Principles of Laboratory Testing for Treatment Decision-Making in Metastatic Breast Cancer

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Genomic Landscape in Receptor Subtypes of Breast Cancer

Early primary tumor

Clonal evolution and genetic instability

Early dissemination

Metastases in different organs

Late dissemination

Metastases in different organs

Therapy-induced clonal evolution

Resistant clone

Evolution of resistant clone

The Fluid State of the Tumor Micro-Ecosystem: Genomic Evolution and Adaptive Resistance

Santarpia L et al. The Oncologist 2016
Within Tumours:
- subclones

Between patients: Subtypes and clinical courses

Within a patient: Evolution, divergent paths
# What, How, and Why Do We Test in Metastatic Breast Cancer?

<table>
<thead>
<tr>
<th>Test</th>
<th>Material Measured</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estrogen/Progesterone receptor</td>
<td>Protein</td>
<td>Effectiveness of hormonal (endocrine) therapies</td>
</tr>
<tr>
<td>HER2 receptor</td>
<td>Protein and/or DNA</td>
<td>Effectiveness of HER2-targeted therapies</td>
</tr>
<tr>
<td>PD-L1</td>
<td>Protein</td>
<td>Effectiveness of immunotherapy</td>
</tr>
<tr>
<td>Germline (Hereditary) gene mutations</td>
<td>Germline DNA</td>
<td>Genetic counseling, cancer prevention, Effectiveness of PARP inhibitors therapy</td>
</tr>
<tr>
<td>Tumor Mutations</td>
<td>Tumor DNA</td>
<td>Tumor gene mutations that show potential activity of specific targeted therapies</td>
</tr>
<tr>
<td>Tumor Gene Expression (RNA)</td>
<td>Tumor RNA</td>
<td>Expression profiles to optimize and personalize treatment (experimental)</td>
</tr>
</tbody>
</table>
Recommendations for Testing in Metastatic Breast Cancer

• **Imaging** (CT chest, abdomen, pelvis and bone scan or whole body PET/CT scan, if indicated brain MRI)

• Genetic counseling and germline DNA testing

• Biopsy of metastatic lesion for histological confirmation of cancer, ER, PR and HER2 testing

• After 1st line therapy (or sooner), tumor gene next generation DNA and/or RNA sequencing
Estrogen and Progesterone Receptor Immuno-Staining:
Scoring by % Staining and Intensity

Estrogen Receptor Assay Examples

Negative

Weak

Strong

$0 + 0 = 0$

$2 + 1 = 3$

$3 + 5 = 8$
Estrogen, Estrogen Receptor and Different Endocrine Therapies Activities in HR+ Breast Cancer

- Aromatase
- Aromatase Inhibitor
- Androgen
- Estrogen
- Ovaries

- SERM (Tamoxifen, Toremifene)
- Downregulator (Fulvestrant)

- Chaperone
- Estrogen receptor

- DNA
Primary Drivers of Growth in HR+/HER2-Breast Cancer

These also mediate resistance to endocrine therapy.
The Importance of the PI3K Pathway in HR+ Breast Cancer

PI3K isoforms

Overall Response Rates and Survival in the PIK3CA-Mutant Cohort

Patients with measurable disease:
- Placebo + fulvestrant: 35.7%
- Alpelisib + fulvestrant: 16.2%
  - Significance: p=0.0002

All patients:
- Placebo + fulvestrant: 26.6%
- Alpelisib + fulvestrant: 12.8%
  - Significance: p=0.0006

**Table:**

<table>
<thead>
<tr>
<th></th>
<th>Alpelisib + FUL (n=169)</th>
<th>Placebo + FUL (n=172)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. events, n (%)</td>
<td>87 (51.5)</td>
<td>94 (54.7)</td>
</tr>
<tr>
<td>Censored, n (%)</td>
<td>82 (48.5)</td>
<td>78 (45.3)</td>
</tr>
<tr>
<td>Median OS, mo (95% CI)</td>
<td>39.3 (34.1-44.9)</td>
<td>31.4 (26.8-41.3)</td>
</tr>
<tr>
<td>HR (95% CI)</td>
<td>0.86 (0.64-1.15)</td>
<td></td>
</tr>
<tr>
<td>P value (one-sided)</td>
<td>0.15</td>
<td></td>
</tr>
</tbody>
</table>

**Graphs:**
- Event-free probability over time for Alpelisib + fulvestrant and Placebo + fulvestrant.
- Number of patients still at risk over time.

