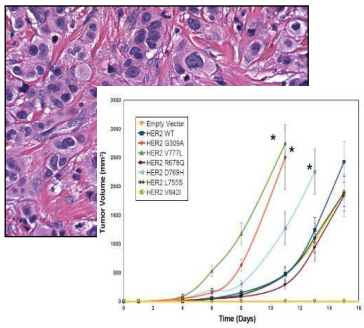

Molecular Diagnostics for Breast Cancer

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Brigham and Women's Hospital
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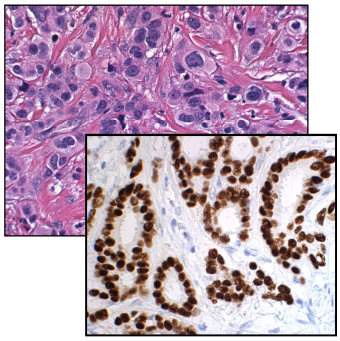
Boston, MA

Disclosures

- Advisory Board for Oncology Analytics, Inc.
- Consult for Novartis
- Research support from Canon, Inc

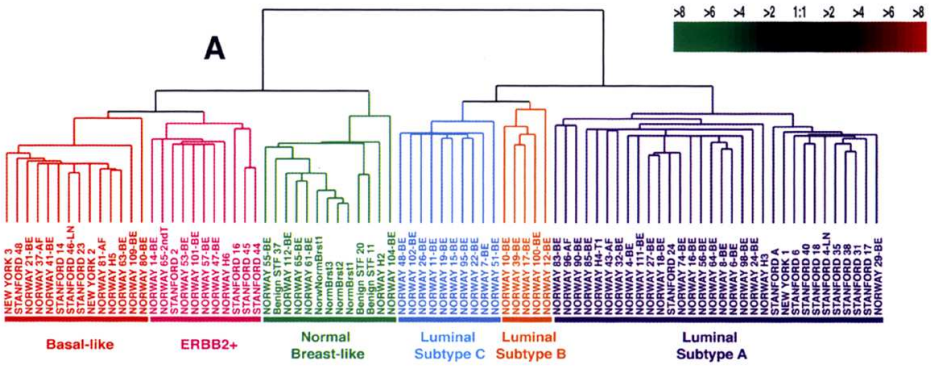
Will discuss the SOLAR-1 trial sponsored by Novartis



- Molecular classification of breast cancer
  - Intrinsic subtypes
  - Multigene predictors
  - Markers of rare TN subtypes
- Biomarkers as tx targets/predictors
  - ER/PR/HER2
  - PIK3CA
  - BRCA 1/2
  - PDL1/TMB/MMR-D
  - NTRK

Molecular Classification of Breast Cancer

Intrinsic Subtypes

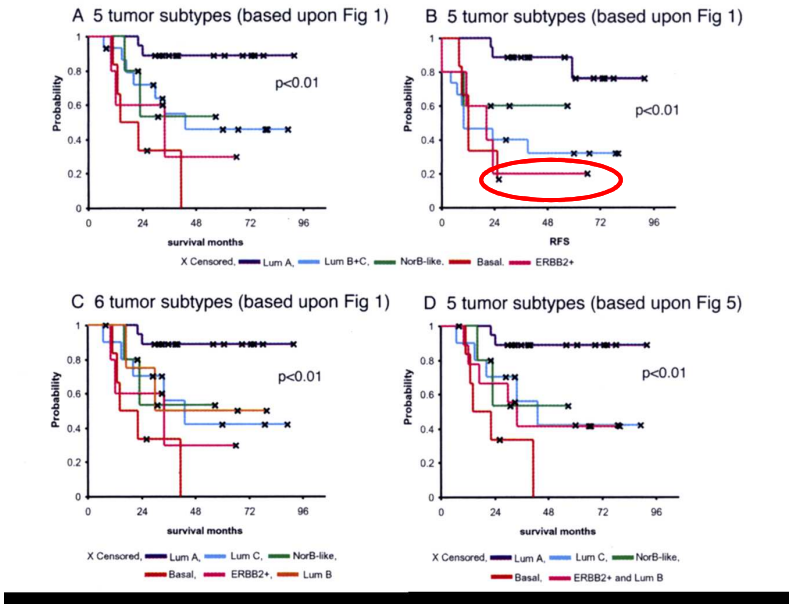


Basal HER2 E Luminal B Luminal A

GE studies done in the early 2000s led to the way we currently think about classification of breast cancer

