Lobular carcinomas: not a single molecular entity

8th of September 2019
European congress of Pathology

Anne Vincent-Salomon
Institut Curie, Paris, FRANCE
Lobular carcinomas: not a single molecular entity

- Introduction:
  - Clinical features
  - Pathological features
  - Prognosis; metastatic pattern; response to treatment
- Phenotype and histo-molecular classes
- Stroma characteristics (TILs)

- Molecular characteristics:
  - Transcriptomic heterogeneity
  - Genomic alterations of primary ILC
  - Genomic alterations of metastatic ILC
- Conclusions
Introduction: Infiltrating lobular carcinomas (ILC)

• Original definition: invasive form of the carcinoma arising in lobules and terminal ducts (Foote and Stewart 1941)

• 2\textsuperscript{nd} most frequent type of invasive carcinomas
  ▪ 10 to 15\% of invasive breast tumors in Western world
  ▪ 5\% in Asia, Africa and Middle East

• Increase of incidence in the past decades (women > 50 years):
  ■ ILC better identified by pathologists
  ■ Impact of the hormone replacement therapies
  ■ Mammography screening & MRI

Clinical presentation

• **Age:**
  - Older than IC-TNS
  - 2% of carcinomas before 35 years old / 11% after 75 years old

• **Hereditary ILC are rare**
  - CDH1 germline mutation (hereditary diffuse gastric cancer syndrome)
  - Possible in BRCA2 patients, but almost never in BRCA1 patients

• **Clinical presentation:**
  - Vague area of thickening or induration without definable margins
  - Skin retraction or nipple retraction
  - More frequently centrally located

• **Higher risk of bilateral cancer (RR = 1.5 to 1.8) (meta-or synchronous)**

• **Multifocality / multicentricity : 31% (x2 >> IC-TNS)**

Christgen *et al* Pathology-Research and Practice 2016; Pestalozzi *et al* JCO 2008
Histopathology: Classical ILC

- Dyscohesive small cells
- Homogeneous aspect
- Intracytoplasmic vacuoles or pseudo-lumens
- No necrosis

Concentric pattern around normal ducts
Single file and linear cords in the stroma
Infiltration of the adipose tissue
Histopathology: ILC variants

Architectural

Solid variant (16%)
- solid sheets of uniform and dyscohesive cells with more mitoses and anisocaryosis

Alveolar (16%)
- Clusters of cells, that lack cell to cell cohesion, separated by thin bands of stroma

Trabecular (7%)

Cytonuclear

Pleomorphic (14%)
- nuclear grade 3, higher rate of mitoses (2.5 to > 10 mitoses / 10 HPF)

Apocrine, Histiocytoïd, Signet ring cells
Histological grade (Elston and Ellis)

### Grade I
Scores 3 to 5

### Grade II
Scores 6 to 7

### Grade III
Scores 8 to 9

#### Distribution of ILC cases among three categories of histological grade.

<table>
<thead>
<tr>
<th>References</th>
<th>Number of ILC cases</th>
<th>Histological Grade 1 (%)</th>
<th>Histological Grade 2 (%)</th>
<th>Histological Grade 3 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sastre-Garrau et al. [39]</td>
<td>726</td>
<td>48</td>
<td>43.5</td>
<td>3</td>
</tr>
<tr>
<td>Talman Moller et al. [6]</td>
<td>860</td>
<td>20</td>
<td>74</td>
<td>6</td>
</tr>
<tr>
<td>Rakha et al. [29]</td>
<td>517</td>
<td>12</td>
<td>76</td>
<td>12</td>
</tr>
<tr>
<td>Iorfida et al. [30]</td>
<td>981</td>
<td>35</td>
<td>52</td>
<td>15</td>
</tr>
<tr>
<td>Toikkanen et al. [42]</td>
<td>217</td>
<td>34</td>
<td>54</td>
<td>12</td>
</tr>
<tr>
<td>Du Toit et al. [32]</td>
<td>171</td>
<td>20</td>
<td>64</td>
<td>16</td>
</tr>
<tr>
<td>Bane et al., 2005 [40]</td>
<td>50</td>
<td>20</td>
<td>66</td>
<td>14</td>
</tr>
<tr>
<td>Mathieu et al. [41]</td>
<td>38</td>
<td>3</td>
<td>97</td>
<td>0</td>
</tr>
</tbody>
</table>

Guiu et al Critical Reviews in Oncology/hematology 2014
• Importance of the mitotic index to determine the prognosis of the pleomorphic (nuclear score 3) ILC
## Prognosis of ILC:

Poorest, better or similar than that of IC-TNS?

<table>
<thead>
<tr>
<th>Prognosis (comparison ILC and IDC)</th>
<th>Number of studies/endpoint</th>
</tr>
</thead>
<tbody>
<tr>
<td>OS</td>
<td>BCSS</td>
</tr>
<tr>
<td>Better outcome of ILC</td>
<td>5</td>
</tr>
<tr>
<td>Similar outcome of ILC</td>
<td>17</td>
</tr>
<tr>
<td>Worse outcome of ILC</td>
<td>1</td>
</tr>
<tr>
<td>According to follow-up duration</td>
<td>1</td>
</tr>
</tbody>
</table>

ILC—invasive lobular carcinoma; IDC—invasive ductal carcinoma; OS—overall survival; BCSS—breast cancer specific survival; DFS—disease-free survival.