References:
CAPItello-291 Trial: Fulvestrant +/- Capivasertib (AKT Inhibitor)

- Men and pre/postmenopausal women
- Recurrence while on or <12 months from end of adjuvant AI, or progression while on prior AI for ABC
- ≤2 lines of prior endocrine therapy for ABC
- ≤1 line of chemotherapy for ABC
- Prior CDK4/6 inhibitors allowed (at least 51% required)
- No prior SERD, mTOR inhibitor, PI3K inhibitor, or AKT inhibitor
- HbA1c <8.0% (63.9 mmol/mol) and diabetes not requiring insulin allowed
- FFPE tumor sample from the primary/recurrent cancer available for retrospective central molecular testing

Dual primary endpoint: Investigator-assessed PFS in the overall population

- Capivasertib + fulvestrant (N=355)
  - Median PFS (95% CI; months): 7.2 (5.5–7.4)
  - Adjusted HR (95% CI): 0.60 (0.51, 0.71); two-sided p-value <0.001

- Placebo + fulvestrant (N=353)
  - Median PFS (95% CI; months): 3.6 (2.8–3.7)

Turner N, et al. NEJM 2023
**HER2 Testing: Technique and Interpretation are Critical**

**Immunohistochemistry**

- 0
- 1+
- 2+
- 3+

2+ cases are “reflexed” to ISH (in situ hybridization)

**Interpretation of Dual Probe ISH based**

- HER2 testing (invasive component) by validated dual-probe ISH assay
- Batch controls and on-slide controls show appropriate hybridization
  - HER2/CEP17 ratio ≥2.0
    - Group 1: Average HER2 copy number ≥4.0 signals/cell
    - Group 2: Average HER2 copy number <4.0 signals/cell
  - HER2/CEP17 ratio <2.0
    - Group 3: Average HER2 copy number ≥6.0 signals/cell
    - Group 4: Average HER2 copy number ≥4.0 and <6.0 signals/cell
    - Group 5: Average HER2 copy number <4.0 signals/cell

*Note – Next-generation tumor DNA sequencing can also call HER2 amplification, but both antibody and ISH analysis should be done to confirm*
Pathway Activation – HER Ligands

HER1/EGFR → HER2 → HER3 → HER4

EGF, TGFα, AR, HRG

PI3K/AKT/mTOR, Ras/MEK/MAPK, (STAT)

Proliferation, Migration, Angiogenesis, Survival

ADCC
Trastuzumab
*Humanized Anti-HER2 Monoclonal Antibody*

- Targets HER2 protein
- Selectively binds with high affinity ($K_d \leq 0.5$ nM)
- 95% human, 5% murine
- IgG1 isotype able to activate antibody-dependent cytotoxicity

Carter P et al. PNAS 1992
Trastuzumab, Pertuzumab, Lapatinib and T-DM1: Complementary Mechanisms

**Trastuzumab Deruxtecan**

**Trastuzumab**
- Inhibits ligand-independent HER2 signaling
- Activates ADCC
- Prevents HER2 ECD shedding

**Pertuzumab**
- Inhibits ligand-dependent HER2 dimerization and signaling
- Activates ADCC

**Lapatinib, Neratinib, Tucatinib**
- Inhibit intracellular kinase domain of HER2, HER1 (EGFR)

**Margetuximab**
- Like trastuzumab, but higher affinity for Fc receptor may contribute more immune effect

**T-DM1**

**Her2**

**Subdomain IV**

**Dimerization domain**
**Exemestane +/- mTOR Inhibitor Everolimus:**
**BOLERO-2 18 Month Followup**

HR = 0.38 (95% CI: 0.31-0.48)
Log-rank *P* value: <.0001

Kaplan-Meier medians
EVE 10 mg + EXE: 11.0 months
PBO + EXE: 4.1 months

### Results for Pivotal CDK 4/6 Inhibitor Trials

<table>
<thead>
<tr>
<th>Trial</th>
<th>CDK Inhibitor</th>
<th>Line of Therapy (Endocrine Rx)</th>
<th>Menopausal Status</th>
<th>PFS HR</th>
<th>Statistical Significance</th>
<th>OS HR</th>
<th>Statistical Significance</th>
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</thead>
<tbody>
<tr>
<td>PALOMA-2</td>
<td>Palbociclib</td>
<td>1st Line/AI</td>
<td>Post</td>
<td>0.56</td>
<td>Yes</td>
<td>0.96</td>
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<tr>
<td>MONALEEESA-2</td>
<td>Ribociclib</td>
<td>1st Line/AI</td>
<td>Post</td>
<td>0.57</td>
<td>Yes</td>
<td>0.76</td>
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<tr>
<td>MONALEEESA-7*</td>
<td>Ribociclib</td>
<td>1st Line/AI or Tam</td>
<td>Pre/Peri</td>
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<td>Yes</td>
<td>0.70</td>
<td>Yes</td>
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<tr>
<td>MONARCH-3</td>
<td>Abemaciclib</td>
<td>1st Line/AI</td>
<td>Post</td>
<td>0.54</td>
<td>Yes</td>
<td>0.75</td>
<td>No (close)</td>
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<tr>
<td>PALOMA-3</td>
<td>Palbociclib</td>
<td>2nd Line/Fulv</td>
<td>Pre/Post</td>
<td>0.46</td>
<td>Yes</td>
<td>0.81</td>
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<tr>
<td>MONARCH-2</td>
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<td>2nd Line/Fulv</td>
<td>Pre/Post</td>
<td>0.55</td>
<td>Yes</td>
<td>0.78</td>
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<tr>
<td>MONALEEESA-3</td>
<td>Ribociclib</td>
<td>1st /2nd Line/Fulv</td>
<td>Pre/Post</td>
<td>0.59</td>
<td>Yes</td>
<td>0.72</td>
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</table>

