Immune checkpoint inhibitors, lymphocytes, and breast cancer

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Stanford Pathology
Objectives

• Recognize the potential prognostic & predictive importance of tumor infiltrating lymphocytes in breast cancer
• Describe the mechanism of action of CTLA-4 and PD-1/PD-L1 inhibitors
  – Understand the advent of immune related Adverse Effects (irAE’s) with immune checkpoint inhibitors
  – Recognize histopathologic features of IrAE’s on tissue biopsy
• Understand the controversy surrounding biomarker analysis for immune checkpoint inhibitor therapy
Lymphocytes in breast

- Physiologic (involution)
- Diabetic mastopathy
- Duct ectasia
- Trauma/Procedure
- Autoimmune disease
- Infection
- Lymphoproliferative/Lymphoma
- Carcinoma
Diabetic mastopathy

• “lymphocytic mastopathy” or “sclerosing lymphocytic lobulitis”
• Presents as mass (esp. palpable)
• Dense lymphocytic infiltrates, circumscribed
  – Mostly B-cells
  – No germinal centers
  – Surround atrophic lobules (or ducts, vessels)
• Dense fibrous stroma
  – “Keloid-like”
  – Rarely, epithelioid fibroblasts

D’Alfonso. JPTM 2015; 49:279-87
Lupus mastitis

- Lobular lymphocytic panniculitis
- Mixed B- T-cell infiltrate with germinal centers
- Vasculitis
- Hyaline fat necrosis
- In 2-3% of lupus patients
- Rx: immunosuppression

Granulomatous mastitis

Differential diagnosis:
- TB/infectious (fungal, cat scratch, **bacterial**)  
  - Bug stains (AFB/Fite, GMS and Gram)
- Idiopathic  
  - Dx of exclusion
- Autoimmune  
  - Sarcoid  
  - Rheumatoid nodules/RA  
  - GPA/vasculitis
- Rule out carcinoma
- Infarct
- SMOLD  
- IgG4 related disease??
- (Biopsy site/foreign body)

Renshaw. AJCP 2011. 136:424-427
Granulomatous & necrotizing mastitis
Distinctive cystic spaces lined by polys
  - Spaces larger than adipocyte
Gram + organisms (corynebacterium)
  - Bugs are IN THE SPACES
  - Lipophilic, requires Tween to grow
  - Rx with tetracycline, doxycycline (2+ weeks)
  - NZ study with culture data
<table>
<thead>
<tr>
<th>Granulomatous mastitis</th>
<th>Pattern</th>
<th>Age (range)</th>
<th>Child bearing</th>
<th>Gram+ bugs?</th>
</tr>
</thead>
<tbody>
<tr>
<td>D’Alfonso AJSP</td>
<td>CNGM</td>
<td>34 (25-49)</td>
<td>nd</td>
<td>5/12</td>
</tr>
<tr>
<td>Troxell AJCP</td>
<td>CNGM</td>
<td>33 (19-47)</td>
<td>4.75 y ago</td>
<td>16/19</td>
</tr>
<tr>
<td>Troxell AJCP</td>
<td>Other GM</td>
<td>47 (29-71)</td>
<td>14 y ago</td>
<td>0/16</td>
</tr>
</tbody>
</table>

CNGM histologic pattern → lipophilic bacteria

TB (T-cell lymphoma)  
Stromal ‘idiopathic’ bilateral  
Post XRT for cancer
Core biopsy: granulomas
Same case: epithelioid histiocytes?
Same case: epithelioid histiocytes?
Lymphoplasmacytic infiltrate, mass-forming

Stromal sclerosis and loss of breast lobules

272-495 IgG4+ cells/hpf

NO GRANULOMAS!!

