NEXT GENERATION LEARNING

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USCAP
Creating a Better Pathologist
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Dr. Ashley Cimino-Mathews declares she has no conflict(s) of interest to disclose.
Immune Targeting in Breast Cancer

Ashley Cimino-Mathews, MD
Department of Pathology and Oncology
The Johns Hopkins Hospital
13 March 2016
Outline and Objectives

• Review the concept of tumor immune microenvironment
• Describe the role of tumor infiltrating lymphocytes (TILs) and immune checkpoints in breast carcinoma
• Present the current immunotherapeutic strategies in breast carcinoma
• Discuss the role of the pathologist in driving the field of cancer immunology and immunotherapy
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Cancer immune surveillance

Tumor elimination

Immune evasion

Equilibrium

Immune system (largely innate, Th1 helper T cells, and CD8+ cytotoxic T cells) recognizes tumor neo-antigens as “foreign” → tumor elimination

Tumor cells gain immune resistance mechanisms and induce immune tolerance and the immune milieu shifts to a pro-tumorigenic Th2 immune response → tumor survival
Figure 1. The Hallmarks of Cancer
Figure 3. Emerging Hallmarks and Enabling Characteristics

Emerging Hallmarks

- Deregulating cellular energetics
- Avoiding immune destruction
- Genome instability and mutation
- Tumor-promoting Inflammation

Enabling Characteristics
Cancer immune surveillance

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Immune system (largely innate, Th1 helper T cells, and CD8+ cytotoxic T cells) recognizes tumor neo-antigens as “foreign” → tumor elimination

Tumor cells gain immune resistance mechanisms and induce immune tolerance and the immune milieu shifts to a pro-tumorigenic Th2 immune response → tumor survival
Figure 1. The inflamed tumor microenvironment.

**Inhibitory interactions** between antigen presenting cells and T cells dampen the T cell immune response

**Stimulatory signals** from T cells activate antigen presenting cells

Co-stimulatory signals from antigen presenting cells activate T cells

Cytokine secretion by antigen presenting cells and T cells stimulate or inhibit the immune response

Co-stimulatory and inhibitory immune signals between antigen presenting cells (grey) and T-lymphocytes (blue) interact to modulate the anti-tumor immune response.

TILs in Cancer

Th = helper CD4+ T
M = macrophage
N = neutrophil
DC = dendritic cell
MDSC = myeloid suppressor

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Figure 6. Therapeutic Targeting of the Hallmarks of Cancer

- EGFR inhibitors
- Cyclin-dependent kinase inhibitors
- Immune activating anti-CTLA4 mAb
- Telomerase Inhibitors
- Selective anti-inflammatory drugs
- Inhibitors of VEGF signaling
- Inhibitors of HGF/c-Met

- Aerobic glycolysis inhibitors
- Proapoptotic BH3 mimetics
- PARP inhibitors
- Deregulating cellular energetics
- Resisting cell death
- Genome instability & mutation
- Inducing angiogenesis
- Activating invasion & metastasis
- Evading growth suppressors
- Avoiding immune destruction
- Enabling replicative immortality
- Tumor-promoting inflammation
Outline and Objectives

• Review the concept of tumor immune microenvironment

• Describe the role of tumor infiltrating lymphocytes (TILs) and immune checkpoints in breast carcinoma
  – Variation across subtypes of invasive breast carcinoma
  – Tumor microenvironment of in situ ductal carcinoma (DCIS)
  – Tumor microenvironment of metastatic breast carcinoma
What do we know about TIL and breast carcinomas?
Medullary breast carcinoma

• The classic histologic criteria used to define medullary carcinomas:
  – Syncytial growth pattern in >75% tumor
  – Lack of tubule formation
  – Cytologically atypical cells with prominent nucleoli and abundant cytoplasm
  – Circumscribed/pushing border
  – Prominent and diffuse lymphoplasmacytic inflammation

• Medullary carcinomas have relatively favorable prognosis
THE RELATIVELY FAVORABLE PROGNOSIS OF MEDULLARY CARCINOMA OF THE BREAST

Oliver S. Moore, Jr., M.D.,* and Frank W. Foote, Jr., M.D.

In the scheme for classification of mammary cancer currently employed in the laboratories of the Memorial Hospital, the term "medullary carcinoma" is used with a special used synonymously by us; but this designation has been abandoned, since by derivation all true breast cancers are adenocarcinomas and not a few are unfortunately bulky, yet they

Fig. 3. Medullary carcinoma with lymphoid stroma. Medium magnification. Fig. 4. Higher power to show detail of lymphoid infiltrate.
MEDULLARY CARCINOMA OF THE BREAST
A Clinicopathologic Study with 10 Year Follow-Up

REN L. RIDOLFI, MD, PAUL PETER ROSEN, MD, ABRAHAM PORT, MS, DAVID KINNE, MD,
VALERIE MIKÉ, PhD

CANCER October 1977
Vol. 40

★ 1964 INfiltrating Duct CA. (251 PTS)
★ NON-MEDULLARY BREAST CA. (56 PTS)
★ ATYPICAL MEDULLARY BREAST CA. (79 PTS)
★ TYPICAL MEDULLARY BREAST CA. (57 PTS)

Fig. 11. Life table analysis of survival.
Questions that arise from observations about medullary carcinomas

• Why do some tumors have TIL and others don’t?
  – The presence of certain tumor neoantigens
  – BRCA1 mutation and genomic instability

• Are the presence of TIL good or bad?
  – Is the impact of TIL the same for all subtype of breast carcinoma
  – Does it depend upon the actual type of TIL?