---

**DFS**

**OS**

![Graphs showing DFS and OS at 6 and 10 years for different groups: ER-, ER+, All patients, 767 ILC, 8607 IDC](image)

*Guiu et al.* Critical Reviews in Oncology / Hematology, 2014

*Pestalozzi et al.* JCO 2008;

IBCSG trials between 1978 and 2002
Distant metastases in ILC

- More bone and less lung metastases
- in atypical sites: peritoneum, ovary, digestive tract, skin, meninges….

- Rate of multiple metastases higher in ILC (25%) than in IDC (15.8%) \((p=0.016)\).

## Surgical treatment

<table>
<thead>
<tr>
<th>Type of surgery</th>
<th>ILC</th>
<th>IDC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mastectomy</td>
<td>29 to 71%</td>
<td>56.2%</td>
</tr>
<tr>
<td>Conservative surgery</td>
<td>28.2 to 71%</td>
<td>44 to 47%</td>
</tr>
<tr>
<td>Positive margins</td>
<td>6.7 to 43*%</td>
<td>2.2 to 16*%</td>
</tr>
</tbody>
</table>

* After neoadjuvant CT

ILC response to therapy

- Low response rate to neoadjuvant chemotherapy: pCR = 7.8% of the cases

- Response to hormonotherapy (BIG -1-98) benefit of Letrozole versus Tamoxifen (ILC n= 324)

- Benefit of adjuvant endocrine therapy + chemotherapy for « high risk » ILC:
  - pathological score based on: size > 2cm; LVI+; pN1
  - DFS: HR=0.52 [0.36-0.75]; p<0.001
  - OS: HR=0.37[0.24-0.57]; p<0.001

ILC biological current features
E-cadherin: adhesion family of transmembrane protein

- E-cadherin loss of expression in 85% of the cases
  - Inactivation by truncating mutations or epigenetic mechanisms
  - Does not preclude the diagnosis of ILC
  - Helpful when diagnosing ILC variants or mixed histological types (ILC + IC-TNS)

ILC phenotype and histo-molecular classes

- ER+ > 90% of the cases
- ER+ PR- HER2 - 10% of the cases
- Low proliferation

- AR + in ~ 70 to 87% of the cases

- Histo-molecular classes:
  - Luminal A: 41 to 83% of the cases
  - Luminal B in up to 77% of the variant ILC (alveolar, solid…)
  - HER2 positive (score 3+) < 5% of the cases
  - ILC triple negative ~ 1,5% of the cases

Transcriptomic prognostic signatures in ILC

• Able to provide prognostic informations but not developed for predicting late recurrence of ILC

• Rate of ILC classified in high risk group:
  - Oncotype DX: 2%
  - Mammaprint: 24%
  - Genomic grade: 17%

Beumer et al Biomark Insights 2016; Metzger-Filho et al Ann Oncol 2013
Stroma characteristics
TILs in ILC (Inst Bordet’s study)

• TILS density is lower compared to IDC (n ILC = 149)
• Mean TILs value: 5%

• Higher in patients < 50 years, > 2cm, N+, high Ki67 (>20%)
• Higher TILs in ILC variants but lower TILs in the alveolar variant

• TILs > 5% associated with worse prognosis in univariate analysis

Desmedt et al JNCI 2018

less or no CD8 and M1 macrophages in ILCs
TILs in ILC (Institut Curie’s study)

- Retrospective quantification of TILs in 459 lobular carcinomas
- 8.8 years of follow-up

- Mean value of 5%

- Higher TILs associated with:
  - < 50 y, higher grade, tumoral size, N+
  - Multinucleation, macronucleolus
  - Her 2 amplification

- Prognostic impact in multivariable analysis:
  - worse RFS (≤ 5% HR: 1.91 versus > 5% HR: 3.10; p=0.004)
  - worse OS (≤ 5% HR: 4.38 versus > 5% HR: 6.15; p<0.001), independently of chemotherapy.

Tille et al, manuscript in preparation
Molecular characteristics of ILC
ILC and luminal IDC have distinct transcriptomic signatures

Transcriptomic heterogeneity of ILC
Different mRNA ILC subtypes: the TCGA ILC study

N= 106 (only LumA)

In the immune-related: upregulation of
- Cytokines Chemokine expression
- Macrophages associated signalling
- T-cell receptor gene expression signatures

Ciriello et al. Cell 2015
Different mRNA subtypes: the RATHER consortium

Immune-related:
- Upregulation of cytokines, chemokines
- Innate immunity
- Tumors TILs rich

Hormone related
- Upregulation of ER, PR
- And cell-cycle genes

Genomic landscape of *primary* ILC
>2000 ILC (primary and met) samples have been sequenced