*PFS/OS data reported for approved AI subset. Abbreviations: CDK=Cyclin-dependent kinase; Rx=therapy; PFS=progression-free survival; HR=hazard ratio; OS=overall survival; AI=aromatase inhibitor; Fulv=fulvestrant; NR=not reported

References:


HER2+ Metastatic Breast Cancer:

Serial Improvements in Survival with Newer Agents and Combinations

BUT…. Rare “Cures”

Updated Data from CLEOPATRA Trial: Docetaxel + Trastuzumab +/- Pertuzumab in First Line

50/406 (12%) patients crossed over from control to pertuzumab after 2nd interim analysis showed survival benefit

Median Progression-Free Survival: THP=18.8 mo; TH=12.4 mo (HR=0.69; 95% CI 0.59-0.81)
Median Overall Survival: THP=57.1 mo; TH=40.8 mo (HR=0.69; 95% CI 0.58-0.82)
Destiny-Breast03 Randomized trial T-DXd vs. T-DM1
Primary Endpoint: PFS by blinded independent central review

Median Progression-free Survival (95% CI)
- Trastuzumab deruxtecan: NR (18.5–NE)
- Trastuzumab emtansine: 6.8 (5.6–8.2)

12-Mo Progression-free Survival (%)
- Trastuzumab deruxtecan: 75.8 (69.8–80.7)
- Trastuzumab emtansine: 34.1 (27.7–40.5)

Hazard ratio for disease progression or death, 0.28 (95% CI, 0.22–0.37) P<0.001

Cortés J, et al. NEJM 2022
**Progression-Free Survival in HR+ and All Patients**

**Hormone receptor-positive**

- **Hazard ratio:** 0.51
- **95% CI:** 0.40-0.64
- **P <0.0001**

- **Δ:** 4.7 mo
- **T-DXd mPFS:** 9.9 mo
- **TPC mPFS:** 5.4 mo

**All patients**

- **Hazard ratio:** 0.50
- **95% CI:** 0.40-0.63
- **P <0.0001**

- **Δ:** 4.8 mo
- **T-DXd mPFS:** 10.1 mo
- **TPC mPFS:** 5.1 mo

**PFS by blinded independent central review.**

HR, hormone receptor; PFS, progression-free survival; T-DXd, trastuzumab deruxtecan; TPC, treatment of physician’s choice.

Modi S, et al. NEJM 2022
Mechanisms of DNA Repair

DNA DAMAGE

MAJOR DNA REPAIR PATHWAYS

Environmental factors (UV, radiation, chemicals)

Normal physiology (DNA replication, ROS)

Chemotherapy (alkylating agents, antimetabolites)

Radiotherapy

Single Strand Breaks
- Nucleotide excision repair
- Base excision repair
  PARP1

Double Strand Breaks
- Non-homologous end-joining
- Homologous recombination
  BRCA1/BRCA2
- Fanconi anemia pathway
- Endonuclease-mediated repair

Replication Lesions
- Base excision repair
  PARP1

DNA Adducts/Base Damage
- Alkyltransferases
- Nucleotide excision repair
- Base excision repair
  PARP1

OlympiAD: Olaparib vs. Chemotherapy of Physician’s Choice*

Progression-Free Survival

Robson M, et al. NEJM 2017

**Capecitabine (45%); eribulin (37%); vinorelbine (18%)**

*Olaparib 300 mg bd
Chemotherapy TPC

Progression/deaths, n (%)
Median PFS, months

<table>
<thead>
<tr>
<th>Olaparib</th>
<th>Chemotherapy TPC</th>
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</thead>
<tbody>
<tr>
<td>163 (79.5)</td>
<td>71 (73.2)</td>
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<tr>
<td>7.0</td>
<td>4.2</td>
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HR 0.58

95% CI 0.43 to 0.80; P=0.0009

No. at Risk

<table>
<thead>
<tr>
<th>Olaparib</th>
<th>Standard therapy</th>
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<tr>
<td>205</td>
<td>97</td>
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<tr>
<td>201</td>
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</table>

*Robson M, et al. NEJM 2017*
KEYNOTE-355 Study Design (NCT02819518)

