Management of breast cancer: 
past, present and future

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Adjuvant chemotherapy: Which patient?
Luminal A-like patients do not seem to derive benefit from adjuvant chemotherapy

N- ER+ pre- and post-menopausal
TAM vs CMF/MF + TAM

N+ ER+ post-menopausal
TAM vs CAF + TAM

Paik S et al, J Clin Oncol 2006
Albain K et al, Lancet Oncol 2010
Main limitations of the presented studies

- Modest sample size $\Rightarrow$ potential risk of false positive/negative results

- Retrospective design $\Rightarrow$ mainly hypothesis-generating studies
Contrasting evidence on the role of adjuvant chemotherapy in ER+ tumors is provided by the EBCTCG overview.

- The most recent results provided by the EBCTCG overview are based on ± 100,000 women and 123 randomised trials.

- The overview results suggest that adjuvant chemotherapy proportional benefits are independent of patient’s age, nodal status, ER status, and histology grade.
Issues limiting the use of the overview results on adjuvant chemotherapy in clinical practice in 2015

- ER + tumors are clinically heterogeneous

- Results for the ER + patients aged < 55 may at least in part be related to ovarian function suppression due to chemotherapy, and they may tend to over-estimate benefits deriving from chemotherapy cytotoxicity

- The population evaluated in the overview does not seem to represent the majority of the population seen in today’s practice
Conclusions: can we avoid the use of adjuvant chemotherapy in Luminal A breast cancer?

- Lack of strong evidence in favour of, or against, adjuvant chemotherapy in Luminal A breast cancer

- Prospectively-designed trials are ongoing (Rx-Ponder, Mindact, TailorX)

- In the meantime

  Adjuvant chemotherapy
  
  $N \text{ neg.}$  $N + 1-3$  $N + \geq 4$
  No      Maybe     Yes

! Consider the patient’s wishes and expectations
Once the decision is made to give adjuvant chemotherapy, should an anthracycline-taxane regimen be the standard of care for all patients?
**Chemotherapy regimens by intrinsic subtype (My personal opinion)**

- Considering the lack of bio-markers with proven predictive value for the activity of individual regimens, risk (pT, pN) may still be an important factor to select the most appropriate regimen.

<table>
<thead>
<tr>
<th>Luminal A</th>
<th>Luminal B</th>
<th>HER-2 +</th>
<th>Triple negative*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low risk</td>
<td>No chemo</td>
<td>TC CMF</td>
<td>TCH +H T+H</td>
</tr>
<tr>
<td>High risk</td>
<td>TC CMF</td>
<td>A → T</td>
<td>A → T+H</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>A → T</td>
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</table>

* Consider platinum compounds if BRCA mutation

Challenges for the future

- Intra-tumor heterogeneity: should we target adjuvant therapy on the most prevalent tumor cell type identified? or the most biologically-aggressive tumor cell type identified?

- Improving the definition of risk of disease relapse
A considerable proportion of early breast cancer patients not receiving adjuvant systemic therapy do not relapse.

CMF

surgery

no adjuvant systemic therapy

N+
Median f-up 28.5 yrs.

N- ER-
Median f-up 20 yrs.

RFS%

Bonadonna G et al, BMJ 2005
A considerable proportion of ER+ “high risk” patients treated with tamoxifen as the only adjuvant therapy do not relapse.

- **N- ER+**
  - Tamoxifen
  - Chemo → tamoxifen

- **N+ ER+**
  - Tamoxifen
  - Chemo → tamoxifen

* Paik S et al, J Clin Oncol 2006; ** Albain K et al, Lancet Oncol 2010
Present and Future

Year 2015

to define risk of disease relapse

to define responsiveness to adjuvant therapies

Year 20...

to define responsiveness to adjuvant therapies (The Predictor)

to define risk of disease relapse (The Detector)
Can metabolomics predict the risk of disease relapse?