3 of 4 with multiple masses, elevated IgG or IgG4

Controls

- Lymphocytic mastopathy 0-5 IgG4+/hpf (n=9)
- Granulomatous mastitis 5-398 IgG4+/hpf (n=6)
IgG4-related sclerosing mastitis

Cheuk. AJSP 2009;33:1058-64
IgG4 in granulomatous mastitis

- IgG4+ plasma cells not specific for IgG4-RSD in other organs
- Granulomas unusual in IgG4-RSD in other organs
- Granulomatous mastitis not IgG4-RSD (in my opinion)

<table>
<thead>
<tr>
<th>IgG4/hpf</th>
<th>median</th>
<th>range</th>
<th># &gt;30/hpf</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNGM</td>
<td>22</td>
<td>6-58</td>
<td>2/9</td>
</tr>
<tr>
<td>Other GM</td>
<td>6</td>
<td>0-22</td>
<td>0/5</td>
</tr>
</tbody>
</table>

Berg “Evaluating breast lymphoplasmacytic infiltrates...” Hum Pathol. 2015. 46:1162-70
Breast lymphoma

• Lymphoma 0.04-1% of breast malignancies
• Breast presentation in <3% of extranodal lymphomas
• Primary breast lymphoma criteria (Wiseman & Liao, Hugh):
  – Lymphomatous infiltrate into breast tissue
  – No antecedent diagnosis of lymphoma
  – Breast as the clinical site of presentation
  – Ipsilateral lymph node involvement allowed if simultaneous
• Secondary breast lymphoma: systemic lymphoma with concurrent or subsequent involvement of breast
### Breast lymphoma subtypes

<table>
<thead>
<tr>
<th>Lymphoma</th>
<th>% 1’ BL</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>DLBCL</td>
<td>56-84%</td>
<td>ABC type 60-70%</td>
</tr>
<tr>
<td>Marginal Zone</td>
<td>9-28%</td>
<td>age ~68</td>
</tr>
<tr>
<td>Follicular</td>
<td>10-19%</td>
<td>Age ~62</td>
</tr>
<tr>
<td>Burkitt</td>
<td>&lt;6%</td>
<td>Esp. bilateral, EBV younger women</td>
</tr>
<tr>
<td>Implant associated ALCL</td>
<td>1/500,000</td>
<td>with implants</td>
</tr>
<tr>
<td>SLL, LPL, T-cell, mantle, Hodgkin</td>
<td>&lt;1% each</td>
<td></td>
</tr>
</tbody>
</table>

Seroma-associated primary anaplastic large-cell lymphoma adjacent to breast implants: an indolent T-cell lymphoproliferative disorder

Anja C Roden¹, William R Macon¹, Gary L Keeney¹, Jeffrey L Myers², Andrew L Feldman¹ and Ahmet Dogan¹

Anaplastic Large Cell Lymphoma (ALCL) In Women with Breast Implants: Preliminary FDA Findings and Analyses

January 2011
Center for Devices and Radiological Health
U.S. Food and Drug Administration

Anaplastic Large Cell Lymphoma Associated With Breast Implants: A Report of 13 Cases

Tariq N. Aladily, MD,* L. Jeffrey Medeiros, MD,* Mital B. Amin, MD,† Nisreen Haideri, MD,‡ Dongjiu Ye, MD,§ Sergio J. Azevedo, MD,∥ Jeffrey L. Jorgensen, MD, PhD,* Mariza de Peralta-Venturina, MD,¶ Eid B. Mustafa, MD,# Ken H. Young, MD, PhD,* M. James You, MD, PhD,* Luis E. Fayad, MD,** Ann Marie Blenc, MD,† and Roberto N. Miranda, MD*
Implant associated ALCL

- First reported 1997 Keech & Creech
- >90 cases reported
- Median 8 years with implants
- Effusion around implant +/-mass
- Phenotype:
  - CD30+, EMA+, ALK-
  - T-cell antigen expression (no B-cell)
    - esp. CD43, CD4, cytotoxic granule
    - CD3, CD5 weak or absent
  - T-cell gene rearrangements
- Treatment
  - surgical excision of implant and capsule
  - chemo, XRT?

Implant ALCL

Distribution:

- Effusion - sparse cells in fibrous capsule
  - 93% remission
- Mass
  - 72% remission
- R/o systemic or cutaneous

Clemens et al. J Clin Oncol 2016;34:160-68
Seroma cytology
Seroma CD30
Fibrous capsule
Lymphocytes & Carcinoma
DCIS: eos & lymphs
DCIS & lymphs
DCIS, lymphocytes & microinvasion
Calponin (p63, SMM similar)
An Examination of the Local Cellular Immune Response to Examples of Both Ductal Carcinoma In Situ (DCIS) of the Breast and DCIS With Microinvasion, With Emphasis on Tertiary Lymphoid Structures and Tumor Infiltrating Lymphocytes

