Neoadjuvant Systemic Treatment: the Rosetta Stone for Breast Cancer?

Daniele Generali, MD DPhil
U.O. Multidisciplinare di Patologia Mammaria
U.S. Terapia Molecolare e Farmacogenomica
AZ. Istituti Ospitalieri di Cremona (Italy)
FDA releases draft guidance on pCR as potential endpoint for breast cancer drugs

Accelerated approval regulations currently require confirmation of a clinical benefit, such as DFS or OS. The ability to use pCR as an endpoint could allow potential clinical benefits of investigational drugs to be determined much quicker, allowing treatments to come on the market up to a decade earlier.

(the FDA defined pCR as “the absence of any residual invasive cancer on hematoxylin and eosin evaluation of the resected breast specimen and all sampled ipsilateral lymph nodes following completion of neoadjuvant systemic therapy.”)

FDA:

“Despite advances in systemic therapy of early-stage breast cancer over the past few decades, there remains a significant unmet medical need for certain high-risk or poor prognosis populations of early-stage breast cancer patients,” the FDA wrote.

“This guidance is intended to encourage industry innovation and expedite the development of breakthrough therapies to treat high-risk early-stage breast cancer.”
Neoadjuvant Systemic Treatment: the Rosetta Stone for Breast Cancer?

- Surrogate Biological Markers
- Trial Design
- Patients Advocate
Surrogate Biological Markers
Putative Predictive Factors of pCR

- Tumor size & Tumor grade
- Histological type
- ER/PgR
- Her2/neu
- Proliferative markers (Ki-67/MIB-1, PCNA)
- Treatment & MDR-1/pgp
Pathologic CR (pCR) was a significant predictor of OS, regardless of treatment.

Outcomes of Neoadjuvant Trials with unselected tumor characteristics

Classification of cases with Residual Disease as “non responders” is an oversimplification and misleading

Impact of treatment characteristics on the pCR

<table>
<thead>
<tr>
<th>Treatment Characteristic</th>
<th>Odds Ratio (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cycles (per 2 additional cycles)</td>
<td>1.18 (1.04 to 1.34)</td>
<td>0.009</td>
</tr>
<tr>
<td>Antracycline (higher vs lower dose)</td>
<td>1.55 (1.18 to 2.03)</td>
<td>0.002</td>
</tr>
<tr>
<td>Taxane (higher vs lower dose)</td>
<td>1.58 (1.12 to 2.22)</td>
<td>0.009</td>
</tr>
<tr>
<td>Capecitabine (yes vs no)</td>
<td>1.62 (1.07 to 2.45)</td>
<td>0.022</td>
</tr>
<tr>
<td>Tamoxifen (yes vs no)</td>
<td>1.12 (0.65 to 1.94)</td>
<td>0.69</td>
</tr>
<tr>
<td>Trastuzumab§ (yes vs no)</td>
<td>3.20 (2.19 to 4.67)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Allevi G et al “Increased pathological complete response rate after a long-term neoadjuvant letrozole treatment in postmenopausal oestrogen and/or progesterone receptor-positive breast cancer” Br J Cancer 2013

Distribution of Clinical Disease Response (Absolute and Percentage Value) According to Months of Treatment.

|                      | 4  |     | 8  |     | 12 |  
|----------------------|----|-----|----|-----|----|---------
| No. of patients      | 117| 75  | 40 |     |    |         
| NA                   | 3  | 5   | 0  |     |    |         
| Progressive Disease  | 5  | 1   | 0  |     |    |         
| Stable Disease       | 54 | 10  | 2  | (5.0%) |    |         
| Partial Response     | 37 | 33  | 15 | (37.5%) |    |         
| Complete Response    | 21 | 31  | 23 | (57.5%) |    |         
| Overall Response Rate| 49.6% | 85.3% | 95.0% |       |    |         

NA: not available.
* Confidence Interval.

Changes in Ki67 Expression for Individual Patients at Baseline and Post-treatment Histology According to Cohort.

The pathCR was confined to:
1) Cohort C (7 out of 40 cases, 17.5%),
2) Cohort B (2 out of 40 cases, 5.0%)
3) Cohort A (1 out of 40 cases, 2.5%)

(P value for trend < 0.04)
Intrinsic sub-types have different prognosis and different response to NACT.

Mosaic plots of the factors that were consistently associated with endocrine response.

