LOCALLY ADVANCED BREAST CANCER

Definition and biology

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Laboratoire National de Santé, Luxembourg
General-Secretary of the International Academy of Cytology
Management of locally advanced breast cancer—perspectives and future directions

Konstantinos Tryfonidis, Elzbieta Senkus, Maria J. Cardoso and Fatima Cardoso

Abstract | Locally advanced breast cancer (LABC) constitutes a heterogeneous entity that includes advanced-stage primary tumours, cancers with extensive nodal involvement and inflammatory breast carcinomas. Although the definition of LABC can be broadened to include some large operable breast tumours, we use this term to strictly refer to inoperable cancers that are included in the above-mentioned categories. The prognosis of such tumours is often unfavourable; despite aggressive treatment, many patients eventually develop distant metastases and die from the disease. Advances in systemic therapy, including radiation treatment, surgical techniques and the development of new targeted agents have significantly improved clinical outcomes for patients with this disease. Notwithstanding these advances, LABC remains an important clinical problem, particularly in developing countries and those without widely adapted breast cancer awareness programmes. The optimal management of LABC requires a multidisciplinary approach, a well-coordinated treatment schedule and close cooperation between medical, surgical and radiation oncologists. In this Review, we discuss the current state of the art and possible future treatment strategies for patients with LABC.

LOCALIZED ADVANCED BREAST CANCER (LABC) DEFINITION

• T0-T3 primary tumours with clinically detectable axillary, ipsilateral infraclavicular, supraclavicular or internal mammary lymph nodes (N2-N3) or tumour extension to the chest wall or skin (T4) regardless of nodal status.

• Inflammatory breast carcinoma (IBC)
LOCALIZED ADVANCED BREAST CANCER (LABC) DEFINITION

- Tumours more than 5 cm in size with regional lymphadenopathy (N1–3)
- Tumours of any size with direct extension to the chest wall or skin, or both (including ulcer or satellite nodules), regardless of regional lymphadenopathy
- Presence of regional lymphadenopathy (clinically fixed or matted axillary lymph nodes, or any of infraclavicular, supraclavicular, or internal mammary lymphadenopathy) regardless of tumour stage

LOCALIZED ADVANCED BREAST CANCER (LABC)
DEFINITION

• “INOPERABLE CASES WITH INFLAMMATORY AND/OR EXTENSIVE SKIN INVOLVEMENT, FIXED OR VERY BULKY AXILLARY NODAL DISEASE AND/OR SUPRACLAVICULAR OR INTERNAL MAMMARY NODAL INVOLVEMENT”.

LOCALIZED ADVANCED BREAST CANCER (LABC)
DEFINITION
8.5% of American and 4% of European patients, however can reach as high as 60% in low-resources countries.

This percentage decrease in populations undergoing regular screening programmes.

Risk factors (IBC): race (black and Hispanic), obesity, young age at first delivery, rural residence and longer cumulative duration of breastfeeding.
For primary IBC the 5-Y survival range from 25% to 48% and it is worst than non-inflammatory LABC.
LOCALIZED ADVANCED BREAST CANCER (LABC)  
HISTOLOGICAL TYPE

Ductal

Lobular

Tubular

Medullary

Micropap

Pleom. lobular

Mucinous

Lipid-rich

Metaplastic

Secretory
LOCALIZED ADVANCED BREAST CANCER (LABC) MOLECULAR SUBTYPE

LUMINAL A: ER+/PgR+/HER2-
LUMINAL B: ER+/PgR+/HER2+ and or Ki67+
HER-OE: ER-/PgR-/HER2+
BASAL-LIKE: ER-/PgR-/HER2-/Basal Markers
CLAUDIN-LOW: ER-/Pg-/HER2-/Claudinlow

Phenotypic and molecular characterization of the claudin-low intrinsic subtype of breast cancer

Alex Prat1,2, Joel S Parker1,2, Olga Karginova1,2,3, Cheng Fan1, Chad Livasy1,3, Jason I Herschcowitz4, Xiaping He1,2,3, Charles M Perou1,2,3

Graph showing relapse-free survival by molecular subtype with p=7.67e-06.
INFLAMMATORY BREAST CARCINOMA (IBC) DEFINITION

• “When a purple colour is on the skin over the tumour it is a very unpropitious beginning”. Charles Bell, 1807

• 1924: Lee and Tannenbaum proposed the currently used term inflammatory carcinoma.