Kim et al. AJCP. 2016; 146:137-44

<table>
<thead>
<tr>
<th>Feature</th>
<th>mi Absent (177)</th>
<th>Mi Present (27)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphoid aggregates around tumor (p=0.002)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>81.4%</td>
<td>3.7%</td>
</tr>
<tr>
<td>Present</td>
<td>69.5%</td>
<td>96.3%</td>
</tr>
<tr>
<td>Breast cancer subtype (p=0.001)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ER+/HER2-</td>
<td>59.9%</td>
<td>7.4%</td>
</tr>
<tr>
<td>ER+/HER2+</td>
<td>13.6%</td>
<td>14.8%</td>
</tr>
<tr>
<td>ER-/HER2+</td>
<td>20.9%</td>
<td>59.3%</td>
</tr>
<tr>
<td>Triple neg</td>
<td>5.6%</td>
<td>18.5%</td>
</tr>
</tbody>
</table>

Not shown: significant difference by DCIS necrosis, nuclear grade, germinal center in TLS
Calponin stain
(p63, SMM similar)

All DCIS?
Microinvasive CA (CAP/AJCC)

- Invasion measuring 1mm or less
- If multiple:
  - Do not add sizes together
  - Estimate # of foci, or ‘too numerous to quantify’
- IHC for myoepithelial cells
- IHC for ERPgRHer2 (?)
Tumor-immune interactions

- Immune response can eliminate incipient tumors
- Advanced tumors have ‘escaped’ host immunity
  - Immune parameters may still influence survival
    - Abundant CD8+ infiltration associated with survival
    - Degree of lymphoid infiltrate associated with survival for triple-negative & Her2+
  - Hormone receptor+ tumors have least lymphoid infiltrate
  - Immunologic parameters related to pCR in neoadjuvant setting
  - Lymphocyte predominant breast cancer: ‘more lymphocytes than tumor cells’; 50+% stromal TILS
- Immune cells shape microenvironment
- Immune modulation in cancer therapy

The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendations by an International TILs Working Group 2014


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**Abbreviations:**
- TILs: tumor-infiltrating lymphocytes
- M1: macrophage activated pro-inflammatory
- M2: macrophage activated anti-inflammatory
- N1: neutrophil activated pro-inflammatory
- N2: neutrophil activated anti-inflammatory
- Th: helper CD4+ T cells
- TGFβ: transforming growth factor beta
- DC: dendritic cell
- MDSC: myeloid suppressor cell
- IFNγ: interferon gamma
- IL21: interleukin 21
- IgG: immunoglobulin G
- Treg: regulatory T cell

**Figure:**
- Diagram illustrating the interaction between different cell types in tumor suppression and progression.
Immune microenvironment in breast cancer
TILS: invasive carcinoma

Workgroup document with guidelines, tutorial for stromal TILs

Immune checkpoint inhibitors

Takes the brakes off of the immune system, allowing enhanced attack on cancer cells

Overcomes tumor immune evasion
## Immune checkpoint: alphabet soup

<table>
<thead>
<tr>
<th>Agent</th>
<th>Trade</th>
<th>Target</th>
<th># Trials</th>
<th># breast ca</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ipilimumab</td>
<td>Yervoy</td>
<td>CTLA-4</td>
<td>294</td>
<td>17</td>
</tr>
<tr>
<td>Tremelimumab</td>
<td>In trials</td>
<td>CTLA-4</td>
<td>82</td>
<td>10</td>
</tr>
<tr>
<td>Nivolumab</td>
<td>Opdivo</td>
<td>PD-1</td>
<td>241</td>
<td>15</td>
</tr>
<tr>
<td>Pembrolizumab</td>
<td>Keytruda</td>
<td>PD-1</td>
<td>345</td>
<td>34</td>
</tr>
<tr>
<td>Atezolizumab</td>
<td>Tecentriq</td>
<td>PD-L1</td>
<td>81</td>
<td>11</td>
</tr>
<tr>
<td>Durvalumab</td>
<td>In trials</td>
<td>PD-L1</td>
<td>118</td>
<td>23</td>
</tr>
<tr>
<td>Avelumab</td>
<td>In trials</td>
<td>PD-L1</td>
<td>18</td>
<td>2</td>
</tr>
</tbody>
</table>

Immune checkpoint inhibitors
Takes the brakes off immune system, restoring attack on cancer cells