• Can we harness the “good” (anti-tumor) TIL and block the inhibitory checkpoints of the “bad” (anti-tumor) TIL for therapy?
What do we know about TIL and primary (non-medullary) invasive breast carcinomas?
Triple negative and HER2+ carcinomas are more immunogenic than luminal (ER+) carcinomas

- Higher number of TIL in ER- than ER+ carcinomas [1-3]
- Expression of immune response gene signature is associated with improved survival in ER-HER2- and HER2+ carcinomas but not ER+/HER2- carcinomas [4]
- A T-cell metagene profile correlates with improved response to chemotherapy in ER- and Her2+ carcinomas [5]
- B-cell gene profiles confer a favorable prognosis in TNBC, ER- and ER+Ki67^{high} carcinomas, but not in ER+Ki67^{low} carcinomas [6]

Tumor infiltrating lymphocytes in a triple negative breast carcinoma

What is the association of TIL with survival in (non-medullary) invasive breast carcinomas?
The presence of TIL and lymphoid aggregates are associated with improved survival

- Several series have shown TIL in treatment naïve TNBC to be independent prognostic factors for overall survival, decreased metastasis, and increased metastasis free survival [1-5]
- Brisk TIL in treatment naïve HER2+ carcinomas correlates with survival [1]
- In addition, brisk TIL in TNBC after chemotherapy correlates with overall survival [6]
- Increased density of high endothelial venules (see in lymphoid aggregates) is associated with overall survival in treatment naïve carcinomas [7]

Relationship between stromal lymphocytic infiltration, treated as a continuous variable and modeled using restricted cubic splines, and the relative risk of any event, distant event and death.

Disease free survival

Distant-disease free survival

Overall survival
Among TIL subtypes, CD8+ cells are favorable and FoxP3+ cells are unfavorable

- High numbers of CD8+ T-cells predicts patient survival across breast cancer subtypes [1]
- High numbers of FoxP3+ regulatory T cells are associated with higher tumor grade [2], ER negativity [2], shorter relapse-free survival [3] and shorter overall survival [3]

Does the presence of TIL have an association with treatment response?
The presence of TIL predicts favorable response to neoadjuvant therapy

- High numbers of TILs correlate with pCR to neoadjuvant chemotherapy across subtypes, particularly TNBC and HER2+ [1,2]
- A greater reduction in FoxP3+ cells is reported in patients with ER+ tumors responding to the aromatase inhibitor letrozole [3]
- The presence of TIL after neoadjuvant therapy is favorable (improved recurrence-free and overall survival), and decreased FoxP3+ cells I seen in patients with pCR [4-6]

What is the role of immune checkpoint pathways in modulating the immune response in breast carcinoma?

The **PD-1 Pathway**
PD-1 is expressed on immune cells. Its ligand PD-L1 is expressed by immune cells and can be induced on tumor cells by inflammatory cytokines or by mutations.
PD-L1 is expressed by breast carcinoma cells and tumor infiltrating lymphocytes

- PD-L1 expression first reported in 34% primary breast carcinomas and in 41% TIL [1]
- Rates of positive vary with breast carcinoma subtypes
- PD-L1 positivity is more common in TNBC than other subtypes [1,2]
- We reported PD-L1 expression in 21% primary carcinomas and in 78% TIL, with more common expression in HER2+ and basal like carcinoma [3]
- PD-L1 labeling on carcinoma cells and by TIL correlate with pCR [4]
- PD-L1 mRNA has been associated with improved clinical outcome [5]

The distribution of PD-L1 labeling between TIL and neoplastic cells differs across organ types, but in breast carcinomas PD-L1 labeling is seen *both* on TIL and carcinoma cells
PD-L1 labeling on carcinoma cells and TIL in a HER2+ primary breast carcinoma.
Spectrum of PD-L1 expression on tumor and/or immune cells

Melanoma

HNSCC

Breast Carcinoma

Gastric Carcinoma

What about the immune microenvironment in other special types of breast carcinoma?
PD-L1 Expression and the Immune Microenvironment in Primary Invasive Lobular Carcinomas of the Breast

Elizabeth Thompson MD PhD, Janis M. Taube MD, Rebecca Asch-Kendrick MD, Haiying Xu BS, Rajni Sharma PhD, Alan Meeker PhD, Pedram Argani MD, Leisha A. Emens MD PhD, Ashley Cimino-Mathews MD

