THE RIGHT BIOMARKER FOR THE RIGHT PATIENT
From immunohistochemistry to predictive molecular pathology of breast cancer.

Nicola Fusco
European Institute of Oncology (IEO)
University of Milan, Italy
DISCLOSURES

I have received honoraria for consulting/advisory role from Merck Sharp & Dohme (MSD) and Boehringer Ingelheim.
Hormone receptors: ER & PgR

75%-80% of invasive breast cancers are ER⁺/PgR⁺

Rationale for clinical testing: to identify patients who may benefit from hormonal therapy
  >> substantial survival benefits in ER⁺
  >> weak prognostic factor

METHOD:
  - IHC on FFPE tissue sections
  - Only nuclear staining
  - Single-gene expression assays are not recommended
  - False-negative results: still ~15% of the cases
    >> patients may not receive effective therapy
    >> internal and external controls

Allison et al. Estrogen and progesterone receptor testing in breast cancer: ASCO/CAP Guideline Update. JCO, Jan 2020
PREDICTIVE MARKERS

**ER⁺/PgR⁻ invasive breast cancers**

5% of all invasive breast cancers

- Subset of Luminal B tumors
- Preferentially post-menopause
- Clinically heterogeneous
- Larger tumor size than PgR⁺
- Worse prognosis than PgR⁺
- Higher response but also worse long-term outcome after neoadjuvant chemotherapy
- Genomic instability

>> Enriched for mutations in cancer genes (e.g. **TP53, PIK3CA, CDH1, HER2, BRAF**)

PREDICTIVE MARKERS

ER-low invasive breast cancers

Invasive carcinomas with low level (1-10%) of ER expression

2-3% of ER+ invasive breast cancers

Clinically challenging
  >> Heterogeneous behavior and biology
  >> Gene expression profiles more similar to ER-cancers
  >> Eligible for HT but limited data on the benefit

Diagnostically challenging
  >> Usually weak/very weak nuclear staining
  >> Pre-analytical issues
  >> Inter-observer reproducibility
  >> An additional comment should be provided in the pathology report

Allison et al. Estrogen and progesterone receptor testing in breast cancer: ASCO/CAP Guideline Update. JCO, Jan 2020
HER2

~15-20% of invasive breast cancers overexpress HER2

Rationale for clinical testing: to determine patient eligibility for anti-HER2 therapy

METHOD:

- IHC on FFPE tissue sections
  >>Only membrane staining
- In situ hybridization (ISH) in IHC 2+
- In both IHC and ISH the pre-analytic phase is crucial

“HER2-enriched” by transcriptomic analysis
  >> super-responders

HER2 intra-tumor heterogeneity

2% of HER2+ breast cancers show intra-tumor heterogeneity of HER2 expression

Patterns of HER2 heterogeneity:
- “clustered”, topographically distinct HER2+ and HER2- tumor clones
- “scattered”, isolated HER2+ cells in a HER2- tumor
- “mosaic”, diffuse intermingling of cells with different HER2 statuses (ISH)

• Lower pCR after neoadjuvant treatment with TTZ+chemo
• No pCR in stage II/III after neoadjuvant T-DM1 and pertuzumab

Marchio et al.. Semin Cancer Biol 2020
**PREDICTIVE MARKERS**

**HER2-low invasive breast cancers**

Spectrum of carcinomas with different degrees of HER2 expression (1+ to 2+/ISH\(^{\text{NEG}}\))

- 45%-55% of all invasive breast cancers
- Poorer prognosis compared to HER2-negative breast carcinomas
- TTZ duocarmazine (SYD-985) and TTZ deruxtecan (DS-8201) have shown encouraging response rates in HER2-low breast cancer

**Diagram:**

- HER2 testing by validated IHC assay
- Circumferential membrane staining that is complete, intense, and in >10% of tumor cells → (IHC 3+)
- Weak to moderate complete membrane staining in >10% of tumor cells → (IHC 2+)
- Incomplete membrane staining that is faint/barely perceptible and in >10% of tumor cells → (IHC 1+)
- No staining is observed HER2-null or membrane staining that is incomplete and is faint/barely perceptible and in ≤10% tumor cells → (IHC 0+)

**Pie Chart:**

- HER2-positive BC 15%
- HER2-low BC 45-55%
- HER2-negative BC 30-40%

**Tarantino et. JCO 2020**
Programmed death-ligand 1 (PD-L1)

- PD-L1 is expressed in 40-65% of TNBC
- Expression is restricted, in most cases, to immune cells
- PD-L1 expression is predictive of response to Atezolizumab (anti-PD-L1)
- Chemotherapy may enhance tumor-antigen release and antitumor responses to immune checkpoint inhibition
- **IMpassion130 Study**: Atezolizumab + nab-paclitaxel prolonged PFS in PD-L1 TNBC patients

Schmid et al. NEJM 2018; Marra et al. BMC Med 2019
**IMMUNE-RELATED MARKERS**

**PD-L1 testing method**

IHC staining with VENTANA PD-L1 SP142 Assay (CDx) demonstrates staining in TILS and occasionally in tumor cells.