- To explore the value of metabolomic spectra from serum samples of early breast cancer patients in predicting disease relapse

- Potential advantage of metabolomics: data are collected from peripheral sites and are host- and tumor- derived
Key-message from our pilot study: early and advanced breast cancer patients have different metabolomic profiles

- Of 48 pre-operative cases, 36 are correctly classified
- Of 51 advanced cases, 35 are correctly classified
  (accuracy 71.7%)

The second study

Breast Medicine Service  
Memorial Sloan-Kettering  
Cancer Center  
New York

clinical data

“Sandro Pitigliani” Dept. of Medical Oncology, Prato, Istituto Toscano Tumori

Data-analysis and study reports

Serum samples from breast cancer patients

Magnetic Resonance Center (CERM)  
University of Florence

Metabolomic data

Study design

Early breast cancer (EBC) patients (N = 80) → breast surgery → serum samples → $^1$H-NMR-based metabolomic spectra*

Metastatic breast cancer (MBC) patients (N = 95) → Serum samples (before a new line of therapy) → $^1$H-NMR-based metabolomic spectra*

EBC pts. split to form a training set (N = 40 pts.) and a validation set (N = 40 pts.)

* by a Bruker 600 MHZ metabolic profiler
Methods

- First step: to establish if metabolomic spectra could distinguish between EBC and MBC pts.

- Second step: to assign a metabolomic score (MS) of relapse to each EBC pt. from the training set using a Random Forest classifier

- Third step: to correlate the MS with actual clinical outcome (performance of the MS evaluated by ROC curves)

- Fourth step: to replicate the analysis using the spectra from the validation set
Metabolomic spectra from MBC and EBC patients

Discrimination between metastatic (green) N = 95 and early (red) N = 40

Accuracy: 83.7%

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Random forest: steps

1) Each sample from EBC patients evaluated through 100 decisional algorythms

2) Each algorythm made of different metabolites

3) Each sample from EBC pts. classified by each algorythm as “early” or “advanced”, based on metabolites level of expression

4) The number of times a given sample from EBC pts. is miss-classified as “advanced” indicates the “Metabolomic score”
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ROC curve for the Metabolomic Score from EBC patients (training set)

AUC = 0.863

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ROC curve for the Metabolomic Score from EBC patients (validation set)

AUC = 0.824

Next steps

- Repeat the study in a series of EBC patients treated at a different center and, possibly not treated with adjuvant systemic therapies

- Use the MS information to stratify patients classified as low score or high score by Oncotype Dx® (ongoing in collaboration with the Memorial Sloan-Kettering Cancer Center)
A new challenge for clinicians: the biopsy of a metastatic site
Size of the problem

Analysis of 29 studies comparing ER and HER-2 status between matched primary and metastatic tumor samples

Pitfalls for most of the reported studies

• Most of the studies have a retrospective design and a limited sample size

• Importantly, in most of these studies ER and HER-2 status from the primary and the metastatic sites has not been re-assessed at the same time using the same technical procedures
Impact of a change in ER or HER-2 status in physician’s treatment decisions

<table>
<thead>
<tr>
<th></th>
<th>ER loss</th>
<th>ER gain</th>
<th>HER-2 loss</th>
<th>HER-2 gain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases</td>
<td>37</td>
<td>30</td>
<td>69</td>
<td>80</td>
</tr>
<tr>
<td>Decision</td>
<td>stop hormonotherapy</td>
<td>start hormonotherapy</td>
<td>stop anti-HER-2</td>
<td>start anti-HER-2</td>
</tr>
<tr>
<td>Percentage</td>
<td>22/37 (59%)</td>
<td>13/30 (43%)</td>
<td>41/69 (59%)</td>
<td>61/80 (76%)</td>
</tr>
</tbody>
</table>

Evaluated in 14 studies

Is the rate of change in physician’s treatment decisions the most clinically relevant parameter?
Impact on clinical outcome derived from treatment change (HER-2 negative → HER-2 positive treated with anti-HER-2 based therapy)