March 14th, 2016 Monday morning poster session

- Here we evaluate TIL and PD-L1 expression in 47 ILC including ER+, triple negative and HER2+ tumors.
- Overall, all ILC contained TIL, but no case had diffuse TIL (>50%).
- 17% ILC contained PD-L1+ carcinoma cells, and all cases had PD-L1+ TIL, but the majority were focal-moderate.
- TIL infiltrate density correlated with TIL PD-L1 status, as tumors with moderate TIL infiltrate had higher PD-L1+ TIL than those with rare TIL (p=0.004).
- PD-L1+ ILC had more diffuse PD-L1+ TIL (63%) than did PD-L1- ILC (23%) (p=0.04).
- ER- ILC had more diffuse PD-L1+ TIL (50%) than ER- ILC (27%) (p=0.03).
- In contrast to previous studies in invasive ductal carcinomas correlating TIL density and carcinoma PD-L1 positivity with ER negativity and high tumor grade, there was no correlation between TIL infiltrate density and carcinoma PD-L1 status and the ER status, HER2 status, or tumor grade of the lobular carcinomas in our series.
- **These results support exploring immunotherapy including immune checkpoint blockade in primary breast ILC.**
What is the immune microenvironment of ductal carcinoma in situ?
All PD-L1+ invasive carcinomas with associated DCIS showed PD-L1 expression by the DCIS carcinoma cells. (Conversely all PD-L1- invasive carcinomas with associated DCIS had PD-L1- DCIS).
The Immune Microenvironment of Breast Ductal Carcinoma in Situ
Mod Pathol. 2016 Jan 15. [Epub ahead of print]

• Here we evaluate the tumor microenvironment in 27 cases of DCIS
• None of the DCIS cells were PD-L1+, but 81% of DCIS contained PD-L1+ TIL.
• DCIS with moderate-diffuse TIL were more likely to have PD-L1+ TIL (p=0.004).
• TIL with high levels of PD-L1 expression (>50% cells) were seen only in triple negative DCIS (p=0.0008).
• Findings suggest an active immune response within breast DCIS and supports TIL PD-L1 expression as a marker of downregulation of the body’s immune response within DCIS.
• The presence of PD-L1+ TIL in DCIS suggests that investigation of immune-based therapies may be warranted even in pre-invasive disease.
Most cases of DCIS (81%) contain PD-L1+ TIL, and the presence of PD-L1+ TIL is associated with greater numbers of all TIL subsets.
A subset of DCIS (19%) have PD-L1- TIL, with a small number of all TIL subsets.
All cases of triple negative DCIS contained TIL with high PD-L1 expression (>50% cells), whereas ER+ DCIS contain TIL with low (<50%) or absent PD-L1 expression.

Thompson. Mod Pathol. 2016 Jan 15. [Epub ahead of print]
What is the immune microenvironment of metastatic breast carcinoma?
Metastatic carcinomas have fewer TIL than matched primary carcinomas

- In a pilot study of 16 paired primary and metastatic breast carcinomas and subsequent expansion to 26 pairs, metastatic breast carcinomas overall contained fewer TILs than their matched primary carcinomas
- Triple negative metastases have fewer CD3+ TIL compared to the luminal metastases
- Brain metastases had fewer TILs relative to metastases from other sites
- PD-L1 labeling can differ between the primary and metastatic carcinoma.

Overall, metastatic breast carcinomas contain fewer TIL than their matched primary carcinomas, across the T cell subtypes.
The pattern of tumor infiltrating T-cell lymphocytes in matched primary and metastatic breast carcinomas differs in triple negative and luminal breast carcinoma subtypes.

Abstract from SABCS 2015

Triple-negative (TN) and HER2+ breast cancers (BC) have different immune milieu in primary and metastatic tumors


Conclude that “TN and HER2+ MBCs have a ‘colder’ immune microenvironment than primary tumors, with significantly lower expression of genes related to immune response and to antigen presentation.”
One triple negative carcinoma gained PD-L1 labeling in the metastatic tumor

Overall these findings suggest that evaluating the immunologic microenvironment of both primary and metastatic carcinoma may yield important clinical information to guide breast cancer prognosis and therapy.
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• Describe the role of tumor infiltrating lymphocytes (TILs) and immune checkpoints in breast carcinoma
• Present the current immunotherapeutic strategies in breast carcinoma
• Discuss the role of the pathologist in driving the field of cancer immunology and immunotherapy
Immunotherapy in breast cancer

Immune checkpoint antagonists

Target **molecules** expressed by tumor or immune cells

Breast cancer vaccines

Target **tumor antigens** on tumor cells

Both ultimately target the tumor-specific T cell (to activate anti-tumor immunity)
Immunotherapy in breast cancer

Immune checkpoint antagonists

- PD-1/PD-L1 pathway
- CTLA4 pathway
- LAG3 pathway

Target molecules expressed by tumor or immune cells

Immune checkpoint: Inhibitory interactions between antigen presenting cells and T cells dampen the anti-tumor T cell response

Resulting in anti-tumor T cell activation

Antigen Presenting Cell

Antitumor T cell

TCR

MHC Class I/II

KIR

Signal 1

CD80/86

CTLA4

PD-1

PD-L1

LAG3

Antigen specific (HER2, MUC-1, hTERT, survivin, mammaglobin)

Cell-based vaccines (dendritic cells, allogeneic tumor cells)

Preventative

Secondary (patients with a history of breast cancer)

Primary (patients at risk for developing breast cancer)
FDA-approved checkpoint inhibitors in solid organ tumors

• **Nivolumab** (Bristol-Myers Squibb)
  – Anti-PD-1 antibody
  – Melanoma and squamous lung carcinoma

• **Pembrolizumab** (Merck)
  – Anti-PD-1 antibody
  – Melanoma

• **Ipilimumab** (Bristol-Myers Squibb)
  – Anti-CTLA4 antibody
  – Melanoma
Trials targeting immune checkpoint pathways in breast cancer

