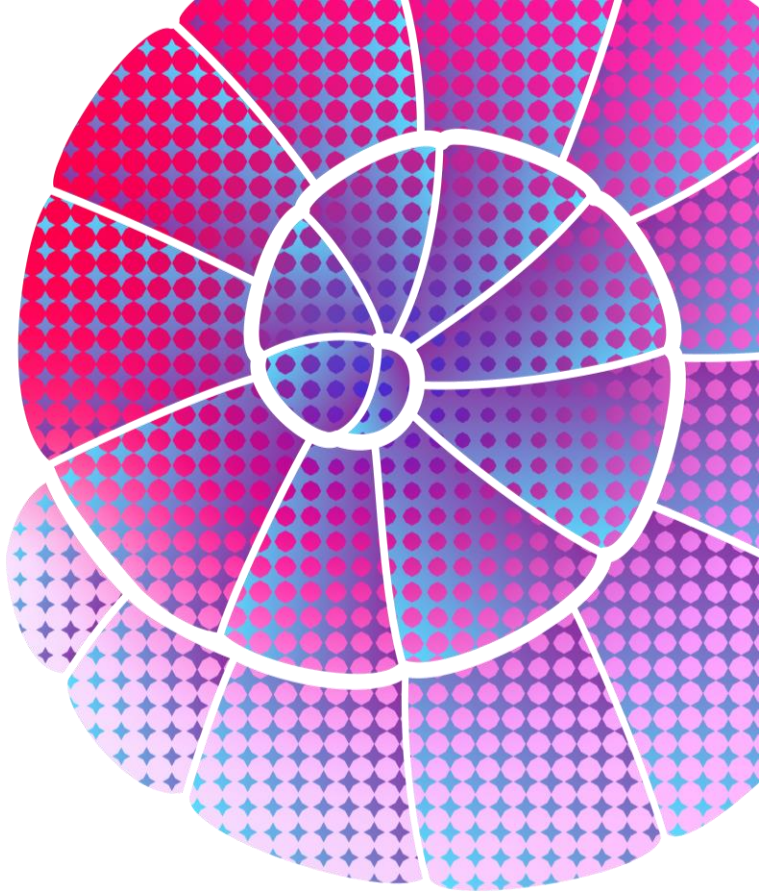
ESMO BREAST CANCER
VIRTUAL MEETING

THE RIGHT BIOMARKER FOR THE RIGHT PATIENT

From immunohistochemistry to predictive molecular pathology of breast cancer.



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DISCLOSURES

I have received honoraria for consulting/advisory role from Merck Sharp & Dohme (MSD) and Boehringer Ingelheim

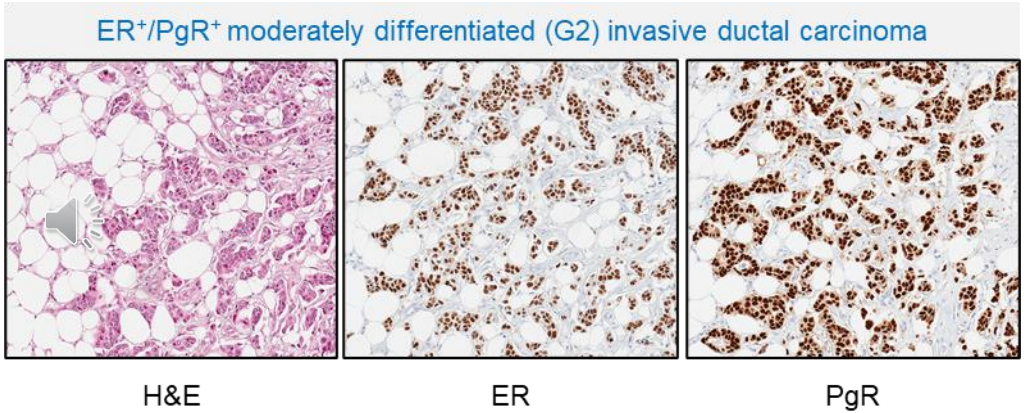
Hormone receptors: ER & PgR

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

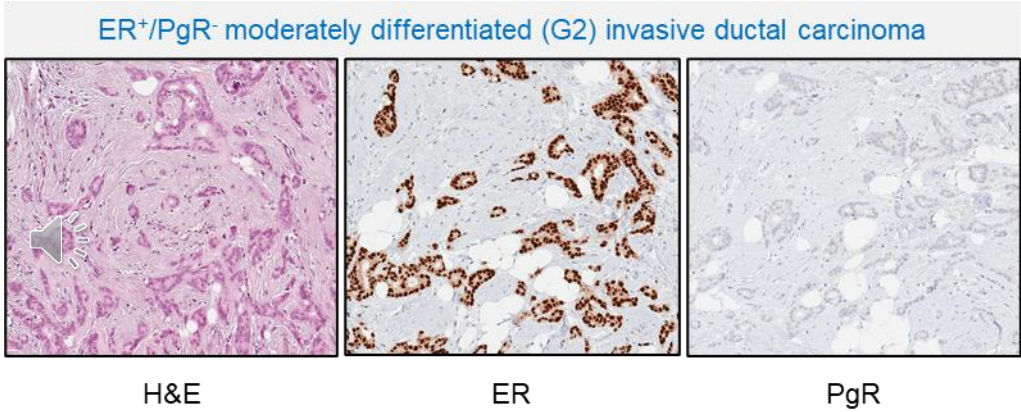
>> internal and external controls

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

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*)