• « A clinical and pathological entity characterized by erythema and oedema involving a third or more of the skin of the breast » - AJCC definition.
INFLAMMATORY BREAST CARCINOMA (IBC)

- 1-5% of invasive breast carcinomas
- 8.5% of LABC
INFLAMMATORY BREAST CARCINOMA (IBC)
PATHOLOGY
INFLAMMATORY BREAST CARCINOMA (IBC) PATHOLOGY
INFLAMMATORY BREAST CARCINOMA (IBC)
PATHOLOGY

• Macroscopically the tumours are frequently indistinct, present throughout the breast.

• Histologically are IDC, grade 3, frequently present in lymphatic spaces in dermis.

• Cutaneous ulceration and Paget disease are rare.
INFLAMMATORY BREAST CARCINOMA (IBC) IMMUNOHISTOCHEMICAL PROFILE

• More frequently ER and PR negative.
• HER2 more frequently positive (41%) than non IBC.
• P53 positive in up to 84% of the cases.
• High KI 67 index
• E-cadherin positive
• High angiogenic index
INFLAMMATORY BREAST CARCINOMA (IBC)
E-CADHERIN EXPRESSION
Classical Cadherin family

Epithelial cadherin — E-cadherin
Placental cadherin — P-cadherin

Cadherin/catenin complexes

- Cell Shape and polarity
- Cytoskeleton Organization
- Differentiation
- Cell Motility
- Signaling pathway

P-Cadherin Overexpression Is an Indicator of Clinical Outcome in Invasive Breast Carcinomas and Is Associated with CDH3 Promoter Hypomethylation

Joana Paredes,1 André Albergaria,1 João T. Oliveira,1 Carmen Jerónimo,2,3 Fernanda Milanezi,1,5 and Fernando C. Schmitt1,4

Clin Cancer Res 2005;11 (16) August 15, 2005

Disease Free-Survival

Overall Survival

P-cadherin expression is significantly associated with decreased survival in a short-term follow-up (≈ 5 years after diagnosis)
E- and P-cadherin role in cancer progression

Mechanisms involved in the process of protein inactivation or downregulation:
• gene mutations
• loss of heterozygosity
• expression of E-cadherin repressors

Mechanisms involved in the process of protein aberrant expression:
• promoter hypomethylation
• inactivation of P-cadherin transcription repressors (BRCA1, ER-α)
• induction of P-cadherin transcription activators (cEBP-β, p63 and β-ctn)

Promoter hypermethylation

TUMOR/INVASIVE PROMOTING FUNCTION

P-cadherin

TUMOR/INVASIVE SUPPRESSIVE FUNCTION

E-cadherin
Invasive Lobular Carcinoma
- Represent about 10-15% of all invasive breast carcinomas
- Loss of cell-cell adhesion (E-cadherin negative)

Invasive Ductal Carcinoma
- Most common type of breast cancer
- Represent 85–90% of all breast cancers
- Maintenance of cell-cell adhesion (E-cad positive)

E-cadherin expression is retained:
- mouse 4T1 highly metastatic breast cancer cell model
- inflammatory breast cancer
- derivative metastases

E- and P-Cadherin co-expression in breast tumors is associated with a decreased survival time and is a significant poor prognostic predictor for breast cancer patients.
IN VITRO BREAST CANCER CELL MODELS

B.

<table>
<thead>
<tr>
<th>Immunofluorescence</th>
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<th>siPcad siEcad</th>
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<th>E/P-</th>
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Breast Carcinoma
Primary Inflammatory Breast cancer
E and P-Cadherin co-expression: invasion and growth

P-cad expression in a E-cad wt background, promotes cell invasion

E- and P-cadherin expressing or none cadherin expressing cells show similar invasive behaviour

E and P-cadherin interactions in breast cancer

Ribeiro AS et al. J Pathol 2013

E- and P-cadherin proteins are in close proximity in co-expressing tumors

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<td>DAPI</td>
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E-cadherin/P-cadherin tumor

Normal mammary gland
P-Cadherin expression delocalizes p120ctn to the cytoplasm and activates signaling pathways.

P-cad overexpressing cells show increased delocalization of p120ctn to cytoplasm, activation of Src Kinase and Rac.
Cadherin/catenin complex in tissue samples

149 invasive breast carcinomas

E- and P-cadherin co-expressing tumors show increased catenins cytoplasmic localization

P-cadherin and metastasis
4T1 metastatic breast cancer model

Orthotopic injection of 4T1 cells (100,000) in the mammary fat pad.