<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>Metastatic breast cancer</td>
<td>Metastatic hormone-responsive breast cancer</td>
<td>Metastatic triple-negative breast cancer</td>
<td>Metastatic triple-negative breast cancer</td>
<td></td>
</tr>
<tr>
<td>Sample Size</td>
<td>30</td>
<td>26</td>
<td>32</td>
<td>54(^a) (21 evaluable for efficacy)</td>
</tr>
<tr>
<td>Clinical Activity</td>
<td>6-month PFS: 90%</td>
<td>SD ≥ 12 weeks: 42%</td>
<td>ORR: 18.5% 1 CR and 2 PRs 6-month PFS: 23.3%</td>
<td>ORR: 19% 2 CRs and 2 PRs 6-month PFS: 27%</td>
</tr>
<tr>
<td>Biomarkers</td>
<td>Increased activated APCs Higher % NK cells Higher % CD8+ EMT cells</td>
<td>Increased ICOS+ T cells Increased ICOS+ T cell/ FoxP3+ Treg ratio in peripheral blood</td>
<td>Not reported</td>
<td>Increased number of activated proliferating CD8+ T cells in peripheral blood Increased serum IL-18 levels</td>
</tr>
</tbody>
</table>

\(^a\)Trial continues to accrue patients with metastatic triple-negative breast cancer.

APCs = antigen-presenting cells; CR = complete response; CTLA-4 = cytotoxic T-lymphocyte antigen-4; EM = effector memory; FoxP3 = forkhead box–binding protein–3; ICOS = inducible costimulator of T-cell activation; Ig = immunoglobulin; IL = interleukin; LAG-3 = lymphocyte activation gene–3; NK = natural killer; ORR = objective response rate; PD-1 = programmed cell death–1; PD-L1 = programmed cell death ligand–1; PFS = progression-free survival; PR = partial response.
Inhibition of PD-L1 by MPDL3280A leads to clinical activity in patients with metastatic triple-negative breast cancer (TNBC)

Leisha A. Emens,1 Fadi S. Braiteh,2 Philippe Cassier,3 Jean-Pierre DeLord,4 Joseph Paul Eder,5 Marcella Fassò,6 Yan Wang,6 Wei Zou,6 Luciana Molinero,6 Daniel S. Chen,6 Ian Krop7

1 Johns Hopkins University, Baltimore, MD; 2 Comprehensive Cancer Centers of Nevada, Las Vegas, NV; 3 Centre Leon Berard, Lyon, France; 4 Institut Claudius Regaud, Toulouse, France; 5 Yale School of Medicine, New Haven, CT; 6 Genentech, Inc., South San Francisco, CA; 7 Dana-Farber Cancer Institute, Boston, MA
MPDL3280A: Phase Ia Trial Schema

Phase Ia Expansion Ongoing

<table>
<thead>
<tr>
<th>TNBC</th>
<th>UBC</th>
<th>Melanoma</th>
<th>NSCLC</th>
<th>RCC</th>
<th>Other Tumor Types</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. PD-L1–selected</td>
<td>1. PD-L1–selected</td>
<td>All-comers</td>
<td>1. All-comers</td>
<td>1. All-comers</td>
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<tr>
<td>2. All-comers</td>
<td>2. All-comers</td>
<td></td>
<td>2. PD-L1–selected</td>
<td>2. PD-L1–selected</td>
<td>2. All-comers</td>
</tr>
</tbody>
</table>

MPDL3280A doses: IV q3w 15 mg/kg, 20 mg/kg or 1200 mg
Key eligibility criteria: measurable disease per RECIST v1.1 and ECOG PS 0 or 1

- **TNBC cohort objective:** to explore the safety, efficacy and biomarkers of MPDL3280A in women with metastatic TNBC in an ongoing Phase Ia trial in solid tumors
- The TNBC cohort originally enrolled PD-L1–selected patients and then all-comers
- PD-L1 expression was centrally evaluated on tumor-infiltrating immune cells (ICs) based on an immunohistochemistry (IHC) assay using the SP142 antibody assay
MPDL3280A: Summary of Efficacy

Efficacy-evaluable patients (n = 21) with TNBC in Phase Ia expansion

<table>
<thead>
<tr>
<th>IC2/3 patients, n(^a)</th>
<th>ORR (95% CI)</th>
<th>24-Week PFS (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>19% (5-42)</td>
<td>27% (7-47)</td>
</tr>
</tbody>
</table>

- Responses included 2 CRs (1 IC3 and 1 IC2) and 2 PRs (IC2)
  - 3 of 4 responses were ongoing
- 3 patients recorded as PD appeared to experience pseudoprogression, with durable shrinkage of target and new lesions

\(^a\) IC0/1 patients not yet evaluable for efficacy.
IHC IC3 (≥ 10% of ICs PD-L1+), IHC IC2 (≥ 5% to < 10% of ICs PD-L1+).
Investigator-assessed confirmed ORR per RECIST v1.1.
Efficacy population includes patients dosed by July 21, 2014; clinical data cutoff, December 2, 2014.
Mediator duration of response has not yet been reached (range: 18 to 56+ wks)

• Median duration of survival follow-up is 40 wks (range: 2+ to 85+ wks)
MPDL3280A: Clinical Activity of MPDL3280A in a Patient With Pseudoprogression

- TNBC; s/p salvage chemotherapy (× 3), trial vaccine; MPDL3280A (Mar 2013 to Feb 2014)
- Target lesions responded, and new lesions developed; new lesions eventually responded

SLIDE COURTESY OF: Leisha Emens, MD PhD, AACR Meeting presentation
Intratumoral CD8 and PD-L1 expression were increased after MPDL3280A treatment.

