67 as a continuous variable.
- Establish if different cut-off points are necessary for prognosis and therapy prediction.
- Evaluate the potential of automated image analysis for reducing between-assay variability.
Ki67 protein in breast cancer – how do we assess it?

- International Ki67 reproducibility working group
  - Published results of its first study in 2013\(^1\)
  - Group of 8 laboratories experienced in the use of Ki67 in breast cancer
  - 100 breast cancer cases TMA – one stained by participating lab and one by the central lab, using antibody MIB-1
  - Each laboratory scored Ki67 using its own method
  - Six laboratories repeated scoring of cases on 3 different days
  - Reproducibility was quantified by intra-class correlation coefficient (ICC)

\(^1\) Polley et al. *An international Ki67 reproducibility study*. JNCI 2013; 105: 1897
Ki67 protein in breast cancer – how do we assess it?

• International Ki67 reproducibility working group
  • Inter-laboratory reproducibility was only moderate (central staining: ICC = 0.71, 95% CI = 0.47 to 0.78; local staining: ICC = 0.59, 95% CI = 0.37 to 0.68)
  • Geometric mean of Ki67 values for each laboratory across the 100 cases ranged from 7% to 24% (central staining)
Ki67 protein in breast cancer – how do we assess it?

• International Ki67 reproducibility working group
  • Formal counting methods gave more consistent results than visual estimation
Ki67 protein in breast cancer – how do we assess it?

• International Ki67 reproducibility working group

• Factors contributing to inter-lab discordance included
  • staining methodology
  • tumor region selection
  • counting method
  • subjective assessment of staining positivity
Ki67 protein in breast cancer – how do we assess it?

• International Ki67 reproducibility working group

• Factors contributing to inter-lab discordance included
  • staining methodology
  • tumor region selection
  • counting method
  • subjective assessment of staining positivity
Ki67 protein in breast cancer – how do we assess it?

- International Ki67 reproducibility working group Phase 2 study\(^1\)
  - 16 laboratories
  - 50 breast cancer cases TMA – stained by central lab
  - Simple prescribed scoring method (counted) and staining thresholds for determination of the percentage of stained tumor cells
  - What is ‘positive’ image set
  - Each laboratory calibrated their scoring, using a web-based training set of images

1. Polley et al. *An international study to increase concordance in Ki67 scoring.* Mod Pathol 2015; **28**: 778
Ki67 protein in breast cancer – **how** do we assess it?

- International Ki67 reproducibility working group Phase 2 study
  - 16 laboratories
  - 50 breast cancer cases TMA – stained by central lab, using antibody MIB-1
  - Simple prescribed scoring method (counted) and staining thresholds for determination of the percentage of stained tumor cells
  - Each laboratory calibrated their scoring, using a web-based training set of images

- ICC = 0.95, 95% CI = 0.90 to 0.97 – **job done!**
Ki67 protein in breast cancer – how do we assess it?

- ICC = 0.95, 95% CI = 0.90 to 0.97 — job NOT done!
- Centrally stained cases
- TMA cores – not breast cores, or resection slides
Ki67 protein in breast cancer – how do we assess it?

- International Ki67 reproducibility working group Phase 3 study
  - 22 laboratories
  - 30 breast cancer needle cores – stained by central lab
  - Simple prescribed scoring methods (counted) – hotspot and Global

Ki67 protein in breast cancer – how do we assess it?

• International Ki67 reproducibility working group Phase 3 study
  
  • **Hotspot scoring method**
    
    • Select and count a single high-power field that is judged to contain the region of highest Ki67 expression within the section
    
    • Count 500 invasive tumour nuclei
  
  • **Global scoring method**
    
    • Estimate proportions of tumour that represents ‘negative’, ‘low’, ‘moderate’ and ‘high’ Ki67 expression within the section
    
    • Based on those estimates, select and count up to four fields representative of the range of expression seen
    
    • Count 100 invasive tumour nuclei in each field
Ki67 protein in breast cancer – *how* do we assess it?

- International Ki67 reproducibility working group Phase 3 study
  - **Hotspot scoring method**
    - ICC = 0.84, 95% CI = 0.77 to 0.92
  - **Global scoring method**
    - ICC = 0.87, 95% CI = 0.81 to 0.97 – meets pre-specified success criteria of ICC>0.80 (and CI’s which do not cross that boundary)
Ki67 protein in breast cancer – how do we assess it?

- International Ki67 reproducibility working group Phase 3 study
  - Hotspot scoring method
    - ICC = 0.84, 95% CI = 0.77 to 0.92
  - Global scoring method
    - ICC = 0.87, 95% CI = 0.81 to 0.97 – meets pre-specified success criteria of ICC>0.80 (and CI’s which do not cross that boundary)

- job done!
Ki67 protein in breast cancer – how do we assess it?

- International Ki67 reproducibility working group Phase 3 study - job NOT done!
Using Ki67 scores in a multi-parameter algorithm – IHC4+C

- IHC+C is a prognostic tool which calculates residual risk of distant disease recurrence
- Based on a combination of immunohistochemically assessed makers together with clinical parameters
- Developed and validated in post-menopausal women with early advanced ER-positive breast cancer
  - transATAC and Nottingham series
Using Ki67 scores in a multi-parameter algorithm – IHC4+C

• Combines a clinical treatment score derived from assessment of following parameters
  • Nodal status
  • Tumour size
  • Tumour grade
  • Patient age (<65 years, >=65 years)
  • Treatment type (tamoxifen, aromatase inhibitor)

• With an IHC4 score derived from 4 standard IHC markers
  • ER (H-score)
  • PgR (% positivity)
  • HER2 (status: negative or positive)
  • Ki67 (% positivity)
Using Ki67 scores in a multi-parameter algorithm – IHC4+C

\[
\text{clinical score} = 100 \times \{0.417N_{1-3} + 1.566N_{4+} \\
+ 0.930(0.497T_{1-2} + 0.882T_{2-3} + 1.838T_{>3} + 0.559Gr_2 \\
+ 0.970Gr_3 + 0.130\text{Age} \geq 65 - 0.149\text{Ana})\}
\]

\[
\text{IHC4} = 94.7 \times \{-0.100 \text{ER}_{10} - 0.079 \text{PgR}_{10} \\
+ 0.586 \text{HER2} + 0.240 \ln(1 + 10 \times \text{Ki67})\}
\]
Using Ki67 scores in a multi-parameter algorithm – IHC4+C

<table>
<thead>
<tr>
<th>Involved nodes</th>
<th>0</th>
<th>Involved nodes</th>
<th>&gt;3</th>
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<tbody>
<tr>
<td>Tumour size</td>
<td>&lt;10mm</td>
<td>Tumour size</td>
<td>&gt;30mm</td>
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<tr>
<td>Tumour grade</td>
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<td>Tumour grade</td>
<td>3</td>
</tr>
<tr>
<td>Patient age</td>
<td>&lt;65 years</td>
<td>Patient age</td>
<td>&gt;=65 years</td>
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<td>Treatment type</td>
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<td>Treatment type</td>
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<tr>
<td>Clinical Score</td>
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<td>Clinical Score</td>
<td>430</td>
</tr>
</tbody>
</table>

**CTS**

**IHC4**

<table>
<thead>
<tr>
<th>ER (H-score)</th>
<th>300</th>
<th>ER (H-score)</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>PgR (% positive)</td>
<td>100</td>
<td>PgR (% positive)</td>
<td>0</td>
</tr>
<tr>
<td>HER2 status</td>
<td>Negative</td>
<td>HER2 status</td>
<td>[Positive]</td>
</tr>
<tr>
<td>Ki67 (% positive)</td>
<td>0</td>
<td>Ki67 (% positive)</td>
<td>100</td>
</tr>
<tr>
<td>IHC4 Score</td>
<td>-170</td>
<td>IHC4 Score</td>
<td>192</td>
</tr>
</tbody>
</table>

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3rd NordiQC Conference on Applied Immunohistochemistry. 8th June 2017
Using Ki67 scores in a multi-parameter algorithm – IHC4+C

clinical score = 100 × \{0.417N_{1-3} + 1.566N_{4+} + 0.930(0.497T_{1-2} + 0.882T_{2-3} + 1.838T_{>3} + 0.559Gr_2 + 0.970Gr_3 + 0.130\text{Age} \geq 65 - 0.149\text{Ana})\}

\[
\text{IHC4} = 94.7 \times \{-0.100 \text{ER}_{10} - 0.079 \text{PgR}_{10} + 0.586 \text{HER2} + 0.240 \ln (1 + 10 \times \text{Ki67})\}
\]
Using Ki67 scores in a multi-parameter algorithm – IHC4+C

- International Ki67 reproducibility working group Phase 3 study
Ki67 scores (Global method) – clinically appropriate cases (N = 20)
IHC4+C derived risk scores

- TB016
- TB033
- TB036
- TB040
- TB077
- TB082
- TB083
- TB112
- TB192
- TB193
- TB196
- TB203
- TB245
- TB286
- TB310
- TB319
- TB374
- TB381
- TB468
- TB469
- TB634
- TB634
- TB634
- TB634
- TB634
Using Ki67 scores in a multi-parameter algorithm – IHC4+C