Van Mackelenbergh et al. Breast Cancer Res. Treat. 2018; Lopez et al. Int J Mol Sci 2019

ER-low invasive breast cancers

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

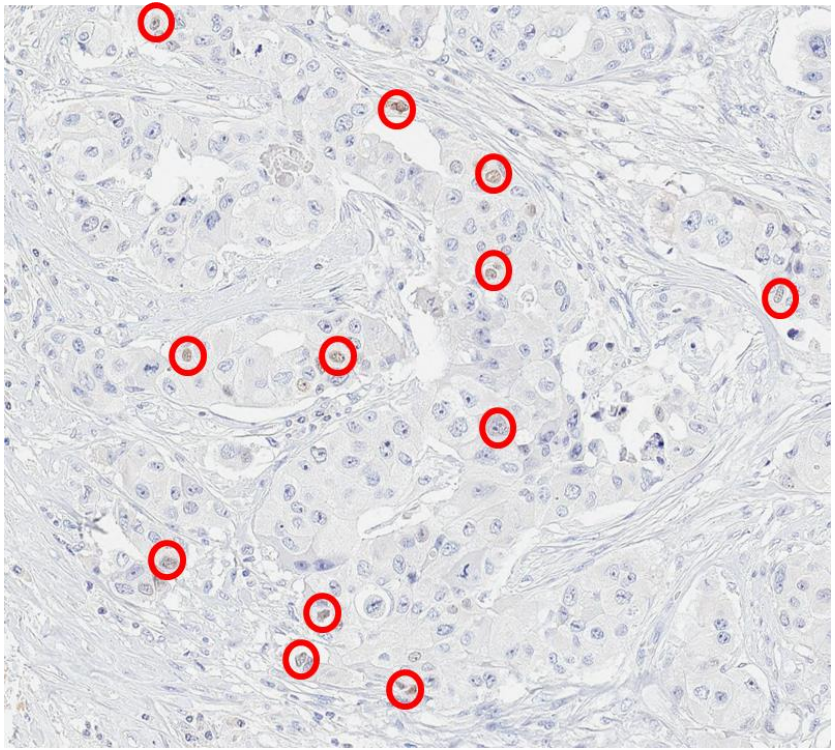
2-3% of ER$^+$ invasive breast cancers

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

PREDICTIVE MARKERS

HER2

~15-20% of invasive breast cancers overexpress HER2

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

METHOD:

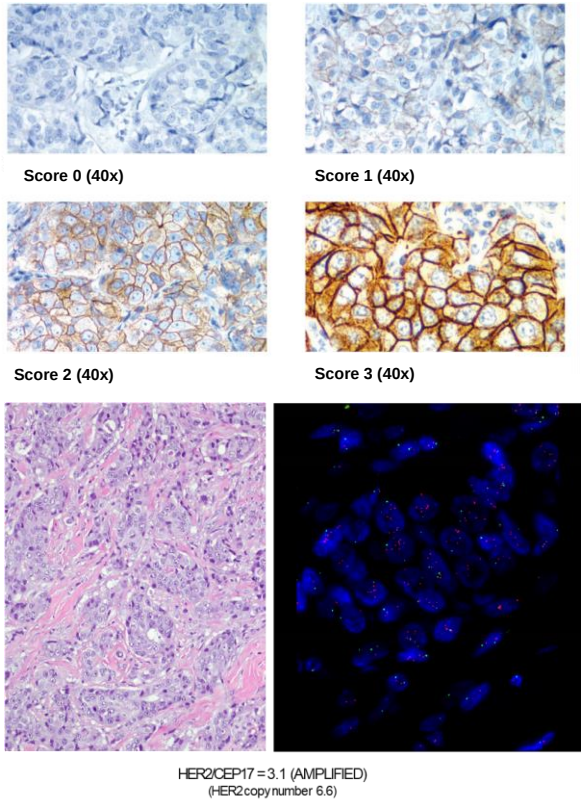
- IHC on FFPE tissue sections

  >>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



Metzeger Filho et al. ASCO 2019; Prat et al. J. Natl. Cancer Inst. 2019; Perez et al. BMC Cancer 2019; Song et al; Rye et al. Mol. Oncol. 2018; Modi et al. JCO 2020; Modi et al. NEJM 2019; Modi et al. SABCS 2019; Banerji et al. Lancet Oncol 2019; Fehrenbacher et al. Cancer Res 2018; von Minckwitz et al. NEJM 2019