Key Eligibility Criteria
- Age ≥18 years
- Central determination of TNBC and PD-L1 expression
- Previously untreated locally recurrent inoperable or metastatic TNBC
- Completion of treatment with curative intent ≥6 months prior to first disease recurrence
- ECOG performance status 0 or 1
- Life expectancy ≥12 weeks from randomization
- Adequate organ function
- No systemic steroids
- No active CNS metastases
- No active autoimmune disease

Stratification Factors:
- Chemotherapy on study (taxane vs gemcitabine/carboplatin)
- PD-L1 tumor expression (CPS ≥1 vs CPS <1)
- Prior treatment with same class chemotherapy in the neoadjuvant or adjuvant setting (yes vs no)

Pembrolizumab* + Chemotherapy*  
Placebo* + Chemotherapy*

Progressive disease**/cessation of study therapy

R 2:1

*Cortes J, et al. ASCO 2020

*Pembrolizumab 200 mg intravenous (IV) every 3 weeks (Q3W)
*Chemotherapy dosing regimens are as follows:
  - Nab-paclitaxel 100 mg/m² IV on days 1, 8, and 15 every 28 days
  - Paclitaxel 90 mg/m² IV on days 1, 8, and 15 every 28 days
  - Gemcitabine 1000 mg/m²/carboplatin AUC 2 on days 1 and 8 every 21 days

**Normal saline
***Treatment may be continued until confirmation of progressive disease
CNS=central nervous system; ECOG=Eastern Cooperative Oncology Group; PD-L1=programmed death ligand 1; R=randomized; TNBC=triple-negative breast cancer
Progression-Free Survival: PD-L1 CPS ≥10


---

Hazard ratio (CI) analyzed based on a Cox regression model with treatment as a covariate stratified by the randomization stratification factors. Data cutoff December 11, 2019.

*Prespecified P value boundary of 0.00411 met.
## Genomic Aberrations in Breast Cancer that Guide Precision Medicine in Breast Cancer

<table>
<thead>
<tr>
<th>Gene</th>
<th>Aberration</th>
<th>Frequency</th>
<th>Targeted Drug(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EVIDENCE BASED (from randomized or Phase II trials)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HER2</td>
<td>Amplification</td>
<td>20%</td>
<td>Trastuzumab, pertuzumab, T-DM1, lapatinib, neratinib, more</td>
</tr>
<tr>
<td>BRCA 1/2</td>
<td>Inactivation mutations (germline)</td>
<td>5%</td>
<td>Olaparib, talozoparib</td>
</tr>
<tr>
<td>PIK3CA</td>
<td>Activating mutations</td>
<td>30-40%</td>
<td>Alpelisib</td>
</tr>
<tr>
<td>BRAF</td>
<td>Activating mutation (V600E)</td>
<td>2-3% (TNBC)</td>
<td>Dabrafenib and trametinib</td>
</tr>
<tr>
<td>Several</td>
<td>Microsatellite instability</td>
<td>1-2%</td>
<td>Pembrolizumab</td>
</tr>
<tr>
<td>NTRK</td>
<td>Gene fusion</td>
<td>&lt;1%</td>
<td>Larotrectinib</td>
</tr>
<tr>
<td>RET</td>
<td>Gene fusion/rearrangements/SNV</td>
<td>&lt;1.5%</td>
<td>Selpercatinib</td>
</tr>
<tr>
<td>HER2</td>
<td>Activating mutations (non-amplified HER2)</td>
<td>2-10%</td>
<td>Neratinib (not FDA-approved)</td>
</tr>
</tbody>
</table>

| **EMERGING** | | | |
| FGFR1-4 | Amplification | 10% | FGFR inhibitors |
| ESR1 | Mutation (after AI exposure) | 30-40% | Fulvestrant, other SERDs (elacestrant in randomized trial) |
| AKT | Activating mutations | 2% | AKT, mTOR inhibitors (capivasertib, everolimus) |
| PTEN | Inactivating mutations/silencing | 20% | PI3K (non alpha-selective), Akt, mTOR inhibitors |
| Myc | Amplification | 16% | BET inhibitors |
| c-MET | Amplification/mutation | 15% | Met inhibitors (cabozantinib) |
| CDH1 | Inactivating mutations/silencing | 7% | Wnt inhibitors, ALK/ROS inhibitors |

Abbreviations: IHC=immunohistochemistry; AI = aromatase inhibitor; SERD=selective estrogen receptor downregulator
Elacestrant demonstrated a significant improvement versus Fulvestrant as SOC in patients with ER+/HER2-advanced/metastatic breast cancer and mESR1 following CDK4/6i therapy.

Approved on 1/27/2023 for HR+/HER2, 2nd/3rd line, ESR1-mutated cancer

THANK YOU