Plasma cancer antigens CA19.9, CA125 and CA15.3 were reduced over time.
MPDL3280A: Conclusions

- MPDL3280A was generally well tolerated in patients with TNBC
- MPDL3280A monotherapy demonstrated promising clinical activity in PD-L1–selected patients with TNBC
  - Four of 21 PD-L1–selected patients with TNBC responded, including 2 patients with CRs
  - An additional 3 of 21 patients experienced pseudoprogression
- A patient experiencing pseudoprogression had increased intratumoral CD8 and PD-L1 as well as reduced plasma cancer antigens
- Further evaluation of MPDL3280A is ongoing in both PD-L1 IC2/3 and PD-L1 IC0/1 patients with TNBC (NCT01375842)
Immunotherapy in breast cancer

Immune checkpoint antagonists
- PD-1/PD-L1 pathway
- CTLA4 pathway
- LAG3 pathway

Breast cancer vaccines
- Therapeutic
  - Antigen specific (HER2, MUC-1, hTERT, survivin, mammaglobin)
  - Cell-based vaccines (dendritic cells, allogeneic tumor cells)
- Preventative
  - Secondary (patients with a history of breast cancer)
  - Primary (patients at risk for developing breast cancer)
Trials of Therapeutic Breast Cancer Vaccines (1/3)

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Patient Population</th>
<th>Sample Size</th>
<th>Biomarkers</th>
<th>Clinical Activity</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HER2 peptide/protein vaccines</td>
<td></td>
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</tr>
<tr>
<td>HER2 peptide + GM-CSF adjuvant</td>
<td>Stage 3,4 HER2+ breast, lung, and ovarian cancer</td>
<td>64</td>
<td>HER2-specific DTH, T cells, Ab</td>
<td>NR</td>
<td>Disis 1999[70]</td>
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<td></td>
<td></td>
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<td>Knutson 2002[71]</td>
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<td></td>
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<td>Disis 2000[72]</td>
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<td>Knutson 2001[73]</td>
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<td>Disis 2002[74]</td>
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<td></td>
<td>Disis 2004[75]</td>
</tr>
<tr>
<td>HER2 ICD protein + GM-CSF adjuvant</td>
<td>Stage 2,3,4 HER2+ breast and ovarian cancer</td>
<td>29</td>
<td>HER2-specific Ab and T cells</td>
<td>NR</td>
<td>Disis 2004[76]</td>
</tr>
<tr>
<td>HER2 peptide + GM-CSF adjuvant and trastuzumab</td>
<td>Stage 4 HER2+ breast cancer</td>
<td>22</td>
<td>HER2-specific T cells, decreased serum TGF-β</td>
<td>NR</td>
<td>Disis 2009[77]</td>
</tr>
<tr>
<td>HER2 protein + AS15 adjuvant and lapatinib</td>
<td>Stage 4 HER2+ breast cancer</td>
<td>12</td>
<td>HER2-specific Ab and T-cell responses</td>
<td>NR</td>
<td>Hamilton 2012[78]</td>
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<tr>
<td>HER2 peptide + GM-CSF adjuvant</td>
<td>Stage 4 HER2+ breast and ovarian cancer</td>
<td>14</td>
<td>HER2-specific DTH, CD8+ T cells that secrete IFNγ and lyse tumor cells</td>
<td>NR</td>
<td>Murray 2002[79]</td>
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</table>
## Trials of Therapeutic Breast Cancer Vaccines (3/3)

### Table 2: Clinical Trials of Therapeutic Breast Cancer Vaccines

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Patient Population</th>
<th>Sample Size</th>
<th>Biomarkers</th>
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<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast cancer vaccines targeting other antigens</td>
<td></td>
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</tr>
<tr>
<td>STn-KLH + CY vs KLH + CY</td>
<td>Stage 4 breast cancer</td>
<td>1,028</td>
<td>New vaccine-specific Ab</td>
<td>No difference</td>
<td>Miles 2011[80]</td>
</tr>
<tr>
<td>hTERT peptide + montanide and GM-CSF adjuvant</td>
<td>Stage 4 breast cancer</td>
<td>19</td>
<td>New TILs post vaccine, hTERT CD8+ T cells</td>
<td>Improved survival with hTERT immunity</td>
<td>Emens 2012[2]</td>
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<tr>
<td>GLOBO-H-KLH + QS21 adjuvant</td>
<td>Stage 4 breast cancer</td>
<td>27</td>
<td>IgM, CDC, ADCC; no IgG</td>
<td>2-year DFS in 56%</td>
<td>Emens 2012[2]</td>
</tr>
<tr>
<td>CEA-MUC1-TRICOM poxvirus</td>
<td>Stage 4 breast and ovarian cancer</td>
<td>26</td>
<td>Inconsistent</td>
<td>Possible CB in patients with MRD</td>
<td>Emens 2012[2]</td>
</tr>
<tr>
<td>Survivin peptide + IFA</td>
<td>Advanced/recurrent breast cancer</td>
<td>14</td>
<td>Survivin-specific T cells</td>
<td>SD in 14%</td>
<td>Emens 2012[2]</td>
</tr>
<tr>
<td>Mammaglobin cDNA</td>
<td>Stage 4 breast cancer</td>
<td>14</td>
<td>Mammaglobin-specific T cells</td>
<td>Possible benefit</td>
<td>Tiriveedhi 2014[81]</td>
</tr>
</tbody>
</table>
### Table 2: Clinical Trials of Therapeutic Breast Cancer Vaccines