HER2 intra-tumor heterogeneity

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

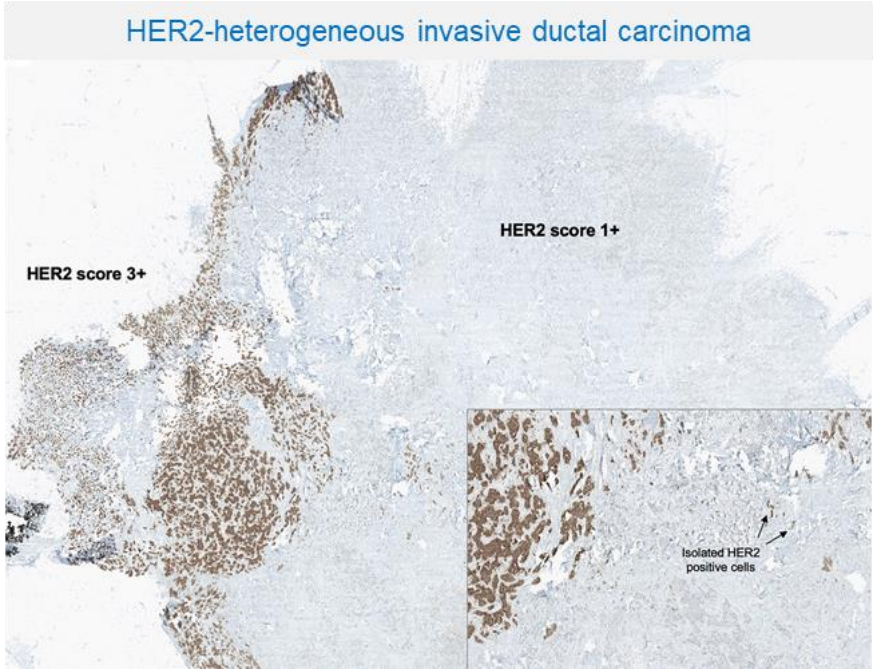
Patterns of HER2 heterogeneity:

>> “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

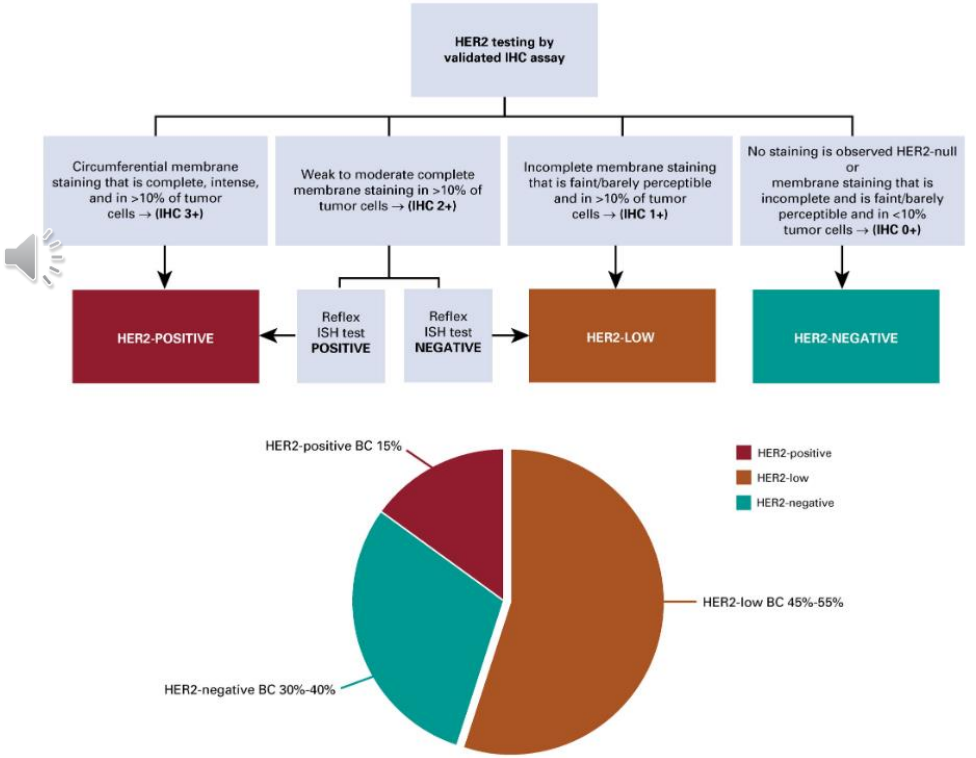


Marchio et al.. Semin Cancer Biol 2020

HER2-low invasive breast cancers

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

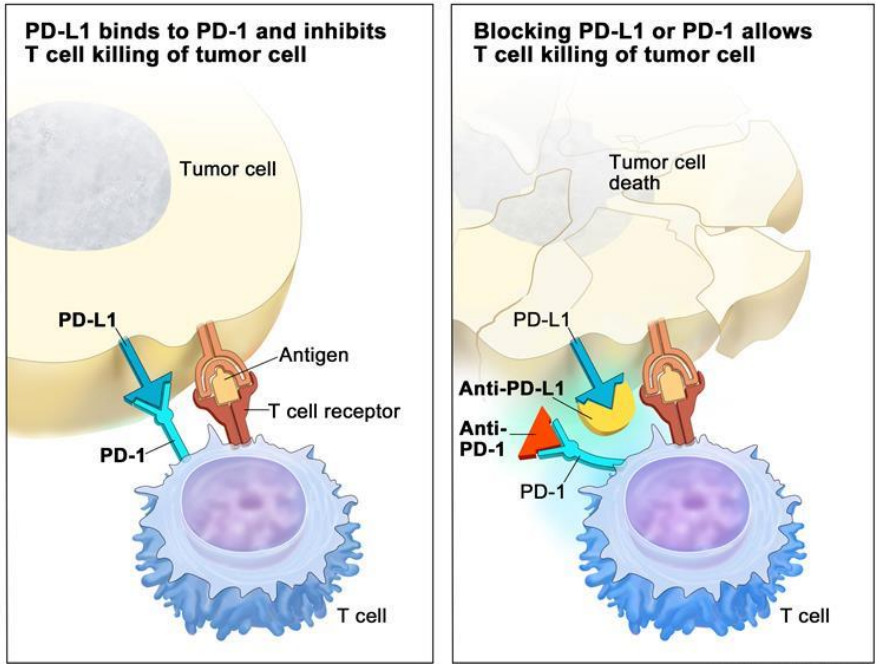
- 45%-55% of all invasive breast cancers
- Poorer prognosis compared to HER2-negative breast carcinomas
- TTZ duocarmazine (SYD-985) and TTZ deruxtecan (DS-8201) have shown encouraging response rates in HER2-low breast cancer



Tarantino et. JCO 2020

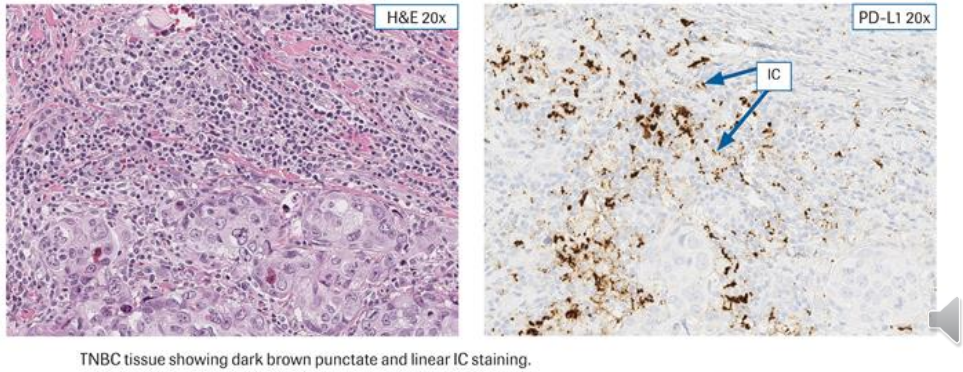
Programmed death-ligand 1 (PD-L1)

- 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

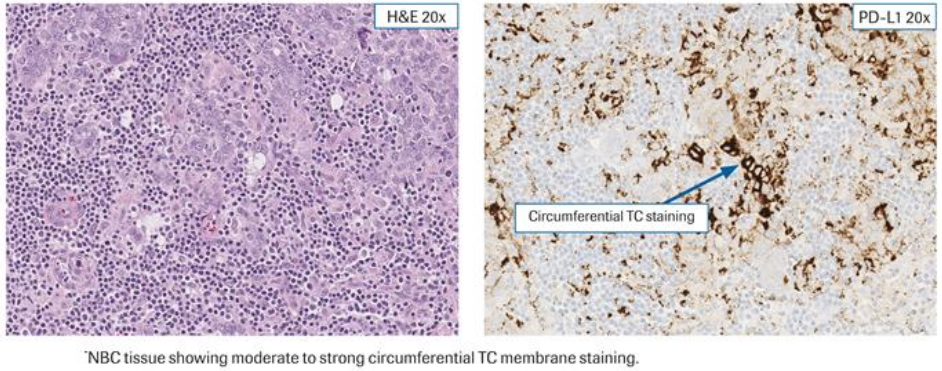


Schmid et al. NEJM 2018; Marra et al. BMC Med 2019

PD-L1 testing method



TNBC tissue showing dark brown punctate and linear IC staining.



TNBC tissue showing moderate to strong circumferential TC membrane staining.

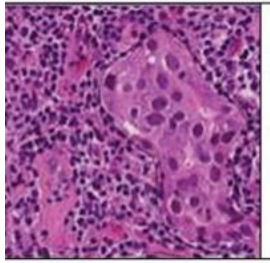
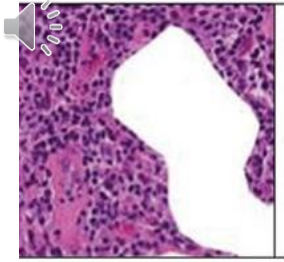
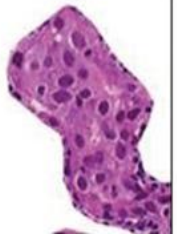
IHC staining with VENTANA PD-L1 SP142 Assay (CDx) demonstrates staining in TILS and occasionally in tumor cells

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

Pagni et al. Int J Mol Sci 2019

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

Lymphocyte-predominant breast cancer (LPBC)		
	Working category to describe tumors with “more lymphocytes than tymor cells”.	Definitions vary across studies with stromal TILs of 50–60% 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.
Stromal TILs		
	Indicator of increased accumulation of immune-cells in tumor tissue	Stromal TILs have been shown to be predictive for increased response 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.
Intratumoral TILs		
	TILs with direct cell-cell contact with carcinoma cells, might be an indicator of direct cell-based anti-tumor effects.	Several studies have shown that intratumoral TILs and more difficult to evaluate and do not provide additional predictive/prognostic information compared to stromal TILs.

