PATHOLOGY AND MOLECULAR BIOLOGY OF BREAST CANCER

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SPLIT MEDICAL UNIVERSITY
TOPICS

• Pathology in neoadjuvant setting
• Prognostic multigene signatures in breast cancer
HANDLING AND REPORTING A BREAST SPECIMEN AFTER NACT
NEODJUVANT CHEMOTHERAPY

- Indications:
  - Management of locally advanced invasive breast cancers including inflammatory breast cancer
  - Down-staging of large inoperable cancers to permit surgical resection
  - Routine management of women with high risk disease who would require adjuvant chemotherapy based on biological tumour characteristics and clinical-radiological findings

Surgery after primary systemic treatment

NEOADJUVANT SYSTEMIC THERAPY BENEFITS

- Reduce local disease burden
  - Non operable to operable
  - Mastectomy to breast conserving therapy
  - Axillary dissection to sentinel lymph node biopsy
- In vivo assessment of tumor response to treatment
GOAL OF THE NACT IS PATHOLOGIC COMPLETE RESPONSE

- Accurate assessment of treatment response can only be made by careful macroscopic and microscopic examination of breast and lymph nodes.
Before slicing the post NASCT sample pathologist MUST have information about:

1. NAST
2. Location of tumour/s within the breast before and after the treatment
3. Pre and post treatment size on imaging
4. Number and location of clips

IN THE REAL WORLD PATHOLOGIST SOMETIMES DON‘T KNOW THAT PATIENT WAS TREATED WITH NACT

No tumor
Strange tumor histology
Look at the date of prior core biopsy
• Specimen should be sent fresh and orientated to the histopathology laboratory as quickly as possible for slicing into 1-2 cm thick slices to aid fixation – good fixation is critical for accurate assessment. ¹

TYPES OF RESPONSE TO NAST

MACROSCOPICALLY EVIDENT TUMOUR: EXTENSIVE SAMPLING IS NOT NECESSARY

Tan and Sahin, Atlas of Differential Diagnosis in Breast Pathology, 2017
Assessment of treatment response requires identification and sampling of the tumor bed. Failure to identify the tumor bed can result in erroneous categorisation as pCR.
Placement of a marker clip at the time of diagnosis is very helpful in the event of an excellent response to treatment.

SPECIMEN RADIOGRAPH IS HELPFUL IN LOCATING TUMOR BED/CLIP IN PATIENTS WITHOUT GROSSLY OBVIOUS RESIDENTIAL DISEASE.
WHEN RESIDUAL TUMOR AND/OR TUMOR BED IS NOT IDENTIFIED
MORE EXTENSIVE SAMPLING IS REQUIRED – PROVING A NEGATIVE

Radiograph slices
Diagram slices
Note from where sections are taken for histologic examination

<table>
<thead>
<tr>
<th>Microscopic Examination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size and focality of the residual tumor</td>
</tr>
<tr>
<td>Existence and extent of DCIS</td>
</tr>
<tr>
<td>Cellularity of the residual tumor</td>
</tr>
<tr>
<td>LVI</td>
</tr>
<tr>
<td>Resection margins</td>
</tr>
<tr>
<td>Lymph node evaluation</td>
</tr>
<tr>
<td>Pathological response to NACT</td>
</tr>
<tr>
<td>Biomarker retesting</td>
</tr>
</tbody>
</table>

RESIDUAL CANCER BURDEN

Standardization of pathologic evaluation and reporting of postneoadjuvant specimens in clinical trials of breast cancer: recommendations from an international working group

Elena Provenzano1, Veerle Bossuyt2, Giuseppe Viale3, David Cameron4, Sunil Budhu5, Carsten Denkert6, Gaétan MacGrogan7, Frédérique Penault-Llorca8, Judy Boughey9, Giuseppe Curigliano10, I Michael Dixon11, Laura Esserman12, Gerd Fastner13, Thorsten Kuehn14, Florentia Peintinger13,16, Gunter von Minckwitz17, Julia White18, Wei Yang19 and W Fraser Symmans20 on behalf of the Residual Disease Characterization Working Group of the Breast International Group-North American Breast Cancer Group (BIC-NABCG) collaboration