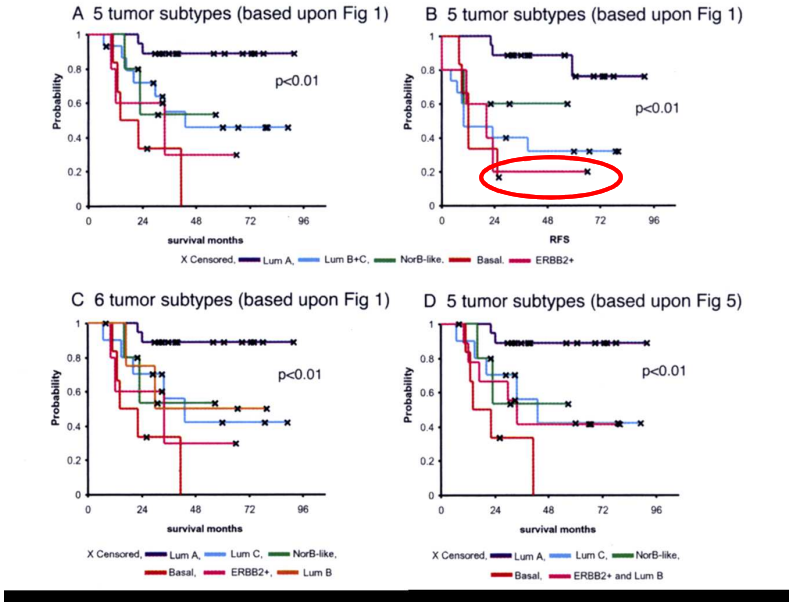
Perou CM et al (2000) Nature 406:747-752.
Sorlie T (2001) PNAS 98:10869-10874.