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<tbody>
<tr>
<td><strong>Cell-based breast cancer vaccines</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>HER2-DC (lapuleucel-T)</td>
<td>Stage 4 HER2+ breast cancer</td>
<td>18</td>
<td>HER2-specific T-cell proliferation</td>
<td>SD in 16.7%</td>
<td>Park 2007[82]</td>
</tr>
<tr>
<td>p53-DC</td>
<td>Stage 4 breast cancer</td>
<td>26</td>
<td>p53-specific T cells and Ab in 38% and 42%</td>
<td>SD in 42%</td>
<td>Svane 2007[83]</td>
</tr>
<tr>
<td><strong>Allogeneic GM-CSF-secreting breast tumor cells + low-dose CY and DOX</strong></td>
<td>Stage 4 breast cancer</td>
<td>28</td>
<td>HER2-specific DTH and Ab; best chemo doses CY 200 mg/m², DOX 35 mg/m²</td>
<td>NR</td>
<td>Emens 2009[86]</td>
</tr>
<tr>
<td><strong>Allogeneic GM-CSF-secreting breast tumor cells + low-dose CY and trastuzumab</strong></td>
<td>Stage 4 HER2+ breast cancer</td>
<td>20</td>
<td>HER2-specific DTH and polyfunctional CD8+ T cells, decreased Tregs and MDSCs</td>
<td>6-month CB 55%; PFS 7 months; OS 42 months</td>
<td>Chen 2013[87]</td>
</tr>
<tr>
<td>DC-autologous tumor fusion</td>
<td>Stage 4 breast and renal cancer</td>
<td>23</td>
<td>Increased CD4+ and CD8+ T cells</td>
<td>SD or PR in 43%</td>
<td>Emens 2012[2]</td>
</tr>
</tbody>
</table>

Ab = antibody; ADCC = antibody-mediated cytotoxicity; CB = clinical benefit; CDC = complement-mediated cytotoxicity; CEA = carcinoembryonic antigen; CY = cyclophosphamide; DC = dendritic cell; DFS = disease-free survival; DOX = doxorubicin; DTH = delayed-type hypersensitivity; GM-CSF = granulocyte-macrophage colony-stimulating factor; HER2 = human epidermal growth factor receptor type 2; hTERT = human telomerase reverse transcriptase; ICD = intracellular domain; IFA = incomplete Freund’s adjuvant; IFN-γ = interferon-γ; Ig = immunoglobulin; KLH = keyhole limpet hemocyanin; MDSCs = myeloid-derived suppressor cells; MRD = minimal residual disease; MUC1 = mucin-1; NR = not reported; OS = overall survival; PFS = progression-free survival; PR = partial response; SD = stable disease; STn = sialyl-Tn; TGF-β = transforming growth factor–β; TILs = tumor-infiltrating lymphocytes; Treg = regulatory T cells; TRICOM = TRIad of Costimulatory Molecules.
### Trials of Preventative Breast Cancer Vaccines

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Patient Population</th>
<th>Sample Size</th>
<th>Biomarkers</th>
<th>Clinical Activity</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HER2 E75 peptide vaccine + GM-CSF adjuvant</td>
<td>Early HER2+ breast cancer NED after adjuvant therapy</td>
<td>108 vaccinated; 79 controls</td>
<td>HER2-specific memory T cells Decreased Tregs Decreased TGF-β</td>
<td>5-year DFS: 89.7% vaccinated vs 80.2% controls</td>
<td>Mittendorf 2014[89]</td>
</tr>
<tr>
<td>HER2 DC intranodal</td>
<td>DCIS</td>
<td>27</td>
<td>HER2-specific T cells; loss of HER2 expression in 50% of residual DCIS at surgery</td>
<td>18.5% with NED at surgery</td>
<td>Czerniecki 2007[91] Sharma 2012[92]</td>
</tr>
</tbody>
</table>

DC = dendritic cells; DCIS = ductal carcinoma in situ; DFS = disease-free survival; GM-CSF = granulocyte-macrophage colony-stimulating factor; HER2 = human epidermal growth factor receptor type 2; NED = no evidence of disease; TGF-β = transforming growth factor-β; Tregs = regulatory T cells.
Outline and Objectives

• Discuss the role of the pathologist in driving the field of cancer immunology and immunotherapy
  – Scoring TIL in breast carcinoma
  – Impact of tissue sampling
  – Impact of antibody choice and stain interpretation
  – The TMN-Immune staging system
How do we score TILs in breast carcinoma?
The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendations by an International TILs Working Group 2014


