What the clinician needs to know from the breast pathologist

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Outline

Adjuvant setting, predictive biomarkers

Metastatic setting, targeted therapy for actionable mutations

Breast cancer immunotherapy
Outline

Adjuvant setting, predictive biomarkers

Metastatic setting, targeted therapy for actionable mutations

Breast cancer immunotherapy
Clinically useful characteristics in BC pts

- Patient characteristics
- Disease characteristics
- Biomarkers

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Ethnicity)</td>
<td></td>
</tr>
<tr>
<td>Tumor type</td>
<td></td>
</tr>
<tr>
<td>Tumor size</td>
<td></td>
</tr>
<tr>
<td>Axillary status</td>
<td></td>
</tr>
<tr>
<td>Tumor grade</td>
<td></td>
</tr>
<tr>
<td>Peri-tumoral vascular invasion</td>
<td></td>
</tr>
<tr>
<td>Margin status</td>
<td></td>
</tr>
<tr>
<td>ER/PgR/HER2/Ki-67</td>
<td></td>
</tr>
<tr>
<td>Multigene prognosticators ER+/HER2-</td>
<td></td>
</tr>
</tbody>
</table>
Breast inter-tumor heterogeneity
Morphology & phenotype

[Graphs and images showing disease free survival and overall survival for different breast cancer subtypes, with statistical data shown for log-rank tests.]
Clinically useful characteristics in BC pts

- **Patient characteristics**
  - Age
  - (Ethnicity)

- **Disease characteristics**
  - Tumor type
  - Tumor size
  - Axillary status
  - Tumor grade
  - Peri-tumoral vascular invasion
  - Margin status

- **Biomarkers**
  - ER/PgR/HER2/Ki-67
  - Multigene prognosticators ER+/HER2-
Multigene tests

- Gene expression – based assay to predict the risk of recurrence
- Oncotype DX has been validated to predict benefits from chemotherapy in early stage, hormone receptor positive breast cancer patients
- Expensive tests
- Combined approach allowed
Oncotype DX

- RS predictive value evaluated in the NSABP B20 trial (TAM vs TAM+CMF)
- Patients with RS≥31 derive significant benefit from the adjunct of CHT
- Intermediate RS further evaluated in the TAILORx Trial
- Oncotype DX has been demonstrated to select
Multigene tests

- Gene expression-based assay to predict the risk of recurrence
- Oncotype DX has been validated to predict benefits from chemotherapy in early stage, hormone receptor positive breast cancer patients
- Expensive tests
- Combined approach allowed
**BONDx Study:** 394 pts in 4 centers in Lombardy ((1428 consecutive eBC pts → 1082 HR+/HER2-/N0 & N1 → 398 Intermediate risk → 394 enrolled)

1428 eBC

1082 HR+/HER2-

398 Intermediate clinical-pathologic risk

**Intermed/Low Risk (≥ 4)**
- G1
- T1a-b
- Ki67 < 15%
- NO
- ER > 80%

**Intermed/High Risk (≥ 4)**
- G3
- T > 2
- Ki67 > 30%
- N+
- ER < 30%
BONDx Study

Pre Test | Post Test
---|---
CT+HT | HT
N=97 | N = 48
25% of total | 49% 49%
CT+HT | CT+HT
N= 49 | N=12
51% | - 4%

49% CT reduction
4% CT increment

Treatment recommendation changed in 15% cases (61 pts)

Courtesy of Dr. Tondini
To be prescribed by a breast unit (150 pts/year)

<table>
<thead>
<tr>
<th>Low risk (at least 4 of the following:)</th>
<th>Intermediate risk</th>
<th>High risk (at least 4 of the following:)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>T1a/T1b</td>
<td>G3</td>
</tr>
<tr>
<td>Ki-67 &lt;15%</td>
<td>1500 patients/year</td>
<td>T3/T4</td>
</tr>
<tr>
<td>ER &gt;80%</td>
<td>50-75% CHT reduction</td>
<td>Ki-67 &gt;30%</td>
</tr>
<tr>
<td>N0</td>
<td></td>
<td>ER &lt;30%</td>
</tr>
</tbody>
</table>