CLTA-4
“cytotoxic T-lymphocyte-associated protein 4”

Immune checkpoint inhibitors
Takes the brakes off immune system, restoring attack on cancer cells

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Immune checkpoint inhibitors
Takes the brakes off immune system, restoring attack on cancer cells

Breast cancer studies

<table>
<thead>
<tr>
<th>Drug</th>
<th>N</th>
<th>Inclusion (PD-L1 IHC)</th>
<th>% of screen population +</th>
<th>Response (% patients)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atezolizumab</td>
<td>21</td>
<td>5% lymphs</td>
<td>23%</td>
<td>19% TNBC</td>
</tr>
<tr>
<td>Pembrolizumab</td>
<td>32</td>
<td>1% tumor</td>
<td>58%</td>
<td>18.5% TNBC</td>
</tr>
<tr>
<td>Tremelimumab</td>
<td>26</td>
<td></td>
<td></td>
<td>0 (ER+)</td>
</tr>
</tbody>
</table>

Problems with immune checkpoint inhibitors

Immune related adverse effects (irAE)
Side effect: attack on normal tissues
**Immune related adverse effects: irAE’s**


<table>
<thead>
<tr>
<th>Organ System</th>
<th>Ipilimumab (Anti-CTLA-4), Any Adverse Effects(^7) (%)</th>
<th>Ipilimumab Grade 3 + (^7) (%)</th>
<th>Nivolumab &amp; Pembrolizumab (Anti-PD-1), Any Adverse Effects* (%)</th>
<th>Nivolumab (Anti-PD-1), Grade 3 + † (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin (rash, pruritus, vitiligo, esp. in melanoma)</td>
<td>65</td>
<td>&lt; 3</td>
<td>40.7</td>
<td>1.1</td>
</tr>
<tr>
<td>Gastrointestinal (colitis, diarrhea, perforation)</td>
<td>33</td>
<td>10</td>
<td>30.5</td>
<td>1.8</td>
</tr>
<tr>
<td>Respiratory (pneumonitis, sarcoidosis)</td>
<td>Not listed</td>
<td>Not listed</td>
<td>13.1</td>
<td>0.8</td>
</tr>
<tr>
<td>Endocrine (thyroiditis, hypophysitis, adrenalitis)</td>
<td>&lt; 5</td>
<td>&lt; 3</td>
<td>8.5</td>
<td>0.4</td>
</tr>
<tr>
<td>Renal &amp; urinary (interstitial nephritis, immune complex GN)</td>
<td>Case reports</td>
<td>Case reports</td>
<td>1.5</td>
<td>0.6</td>
</tr>
<tr>
<td>Hepatobiliary (hepatitis, biliary pattern)</td>
<td>&lt; 2</td>
<td>1</td>
<td>2.9</td>
<td>0</td>
</tr>
</tbody>
</table>

Enterocolitis in Patients With Cancer After Antibody Blockade of Cytotoxic T-Lymphocyte–Associated Antigen 4

• Stomach, SI, colon
• Lymphoplasmacytic expansion of LP
• Intraepithelial lymphocytes (T-cells)
• Epithelial apoptosis
• Cryptitis
• Villous blunting in SI
• “autoimmune enteropathy” like
Marginean Archives 2016;140: 748-58
• GVHD-like pattern, or
• Focal acute colitis
Ipilimumab: Kidney

73 year old woman with melanoma
Brisk AIN with eosinophils
Pembrolizumab: Kidney

66 year old man with urothelial CA
Brisk AIN with eos, tubulitis, ATN
Problems with immune checkpoint inhibitors

Biomarker testing (*)&^%^%$##!!!
Drug & biomarker development

New Drug \[\rightarrow\] Biomarker for drug (target) \[\rightarrow\] Clinical trial \[\rightarrow\] Approval with contingent biomarker

“Companion diagnostics”
Drug & biomarker: PD-L1

- New Drug
- Biomarker for drug (target)
- Clinical trial
- Approval with contingent biomarker

Same drug mechanism, target & biomarker:
- Nivolumab
  - 28-8 Dako Link
- Pembrolizumab
  - 22C3 Dako Link
- Atezolizumab
  - SP142 Ventana Ultra
- Durvalumab
  - SP263 Ventana Ultra
- Doka Link