Expression of markers of resistance (HIF1α, p44/p44) and responsiveness (ERα)
Milani M et al, “Hypoxia-related biological markers as predictors of epirubicin-based treatment responsiveness and resistance in locally advanced breast cancer”

Distribution of Clinical Disease Response
(Absolute and Percentage Value)
According to Type of Treatment.

<table>
<thead>
<tr>
<th>Response</th>
<th>EPI 120</th>
<th></th>
<th>EPI 120 + E</th>
<th></th>
<th>EPI 40 + EP0</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Progression</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>5.2</td>
</tr>
<tr>
<td>Stable Disease</td>
<td>6</td>
<td>9.5</td>
<td>1</td>
<td>1.8</td>
<td>3</td>
<td>5.2</td>
</tr>
<tr>
<td>Partial Response</td>
<td>41</td>
<td>65.1</td>
<td>36</td>
<td>65.5</td>
<td>26</td>
<td>44.9</td>
</tr>
<tr>
<td>Complete Response</td>
<td>16</td>
<td>25.4</td>
<td>18</td>
<td>32.7</td>
<td>26</td>
<td>44.8</td>
</tr>
<tr>
<td>Overall Response Rate</td>
<td>57</td>
<td>90.5</td>
<td>54</td>
<td>98.2</td>
<td>52</td>
<td>89.7</td>
</tr>
</tbody>
</table>

Pretreatment haemoglobin levels significantly predict the tumour response to primary chemotherapy in human breast cancer.

Br J Cancer. 2003 Sep 15;89(6):977-82

HB basal levels and HIF-1 alpha nuclear expression intensity were both significantly negatively correlated with pCR

HB basal levels and HIF-1 alpha nuclear expression intensity were both significantly negatively correlated with a higher risk of relapse and with OS
Nomogram: Impact of pre-op on pCR (638/702)
Nomogram:
Impact of pre-op on DFS at 5yrs

Points
NeoAdjuvantT
Histology
Age in Years
KI67
PgR post
Grading post
T
pathCR
Total Points
5 years Disease Free Survival
Adaptive neurofuzzy-based method for the determination of pCR

Surface graph showing relationship of Ki67 and Stage/ Ki67 and Age with predicted output (pCR).
Trial Design
TOPICS TO BE COVERED

• The drug discovery pipeline is inefficient and *very* costly
• Averages ~10-15yrs to complete
• Clinical trials information infrastructure (the pipeline) is paper-based and disjointed -- making it difficult to be more efficient.
• Biomarkers are showing promise in informing treatment choices, but validation of biomarkers in the clinical trial process has proven to be difficult - the “biomarker barrier”
ERA of Molecular Biology

“Molecular signature of cancer”

• “Omics” technologies for discovery of new prognostic and predictive markers

• Development of targeted therapies

  Only treat those who need treatment

  Only treat those who will benefit from treatment

Genomics

Proteomics
How do we do clinical trials today?
Molecular Therapy and Pharmacogenomic Unit

develop protocols
create forms
enroll and care for patients

the science
chart clinical care

archive records
store records
### Theraprint profile as a predictor of tumor response in neoadjuvant approach in breast cancer

**Cappelletti MR et al.**

<table>
<thead>
<tr>
<th>BluePrint</th>
<th>Luminal A (N=20)</th>
<th>Luminal B (N=14)</th>
<th>Her2 (N=4)</th>
<th>Basal (N=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CT_tras</td>
<td>1</td>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>CT+ET</td>
<td>7</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CT+ET+trast</td>
<td>2</td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>CT+ET+sorafenib</td>
<td>6</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CT+ET+zometa</td>
<td>4</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pCR</td>
<td>0/19 (0%)</td>
<td>1/13 (8%)</td>
<td>1/4 (25%)</td>
<td>1/3 (33%)</td>
</tr>
<tr>
<td>CR</td>
<td>7/19 (37%)</td>
<td>4/13 (31%)</td>
<td>1/4 (25%)</td>
<td></td>
</tr>
<tr>
<td>PR</td>
<td>8/19 (79%)</td>
<td>6/13 (46%)</td>
<td>3/4 (75%)</td>
<td>1/3 (33%)</td>
</tr>
<tr>
<td>SD</td>
<td>4/19 (21%)</td>
<td>2/13 (15%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PD</td>
<td></td>
<td></td>
<td></td>
<td>1/3 (33%)</td>
</tr>
<tr>
<td>ET only</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PR</td>
<td>1/1 (100%)</td>
<td>1/1 (100%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
TheraPrint - Unbiased Gene Selection