• Agreement of risk scores in clinically appropriate cases (N = 20):
  • ICC = 0.99 (95% CI, 0.99 - 1.00)
Using Ki67 scores in a multi-parameter algorithm – IHC4+C

- Agreement of risk scores in clinically appropriate cases (N = 20)
Using Ki67 scores in a multi-parameter algorithm – IHC4+C

- Agreement of risk scores in clinically appropriate cases (N = 20)

| Case   | A | B | C | D | E | F | G | H | I | J | K | L | M | N | O | P | Q | R | S | T | U | V |
| TB016  | 13| 13| 14| 14| 14| 14| 14| 14| 15| 13| 15| 14| 14| 14| 14| 12| 13| 13| 13| 14| 15| 13| 13 |
| TB033  | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 4 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| TB036  | 6 | 6 | 6 | 6 | 7 | 6 | 6 | 7 | 7 | 6 | 6 | 7 | 7 | 6 | 6 | 5 | 6 | 6 | 6 | 6 | 6 | 6 |
| TB040  | 4 | 5 | 5 | 4 | 5 | 5 | 4 | 5 | 6 | 4 | 5 | 6 | 5 | 4 | 5 | 5 | 5 | 4 | 5 | 5 | 5 | 5 |
| TB077  | 40| 44| 44| 41| 44| 43| 42| 40| 42| 42| 42| 43| 42| 46| 45| 41| 43| 41| 42| 42| 44| 44| 43 |
| TB082  | 12| 13| 14| 13| 13| 12| 13| 14| 12| 14| 14| 14| 15| 13| 15| 13| 12| 14| 14| 14| 14| 14 | 14 |
| TB083  | 12| 13| 14| 13| 14| 13| 15| 15| 13| 14| 15| 15| 15| 13| 14| 14| 13| 12| 14| 16| 14| 14 | 13 |
| TB112  | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| TB122  | 23| 29| 29| 29| 29| 29| 29| 29| 29| 29| 29| 29| 29| 29| 29| 29| 29| 29| 29| 29| 29 | 29 |
| TB193  | 4 | 6 | 6 | 6 | 7 | 6 | 7 | 7 | 6 | 7 | 7 | 6 | 7 | 5 | 6 | 6 | 6 | 6 | 6 | 7 | 6 | 6 |
| TB196  | 5 | 5 | 5 | 5 | 5 | 4 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 4 | 7 | 4 | 5 |
| TB203  | 20| 22| 22| 22| 22| 22| 22| 22| 22| 22| 22| 22| 22| 22| 22| 22| 22| 22| 22| 22| 22 | 22 |
| TB245  | 13| 13| 13| 13| 13| 13| 13| 13| 13| 13| 13| 13| 13| 13| 12| 13| 12| 13| 13| 13| 13 | 13 |
| TB286  | 14| 15| 15| 16| 15| 15| 15| 16| 15| 16| 15| 16| 15| 16| 15| 16| 15| 16| 15| 16| 16 | 15 |
| TB310  | 31| 34| 34| 34| 34| 32| 35| 35| 33| 33| 33| 32| 32| 34| 38| 32| 31| 33| 33| 33| 33 | 32 |
| TB319  | 12| 14| 13| 13| 13| 13| 13| 13| 13| 13| 12| 13| 13| 12| 14| 14| 12| 13| 13| 13| 13 | 13 |
| TB374  | 7 | 9 | 8 | 8 | 8 | 8 | 9 | 7 | 8 | 8 | 8 | 8 | 8 | 8 | 7 | 8 | 8 | 7 | 8 | 7 | 6 |
| TB468  | 32| 35| 34| 34| 34| 35| 34| 34| 33| 33| 34| 32| 35| 36| 34| 34| 32| 31| 33| 34| 34 | 34 |
| TB654  | 4 | 5 | 5 | 5 | 5 | 4 | 5 | 6 | 5 | 5 | 5 | 5 | 6 | 5 | 5 | 5 | 5 | 5 | 5 | 6 | 5 | 5 |
Using Ki67 scores in breast cancer – next steps

• International Ki67 reproducibility working group
  • Automated image analysis arm
  • Global method and hotspot in resection slides
  • Clinical validation study – POETIC
Acknowledgements

• International Ki67 reproducibility working group members
  • Mitch Dowsett
  • Torsten Nielsen, Sam Leung
• IHC4+C development
  • Jack Cuzick, Iva Sestak

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