## Immune checkpoint: alphabet soup

<table>
<thead>
<tr>
<th>Agent</th>
<th>Trade</th>
<th>Drug Target</th>
<th>Antibody (PD-L1)</th>
<th>Platform</th>
<th>Defn + (tumor cells unless noted)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nivolumab</td>
<td>Opdivo</td>
<td>PD-1</td>
<td>Dako 28-8</td>
<td>Link 48 Envision FLEX</td>
<td>5% ‘complementary diagnostic’</td>
</tr>
<tr>
<td>Pembrolizumab</td>
<td>Keytruda</td>
<td>PD-1</td>
<td>Dako 22C3</td>
<td>Link 48 Envision FLEX</td>
<td>Strong= &gt;50%  Weak= 1-49%</td>
</tr>
<tr>
<td>Atezolizumab</td>
<td>Tecentriq</td>
<td>PD-L1</td>
<td>SP142</td>
<td>Ventana Ultra/Optiview</td>
<td>Tumor (1,5,50%) Immune (1, 5, 10%)</td>
</tr>
<tr>
<td>Durvalumab</td>
<td></td>
<td>PD-L1</td>
<td>SP263</td>
<td>Ventana Ultra/Optiview</td>
<td>25%</td>
</tr>
<tr>
<td>Avelumab</td>
<td></td>
<td>PD-L1</td>
<td>Dako?</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Assessment of the PD-L1 status by immunohistochemistry: challenges and perspectives for therapeutic strategies in lung cancer patients

Virchows Arch 2016;468:511–525

Marius Ilie1,2,3,4 • Véronique Hofman1,2,3,4 • Manfred Dietel5,6 • Jean-Charles Soria7,8 • Paul Hofman1,2,3,4
CA & immune cells +

CA neg; immune cells +
Ilie et al. Virchows Arch 2016;468:511–525

28-8
E1L3N
SP142
SP263
PD-(L)1 assays: issues

- Pre-analytic
  - Ischemic time
  - Fixation type (cytology, FFPE)
  - Triage of small tissue for dx, ALK, ROS, molecular, etc.
- Which, if any, assay for your lab?
- Tumor heterogeneity
  - Spatial: small bx
  - Temporal: primary vs. met, ?retest
- VALIDATION/VERIFICATION
  - Predictive marker
- Controls
  - Normal tonsil, placenta

- Interpretation, reporting
  - Training
  - Must list assay details
  - Score tumor vs. lymphs?
  - Semi-quantitation (%)
  - And overall pos/neg per kit
- Proficiency testing
- ?digital analysis
- LDT regulation?
- Future: drug combinations
- Payment for unmatched drug-tests? Malpractice?
“Complexities in Personalized Medicine: Harmonizing Companion Diagnostics Across a Class of Targeted Therapies”

- FDA-AACR-ASCO sponsored conference March, 2015
- “Blueprint project” to evaluate comparability of PD-L1 assays (technical equivalence)
  - “The goal of this proposal is to agree and deliver, via cross industry collaboration, a package of information/data upon which analytic comparison of the various diagnostic assays may be conducted, potentially paving the way for post-market standardization and/or practice guideline development as appropriate.”
  - “reduction to a single assay is considered to be neither feasible nor beneficial for the market”
PD-(L)1 IHC, new reality or fad: Other biomarkers?

- PD-L2 inhibitors? Other checkpoints? IFN-gamma expression?
- **Tumor infiltrating lymphocytes**
  - Morphologic or IHC
- **Mutation burden**: neoantigens (MMR-d colon, smoking-lung)
- Lung: EGFR or KRAS mutation
- Immune gene signatures (mRNA)
- Change in peripheral lymphocyte counts during Rx
- Neoantigen specific circulating T-cells
- CTC’s with high PD-L1?
“Pathologists must take the lead in the rational incorporation of these biomarkers into clinical practice. It is imperative that concerned pathology societies gather their experts, consider feasible approaches to addressing these growing logistical and economic challenges, and begin to develop guidelines to inform pathology practice and, ultimately, influence trends in oncology.”
Lymphocytes and the breast

- Inflammation in the breast may be associated with systemic or treatable conditions (CNGM)
- Consider occult carcinoma
- TILs in breast cancer: stay tuned
- Immune checkpoint inhibitors are here to stay
  - irAE’s are becoming characterized, biopsied
  - PD-L1 assays are a mess
Selected References


• See also on-slide citations
End