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Outline

Adjuvant setting, predictive biomarkers

Metastatic setting, targeted therapy for actionable mutations

Breast cancer immunotherapy
Putative involvement of genomic Heterogeneity in ET resistance


Razavi, Cancer Cell, 2018
Activating ESR1 mutations lead to hormone therapy resistance
Predictive value of ESR1 mutations

SoFEA

ESR1-m

Time From Random Assignment (months)

Progression-Free Survival (%)

Exemestane

Median PFS, 2.6 months

(95% CI, 2.4 to 6.2)

Fulvestrant-containing regimen

Median PFS, 5.7 months

(95% CI, 3.0 to 8.6)

HR, 0.52 (95% CI, 0.30 to 0.90); P = .02

No. at risk (events)

Exemestane

18 (12) 6 (4) 2 (2) 0 (0) 0

Fulvestrant-containing

45 (23) 22 (12) 6 (6) 6 (6) 0

ESR1-wt

Time From Random Assignment (months)

Progression-Free Survival (%)

Exemestane

Median PFS, 6.0 months

(95% CI, 3.0 to 11.1)

Fulvestrant-containing regimen

Median PFS, 5.4 months

(95% CI, 3.4 to 8.4)

HR, 1.07 (95% CI, 0.68 to 1.69); P = .77

No. at risk (events)

Exemestane

29 (18) 21 (9) 12 (5) 5 (3) 0

Fulvestrant-containing

69 (31) 27 (17) 9 (9) 0 (0) 3

PALOMA3

ESR1-m

Time From Random Assignment (months)

Progression-Free Survival (%)

Fulvestrant + Palbociclib

Median PFS, 8.5 months

(95% CI, 2.9 to 11.1)

Fulvestrant + Placebo

Median PFS, 3.6 months

(95% CI, 2.0 to 5.5)

HR, 0.43 (95% CI, 0.26 to 0.74); P = .002

No. at risk (events)

Fulvestrant + Palbociclib

63 (16) 46 (7) 36 (6) 22 (5) 0

Fulvestrant + Placebo

28 (10) 18 (11) 6 (1) 3 (2) 1

ESR1-wt

Time From Random Assignment (months)

Progression-Free Survival (%)

Fulvestrant + Palbociclib

Median PFS, 8.5 months

(95% CI, 2.9 to 11.1)

Fulvestrant + Placebo

Median PFS, 5.4 months

(95% CI, 3.5 to 7.4)

HR, 0.49 (95% CI, 0.35 to 0.70); P < .001

No. at risk (events)