Intrinsic subtypes are associated with clinical behavior



HER2+ and basal groups associated with worse outcome

Intrinsic subtypes are associated with clinical behavior

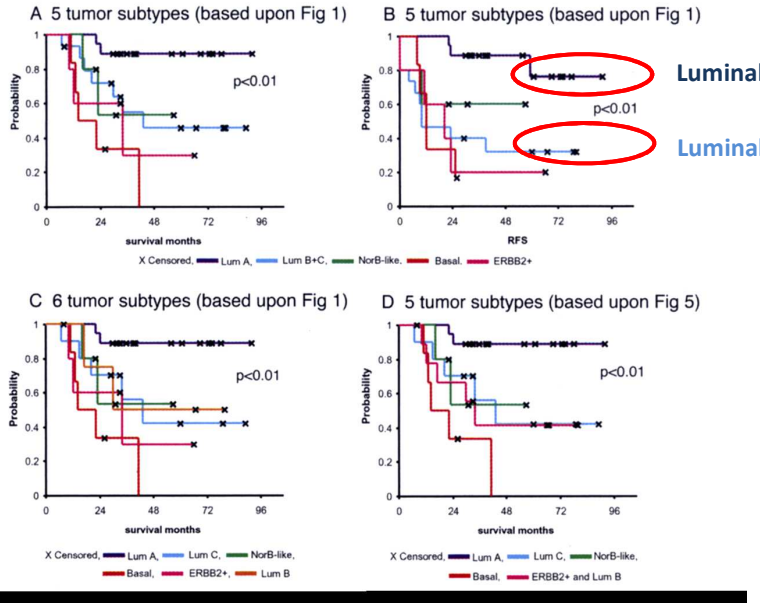


HER2+ and basal groups associated with worse outcome

Most responsive to chemotx

Treated with chemotx if >0.5cm

Intrinsic subtypes are associated with clinical behavior



Luminal A

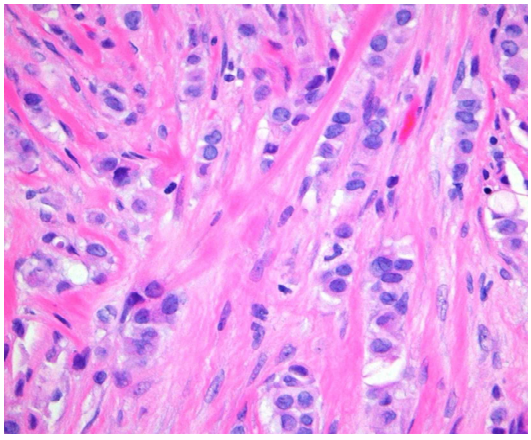
Luminal B

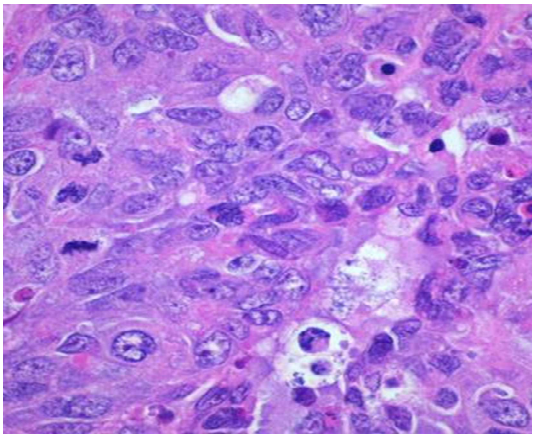
Luminal subtypes associated with better outcome

Luminal A: excellent prognosis

Luminal B: worse prognosis

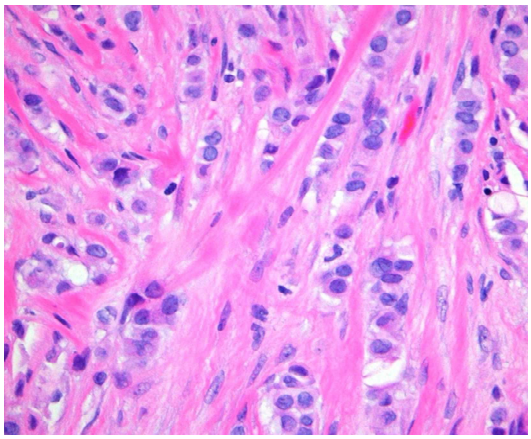
Luminal A	**Luminal B**
Lower nuclear grade	Higher nuclear grade
Lower proliferation	Higher proliferation





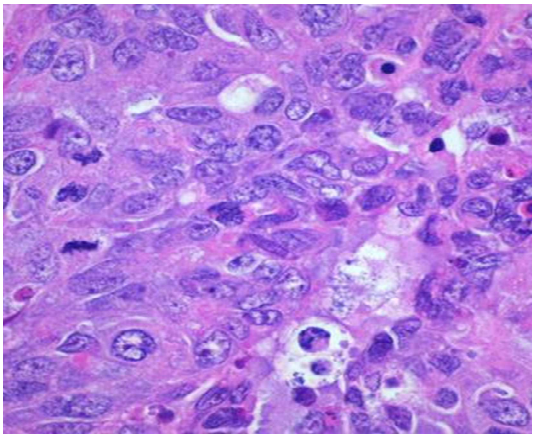
Luminal A

Hormonal Therapy

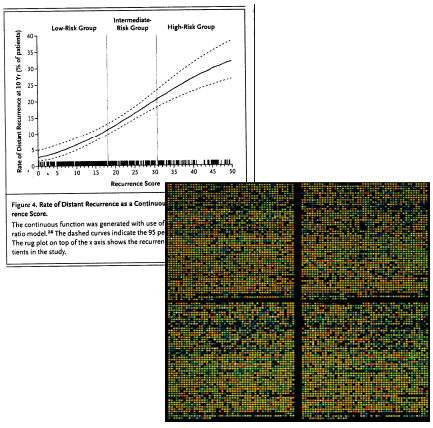


Luminal B

Chemotherapy



The decision about who gets chemotherapy in luminal cancers is usually made using a multigene recurrence predictor.