RESIDUAL CANCER BURDEN

ONLINE CALCULATOR

RCB score is continuous variable which correlates with outcome

Comparation with pre-treatment specimen are not required

Cannot be used if positive lymph node/nodes are removed before treatment

www.mdanderson.org/breastcancer_RBC
FOR CALCULATING RESIDUAL CANCER BURDEN (RCB) WE NEED

Tumor size in two dimension, cellularity (%), extent of DCIS komponent (%), number of positive lymph nodes and size of largest lymph node metastasis

**BEST CASE SCENARIO - PATHOLOGIC COMPLETE RESPONSE**

- No residual carcinoma in breast or lymph nodes, including LVI only, or N0i+
- Presence of residual DCIS do not exclude pCR
- In addition to RCB, ypTN must be included in report
- pCR include: yT0/TISyN0
PATHOLOGIC COMPLETE RESPONSE AND CONNECTION WITH DFS DEPEND ON INTRINSIC BREAST CANCER TYPE

Luminal A

Luminal B, HER2-

Luminal B, HER2+

HER2+

TNBC

RECEPTOR ASSAYS POST NAST

• Receptor status can differ between pre and post-NAST tumor samples.
• Two meta-analyses report discordant results of 13% and 18% for ER, 32% and 26% for PR, and 9% and 6% for HER2 before and after chemotherapy.¹,²
• Reasons for this discordance include:
  • technical failure,
  • intratumoral heterogeneity of marker expression,
  • and changes induced by therapy.

In current practice, the choice of adjuvant therapy is dictated by the results at primary diagnosis.

However, patients with residual disease that originally had negative receptor status can be re-tested to re-evaluate for eligibility for a targeted adjuvant treatment.

TAKE HOME MESSAGES

- Systematic sampling of the correct area of the breast with correlation of gross and microscopic findings allows accurately defining of treatment response which has prognostic and therapeutic implication.

- Specimens should be clearly identified, as neoadjuvant, and the location and size of the tumor pretreatment must be known.

- Pathologic complete response (pCR), residual cancer burden (RCB), and yTyN (American Joint Commission on Cancer) stage are recommended measures of residual disease.
PROGNOSTIC MULTIGENE SIGNATURES
SEVERAL GENES USED TOGETHER IN A FORMULA TO PREDICT OUTCOME
ER+, HER2 -, N0 OR N1
# BREAST CANCER PROGNOSTIC MULTIGENE SIGNATURES THAT ARE COMMERCIALY AVAILABLE

<table>
<thead>
<tr>
<th>Signature</th>
<th>Tissue type</th>
<th>Platform</th>
<th>Gene number</th>
<th>Prospective clinical trials</th>
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</thead>
<tbody>
<tr>
<td>Oncotype DX</td>
<td>FFPE</td>
<td>qRT-PCR</td>
<td>21</td>
<td>TALORX RxPONDER</td>
</tr>
<tr>
<td>Mammaprint</td>
<td>Frozen, FFPE</td>
<td>microarray</td>
<td>70</td>
<td>MINDACT</td>
</tr>
<tr>
<td>PAM50</td>
<td>FFPE</td>
<td>Nanostring technology</td>
<td>50</td>
<td>No</td>
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<tr>
<td>Endopredict assay</td>
<td>FFPE</td>
<td>qRT-PCR</td>
<td>5 P</td>
<td>No</td>
</tr>
<tr>
<td>Genomic grade indeks</td>
<td>Frozen, FFPE</td>
<td>Microarray, qRT-PCR</td>
<td>97</td>
<td>No</td>
</tr>
<tr>
<td>MapQuant /simplyfied</td>
<td></td>
<td></td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Breast cancer index</td>
<td>FFPE</td>
<td>qRT-PCR</td>
<td>2 + 5</td>
<td>No</td>
</tr>
</tbody>
</table>

ONCOTYPE-DX

- One of the best validated, centralised, commercially available breast cancer multigene test
- Based on RNA isolation from FFPE breast cancer tissue followed by RT-PCR
- Provide prognostic information in HR+, HER2- breast cancer

Recurrence score is a continuous variable (0-100), which is a measure of the risk of distant relapse within 10 years in patients with ER+, N0 patients treated with adjuvant tamoxifen.