Fulvestrant + Palbociclib

177 (30) 142 (20) 108 (13) 50 (7) 6

Fulvestrant + Placebo

92 (20) 57 (10) 25 (6) 16 (4) 0

Fribbens, JCO, 2016
<table>
<thead>
<tr>
<th>Gene</th>
<th>Aberration</th>
<th>Frequency</th>
<th>Targeted drug(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Randomized or phase II trials</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BRCA1/2</td>
<td>Inactivating mutation (germiline)</td>
<td>5%</td>
<td>Olaparib, Talazoparib</td>
</tr>
<tr>
<td>PIK3CA</td>
<td>Activating mutation</td>
<td>30-40%</td>
<td>Alpelisib</td>
</tr>
<tr>
<td>Several</td>
<td>Microsatellite instability</td>
<td>1-2%</td>
<td>Pembrolizumab</td>
</tr>
<tr>
<td>HER2</td>
<td>Activating mutation</td>
<td>2%</td>
<td>T,P,T-DM1,L</td>
</tr>
<tr>
<td>NTRK</td>
<td>Gene fusion</td>
<td>&lt;1%</td>
<td>Larotrectinib</td>
</tr>
<tr>
<td><strong>Emerging</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FGFR1-4</td>
<td>Amplification</td>
<td>10%</td>
<td>FGFR-inhibitors</td>
</tr>
<tr>
<td>ESR1</td>
<td>Mutation (after AI exposure)</td>
<td>30-40%</td>
<td>Fulvestrant, SERDs</td>
</tr>
<tr>
<td>AKT</td>
<td>Activating mutation</td>
<td>2%</td>
<td>AKT/mTOR inhibitors (MK2206, everolimus, temsirolimus)</td>
</tr>
<tr>
<td>PTEN</td>
<td>Inactivating mutation or methylation</td>
<td>20%</td>
<td>PI3K (non-α selective), AKT, mTOR inhibitors</td>
</tr>
<tr>
<td>c-MET</td>
<td>Amplification/mutation</td>
<td>15%</td>
<td>MET inhibitors (cabozantinib)</td>
</tr>
<tr>
<td>CDH1</td>
<td>Inactivating mutation</td>
<td>7%</td>
<td>Wnt inhibitors</td>
</tr>
<tr>
<td>Myc</td>
<td>Amplification</td>
<td></td>
<td>BET inhibitors</td>
</tr>
</tbody>
</table>
NGS in the clinical practice of a pathology lab

- Comprehensive genomic analysis in clinical samples

- Technical proficiency

- Druggable/actionable targets
NGS is cost-effective

- $\Delta_{\text{STD} \rightarrow \text{NGS}}$ personnel time savings
- $\Delta_{\text{STD} \rightarrow \text{NGS}}$ cost per patient
Standard approach/NGS break-even

**Minimum Set**
- Break Even: €220
- Volume Centro 3: €260

**Clinical Practice**
- Break Even: €160
- Volume Centro 3: €260

**Future C.P. NO TMB**
- Break Even: €180
- Volume Centro 3: €260

**Future C.P.**
- Break Even: €0
- Volume Centro 3: €260

Pruneri, submitted, 2019
Outline

Adjuvant setting, predictive biomarkers

Metastatic setting, targeted therapy for actionable mutations

Breast cancer immunotherapy
BC immunotherapy goes on stage

- 902 pts R Nab-Paclitaxel/Atezolizumab
- Co-primary end-points PFS in overall and PD-L1+ve population + OS in overall and, if significant, in the PD-L1 population
  - PFS 7.2 vs. 5.5 (median FU 12.9 mo) p=.002
  - PFS 7.5 vs. 5 in PD-L1+ve pts (p<0.001)
  - Median OS 21.3 vs. 17.6 p=.08
  - Median OS 25.0 vs. 15.5 in PD-L1+ve pts (HR 0.62 CI 0.45-0.86)
Biomarkers in IMpassion130 (NCT02425891)

- Ventana PD-L1 (SP142)

- Tumor Infiltrating immune cells (>=1% of the tumor area, any intensity of immunoreaction)

- Test approved by FDA as a companion diagnostic for selecting PD-L1 positive unresectable locally advanced or metastatic TNBC patients for atezolizumab
PD-L1 IHC evaluation: the war of clones