- Oncotype DX™
- MammaPrint ®
- Prosigna ® (PAM50)
- Breast Cancer Index (BCI)
- EndoPredict®
- MammaTyper

The most widely used assay in the US is Oncotype DX

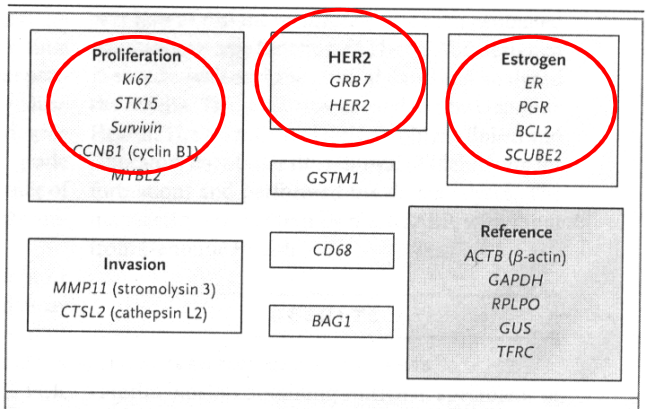
Oncotype DX™

Real-time PCR of 21 genes done in FFPE tissue to predict recurrence and chemotx benefit in ER+ breast cancer.

Algorithm is largely driven by genes related to ER, HER2 and proliferation

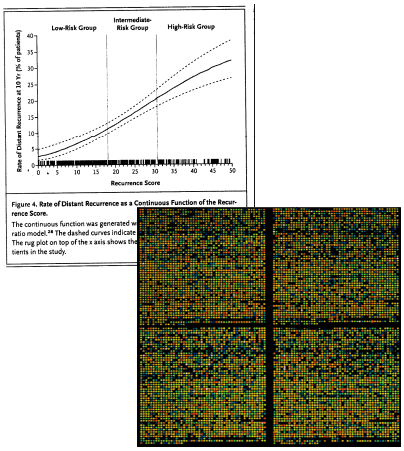
Included in all major clinical guidelines

Assay is performed at Genomic Health



Paik et al, New Engl J Med 2004

Multigene Recurrence Predictors



- Oncotype DX™
- MammaPrint ®
- Prosigna ® (PAM50)
- Breast Cancer Index (BCI)
- EndoPredict®
- MammaTyper

These assays differ from each other and from ODX
- in the technological platforms used
- the patient populations used for their development/validation
- the specific genes included

Multigene Recurrence Predictors

Where are we now in 2021?

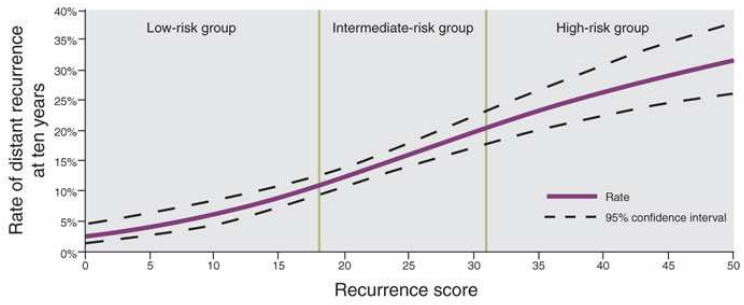
- Prosigna and Mammaprint are both FDA-cleared.
- Oncotype Dx and Mammaprint have been prospectively validated in RCTs to predict chemotherapy benefit.
- Classification of individual patients may vary somewhat in different tests.
- Most oncology guidelines now support the use of a multigene assay to determine chemotx use in ER+ HER2- cancer
  - -->evidence stronger LN neg>LN pos
- Multigene predictors are covered by most major US insurance carriers for pts with early-stage disease.

