How to quantify immune infiltration and tumor cellularity

Carsten Denkert
Translational Cancer Research Group
Institute of Pathology
Charité Universitätsmedizin Berlin

Berlin, Germany

Impakt Meeting, Brussels, 2013
Tumor cellularity as a quality control parameter for molecular analysis

- Option 1:
  - Report „% of tumor cells“
  - „the tumor cells are the most important cells“

100% tumor tissue, 50% tumor cells

- Option 2:
  - Report „% of tumor tissue“
  - „the tumor stroma is also a part of the tumor“

90% tumor tissue, 60% tumor cells
Tumor cellularity – practical recommendations

• Tumor percentage must be documented for each sample used for molecular analysis

• Option 1: tumor tissue area
  – Use a marker pen to circle the tumor on the slide
  – Compare tumor area vs. total area: % tumor tissue
  – Disadvantage: low tumor cell numbers for mutation analysis

• Option 2: if cell numbers are needed
  – Count tumor cells, count total cells
  – Give percentage
  – disadvantage: certain tumor types might be systematically excluded (lobular carcinomas, tumors with many lymphocytes)
Tumor-associated lymphocytes - key message

• Pathologists should get used to report the tumor-associated immuno infiltrate as a standard parameter.

Why??
Clinical data
Biological background

How??
Standardized evaluation
Intratumoral and stromal lymphocytes, LPBC
Histopathological evaluation

- Tumor type
- Tumor grade
- Immune infiltrate
- Hormone receptors
- HER2
- Ki67
Tumor-associated lymphocytes as a predictive marker
neoadjuvant chemotherapy

- GeparQuinto study
- Prospective evaluation
- HER2 neg tumors with EC-T
- N=313

Issa-Nummer et al., submitted
Tumor-associated lymphocytes and pCR – multivariate evaluation

GeparQuinto, prospective evaluation, HER2 neg, EC-T, n=313

<table>
<thead>
<tr>
<th></th>
<th>OR (95% CI)</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPBC (pos. vs. neg)</td>
<td>2.7 (1.5-4.2)</td>
<td>0.003</td>
</tr>
<tr>
<td>HR status IH (ER-/PR- vs. any +)</td>
<td>2.4 (1.2-4.6)</td>
<td>0.01</td>
</tr>
<tr>
<td>Age &lt;50y vs &gt;=50y</td>
<td>1.3 (0.66-2.4)</td>
<td>ns</td>
</tr>
<tr>
<td>Tumor type (ductal/other vs. lobular)</td>
<td>5.0 (0.65-39.1)</td>
<td>ns</td>
</tr>
<tr>
<td>Grade (G3 vs. G1-G2)</td>
<td>1.6 (0.79-3.2)</td>
<td>ns</td>
</tr>
<tr>
<td>Stage (cT12 vs. CT34)</td>
<td>1.6 (0.55-4.5)</td>
<td>ns</td>
</tr>
<tr>
<td>Nodal stat. (cN0 vs. cN+)</td>
<td>1.9 (1.0-3.5)</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Issa-Nummer et al., submitted

Similar results in GeparTrio and GeparDuo, see Denkert et al, JCO 2010
LPBC – Lymphozyten prädominantes MammaCa

LPBC: n=100 (12%); Multivariat iTuLy: p=0.001, Denkert et al, JCO, 2010
Validation strategy – lymphocyte infiltrate

• Hypothesis: peritumoral lymphocytes and chemotherapy response / survival

Training

GeparDuo neoadjuv. EC-Doc (n=218) JCO, 2012

Validation 1

Gepartrio neoadjuv. TAC (n=840) JCO, 2012

GeparQuint o HER2- (prospective) EC-Doc (n=313) submitted

GeparQuint o HER2+ EC-DOC Lap vs. Trast.(n=250)

GeparSixto (prospective) TNBC and HER2+ (n=600)

n=1621

Other studies:

BIG2-98 adjuvant  
n=2009, TNBC: n=256  
(Loi et al, JCO, 2013)

Ono et al, 2012  
neoadjuvant, TNBC: n=96
Prognosis of TNBC – increased lymphocytic infiltrate defines a good prognosis group
Loi et al, JCO 2013 BIG2-98 study (total n=2009, TNBC n=256)

No. at risk
No LPBC 229 202 167 156 146 138 134 116 41 3 0
LPBC 27 26 24 23 22 22 21 18 11 0 0

Log-rank P = .014
Dying tumor cells elicit an immune response that is required for the success of therapy. This immune response mediates the suppression of tumor growth and determines the long-term survival...

Toll-like receptor 4–dependent contribution of the immune system to anticancer chemotherapy and radiotherapy

Lionel Apetoh¹,³,²⁰, François Ghiringhelli¹,⁴,²⁰, Antoine Tesniere¹,²,⁵,²⁰, Michel Obeid¹,²,⁵, Carla Ortiz¹–³, Alfredo Criollo¹,²,⁵, Grégoire Mignot¹–³, M Chiara Maiuri¹,²,⁵,⁶, Evelyn Ullrich¹–³, Patrick Saulnier⁷, Huan Yang⁸, Sebastian Amigorena⁹, Bernard Ryffel¹⁰, Franck J Barrat¹¹, Paul Saftig¹², Francis Levi²,¹³, Rosette Lidereau¹⁴, Catherine Nogues¹⁴, Jean-Paul Mira¹⁵, Agnès Chompret¹⁶, Virginie Joulin¹⁷, Françoise Clavel-Chapelon¹⁸, Jean Bourhis¹⁹, Fabrice André¹⁶, Suzette Delaloge¹⁶, Thomas Tursz³,¹⁶, Guido Kroemer¹,²,⁵,²⁰ & Laurence Zitvogel¹–⁴,²⁰

Nature Medicine 2006
Pathologic Complete Response to Neoadjuvant Chemotherapy of Breast Carcinoma Is Associated with the Disappearance of Tumor-Infiltrating Foxp3⁺ Regulatory T Cells

Sylvain Ladoire,¹,² Laurent Arnould,¹ Lionel Apetoh,³ Bruno Coudert,¹ François Martin,² Bruno Chauffert,¹,² Pierre Fumoleau,¹ and François Ghiringhelli¹,²,³

Research article
T-cell metagene predicts a favorable prognosis in estrogen receptor-negative and HER2-positive breast cancers
Achim Rody¹, Uwe Holtrich¹, Laos Pusztai², Cornelia Liedtke³, Regine Gaetje¹, Eugen Ruckhaeberle¹, Christine Solbach¹, Lars Hanker¹, Andre Ahr¹, Dirk Metzler⁴, Knut Engels⁵, Thomas Karn¹ and Manfred Kaufmann¹

The Humoral Immune System Has a Key Prognostic Impact in Node-Negative Breast Cancer

Marcus Schmidt,¹ Daniel Böhm,¹ Christian von Törne,² Eric Steiner,¹ Alexander Puhl,¹ Henryk Pilch,³ Hans-Anton Lehr,³ Jan G. Hengstler,⁴ Heinz Kölbl,¹ and Mathias Gehrmann²

¹Department of Obstetrics and Gynecology, Medical School, Johannes Gutenberg University, Mainz, Germany; ²Siemens Medical Solutions Diagnostics GmbH, Cologne, Germany; ³Department of Obstetrics and Gynecology and Center for Toxicology, Institute of Legal Medicine and Rudolf-Böheim Institute of Pharmacology and Toxicology, University of Leipzig, Leipzig, Germany; and ⁴Department of Pathology, University of Lausanne, Lausanne, Switzerland

Clinical cancer research 2008
Breast cancer research 2009
Cancer research 2008
A Comprehensive Analysis of Human Gene Expression Profiles Identifies Stromal Immunoglobulin κ C as a Compatible Prognostic Marker in Human Solid Tumors

Marcus Schmidt¹, Birte Hellwig⁶, Seddik Hammad⁷, Amnah Othman⁷, Miriam Lohr⁶, Zonglin Chen¹, Daniel Boehm¹, Susanne Gebhard¹, Ilka Petry¹, Antje Lebrecht³, Cristina Cadenas⁷, Rosemarie Marchan⁷, Joanna D. Stewart⁷, Christine Solbach¹, Lars Holmberg⁸,⁹,¹², Karolina Edlund¹⁰, Hanna Göransson Kultima¹¹, Achim Rody¹³, Anders Berglund⁸,¹⁴, Mats Lambe⁷,⁸, Anders Isaksson¹¹, Johan Botling¹⁰, Thomas Kam¹⁵, Volkmar Müller¹⁶, Aslihan Gerhold-Av², Christina Cotarelo³, Martin Sebastian⁴, Ralf Kronenwett¹⁷.

Message of this presentation

• Pathologists should get used to report the tumor-associated immuno infiltrate as a standard parameter.

Why??
Clinical data
Biological background

How??
Standardized evaluation
Intratumoral and stromal lymphocytes, LPBC
## Definitions for histopathology

<table>
<thead>
<tr>
<th>Description of histological parameter</th>
<th>Intratumoral lymphocytes (iTu-Ly) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>- percentage of tumor cell nests with intraepithelial mononuclear cells.</td>
</tr>
<tr>
<td></td>
<td>- Mononuclear cells (lymphocytes or plasma cells) with direct intercellular contact to invasive tumor cells</td>
</tr>
<tr>
<td></td>
<td>- any granulocyte infiltrate in the area of tumor necrosis is not included.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Description of histological parameter</th>
<th>Stromal lymphocytes (str-Ly) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>- percentage of stromal area with mononuclear inflammatory cells.</td>
</tr>
<tr>
<td></td>
<td>- stromal infiltrate adjacent to intraductal carcinoma or normal breast is not included.</td>
</tr>
</tbody>
</table>

---

Denkert et al, JCO 2010; Denkert et al Breast Care 2011
Lymphocyte-predominant breast cancer (LPBC)

- The lymphocytic infiltrate is a continuous parameter.
  - each 10% increase in lymphocytes leads to a 10-20% increase in response (pCR)
- Some tumors have „more lymphocytes than tumor cells“ – LPBC
  - 12-20% of tumors
  - Definitions:
    - >60% lymphocytes in either tumor cell nests or tumor stroma (Denkert et al, 2010)
    - ≥60% lymphocytes in either tumor cell nests or tumor stroma (Denkert et al, newer studies, more practical for histology)
    - >50% lymphocytes in either tumor cell nests or tumor stroma (Loi et al, 2013)
- For the future: international consensus based on meta-analysis
- For daily practice:
  - „more lymphocytes than tumor cells“
  - report „LPBC“ and the percentages
## Neoadjuvant Systemic Chemotherapy Response Prediction

<table>
<thead>
<tr>
<th>Factor</th>
<th>LoE&lt;sub&gt;2009&lt;/sub&gt;</th>
<th>CTS</th>
<th>LoE&lt;sub&gt;Ox2001&lt;/sub&gt;</th>
<th>GR</th>
<th>AGO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age &lt; 35 yrs</td>
<td>I</td>
<td>B</td>
<td>1a</td>
<td>A</td>
<td>++</td>
</tr>
<tr>
<td>cT1 / cT2 tumors o. N0 o. G3</td>
<td>I</td>
<td>B</td>
<td>1a</td>
<td>A</td>
<td>++</td>
</tr>
<tr>
<td>Negative ER and PgR status</td>
<td>I</td>
<td>B</td>
<td>1a</td>
<td>A</td>
<td>++</td>
</tr>
<tr>
<td>Triple negative breast cancer (TNBC)</td>
<td>I</td>
<td>B</td>
<td>1a</td>
<td>A</td>
<td>++</td>
</tr>
<tr>
<td>Positive HER2 status</td>
<td>I</td>
<td>B</td>
<td>1a</td>
<td>A</td>
<td>++</td>
</tr>
<tr>
<td>Non-lobular tumor type</td>
<td>I</td>
<td>B</td>
<td>1a</td>
<td>A</td>
<td>+/-</td>
</tr>
<tr>
<td>PAM50/Mammaprint</td>
<td>III</td>
<td>C</td>
<td>2b</td>
<td>B</td>
<td>+/-</td>
</tr>
<tr>
<td>Ki-67</td>
<td>I</td>
<td>B</td>
<td>1a</td>
<td>A</td>
<td>+</td>
</tr>
<tr>
<td>Peritumoral lymphocyte infiltration</td>
<td>II</td>
<td>B</td>
<td>2b</td>
<td>B</td>
<td>+</td>
</tr>
<tr>
<td>Early response on sonography</td>
<td>I</td>
<td>B</td>
<td>1b</td>
<td>A</td>
<td>+</td>
</tr>
<tr>
<td>ER/PR status in HER2 positive (CHT+ T/L/P)</td>
<td>I</td>
<td>B</td>
<td>1b</td>
<td>A</td>
<td>+</td>
</tr>
</tbody>
</table>
RESPONSIFY – FP7 Project 2012-2015
(Scientific Coordinator: Sibylle Loibl, 12 European partners)

Aim: „... to develop FFPE based predictive IVD tests for anti-HER2 and anti-angiogenic therapies...“
# RESPONSIFY – workflow

**FFPE tumor samples**

<table>
<thead>
<tr>
<th>Layer</th>
<th>Description</th>
<th>Pathology</th>
<th>Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>12µm</td>
<td>central Pathology 6x2µm</td>
<td></td>
<td>HE, ER, PR, HER2, Ki67, ERCC1</td>
</tr>
<tr>
<td>10µm</td>
<td>HER2 pos only central Pathology 2x5µm</td>
<td></td>
<td>HER2 SISH, CH 17 SISH</td>
</tr>
<tr>
<td>30µm</td>
<td>RNA/DNA samples 6 x 5µm (last two are optional)</td>
<td></td>
<td>DNA – PIK3CA, RNA Isolation, Responsify</td>
</tr>
<tr>
<td>10µm</td>
<td>Optional backup slides 5x2µm</td>
<td>Backup</td>
<td></td>
</tr>
</tbody>
</table>

**Total 62µm**

- **TMA production**
- **Tumorbank**

**Total 400µm**

**Backup**

**Evaluate protein markers on TMA**
Tumor-associated lymphocytes - key messages

• Pathologists should get used to report the tumor-associated immuno infiltrate as a standard parameter.

• Validated as predictive marker for response to neoadjuvant chemotherapy

• Validated as prognostic marker for TNBCs and adjuvant therapy
Charité
Britta Beyer
Jan Budczies
Silvia Darb-Esfahani
Frederick Klauschen
Ines Koch
Berit Müller
Judith Prinzler
Bruno Sinn
Petra Wachs
Stephan Wienert
Manfred Dietel

GBG
Gunter von Minckwitz
Sibylle Loibl
Valentina Nekljudova
Keyur Mehta
Yasmin Issa-Nummer
Christiane Mayr

Translational Subboard
Neoadjuvant Subboard