Sagado et al. Ann Oncol 2015
Dieci et al. Semin Cancer Biol. 2018

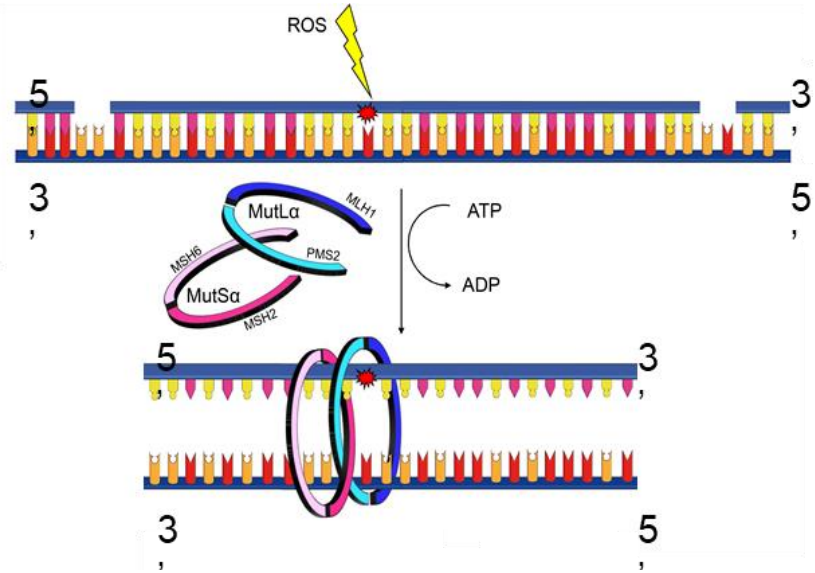
The mismatch repair (MMR) system

- 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

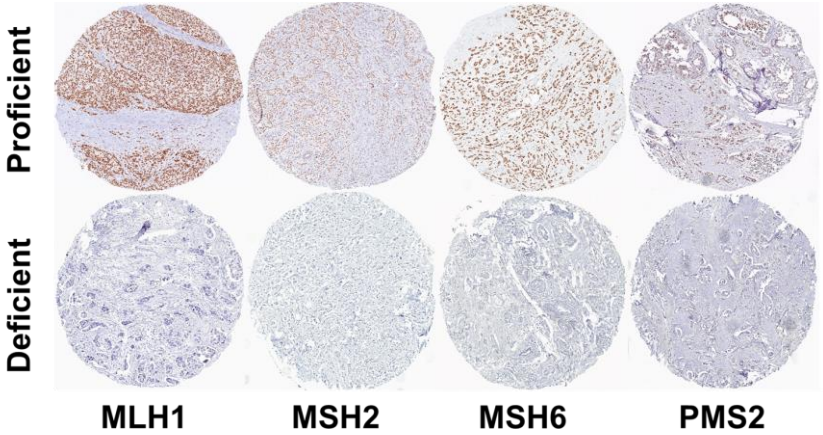


Corti et al. Adv Anat Pathol 2019

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



Pagni et al. Int J Mol Sci 2019

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

Assay	Predictive	Prognostic
21-gene (Oncotype Dx) (for pN0 or node negative)	Yes	Yes
21-gene (Oncotype Dx) (for pN+ or node positive)	N/A* *awaiting results of RxPONDER study	Yes
70-gene (MammaPrint) (for node negative and 1–3 positive nodes)	Not determined	Yes
50-gene (PAM 50) (for node negative and 1–3 positive nodes)	Not determined	Yes
12-gene (EndoPredict) (node negative and 1–3 nodes)	Not determined	Yes
Breast Cancer Index (BCI)	Not determined	Yes