Each drug a platform, an Ab, a scoring system and a threshold

<table>
<thead>
<tr>
<th>Drug</th>
<th>PD-L1 Diagnostic Antibody Clone</th>
<th>PD-L1 Binding Domain</th>
<th>Platform</th>
<th>Second-line Criteria for PD-L1 Positivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nivolumab (Bristol-Myers Squibb)</td>
<td>28-8 (rabbit)</td>
<td>Extracellular</td>
<td>Link 48 Autostainer</td>
<td>≥1% tumor cells</td>
</tr>
<tr>
<td>Pembrolizumab (Merck)</td>
<td>22C3 (mouse)</td>
<td>Extracellular</td>
<td>Link 48 Autostainer</td>
<td>≥50% tumor cells</td>
</tr>
<tr>
<td>Atezolizumab (Genentech/Roche)</td>
<td>SP142 (rabbit)</td>
<td>Cytoplasmic</td>
<td>BenchMark ULTRA</td>
<td>Tumor cells and/or tumor-infiltrating immune cells</td>
</tr>
<tr>
<td>Durvalumab (AstraZeneca/MedImmune)</td>
<td>SP263 (rabbit)</td>
<td>Extracellular*</td>
<td>BenchMark</td>
<td>≥25% tumor cells</td>
</tr>
<tr>
<td>Avelumab (Pfizer/Merck Serono)</td>
<td>73-10</td>
<td>unknown</td>
<td>Dako assay</td>
<td>≥1% tumor cells</td>
</tr>
</tbody>
</table>
PERFORMANCE OF PD-L1 IMMUNOHISTOCHEMISTRY ASSAYS IN UNRESECTABLE LOCALLY ADVANCED OR METASTATIC TRIPLE-NEGATIVE BREAST CANCER: POST HOC ANALYSIS OF IMPASSION130

Hope S. Rugo,1 Sherene Loi,2 Sylvia Adams,3 Peter Schmid,4 Andreas Schneeweiss,5 Carlos H. Barrios,6 Hiroji Iwata,7 Véronique Diéras,8 Eric P. Winer,9 Mark M. Kockx,10 Dieter Peeters,10 Stephen Y. Chui,11 Jennifer C. Lin,11 Anh Nguyen Duc,11 Giuseppe Viale,12 Luciana Molinero,11 Leisha A. Emens13

1University of California San Francisco Comprehensive Cancer Center, San Francisco, CA, USA; 2Peter MacCallum Cancer Centre, Melbourne, VIC, Australia; 3NYU Langone Medical Center, New York, NY, USA; 4Barts Cancer Institute, Queen Mary University London, London, UK; 5University Hospital and German Cancer Research Center Heidelberg, Heidelberg, Germany; 6Centro de Pesquisa Clínica, HSL, PUCRS, Porto Alegre, Brazil; 7Aichi Cancer Center Hospital, Nagoya, Japan; 8Department of Medical Oncology, Centre Eugène Marquis, Rennes, France; 9Dana-Farber Cancer Institute, Boston, MA, USA; 10HistoGeneX NV, Antwerp, Belgium; 11Genentech, Inc., South San Francisco, CA, USA; 12University of Milan, European Institute of Oncology IRCCS, Milan, Italy; 13University of Pittsburgh Medical Center Hillman Cancer Center, Pittsburgh, PA, USA
PD-L1 IHC assays: prevalence and analytical concordance

NPA, negative percentage agreement; OPA, overall percentage agreement; PPA, positive percentage agreement.

* > 97% of SP142+ samples included in 22C3+ or SP263+ samples. *Compared with 41% in ITT (Schmid, New Engl J Med 2018).

≥ 90% OPA, PPA and NPA required for analytical concordance.
Clinical outcomes in PD-L1+ populations per SP142 (IC 1%), 22C3 (CPS 1) and SP263 (IC 1%)

<table>
<thead>
<tr>
<th>Population</th>
<th>Median PFS, mo</th>
<th>HR (95% CI)</th>
<th>Median OS, mo</th>
<th>HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A +nP</td>
<td>P +nP</td>
<td>Δ</td>
<td>A +nP</td>
</tr>
<tr>
<td>SP142</td>
<td>8.3</td>
<td>4.1</td>
<td>4.2</td>
<td>0.60 (0.47, 0.78)</td>
</tr>
<tr>
<td>IC ≥ 1%: 46%</td>
<td>(285/614)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22C3</td>
<td>7.5</td>
<td>5.4</td>
<td>2.1</td>
<td>0.68 (0.56, 0.82)</td>
</tr>
<tr>
<td>CPS ≥ 1: 81%</td>
<td>(497/614)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SP263</td>
<td>7.5</td>
<td>5.3</td>
<td>2.2</td>
<td>0.64 (0.53, 0.79)</td>
</tr>
<tr>
<td>IC ≥ 1%: 75%</td>
<td>(460/614)</td>
<td></td>
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</table>

HR adjusted for prior taxanes, presence of liver metastases, age and ECOG PS.