Include analysis of 16 cancer-related genes and five reference genes.

TAILORx was designed to determine:

1. Whether chemotherapy is beneficial for women with a mid-range recurrence score of 11 to 25.

2. Prospectively confirm that a low recurrence score of 0 to 10 is associated with a low rate of distant recurrence when patients are treated with endocrine therapy alone.

Chemotherapy may be spared in all women older than 50 with RS results of 11 to 25 and all women age 50 or younger with RS results of 11–15.
Oncotype is incorporated in clinical guidelines for early stage breast cancer treatment.
## Table 4. Pathological Prognostic Stage (continued)

<table>
<thead>
<tr>
<th>TNM</th>
<th>Grade</th>
<th>HER2</th>
<th>ER</th>
<th>PR</th>
<th>Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>T4  N0 M0</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>IIA</td>
<td></td>
</tr>
<tr>
<td>T4  N1 M0</td>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
<td>IIB</td>
<td></td>
</tr>
<tr>
<td>T4  N2 M0</td>
<td>Positive</td>
<td>Negative</td>
<td>Negative</td>
<td>IIIB</td>
<td></td>
</tr>
<tr>
<td>Any T N3 M0</td>
<td>Positive</td>
<td>Negative</td>
<td>Negative</td>
<td>IIIB</td>
<td></td>
</tr>
<tr>
<td>G1</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>IIA</td>
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<tr>
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<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>IIB</td>
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</tr>
<tr>
<td>G2</td>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
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<td>IIIB</td>
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<td>Any T Any N M1</td>
<td>Positive</td>
<td>Negative</td>
<td>Negative</td>
<td>IIC</td>
<td></td>
</tr>
</tbody>
</table>

### Notes:
1. For cases with lymph node involvement with no evidence of primary tumor (e.g., T0 N1, etc.) or with breast ductal carcinoma in situ (e.g., Tis N1, etc.), the grade, HER2, ER and PR information from the tumor in the lymph node should be used for assigning stage group.
2. For cases where HER2 is determined to be "equivocal" by IHC (FISH or CISH) testing under the 2013 ASCO/CAP HER2 testing guidelines, HER2 "negative" categorization should be used for staging in the Pathological Prognostic Stage Group.
3. The prognostic value of these Prognostic Stage Groups is based on populations of persons with breast cancer that have been offered and mostly treated with appropriate endocrine and/or systemic chemotherapy (including anti-HER2 therapy).

## Table 5. Genomic Profile for Pathologic Prognostic Staging

When Oncotype DX Score is Less than 11...

<table>
<thead>
<tr>
<th>TNM</th>
<th>Grade</th>
<th>HER2</th>
<th>ER</th>
<th>PR</th>
<th>Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1  N0 M0</td>
<td>Any</td>
<td>Negative</td>
<td>Positive</td>
<td>Any</td>
<td>IA</td>
</tr>
<tr>
<td>T2  N0 M0</td>
<td>Any</td>
<td>Negative</td>
<td>Positive</td>
<td>Any</td>
<td>IA</td>
</tr>
</tbody>
</table>