3 groups injected with:
• 4T1 cell line
• 4T1 shRNA control
• 4T1 shRNA P-cadherin

Tumor volume (mm³)

Metastasis number at the lung surface

In collaboration with Françoise Bono, Sanofi Aventis Recherche & Développement, Toulouse, France
Financial support: SAR&D
Schematic representation of the different types of breast cancer in what concerns cadherin expression

**Tumors:**
- **Good prognostic tumors**
  - Cells with:
    - Intercellular adhesions
    - Low ability to migrate
    - Low ability to invade
    - Sensitive to apoptosis
    - Low tumorigenic potential

- **Under represented group**
  - Cells with:
    - Intercellular adhesions
    - **High ability to migrate**
    - Low ability to invade
    - Resistant to apoptosis
    - Moderate/Low tumorigenic potential

- **Poor prognostic tumors**
  - Cells with:
    - Without intercellular adhesions
    - Low ability to migrate
    - Low ability to invade
    - Resistant to apoptosis
    - High tumorigenic potential

**E-cadherin expressing cells**
- $E^+/P^-$

**P-cadherin expressing cells**
- $E^-/P^+$

**E/P-cadherin co-expressing cells**
- $E^+/P^+$

**E/P-cadherin negative cells**
- $E^-/P^-$

Ribeiro AS et al. J Pathol 2013
INVASION and MIGRATION

Nature Reviews | Molecular Cell Biology

CK 5/6 Immunostaining
Basal epithelial cell associated gene cluster

Epidermal growth factor receptor

P-cadherin

Adherens Junctions

Focal Adhesions

Integrins (cten)

Molecular portraits of human breast tumours

Proximity ligation assays: a recent addition to the proteomics toolbox

Phase II Study of Predictive Biomarker Profiles for Response Targeting Human Epidermal Growth Factor Receptor 2 (HER-2) in Advanced Inflammatory Breast Cancer With Lapatinib Monotherapy


Table 4. Molecular Phenotype of Responding and Nonresponding Patients in Cohort A

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Responders (n = 15)</th>
<th>Nonresponders (n = 15)</th>
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<tr>
<td></td>
<td>No. Affected</td>
<td>Total</td>
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<tr>
<td>HER activation status</td>
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<tr>
<td>pEGFR</td>
<td>5</td>
<td>11</td>
</tr>
<tr>
<td>pH2.2</td>
<td>11</td>
<td>12</td>
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<tr>
<td>pH2.3</td>
<td>10</td>
<td>12</td>
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<tr>
<td>ErbB ligands</td>
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<td>Heresulinin</td>
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<td>TGF-α</td>
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<td>IBC phenotype</td>
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<td>IGF-1R</td>
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<td>PheC</td>
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<td>Apoptosis and tumor</td>
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<td>suppressors</td>
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<td>PTEN deficient</td>
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<td>p53</td>
<td>2</td>
<td>9</td>
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<td>Bcl2</td>
<td>5</td>
<td>10</td>
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<tr>
<td>β-catenin</td>
<td>12</td>
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</table>

Abbreviations: HER, human epidermal growth factor receptor; p-, phosphorlated; EGFR, epidermal growth factor receptor; TGF, transforming growth factor; IBC, inflammatory breast cancer; ER, estrogen receptor; PR, proges- terone receptor; IGF, insulin-like growth factor; PTEN, phosphate and tensin homolog 10.

†P = .031
‡P = .033

Courtesy from Ola Söderberg –Upsalla-Sweden
Identification of motility promoting genes driven by EGF Receptor Signaling

CTEN
SYNJ 2

Amit et al., Nat Gen 2007
Tensin relief facilitates migration

Yuliya Pylayeva and Filippo G. Giancotti

Focal complex  Focal adhesion  Fibrillar adhesion

β₁ cytoskeleton phosphorylation?

TENSIN FAMILY

binds to the side of F-actin

tenzin

ABD Ia b/FAB-N

450

1

1433

1735

ABD II

SH2

PTB

tenzin2

C1

ABD Ia b/FAB-N

125

558

1097

1410

tenzin3

ABD Ia b/FAB-N

435

1123

1459

cten

418

715

mRNA expression (MAS5 values)