National Comprehensive Cancer Network®

NCCN Guidelines Version 3.2020
Invasive Breast Cancer
NCCN Evidence Blocks™

PREDICTIVE MARKERS

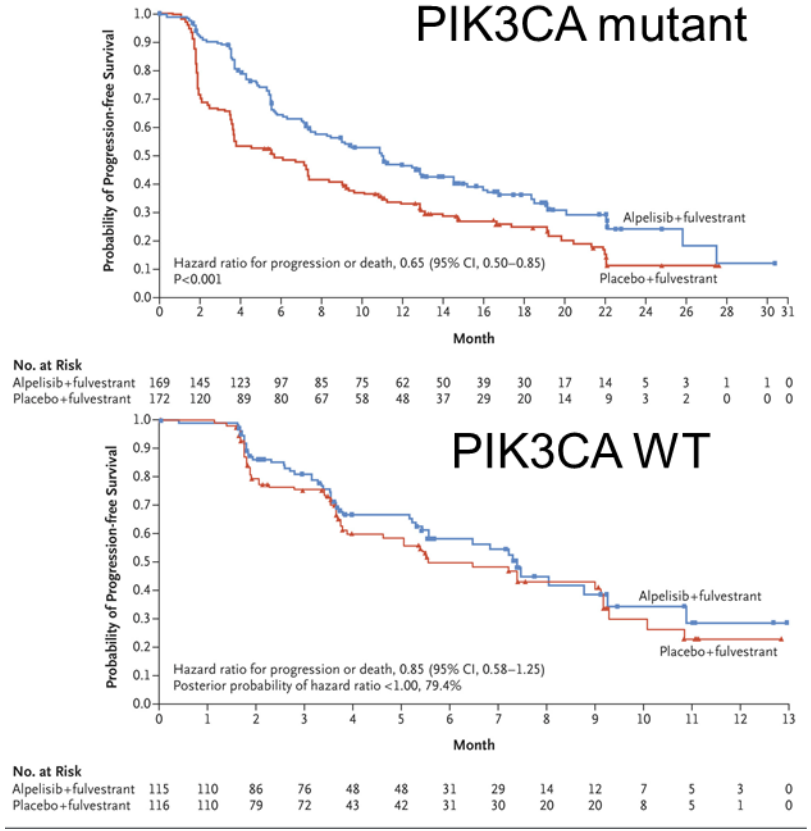
PIK3CA

- 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

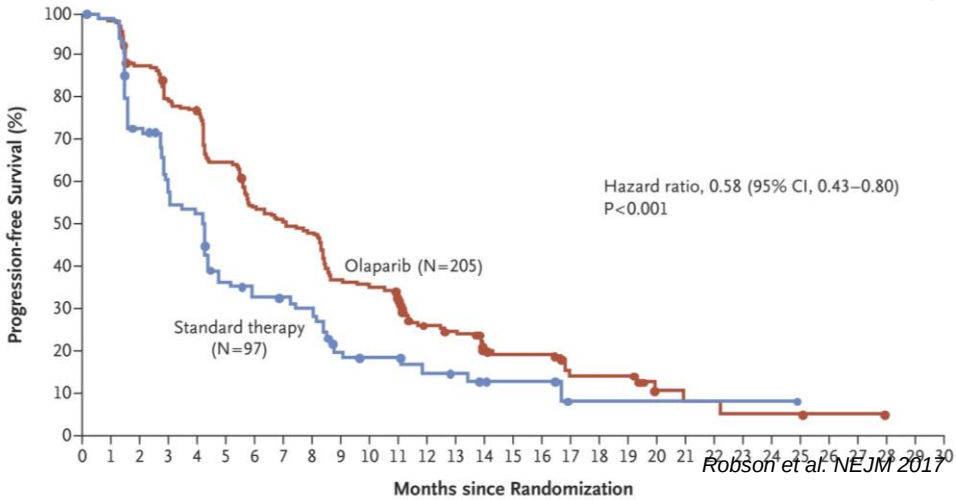


André et al. NEJM 2019
Razavi et al.. Nat Med 2020

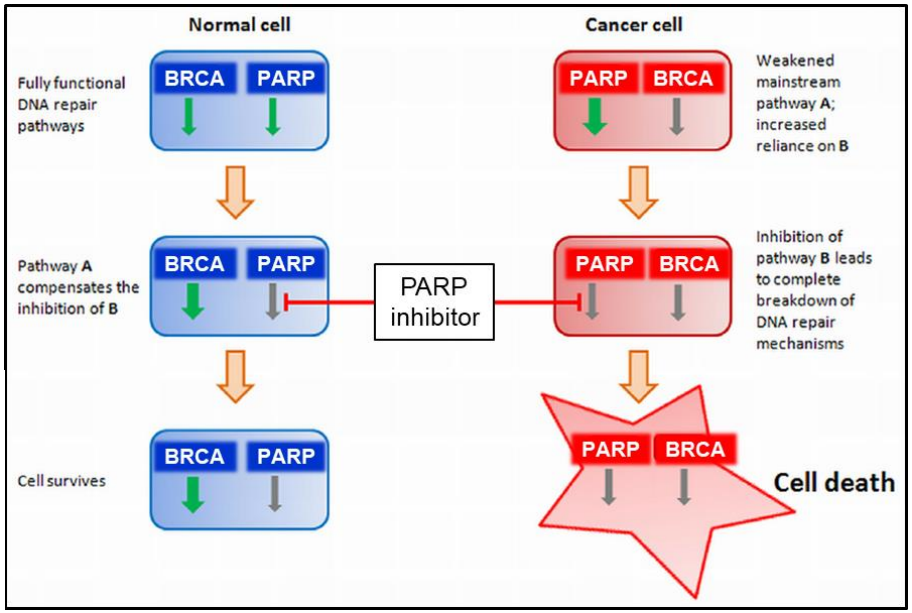
PREDICTIVE MARKERS

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**)