1Breast Cancer Translational Research Laboratory/Breast International Group, Institut Jules Bordet, Brussels; 2Department of Pathology and TCRU, GZA, Antwerp, Belgium; 3Institute of Pathology, Charité - University Hospital, Berlin, Germany; 4Perlmutter Cancer Center, New York University Medical School, New York, USA; 5Department of Pathology, Institut Jules Bordet, Université Libre de Bruxelles, Brussels, Belgium; 6European Institute of Oncology (IEO) and University of Milan, Milan, Italy; 7Department of Pathology GZA, TCRU Hospitals and CORE Antwerp University, Antwerp, Belgium; 8Genomic Health, Inc., Redwood City, USA; 9University of California San Francisco, San Francisco, USA; 10Clermont-Ferrand Biopathology, University of Auvergne, Jean Perrin Comprehensive Cancer Centre, Clermont-Ferrand, France; 11Division of Haematology/Medical Oncology and; 12Department of Cancer Biology, Mayo Clinic Comprehensive Cancer Center, Mayo Clinic, Jacksonville; 13Department of Pathology, The UT M.D. Anderson Cancer Center, Houston; 14Department of Pathology, Brigham and Women’s Hospital, Boston; 15Department of Cancer Biology, Dana Farber Cancer Institute, Boston; 16Department of Cancer Biology, Harvard Medical School, Boston, USA; 17Istituto Europeo di Oncologia, Milan, Italy; 18Department of Medical Oncology, Institut Jules Bordet, Université Libre de Bruxelles, Brussels; 19Department of Pathology, University Hospital Leuven, Leuven, Belgium; 20Department of Medicine, Department of Obstetrics and Gynecology and Women’s Health, Albert Einstein Medical Center, Bronx, USA; 21Laboratory Medicine Program, University Health Network, University of Toronto, Toronto; 22Department of Pathology and Laboratory Medicine, Genetic Pathology Evaluation Centre, University of British Columbia, Vancouver, Canada; 23Department of Pathology, Yale University School of Medicine, New Haven; 24Department of Pathology, Stanford University Medical Center, Stanford; 25Department of Pathology, Memorial Sloan-Kettering Cancer Center, New York, USA; 26German Breast Group, Neu-Isenburg, Germany; 27Department of Pathology, Istituto Europeo di Oncologia, University of Milan, Milan, Italy; 28Department of Pathology and Laboratory Medicine, Indiana University, Indianapolis, USA; 29Molecular Immunology Unit, Institut Jules Bordet, Université Libre de Bruxelles, Brussels, Belgium; 30Division of Research and Cancer Medicine, Peter MacCallum Cancer Centre, University of Melbourne, Victoria, Australia

Received 26 August 2014; accepted 28 August 2014
Table 2. Recommendations for assessing tumor-infiltrating lymphocytes (TILs) in breast cancer

1) TILs should be reported for the stromal compartment (=% stromal TILs). The denominator used to determine the % stromal TILs is the area of stromal tissue (i.e. area occupied by mononuclear inflammatory cells over total intratumoral stromal area), not the number of stromal cells (i.e. fraction of total stromal nuclei that represent mononuclear inflammatory cell nuclei).

2) TILs should be evaluated within the borders of the invasive tumor.

3) Exclude TILs outside of the tumor border and around DCIS and normal lobules.

4) Exclude TILs in tumor zones with crush artifacts, necrosis, regressive hyalinization as well as in the previous core biopsy site.

5) All mononuclear cells (including lymphocytes and plasma cells) should be scored, but polymorphonuclear leukocytes are excluded.

6) One section (4–5 μm, magnification ×200–400) per patient is currently considered to be sufficient.

7) Full sections are preferred over biopsies whenever possible. Cores can be used in the pretherapeutic neoadjuvant setting; currently no validated methodology has been developed to score TILs after neoadjuvant treatment.

8) A full assessment of average TILs in the tumor area by the pathologist should be used. Do not focus on hotspots.

9) The working group’s consensus is that TILs may provide more biological relevant information when scored as a continuous variable, since this will allow more accurate statistical analyses, which can later be categorized around different thresholds. However, in daily practice, most pathologists will rarely report for example 13.5% and will round up to the nearest 5%–10%, in this example thus 15%. Pathologist should report their scores in as much detail as the pathologist feels comfortable with.

10) TILs should be assessed as a continuous parameter. The percentage of stromal TILs is a semiquantitative parameter for this assessment, for example, 80% stromal TILs means that 80% of the stromal area shows a dense mononuclear infiltrate. For assessment of percentage values, the dissociated growth pattern of lymphocytes needs to be taken into account. Lymphocytes typically do not form solid cellular aggregates; therefore, the designation ‘100% stromal TILs’ would still allow some empty tissue space between the individual lymphocytes.

11) No formal recommendation for a clinically relevant TIL threshold(s) can be given at this stage. The consensus was that a valid methodology is currently more important than issues of thresholds for clinical use, which will be determined once a solid methodology is in place. lymphocyte-predominant breast cancer can be used as a descriptive term for tumors that contain ‘more lymphocytes than tumor cells’. However, the thresholds vary between 50% and 60% stromal lymphocytes.
Morphology, definitions, biological and diagnostic relevance of the different immune infiltrates found in breast cancer.