A reciprocal tensin-3–cten switch mediates EGF-driven mammary cell migration

Menachem Katz1, Ido Amit1, Ami Citri1, Tal Shai1, Silvia Corvalho1, Sara Lavi2, Fernanda Milanezi1, Liuba Lyass3, Ninette Amariglio3, Jasmine Jacob-Hirsch4, Nur Ben-Chetrit5, Gabi Tarsic6, Moshe Lindzen7, Reu Arvaham8, Yi-Chun Liou9, Patricia Truski9, Asya Lyass10, Gideon Rechavi11, Neil L. Spector12, Su Hao Lo13, Fernando Schmitt14, Sarah S. Racus15 and Yosef Yarden16
<table>
<thead>
<tr>
<th>Cten correlation to:</th>
<th>Spearman rank correlation (Rho)</th>
<th>P value</th>
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<tr>
<td>EGFR</td>
<td>0.785</td>
<td>0.0006*</td>
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<tr>
<td>HER2/ErbB-2</td>
<td>0.67</td>
<td>0.004*</td>
</tr>
<tr>
<td>Oestrogen receptor</td>
<td>-0.48</td>
<td>0.004*</td>
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<td>Lymph-node metastasis (number of lymph nodes: 0, 1–3, &gt;3)</td>
<td>0.47</td>
<td>0.001*</td>
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<tr>
<td>Histological grade (I, II, III)</td>
<td>0.47</td>
<td>0.001*</td>
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<tr>
<td>Tumour size</td>
<td>0.01</td>
<td>0.84</td>
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The numbers were calculated on the basis of analyses performed using 272 samples derived from invasive breast tumours. Spearman’s rank correlation test was used to determine the correlations. A False Discovery Rate test was used to correct the alpha value for multiple comparisons. Asterisks indicate statistically significant correlations. EGFR, epidermal growth factor receptor.
Breast Cancer Patients: cten undergoes down-regulation upon treatment with an EGFR Kinase Inhibitor
SYNJ2 EXPRESSION IN INVASIVE BREAST CARCINOMAS

Basal-like

HER2-overexpressing

Histological Grade

Estrogen Receptor Subtype

Luminal A  Luminal B  Basal-Like  HER2-Over.

Positive  Negative

I  II  III

0  10  20  30  40  50  60  70  80  90  100

High  Low  Absent

0  10  20  30  40  50  60  70  80  90  100

30  32  48  72

31  58

28  34  42
Navigator-3, a modulator of cell migration, may act as a suppressor of breast cancer progression

Hadas Cohen-Dvashi, Nir Ben-Chetrit, Roslin Russell, Silvia Carvalho, Mattia Lauriola, Sophia Nisani, Maicol Mancini, Nishanth Nataraj, Merav Kedmi, Lee Roth, Wolfgang Köstler, Amit Zeisel, Assif Yitzhaky, Jacques Zylberg, Gabi Tarcic, Raya Elam, Yoav Wigelman, Rainer Will, Sara Lavi, Ziv Porat, Stefan Wiemann, Sara Ricardo, Fernando Schmitt, Carlos Caldas, & Yosef Yarden

Synaptojanin 2 is a druggable mediator of metastasis and the gene is overexpressed and amplified in breast cancer


www.SCIENCESIGNALING.org 20 January 2015  Vol 8 Issue 360 ra7
SYNJ2

- Implicated in the regulation of cytoskeleton;
- Lamellipodia formation;
- Cell migration and invasion.

**In vitro**

MDA-MB-231 cell invasion:

- siRNA:
  - Control
  - Synj2

**In vivo**

- Control
- shSYNJ2
- shSYNJ2 + Wt rescue
Activity of SYNJ2 is necessary for distant metastasis of breast cancer
ALK alteration is a frequent event in aggressive breast cancers

- ALK is amplified in 13.5% of breast carcinomas
- 75% of IBC have ALK amplification
- ALK amplification is related to worst prognosis and high proliferative index
- There is a good correlation between FIS, RT-PCR and IHQ
INFLAMMATORY BREAST CARCINOMA (IBC) MOLECULAR STUDIES

- NGS results in 76 cases of LABC

Park K et al. J Oncontarget 2015
INFLAMMATORY BREAST CARCINOMA (IBC) MOLECULAR STUDIES and RESPONSE TO QT

Park K et al. J OncoTarget 2015
INFLAMMATORY BREAST CARCINOMA (IBC)
MOLECULAR STUDIES and RESPONSE TO QT
LOCALIZED ADVANCED BREAST CANCER (LABC) CONCLUSIONS

• LABC is a heterogeneous disease that includes a wide range of clinical entities including IBC.

• The initial steps in the management of this disease is perform a biopsy to confirm the diagnosis and to study the biological characteristics of the tumour.

• These tumours are more frequently IDC, Grade III, ER-, PR-, HER2 positive and with high Ki67 index.
LOCALIZED ADVANCED BREAST CANCER (LABC)

CONCLUSIONS

• Molecular profiling show that these tumours are more frequently HER2 overexpressing and triple negative.

• P53 mutation is the most common genetic alteration in the LABC.

• E and P-cadherin are co-expressed in IBC and this is related to the prognosis.
LOCALIZED ADVANCED BREAST CANCER (LABC) CONCLUSIONS

• ALK is expressed in 75% of cases of IBC and should be explored as therapeutic target.

• Preliminary NGS studies show that RAS and MET can predict response to chemotherapy.

• Despite the prognosis of LABC is still poor, improvement have been observed with use of multimodal therapy.

• LABC is a good model to follow in vivo evaluation of the efficacy of systemic therapy and creates opportunity to obtain specimens prior, during and after treatment.