The accuracy of these tests is highly dependent on the tissue used.

www.urbanore.com

Oncotype Dx Recurrence Score

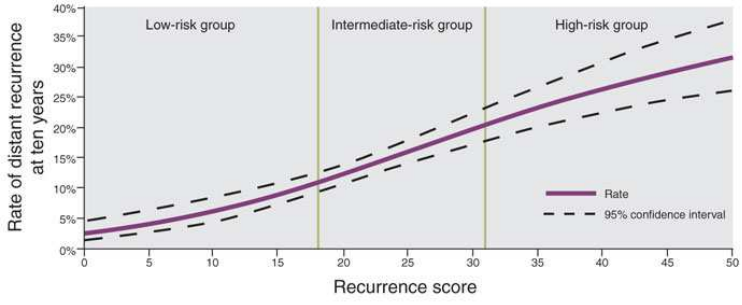
Predicts likelihood of distant recurrence within 10 years if a patient with an ER-positive tumor is treated with tamoxifen.



Oncotype Dx Recurrence Score

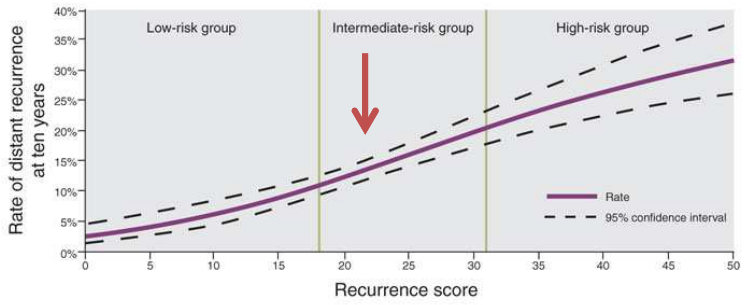
A difficult to interpret complex sclerosing lesion was diagnosed as invasive carcinoma and sent for this assay.

What was the recurrence score?



Oncotype Dx Recurrence Score

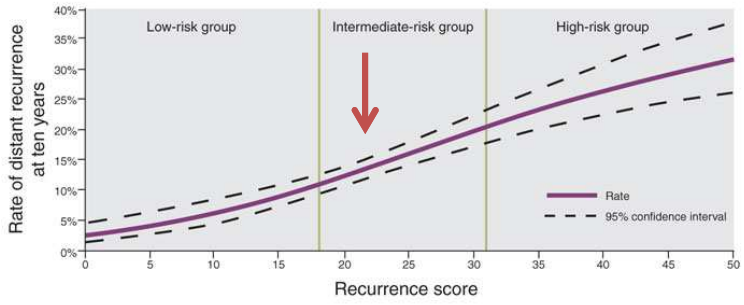
Benign lesion: Recurrence score 21 (13% risk of distant recurrence).



Oncotype Dx Recurrence Score

Benign lesion: Recurrence score 21 (13% risk of distant recurrence).

Recurrence predictors cannot diagnose cancer!



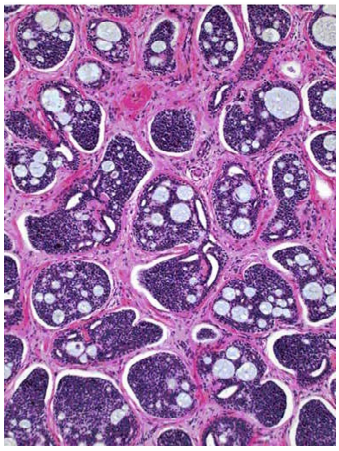
The accuracy of these tests is highly dependent on the tissue used.