<table>
<thead>
<tr>
<th>Initiative</th>
<th>Nr of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCGA (Ciriello Cell 2015)</td>
<td>127</td>
</tr>
<tr>
<td>Rather consortium (Michaut Sci Rep 2016)</td>
<td>144</td>
</tr>
<tr>
<td>METABRIC+ (Pereira Nat Comm 2016)</td>
<td>194</td>
</tr>
<tr>
<td>Bordet &amp; Europe (Desmedt JCO 2016)</td>
<td>414</td>
</tr>
<tr>
<td>Foundation Medicine (Ross Cancer 2016)</td>
<td>315</td>
</tr>
<tr>
<td>MSK-IMPACT (Razavi Cell 2018)</td>
<td>275 (ongoing)</td>
</tr>
<tr>
<td>The Metastatic Breast Cancer project</td>
<td>18 (ongoing)</td>
</tr>
<tr>
<td>Foundation Medicine &amp; Oesterreich’s group (Sokol Ann Oncol 2018)</td>
<td>336 +180</td>
</tr>
<tr>
<td>Meta EuroILC (Desmedt NPJ Breast 2019)</td>
<td>80</td>
</tr>
</tbody>
</table>

Slide: Courtesy of Pr C Desmedt
**CDH1 (E-cadherin) alterations**

- Are across all *E-cadherin* sequence
- Mostly truncating mutations
- Associated with
  - LOH of the 16q in ILC
  - Low protein expression

*Ciriello et al. Cell 2015*
Driver genes in primary ILC

Desmedt et al.  
n=414

Ciriello et al.  
n=127

Michaut et al.  
n=138
Comparison of ILC with IDC

TCG: Ciriello et al Cell 2015
40% to 50% of ILC have an alteration converging to AKT activation

Figure 4. Akt Signaling Is Highest in ILC Tumors
(A) Differential protein and phospho-protein analysis between ILC LumA and IDC LumA reveals significant lower levels of PTEN, and higher levels of Akt, phospho-Akt, EGFR, phospho-EGFR, phospho-STAT3, and phospho-p70S6K in ILC LumA.
**HER2 mutations: higher in grade 3 and pleomorphic ILC**

- TCGA: 7.7% (3/39)
- Desmedt *et al*: 5.1%
- Huang-Chun *et al*:
  - 2% (1/49) in classic ILC
  - 20.8% (5/24) in pleomorphic carcinomas

- Institut Curie’s experience: in grade 3 ILC
  - 15% (6/39) (IC95% = [4;27])
  - 5 out of 8: L755S (exon 19)
  - 2 cases harbouring 2 mutations

TCGA. Nature 2012.
Genomic alterations in classical ILC and sub-types

Desmedt et al JCO 2016
Genomic landscape of metastatic ILC
Targeted sequencing metastatic ILC

Patient Characteristics:
Sex, Age, Site of Collection

Comprehensive Genomic Profiling:
395 genes, MSI status

Mutational Burden:
Sample Level mutations/mb

Sokol et al. Ann Oncol 2018
Targeted sequencing metastatic ILC & IDC

More frequent in ILC:
- **CDH1** (76% vs 6.8%),
- **NF1** (12.2% vs 3.1%),
- **TBX3** (12.8% versus 3.7%)
- **PIK3CA** (52.8% vs 39.8%).

More frequent in IDC:
- **MYC** (23.6% vs 3.9%),
- **GATA3** (15.2% vs 2.2%),
- **FGFR1** (18.3% vs 7.8%),
- **TP53** (37.2% vs 23.9%).
Targeted sequencing primary & metastatic ILC

- More *ESR1* and *NF1* mutations in Meta ILC
- Are mutually exclusive
- Both involved in resistance to hormonotherapy
**ESR1 mutations in metastatic ILC**

No difference in type or prevalence of *ESR1* mutations in metastatic ILC or IDC patients.
Conclusions: ILC are not a single entity!

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Older patients</td>
<td>E-cadherin – (can be + in 15%)</td>
</tr>
<tr>
<td>Non-cohesive cells</td>
<td>Histological subtypes with molecular specificities</td>
</tr>
<tr>
<td>Low number of TILs</td>
<td>ER+ , Grade II, Low Ki67, HER2 - , AR+</td>
</tr>
<tr>
<td>TILs rich ILC: poor outcome</td>
<td>Lum A &amp; B (but HER2 or TN exist)</td>
</tr>
<tr>
<td>Bone and Atypical metastatic sites</td>
<td>2 or 3 transcriptomic sub-groups (one being immune enriched)</td>
</tr>
<tr>
<td>Poor long term outcome</td>
<td>Activation of PIK3CA / AKT pathway</td>
</tr>
<tr>
<td>Benefit from Letrozole and CT</td>
<td>HER2 mutation in grade 3 and non-classic subtypes</td>
</tr>
<tr>
<td></td>
<td>Metastatic ILC: NF1 &amp; ESR1 mutually exclusive mutations</td>
</tr>
</tbody>
</table>
Thank you!