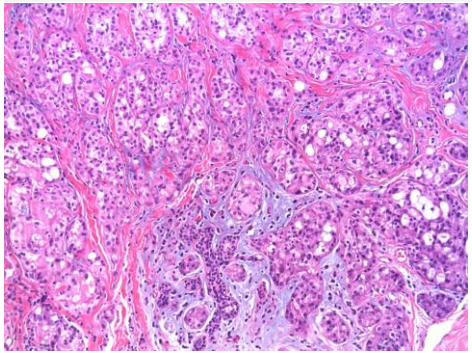
Robson et al. NEJM 2017

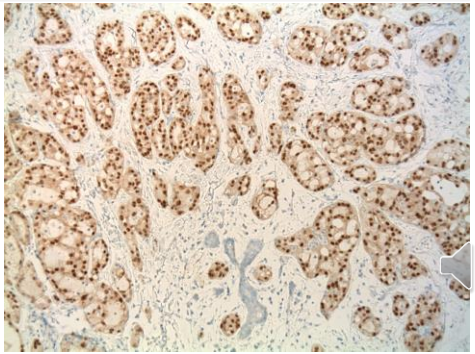


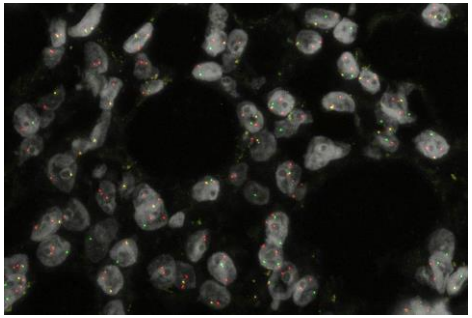
Adapted from Liu et al. Nucleic Acids Res. 2014

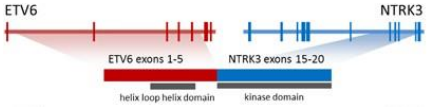
ETV6-NTRK3 fusion gene

Secretory breast carcinoma









Adapted from: Church et al. Mod Pathol 2017

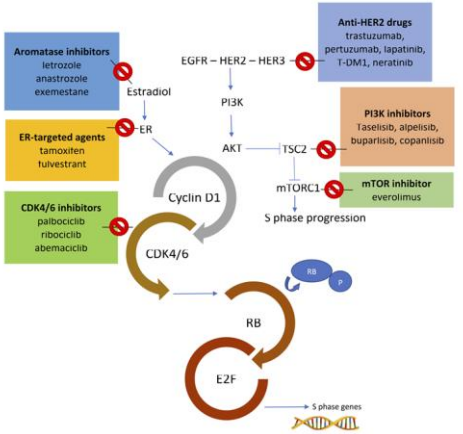
- *NTRK* fusions occur in many very different tumors
- There are a few tumors like secretory breast cancer and congenital fibrosarcoma for which *NTRK* fusions are pathognomonic
- TRK inhibitors offer now the possibility to use *NTRK* fusion as targets in a tumor agnostic fashion

Märkl et al. Pathol Res Prac 2019

Coming soon?

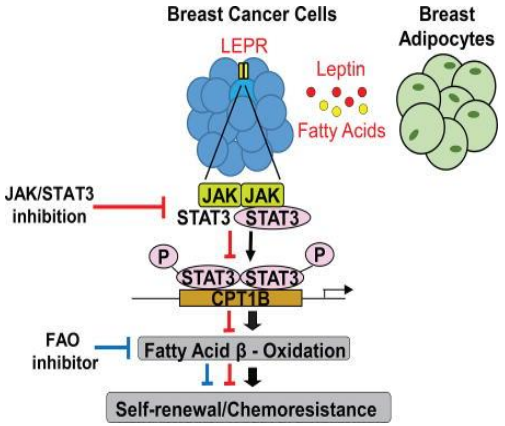
Phosphoinositide 3-kinases (PI3Ks)

- 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



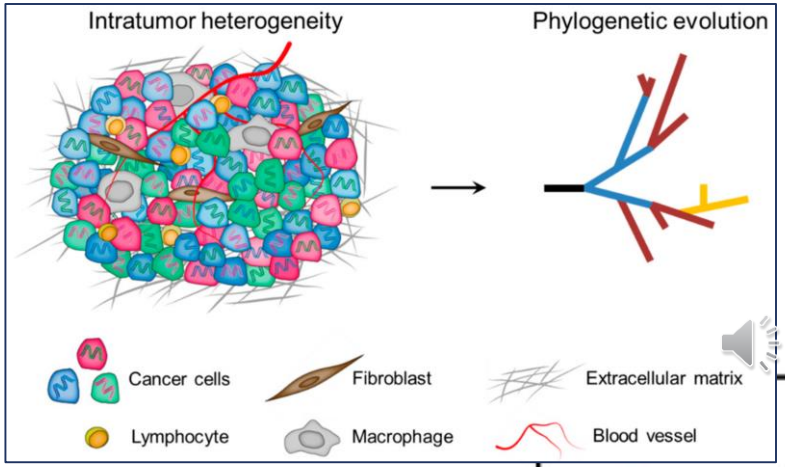
Janus kinase 2 (JAK2)