The PD-L1 tumor-infiltrating immune cell (IC) status is defined by the percentage of tumor area occupied by PD-L1-positive ICs.
IMMUNE-RELATED MARKERS

Tumor-infiltrating lymphocytes (TILs)

- TILs should be routinely characterized in TNBC because of their prognostic value (St Gallen 2019, WHO Breast Tumours 2019)
- Data are inadequate to recommend TILs to guide neo/adjuvant treatment choices in TNBC (St Gallen 2019)
- Stromal TILs are prognostic in TNBC and HER2+ breast cancer
- Not prognostic in ER+ tumors
The mismatch repair (MMR) system

- Major contributor to DNA integrity
- Four main proteins
  >> MLH1, MSH2, MSH6, and PMS2
- Genomes of MMR deficient (dMMR) cancers contain extraordinarily high numbers of somatic mutations
  Tumor mutational burden (TMB)
  Microsatellite instability (MSI)
- FDA approves pembrolizumab for dMMR and/or MSI-H cancers regardless of the tumor site
  >> histology agnostic approval
  >> no CDx
IMMUNE-RELATED MARKERS

MMR testing methods

What is the optimal MMR testing method for breast cancer?

- **IHC >>** MLH1, MSH2, MSH6, PMS2
- **MSI >>** PCR (BAT25, BAT26, D2S123, D5S346, D17S250, …) vs. NGS
- **Sequencing/methylation assays**
- **TMB >>** targeted panels, WES
PROGNOSTIC OR PREDICTIVE?

**Multigene Tests**

- Useful complementary information in ER\(^+\) breast cancers.
- Since ER\(^-\) cancers tend to have higher proliferation rates, the prognostic value of current multigene tests in these cancers is limited.
- May help informing chemotherapy decision in ER\(^+\)/HER2\(^-\) N0/N1a breast cancers.

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<th>Assay</th>
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<td>21-gene (Oncotype Dx)</td>
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NCCN Guidelines Version 3.2020 Invasive Breast Cancer NCCN Evidence Blocks™

NCCN Guidelines Invasive Breast Cancer 2020 NCCN Evidence Blocks™
Predictive Markers

PIK3CA

• Activating mutations of **PIK3CA** occur in 40% of ER+/HER2- breast cancer
  >> Hyperactivation of the alpha isoform of phosphatidylinositol 3-kinase (PI3Kα)
  >> Real Time PCR (exons 7, 9, and 20)

• Alpelisib is a selective inhibitor of PI3Kα

• **SOLAR-1 Trial** >> longer PFS and greater response with alpelisib–fulvestrant than with placebo–fulvestrant in patients with **PIK3CA**-mutated, ER+/HER2-advanced breast cancer

• Resistance to Alpelisib can be related to alterations in **PTEN** and **ESR1** genes

**PIK3CA** mutant

**PIK3CA** WT
PREDICTIVE MARKERS

BRCA1&2

- PARP1 inhibition in BRCA-mutated breast cancers >> synthetic lethality
- Olaparib is a PARP-inhibitor with antitumor activity in BRCA-mutated metastatic breast cancers (OlympiAD trial)

Adapted from Liu et al. Nucleic Acids Res. 2014
**ETV6-NTRK3 fusion gene**

- *NTRK* fusions occur in many very different tumors
- There are a few tumors like secretory breast cancer and congenital fibrosarcoma for which *NTRK* fusions are pathognomonic
- TRK inhibitors offer now the possibility to use *NTRK* fusion as targets in a tumor agnostic fashion

Adapted from: Church et al. Mod Pathol 2017

Märkl et al. Pathol Res Prac 2019
Phosphoinositide 3-kinases (PI3Ks)

- ER transcriptional activity and signaling through HER2/PI3K/AKT/mTOR increase cyclin D1 levels, activating CDK4/6 and promoting cellular progression to the S phase.
- Inhibition of CDK4/6 in the PI3K pathway can suppress mTORC1

Janus kinase 2 (JAK2)

- JAK2/STAT3 regulates lipid metabolism through fatty acid β-oxidation (FAO), promoting breast cancer stemness and chemoresistance.
- Blocking FAO re-sensitize cancer cells to chemotherapy while reducing cancer stemness in vivo.
PROBLEMS TO BE ADDRESSED

Intra-tumor heterogeneity

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