Breast Cancer

Analysis of Entire Human Genome ~25,000 Genes

Prognostic & Predictive Breast Cancer Genes Identified

Gene expression analysis for drug sensitivity
TheraPrint: a new research tool

- Offers read-out of gene expression for 56 genes
- Genes might have relevance in breast cancer therapy and prognosis
- No claims about mRNA level and response can be made

Genes Included:

<table>
<thead>
<tr>
<th>AKT1</th>
<th>CCNE1</th>
<th>EGFR</th>
<th>FLT4</th>
<th>KRT17</th>
<th>PDGFRB</th>
<th>RAD51L3</th>
</tr>
</thead>
<tbody>
<tr>
<td>AURKA</td>
<td>CDH1</td>
<td>EGFR</td>
<td>FRAP1</td>
<td>KRT5</td>
<td>PIK3CA</td>
<td>RAF1</td>
</tr>
<tr>
<td>BCL2</td>
<td>CDH3</td>
<td>ERBB3</td>
<td>GSDML</td>
<td>KRT8</td>
<td>PIK3R1</td>
<td>TRIM29</td>
</tr>
<tr>
<td>BRAF</td>
<td>CRYAB</td>
<td>ERBB4</td>
<td>IGF1R</td>
<td>MAP2K1</td>
<td>PI3X2</td>
<td>TYMS</td>
</tr>
<tr>
<td>BRCA1</td>
<td>CSK</td>
<td>ESR2</td>
<td>IGF2R</td>
<td>MAP2K2</td>
<td>PRKCB1</td>
<td>VEGFA</td>
</tr>
<tr>
<td>BRCA2</td>
<td>CXCL12</td>
<td>FANCF</td>
<td>KDR</td>
<td>NFKB1</td>
<td>PTHLH</td>
<td>VEGFB</td>
</tr>
<tr>
<td>C11orf30</td>
<td>CXCL14</td>
<td>FLT1</td>
<td>KIT</td>
<td>NFKB2</td>
<td>RAD51C</td>
<td>XRCC2</td>
</tr>
<tr>
<td>CCND1</td>
<td>DHFR</td>
<td>FLT3</td>
<td>KRAS</td>
<td>PDGFR</td>
<td>RAD51L1</td>
<td>XRCC3</td>
</tr>
</tbody>
</table>
Differential TheraPrint gene expression between responders (pCR/PR) and non-responders (SD/PD)

• There are no significant genes (also with multiple test correction) when using an ANOVA (assumes the data is normally distributed). Almost all genes fail this distribution test (Kolmogirnov-Smirnof) warranting a non parametric test to test the mean of the two groups.

• Mann Whitney U test gives only a few genes significant genes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Pvalue</th>
<th>pvalue corrected for ties</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAD51L1</td>
<td>0.0138121</td>
<td>0.0137555</td>
</tr>
<tr>
<td>BRCA2</td>
<td>0.014354</td>
<td>0.0141902</td>
</tr>
<tr>
<td>XRCC3</td>
<td>0.0618556</td>
<td>0.0616858</td>
</tr>
<tr>
<td>KRAS</td>
<td>0.063811</td>
<td>0.063775</td>
</tr>
</tbody>
</table>

** difficult to give any conclusion as the sample sizes are small
Box plots of significant gene expression values between pre and post samples. * Plotted for both responders and non-responders.

- **RAD51L1**
- **KRAS**
- **BRCA2**
- **XRCC3**
“WINDOW of OPPORTUNITY”
Clinical Trials

- A clinical trial design that attempts to evaluate the biological role of a certain drug *in vivo*

- It could facilitate rational drug selection, identify therapeutic failures early, and compress timelines for anticancer drug development.

- It could provide initial rationale and guiding principles for further drug development based on studies in humans (rather than xenografts, where tissues of one species are transplanted to another species).

- As it focuses on extensively characterizing how a drug works and whether it hits its intended target (including molecular imaging studies) in a limited number of patients it could yield results that would optimally inform and expedite the subsequent development of molecularly-targeted agents.

- The results can improve the efficiency and chance of success of subsequent trials.