Evaluated in 2 of the 14 studies assessing the impact on physician’s treatment decisions

<table>
<thead>
<tr>
<th>Chang et al.</th>
<th>Fabi et al.</th>
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<tbody>
<tr>
<td>• +/- vs. -/+</td>
<td>• +/- vs. -/+</td>
</tr>
<tr>
<td>• Median OS 32 vs. 11 mos.</td>
<td>• Median TTP 10.3 vs. 5.2 mos.</td>
</tr>
<tr>
<td>• ORR 69 vs. 40%</td>
<td></td>
</tr>
</tbody>
</table>

Chang HJ et al, Jpn J Clin Oncol 2011; Fabi A et al, Clin Cancer Res 2011
Conclusions

• Despite the evidence of a shift in ER and/or HER-2 status between the primary and the metastatic sites is well documented,

• Despite the change in physician’s treatment decisions due to the shift in receptors status is also well documented,

We are lacking good evidence on the clinical activity of the treatment given because of a documented receptors shift
Current indications in clinical practice: my opinion

- Doubts on the presence of metastatic disease (single lesion)

- No (reliable) data on HRs and HER-2 status at the primary tumor site

- HER-2 negative (primary tumor) patient heavily pre-treated, still fit for additional therapies and lack of valid options (???)
The future: free-tumor DNA (ft DNA) and circulating tumor cells (CTC)

In favour of ft DNA

• reduced cost

• ft DNA found in the majority of pts.

In favour of CTC

• comprehensive evaluation of DNA and RNA

• assessment of tumor clonality
**DNA extraction from circulating tumor cells and gene sequencing is feasible**

MBC patients untreated for at least 3 weeks

- **Whole blood**
- **DEPArray™ system:** CTCs count and sorting: single CTCs or a poll of pure CTCs, putative CTCs and WBC

- **CellSearch**
  - CTCs enrichment
  - EpCAM
  - EpCAM + CD146

- **Analyser**
  - CTCs count

- Cut off CTC $\geq 5$
- 900 $\mu$l
- 14 $\mu$l

- **Whole genome amplification**
  - Ampli1 WGA Kit

- **Ion Torrent** (Ion AmpliSeq technology) to detect mutations in a panel of 50 target genes and/or in other genes (customised probes)

**Whole genome amplification**

- Ampli1 WGA Kit

- **Ion Torrent** (Ion AmpliSeq technology) to detect mutations in a panel of 50 target genes and/or in other genes (customised probes)
A pilot study from our group testing the feasibility of detecting PIK3CA gene mutations from CTC of advanced breast cancer patients

- 21 ER+ pts. with $\geq 5$ CTC/7.5 ml blood sample (by CellSearch®)

  15 pts.

  No PIK3CA mutations

  6 pts. with PIK3CA mutations

    4 pts. with mutations at exons 9 or 20 (confirmed in all recovered CTC, range 1-4)

    1 pt. with LOH (8 CTC)

    1 pt. with heterogeneity between CTC (19 CTC)

Next Generation Sequencing

• 10 ng of DNA obtained from primary tumor tissue, plasma and single CTCs
• Ion Torrent platform
• The Ion AmpliSeq™ Cancer Panel targets 50 cancer related genes

• The presence and frequency of a variant were interpreted through the dbSNP, 1000genomi, esp6500 and COSMIC systems
• The pathogeneity of the mutation was analysed with PolyPhen and SNPeff
NGS

Analysis of single CTCs, plasma and primary tumor tissue from pt 11; pt 12 and pt 18
Our current Phase II randomized study comparing DNA damaging vs. DNA non-damaging cytotoxics in advanced breast cancer patients

Eligible pts
- advanced disease
- pre-treated or not (0-2 lines)
- CTC + blood sample
- access to primary tumor sample

Treatment arms
- DNA non-damaging regimen (capecitabine + oral vinorelbine)
- DNA damaging regimen (Cisplatin + cyclophosphamide)
Acknowledgments