<table>
<thead>
<tr>
<th>Morphology</th>
<th>Definition and biological relevance</th>
<th>Diagnostic relevance</th>
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<tbody>
<tr>
<td><strong>Lymphocyte-predominant breast cancer (LPBC)</strong></td>
<td>Working category to describe tumors with &quot;more lymphocytes than tumor cells.&quot;</td>
<td>Definitions vary across studies with stromal TILs of 50–65% used as a threshold. LPBC can be used for predefined subgroup analyses and for description of tumors with a particularly high immune infiltrate, however, keep in mind that TILs are a continuous parameter and the threshold for LPBC is still arbitrary.</td>
</tr>
<tr>
<td><strong>Stromal TILs</strong></td>
<td>Indicator of increased accumulation of immune-cells in tumor tissue</td>
<td>Stromal TILs have been shown to be predictive for increased responses to neoadjuvant chemotherapy as well as improved outcome after adjuvant chemotherapy. Based on current data, this parameter is the best parameter for characterization of TILs.</td>
</tr>
<tr>
<td><strong>Intratumoral TILs</strong></td>
<td>TILs with direct cell-cell contact with carcinoma cells, might be an indicator of direct cell-based anti-tumor effects.</td>
<td>Several studies have shown that intratumoral TILs are more difficult to evaluate and do not provide additional predictive/prognostic information compared to stromal TILs.</td>
</tr>
<tr>
<td><strong>TILs at the invasive margin</strong></td>
<td>The localization of TILs are the invasive edge is included in the evaluation approach presented in this guideline.</td>
<td>For breast cancer there are no studies with a separate evaluation of TILs at the invasive edge. For practical purposes, the reliable evaluation of the invasive edge might be difficult when using core biopsies in the neoadjuvant setting.</td>
</tr>
<tr>
<td><strong>Tertiary lymphoid structures (TLS)</strong></td>
<td>Typically localized in the surrounding area of the tumor. TLS might be localized in normal tissue directly adjacent to the tumor, consisting of a T cell zone next to a B cell follicle, often with germinal centers.</td>
<td>While those structures may be important for the biology of tumor-immune reactions, they are not yet optimized for non-research based assessments. The main problem is that TLS have a spatial heterogeneity and are principally localized in areas surrounding the tumor. They might not be in the plane of the tissue section that is being evaluated, in particular when using core biopsies. Furthermore, it might be difficult to distinguish lymphoid aggregates from true TILs, in particular when the germinal center is not in the plane of the section.</td>
</tr>
</tbody>
</table>

Standardized approach for TILs evaluation in breast cancer.

Step 1: Select tumor area

Step 2: Define stromal area

Step 3: Scan at low magnification

Step 4: Determine type of inflammatory infiltrate

Step 5: Assess the percentage of stromal TILs (examples of percentages shown in figure 4)

Standardization and guidelines for TILs assessment.
What is the impact of tissue sampling on our assessment of the tumor microenvironment?
Full face sections are preferred but tissue microarrays can be useful

- Full face sections are preferred
- Tissue microarrays (TMAs) are increasingly common
  - Advantages: lower cost; increased case number
  - Disadvantages: smaller sample area
- TIL working group: “TMAs may be a good option for future studies, particularly for the rapid evaluation of large clinical cohorts. More investigation is needed before firm methodological recommendations can be offered.”
But not all tissue microarrays (TMAs) are created equal...

- Tissue sampling matters
- Number and size of cores sampled per tumor
- Geographic sampling of tumors

We use TMAs with 5-10 cores (of 1.6 mm diameter each) per tumor, sampled from the center and periphery of the primary tumor.
What is the impact of antibody choice and staining interpretation in assessing PD-L1 labeling?
PD-L1 labeling should be membranous

Membranous PD-L1 labeling in a metastatic breast carcinoma (5H1 antibody)
Will there one day be incorporation of an immune score into the standard TNM staging system to create the TNM-Immune stage (TNM-I)?
Towards the introduction of the ‘Immunoscore’ in the classification of malignant tumours
The current staging system is based on characteristics of the tumor and includes the depth of tumor invasion (T), presence of tumor in lymph nodes (N), and evidence of metastases (M).
Tumor Immunology: Implications for TNM Staging and Therapeutics

Room CC Ballroom 6 E, March 14 2016, 1:00pm to 5:00pm

Description

Special Course

Tumor Immunology: Implications for TNM Staging and Therapeutics
Monday, March 14, 2016
1:00 PM-5:00 PM

Course Directors: Janis M. Taube, MD, and Robert Anders, MD, PhD, Johns Hopkins University School of Medicine, Baltimore, MD

Course Description:
The focus of this course is TNM-Immune staging and the emerging use of surgical pathology specimens for immune-based assays, including immunologic biomarkers for therapeutic selection and monitoring. With the recent FDA approvals for PD-1/PD-L1 checkpoint inhibitors and their associated companion and complimentary diagnostics, surgical pathologists are being asked to incorporate such tests into their routine practice.

Upon completion of this educational activity, participants should be able to:

1. Summarize how the activity of checkpoint agents differs from that of more traditional therapeutics.
2. Summarize the latest concepts regarding the immune contexture of malignant neoplasms.
3. Discuss the histologic features associated with adaptive immune resistance.
4. Understand the limitations of PD-L1 evaluation.
5. Identify additional markers and associated detection techniques that will likely be employed in future Immunopathology assays.
Summary

• The tumor immune microenvironment contains counteracting forces that inhibit or promote tumor growth
• Triple negative and HER2+ breast carcinomas are immunogenic with active immune tumor microenvironments, presenting an opportunity for immune targeting
• Immunotherapeutic strategies in breast carcinoma consist of checkpoint antagonists and vaccines
• Pathologists will have a pivotal role in driving the field of cancer immunology and immunotherapy forward
As we look to the future....

How can we best identify patients and tumors likely to respond to immunotherapy?

What will be the optimal biomarker to identify patients likely to respond to immunotherapy?
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ASIP Companion Meeting, USCAP 2016

References/Suggested Reading: