Diagnostic and therapeutic implications of tumour-infiltrating lymphocytes in breast cancer

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Disclosure slide

• Sividon Diagnostics: research funding, co-founder and shareholder
Heterogenous immune infiltrate in breast cancer

Lymphocyte-predominant breast cancer
(LPBC = more lymphocytes than tumor cells)
Tumor-associated lymphocytes

Clinical relevance
TILs and chemotherapy response in GeparSixto

n=580
pCR rates in GeparSixto: LPBC vs non-LPBC

San Antonio Breast Cancer Symposium
- Cancer Therapy and Research Center at UT Health Science Center – December 10-14, 2013

pCR rates

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>pCR Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients</td>
<td>580</td>
<td>40%</td>
</tr>
<tr>
<td>PM - therapy</td>
<td>290</td>
<td>37%</td>
</tr>
<tr>
<td>PM Carbo - therapy</td>
<td>290</td>
<td>44%</td>
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<tr>
<td>PM - therapy</td>
<td>438</td>
<td>34%</td>
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<tr>
<td>PM Carbo - therapy</td>
<td>221</td>
<td>34%</td>
</tr>
<tr>
<td>PM - therapy</td>
<td>142</td>
<td>60%</td>
</tr>
<tr>
<td>PM Carbo - therapy</td>
<td>69</td>
<td>75%</td>
</tr>
</tbody>
</table>

pCR: ypT0ypN0

Test for interaction:
- PM - therapy: P = 0.09
- PM Carbo - therapy: P < 0.0005

Contact: carsten.denkert@charite.de
STEPP analysis – pCR rate in GeparSixto

- **All patients**
- **PM vs PMC therapy**
Clinical evaluation of TILs in breast cancer

Training:
- GeparDuo neoadjuv. EC-Doc (n=218) JCO, 2010
- Gepartrio neoadjuv. TAC (n=840) JCO, 2010

Validation 1:
- GeparQuint o HER2- (prospective) EC-Doc (n=313)

Validation 2:
- GeparQuint o HER2+ EC-DOC Lap vs. Trast.(n=250)

Validation 3:
- GeparSixto (prospective) TNBC and HER2+ (n=595)

Validation 4:
- Adjuvant TNBC n=737

-Ono et al, 2012 neoadjuvant, TNBC: n=96

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

-ECOG 2197 and ECOG 1199 adjuvant
  Adams et al., SABCS 2013, TNBC: n=481
Tumor-associated lymphocytes- clinically relevant questions

• Clinical validity: Results of clinical biomarker studies
  – prediction of response to neoadjuvant therapy
  – improved prognosis
  – relevant subtypes (TNBC, HER2+, luminal?)

  – consistent results in several studies
TILs vs. molecular markers

• „Counting little blue cells in the tumor tissue."
• Can this reflect the complexity of the immune system?
Further molecular characterization of immune infiltrate

**morphological classification**
Lymphocyte-predominant breast cancer (LPBC) = more than 60% TILs

**molecular characterization**

**Hypothesis:**

Immunosuppressive regulators:
- PD1, PDL1,
- CTLA4, IDO1, FOXP3

Immune activation:
- T-Cells: CD8A, CCL5
- B-Cells: IGKC, CD21, CD80

Chemoattractants:
- CXCL9, CXCL13

Presented by: Carsten Denkert
Immune markers were significantly linked to increased pCR rates – all cases (n=481)

<table>
<thead>
<tr>
<th>Marker</th>
<th>Stromal TILs</th>
<th>CCL5</th>
<th>CXCL9</th>
<th>CXCL13</th>
<th>CD8A</th>
<th>PD1</th>
<th>PDL1</th>
<th>CTLA4</th>
<th>FOXP3</th>
<th>IDO1</th>
<th>IGKC</th>
<th>CD80</th>
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<tr>
<td></td>
<td>OR (95% CI)</td>
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<td>OR (95% CI)</td>
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<tr>
<td>Stromal TILs</td>
<td>1.26 (1.16-1.36)</td>
<td>0.00000001</td>
<td>1.41 (1.23-1.62)</td>
<td>0.000001</td>
<td>1.25 (1.14-1.38)</td>
<td>0.000006</td>
<td>1.16 (1.06-1.26)</td>
<td>0.001</td>
<td>1.29 (1.13-1.48)</td>
<td>0.0002</td>
<td>1.43 (1.24-1.66)</td>
<td>0.000001</td>
<td>1.57 (1.34-1.86)</td>
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Stromal TILs: OR per 10% change, mRNA markers: OR per 1 dCt value (l≈ doubling of mRNA)

Presented by: Carsten Denkert
Immune markers were significantly linked to increased pCR rates – all cases (n=481)

<table>
<thead>
<tr>
<th>Markers</th>
<th>OR (95% CI)</th>
<th>p-value</th>
<th>p-value univariate</th>
<th>p-value multivariate</th>
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<tbody>
<tr>
<td>Stromal TILs</td>
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<tr>
<td>CCL5</td>
<td>1.43 (1.24-1.66)</td>
<td>0.000001</td>
<td>0.00002</td>
<td>0.02</td>
</tr>
<tr>
<td>CXCL9</td>
<td>1.57 (1.34-1.86)</td>
<td>0.00000003</td>
<td>0.000001</td>
<td>0.09</td>
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<tr>
<td>CXCL13</td>
<td>1.38 (1.19-1.60)</td>
<td>0.00001</td>
<td>0.0001</td>
<td>0.06</td>
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<tr>
<td>CD8A</td>
<td>1.23 (1.003-1.50)</td>
<td>0.05</td>
<td>0.02</td>
<td>ns</td>
</tr>
<tr>
<td>PD1</td>
<td>1.25 (1.14-1.36)</td>
<td>0.0000005</td>
<td>0.00003</td>
<td>0.03</td>
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<td>PDL1</td>
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<td>CTLA4</td>
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Stromal TILs: OR per 10% change, mRNA markers: OR per 1 dCt value (l= doubling of mRNA)
“immunosuppressive”

GeparSixto $n=481$
Models for immune interaction in breast cancer

Original model
“balance”

**Immunosuppressive regulators:**
- PD1, PDL1,
- CTLA4, IDO1, FOXP3

**Immune activation:**
- T-Cells: CD8A, CCL5
- B-Cells: IGKC, CD21,
  - CD80
  - CXCL9, CXCL13
Models for immune interaction in breast cancer

Original model
“balance”

Immunosuppressive regulators:
PD1, PDL1, CTLA4, IDO1, FOXP3

Immune activation:
T-Cells: CD8A, CCL5
B-Cells: IGKC, CD21, CD80
CXCL9, CXCL13

Modified model
“feedback loop”

Activation:
Immune activation:
CD8A, CCL5, IGKC, CD21, CD80
CXCL9, CXCL13

Immuno-suppressive regulators:
PD1, PDL1, CTLA4, IDO1, FOXP3

Inhibition:
Immune biomarkers vs. immune subtypes

**Immune biomarkers**
- investigate key immunological molecules or combinations
- determine pro- and anti-immune activation states
- approach based on knowledge about the function of the immune system

- so far no clear clinically relevant pro- and anti-immune groups
- most markers reflect the presence of immune cells
Immune biomarkers vs. immune subtypes

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- investigate key immunological molecules or combinations
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- most markers reflect the presence of immune cells

**Immune subtypes**
- the immunogenicity of individual tumors is different
- this can be monitored by analysis of
  - TILs
  - immune signatures
- determine groups for therapeutic stratification
- different immunogenicity
  - poor
  - moderate
  - strong
The immunological heterogeneity of breast cancer
Three different immune subtypes by unsupervised hierarchical clustering (481 tumors, 12 immune genes)

Presented by: Carsten Denkert
Three different immune subtypes: correlation with TIL morphology

Presented by: Carsten Denkert
Three different immune subtypes: correlation with response rate

Presented by: Carsten Denkert
Therapeutic strategies for different types of immune reactions

- strong immune reaction, but tumor still growing
- good prognosis with chemotherapy
- immunogenic effects of chemotherapy present
  can they be enhanced by checkpoint inhibition?
- no evidence of immune activation
- immune therapy approaches not useful?
- partial immune activation
- immune heterogeneity
- immune escape?
- enhancement of response by immune therapy / checkpoint inhibition?
Comparison of immune mRNAs and TILs for response prediction

Exploratory multivariate analysis including TILs, mRNA markers and clinical markers:

- TILs are significant in all analyses
- immune mRNAs are only significant in selected analyses

TILs contain similar information as immune mRNAs

<table>
<thead>
<tr>
<th>Protein</th>
<th>all cases p-value for immune mRNA</th>
<th>TNBC p-value for immune mRNA</th>
<th>HER2+ p-value for immune mRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCL5</td>
<td>0.04</td>
<td></td>
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<td>CD8A</td>
<td>0.09</td>
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<tr>
<td>PD1</td>
<td>0.005</td>
<td>0.04</td>
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<td>CD21</td>
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</table>

Presented by: Carsten Denkert
Tumor-associated lymphocytes

Analytical validity and strategies for standardization

- We do not observe an improved prediction with molecular markers...
- ... if we measure TILs by H&E at the same time.
- Focus on TILs using H&E sections.
Predefined parameters for TIL evaluation

intratumoral TILs = direct contact to tumor cells

stromal TILs = between the tumor cells

LPBC = Lymphocyte-predominant breast cancer
„more lymphocytes than tumor cells“ (≥60% TILs)

Predefined parameters for TIL evaluation

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Predefined parameters for TIL evaluation

intratumoral TILs = direct contact to tumor cells

stromal TILs = between the tumor cells

LPBC = Lymphocyte-predominant breast cancer

„more lymphocytes than tumor cells“ (≥60% TILs)

Best parameter: stromal TILs

Tumor-associated lymphocytes are a continuous parameter

LPBC = lymphocyte predominant breast cancer

increased TIL levels
Tumor-associated lymphocytes are a continuous parameter

GeparSixto – sorted by increased TIL levels
Standardization of TIL-evaluation in Breast Cancer
Salgado, Denkert et al., Annals of Oncology 2014

1: select tumor area
include area within tumor borders
TLS

de not include immune infiltrate outside of the tumor

2: define stromal area
TLS
do not include TILs in this area
evaluate only TILs in this area stromal TILs

3: scan at low magnification

4: exclude granulocytes

5: assess range of stromal TILs

For intermediate group evaluate different areas at higher magnification.

0-10% stromal TILs
20-40% stromal TILs
50-90% stromal TILs

Standardized evaluation of Tumor-Infiltrating Lymphocytes (TIL) in Breast Cancer for daily clinical and research practice or clinical trial setting
A tutorial prepared by the International Working Group for TIL in breast cancer - 2014

Carsten Denkert
Roberto Salgado
Sandra Demaria

26-30 September 2014, Madrid, Spain
Standardization of TIL-evaluation in Breast Cancer
Salgado, Denkert et al., Annals of Oncology 2014

1: select tumor area
- Include area within tumor borders
- Do not include immune infiltrate outside of the tumor

2: define stromal area
- Do not include TILs in this area
- Evaluate only TILs in this area = stromal TILs

3: scan at low magnification

4: exclude granulocytes
- Do not include granulocytes in necrotic areas

5: assess range of stromal TILs
- For intermediate group evaluate different areas at higher magnification.

6: determine percentage of TILs (in 5-10% steps)
- 0-10% stromal TILs
- 20-40% stromal TILs
- 50-90% stromal TILs

26-30 September 2014, Madrid, Spain
1\textsuperscript{st} TIL breast cancer ring trial

- kickoff meeting scheduled for SABCS 2014
- evaluation of digital slides by different pathologists
- determination of concordance and interclass correlation coefficient
- development of image analysis approaches
Tumor-associated lymphocytes – options for clinical utility

• Conclusions for clinical practice
  – immune signals are strong and easily detectable
  – but there is no clear clinical utility so far

• Option 1: neoadjuvant carboplatin in TNBC
  – high complete response rates in GeparSixto with increased TILs
  – might be an additional factor for therapy decisions
  – validation in other Platin trials pending
  – GeparOcto: dose-dense conventional vs. dose-dense carboplatin

• Option 2: HER2 positive BC
  – trastuzumab effect dependent on TILs (Finher)
  – other validations pending

• Option 3: immune therapies ... prediction of response
Summary – immune infiltration in breast cancer

1. TILs are a predictive marker for response to neoadjuvant therapy – several studies with >2000 patients.
2. TILs are prognostic in TNBC (n>700, two studies).
3. Interaction with therapy:
   – In GeparSixto, the predictive effect of TILs for pCR was particularly high in patients treated with carboplatin.
   – In Finher, the effect of trastuzumab was increased in TIL+ tumors.
   – Validation studies are needed for both questions.
4. Stromal TILs are the most useful parameter.
5. Immune marker signatures are correlated with the presence of immune cells
   – no clear pro- and anti-immune signatures
6. The next steps:
   – include TILs in clinical trial parameters (as well as routine histology).
   – clinical studies for immune checkpoint inhibitors
We would like to thank all patients, clinicians, and pathologists participating in the clinical studies and the biomaterial collection.

**GBG**
- Gunter von Minckwitz
- Sibylle Loibl
- Valentina Nekljudova
- Keyur Mehta
- Stephan Gade
- Christiane Rothhaar
- Translational Subboard of GBG
- Neoadjuvant Subboard of GBG

**RESPONSIFY partners**
- Sherene Loi
- Christos Sotiriou
- Fabrice André

**Charité**
- Britta Beyer
- Jan Budczies
- Silvia Darb-Esfahani
- Sylwia Handzik
- Frederick Klauschen
- Ines Koch
- Berit Pfitzner
- Judith Prinzler
- Bruno Sinn
- Wolfgang Schmitt
- Petra Wachs
- Stephan Wienert
- Manfred Dietel

EU FP7 No 278659

26-30 September 2014, Madrid, Spain