- It could help to evaluate the effects of an agent at the molecular level.
Biological window design

Newly Diagnosed BC
Baseline
Day 14

Biopsy
Blood
TruCut
Blood

Zoledronic Acid or Trastuzumab infusion

Treatment

Tumor Ki67 assessment
Gene Profile
Blood M30/M65 ratio (apoptosis)
CTCs
Foroni C et al, “Pure anti-tumor effect of zoledronic acid in naïve bone-only metastatic and locally advanced breast cancer: proof from the “biological window therapy”
Strina C et al, “TRUP Study: Trastuzumab upfront in HER2+ve LABC”

- HER2/Trastuzumab: pCR rate 45%
- HER2/Trastuzumab: pCR rate 67%

Primary Resistance to Trastuzumab?
Adaptive Clinical Trials

- A clinical trial design that attempts to reduce cost and determine efficacy faster
- Describes class of trial designs where data is used to modify dosing or other parameters -- group sequential, staged protocols, Bayesian designs
- Bayesian Design
  - Efficacy is a probability, and the probability is re-calculated with new information on response to therapy in the trial
I-SPY 2: A Bayesian Adaptive Trial

- A neoadjuvant Phase 2 trial in women with large primary cancers of the breast (>3.0cm).
- **Compare efficacy of novel drugs in combination with standard adjuvant chemotherapy**
- The goal is to identify improved neoadjuvant treatment regimens for patient subsets on the basis of molecular characteristics (biomarker signatures)
- Regimens showing a high Bayesian predictive probability of being more effective graduate from the trial - with their biomarker signatures
- Regimens with low probability of being effective, are dropped
Tumor biopsy

Histology, Tumor Classification
Gene and Protein Profile

Routine Procedures
- Clinical Examination
- Radiological Examination
- Blood exams

PET-CT and MRI
1) basal, 2) intermediate point
3) Before definitive surgery to monitor success of the therapy

Translational Research Procedures
- Tissue, cell and blood samples collection/storage
- Protein MicroArray
- Gene Array, miRNA, SNPs, Proteomics, CTCs...
- New markers, Drugable targets
- Tumor characterization
to choose the best combination therapy suited to the molecular network defect

If the therapy fails, repeat the process and choose a new therapy combination tailored to the obtained information related to the resistant tumor

Definitive Surgery
Patient Advocate
Factors Influencing Decision to Use Neoadjuvant Chemotherapy in Operable Breast Cancer

• Does the patient need adjuvant chemotherapy based on information known prior to surgery?
• Would neoadjuvant chemotherapy potentially alter the extent of resection?
• Does the patient desire breast preservation?
• Would treatment benefit from knowledge of in vivo chemosensitivity?
## Ethical Concerns

<table>
<thead>
<tr>
<th>Research</th>
<th>Prevention</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Informed Consent for biomarkers studies</td>
<td>Impact of individual well-being</td>
<td>Higher risk for patients</td>
</tr>
<tr>
<td>Confidentiality and data-protection</td>
<td>Confidentiality and data-protection</td>
<td>Confidentiality and data-protection</td>
</tr>
<tr>
<td>Right to know/right not to know</td>
<td>Right to know/right not to know</td>
<td></td>
</tr>
<tr>
<td>Study design</td>
<td>Responsibility for health</td>
<td></td>
</tr>
</tbody>
</table>
Q1 How important is it for you to know about which drugs are available for your disease?
Q2 Do you know which treatments are available for your disease?
Q3 Do you know what a clinical study is and if there are any available for your disease?
Q4 Do you believe that a drug in a clinical study is better than what you will receive with standard treatment?
Q5 Do you know that maybe the experimental drug won't work?
Q6 Are you aware of the purposes of the samples stored from you?
Q7 Do you agree to receive multiple radiological and pathological evaluations?
"Translational research transforms scientific discoveries arising from laboratory, clinical, or population studies into clinical applications to reduce cancer incidence, morbidity, and mortality."
Acknowledgements

Pharmacogenomic and molecular Lab
- Maria Rosa
- Laura
- Michela

Clinical Trial Office
- Vanessa
- Maria Elena
- Francesca

Nurses
- Sergio
- Francesco
- Daniele
- Manuela

Clinical Unit
- Carla
- Mara
- Alberto
- Giovanni
Acknowledgements

University of Melbourne
SB Fox & H Thorne

University of Oxford
W.I.M.M
AL Harris

University of Brescia
UO Oncologia Medica
A Berruti

Casa di Cura
Figlie San Camillo
F Ferrozzi

University of Texas
MD Anderson
DJ Reuben

University of Turin
UO Oncologia Medica
L Dogliotti

Istituto Mario Negri
M Broggin & G Damia

University of Brescia
Dip Oncologia Sperimentale
PG Petronini et al

Unità di Patologia Mammaria
U.S Terapia Molecolare e Farmacogenomica
A.O. Istituti Ospitalieri di Cremona