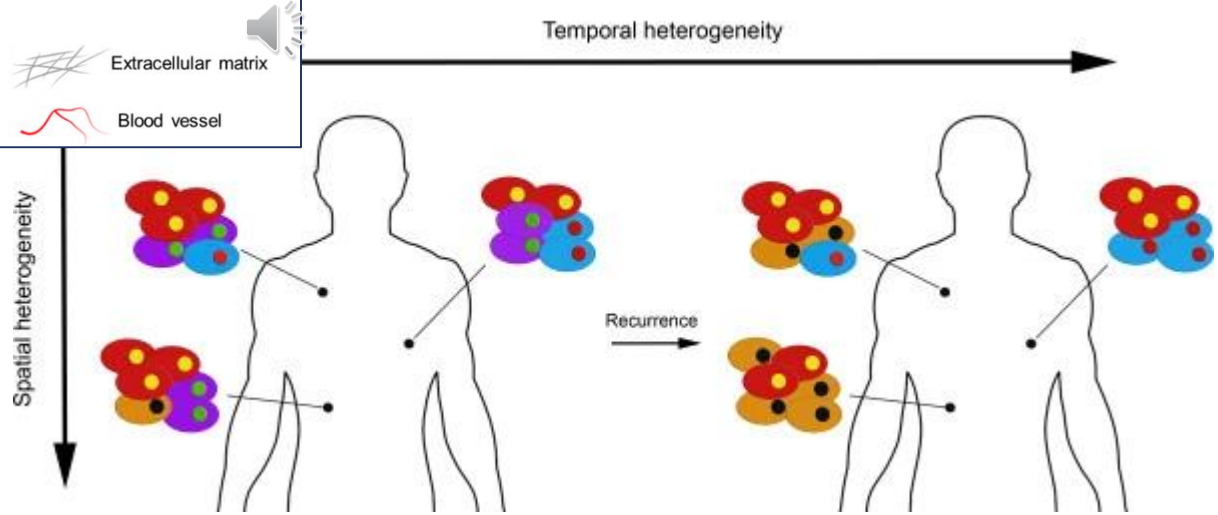
- JAK2/STAT3 regulates lipid metabolism through fatty acid β-oxidation (FAO), promoting breast cancer stemness and chemoresistance.
- Blocking FAO re-sensitize cancer cells to chemotherapy while reducing cancer stemness in vivo.



Grizzi et al. Cancer Management and Research 2020; Wang et al. Cell Metab 2019

Intra-tumor heterogeneity





Martelotto et al. Breast Cancer Res 2014; Lin et al. Cancers 201

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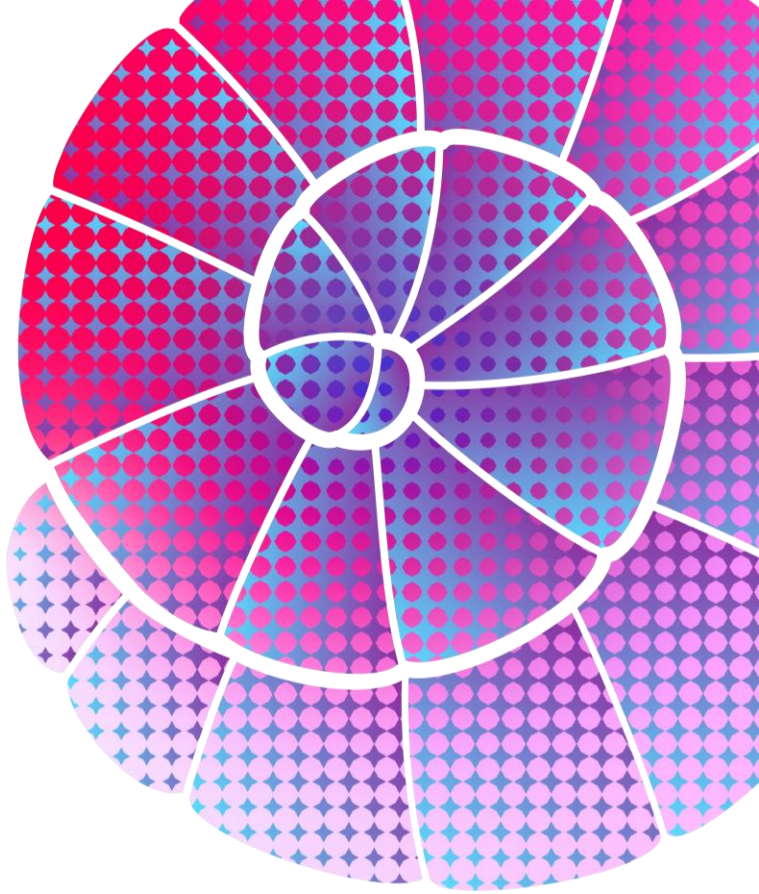
Acknowledgements

Giuseppe Viale, Giuseppe Curigliano
Elena Guerini Rocco, Elham Sajjadi, Konstantinos Venetis, Gianluca Lopez



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