Problems can arise typically for cancers with

- Low cellularity (especially lobular carcinomas)
- A prominent lymphocytic infiltrate
- DCIS of a different type (e.g HER2 pos w/ neg IDC)
- large core needle biopsy site relative to tumor size

Diagnostic Biomarkers in Breast Cancer

- Most TNBC is high-grade and is treated with chemotx
- Several rare low-grade histologic subtypes of TNBC have characteristic molecular alterations



•**Adenoid Cystic Carcinoma**

•most common of the low-grade TNBCs

• 85% have t(6;9)(q22-23;p23-24)

• usually involving **MYB-NFIB**

Muslimani et al. Int Sem Surg Oncol 2006, **3**:17

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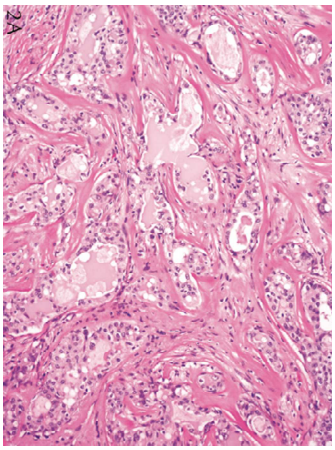

- **Secretory carcinoma**
- Rare TN tumor
- Majority have t(12;15)(p13q25)
- **ETV6-NTRK3** fusion gene

Vasudev et al. Arch Pathol Lab Med. 2011;135:1606

Diagnostic Biomarkers in Breast Cancer

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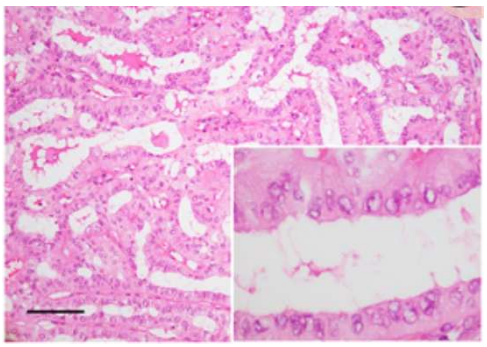
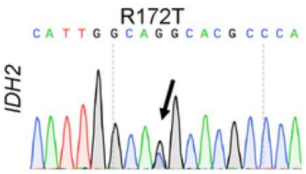


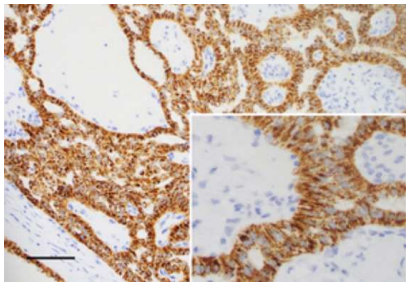
- **Tall cell carcinoma with reverse polarity (TCCRP)**
- **IDH2 R172** hotspot mutations

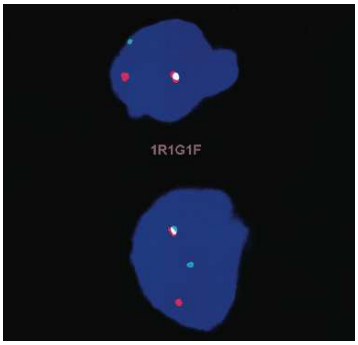
Pareja F (Brogi E). Mod Pathol. 2020 Jun; 33(6): 1056–1064.

Diagnostic Biomarkers in Breast Cancer

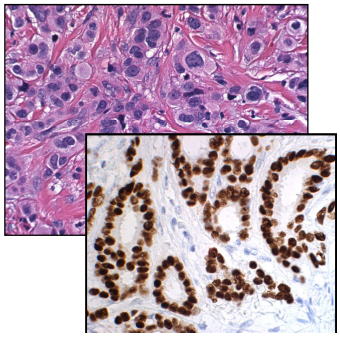
- Often not needed, but can be detected by IHC or sequencing in diagnostically challenging cases (FISH for translocations)
- Clinically important because these low-grade TN cancers usually do not require chemotherapy