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Stromal TILs predicted PFS benefit, but not OS benefit

<table>
<thead>
<tr>
<th>TILs+/PD-L1 IC+ (n = 190)</th>
<th>HR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFS</td>
<td>0.53 (0.38, 0.74)</td>
<td>≤ 0.005</td>
</tr>
<tr>
<td>OS</td>
<td>0.57 (0.35, 0.92)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TILs−/PD-L1 IC+ (n = 176)</th>
<th>HR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFS</td>
<td>0.74 (0.54, 1.03)</td>
<td>0.07</td>
</tr>
<tr>
<td>OS</td>
<td>0.65 (0.41, 1.02)</td>
<td>0.06</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TILs+/PD-L1 IC− (n = 94)</th>
<th>HR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFS</td>
<td>0.99 (0.62, 1.57)</td>
<td>0.97</td>
</tr>
<tr>
<td>OS</td>
<td>1.53 (0.76, 3.08)</td>
<td>0.24</td>
</tr>
</tbody>
</table>

Be aware of subgroup-analysis, comparing variables scored using different scoring-methods.
- TILs scored as a continous variable, compared with PDL1 scored a categorical variables is not appropriate. This applies also to comparing or pooling different antibodies that were scored using different methods. Stromal TILs should be assessed as a continuous variable on its own.

BEP (TILs): n = 893. Cutoff of 10% was used to distinguish low vs intermediate/high levels of TILs (Denkert Lancet Oncol 2018).
* 2x2 Fisher's exact test.

TILs+ were enriched for PD-L1 IC+ (P < 0.0001) but PD-L1 IC+ were not enriched for TILs+ (P = ns)
* Patients with TILs+ tumors only derived clinical benefit if their tumors were also PD-L1 IC+

Conclusions

- With overall percentage agreements of 64% (22C3) and 69% (SP263), the analytical concordance was subpar (<90%) and the assays are not equivalent
  - 22C3 (CPS ≥ 1) and SP263 (IC ≥ 1%) PD-L1 assays identified a larger patient population of which SP142+ (IC ≥ 1%) is a subgroup
- The clinical benefit in 22C3+ and SP263+ subgroups was driven by the SP142+ subgroup
  - The SP142 assay identified patients with the smallest HR point estimates and longest median PFS and OS from atezolizumab + nab-paclitaxel
- The SP142 assay at IC 1% cutoff is the approved diagnostic test used to identify patients with mTNBC most likely to benefit from the addition of atezolizumab to nab-paclitaxel
Immune check-point inhibitor biomarkers

Tumor antigens
(e.g., tumor mutation burden, MSI, neoantigens)

Inflamed tumor
(gene signatures, TILs, & TAICs)

Tumor immune suppression
(PD-L1, CTLA4, Tregs, MDSCs, IDO, LAG-3)

Host
(microbiome & SNPs)

Früh and Peters, Cancer Cell, 2018
Tumors with >150 non-syn mut (10 mut/mb DNA) form neoantigens
BC mutational load inter-tumor heterogeneity
TMB in CheckMate-012 Nivo + Ipi NSCLC
Immunotherapy in breast cancer, take-home

- Immunotherapy for BC pts entered the clinical arena
- Ventana SP142 is a reliable biomarker for atezo (other drugs and Abs?)
- TILs should be included among predictive/prognostic biomarkers
- Keep addressing clinical utility of other biomarkers (GEP, TILs, TMB)
- Tissue workflow and pre-analytical steps critical
- Improve and check reproducibility among pathologists (digital pathology, automated analysis)
Thank you for the attention