### Notes:
1. Obtaining genomic profiles is NOT required for assigning Pathological Prognostic Stage. However, genomic profiles may be performed for use in determining appropriate treatment. If the OncotypeDX® test is performed in cases with a T1N0M0 or T2N0M0 cancer that is HER2-negative and ER-positive, and the recurrence score is less than 11, the case should be assigned Pathological Prognostic Stage Group IA.
2. If OncotypeDX® is not performed, or if it is performed and the OncotypeDX® score is not available, or is 11 or greater for patients with T1–2 N0 M0 HER2-negative, ER-positive cancer, then the Prognostic Stage Group is assigned based on the anatomic and biomarker categories shown above.
3. OncotypeDX® is the only multigene panel included to classify Pathologic Prognostic Stage because prospective Level I data supports this use for patients with a score less than 11. Future updates to the staging system may include results from other multigene panels to assign coherently to patients to Prognostic Stage Groups based on the then available evidence. Inclusion or exclusion of specific cases in this staging table of a genomic profile assay is not an endorsement of any specific assay and should not limit appropriate clinical use of any genomic profile assay based on evidence available at the time of treatment.

**N1 includes N1mi, T2, T3, and T4 cancers and N1mi are included for prognostic staging with T2 N1, T3 N1 and T4 N1, respectively.**
EXTENDED USE OF ONCOTYPE DX FOR N1 PATIENTS WITH BREAST CANCER?
• 70 gene signature approved by the FDA to stratify patients with ER+ or ER- N0/N1 breast cancer into high vs. low risk of relapse

• The prognostic risk discrimination is good among ER-positive cancers
• Almost all ER-negative and/or HER2 positive cancers are classified as high risk.

MINDACT

- MammaPrint was tested in prospective randomised trial which include 6 000 ER+, N0/N1 patients to answer on clinical very important question:

- Can Microarray in Node negative and 1-3 positive Disease Avoid ChemoTherapy (MINDACT) spare more patients from chemotherapy than usual clinical-pathological risk assessment by Adjuvant!

This study showed that chemotherapy could be spared in women who had a low genomic risk for recurrence according to MammaPrint and who were at high clinical risk for relapse defined using Adjuvant!
Mammaprint is endorsed in clinical guidelines for early stage breast cancer treatment.
PAM50/RISK OF RECCURENCE/PROSIGNA KIT

- 50 genes molecular test using Nanostring nCounter technology in patient analysis

- Approved to estimate distant recurrence free survival in women with ER+ breast cancer treated with adjuvant endocrine therapy

- Validate in TransATAC1 and ABCSG-82 study

- Lack of prospective clinical studies that show predictive value of this signature


• ROR (risk of recurrence score) that takes into account
• PAM50 profile\(^1\) and
• clinical features e.g. tumor size and proliferation score \(^2\)
• ROR is stratified into:
  • Low (10 years distant recurrence <10%)
  • Intermediate (10 years distant recurrence 10-20%)
  • High (10 years distant recurrence >20%)
• Provides breast cancer intrinsic subtype classification
• Test can be performed by local pathology laboratories

Chemotherapy should be considered for patients in the PAM50 high-risk group and it is not indicated for patients in the low-risk group.

Additional studies are needed to support recommendations about adjuvant chemotherapy in patients with an intermediate Prosigna/PAM50 ROR score.

Regarding node positive ER/PgR-positive, HER2-negative breast cancer, more data are required to determine whether PAM50-ROR can be used with confidence in guiding the use of adjuvant systemic therapy.

THE CLINICAL APPLICATION OF GENETIC TESTS
PROGNOSTIC INFORMATION,
THERAPY DECISION,
MOLECULAR SUBTYPING
PATIENT STAGING

MULTIGENE PROGNOSTIC PREDICTORS - LIMITATIONS

- Useless in the HER2+ and triple negative patients
- No robust new, prognostic genes have been identified that are unrelated to proliferation or ER signaling
- Some powerful anatomical–pathologic prognostic risk factors such as tumor size and nodal status are not captured by gene signatures

**Molecular tests complement rather than replace the traditional pathological variables, to define the optimal therapy for patients with breast cancer.**
Thank you for attention!!!!