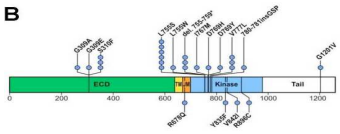






Vasudev et al. Arch Pathol Lab Med. 2011;135:1606





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  - Multigene predictors
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ER/PR/HER2

Traditional Breast Biomarkers

ER/PR/HER2 (IHC+/-FISH)

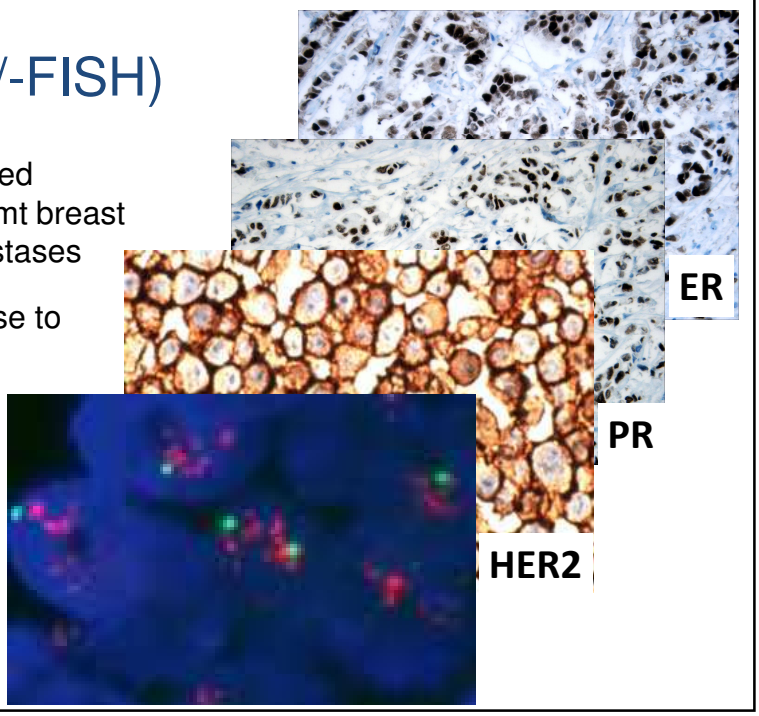
Testing done on all newly diagnosed invasive breast cancers, all post-tmt breast cancers, all recurrences, all metastases

ER/PR expression predict response to ER pathway targeting

- 80% response in ER+/PR+
- 40% response in ER+/PR-

HER2 overexpression and amplification predict response to HER2 targeting

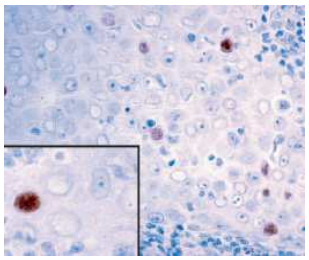
- 65% pCR ER-/HER2+ monotx
- 40% pCR ER+/HER2+ monotx
- 80% with addition of chemotx



ER/PR Scoring is based on % positive tumor cell nuclei

Since 2010 cutoff for positive result is 1%
IHC interpreted by surgical pathologists
Updated ASCO/CAP guidelines (2020)

POSITIVE	>10% positive cells
LOW POSITIVE	**1-10% positive cells**
NEGATIVE	<1% positive cells (any intensity)



- acknowledge the more limited data on endocrine responsiveness in this group
- increasing evidence that at least some low-expressors may be more like TNBC

→Many clinical trials for TNBC now set thresholds at 5% or 10%, to be able to enroll low expressors

www.asco.org/guidelines/erpr

Reisenbichler et al, Am J Clin Pathol 132:396-401, 2009.

Issues with HER2 Evaluation:

How to avoid false positive results (IHC)

What is the best cutoff point for positive result (FISH)

What do we do about heterogeneity and non-classical FISH results

HER2 IHC: Causes of false positive 3+

Overstaining
normal breast tissue should be negative (except apocrine metaplasia which can be 1+ to 2+)

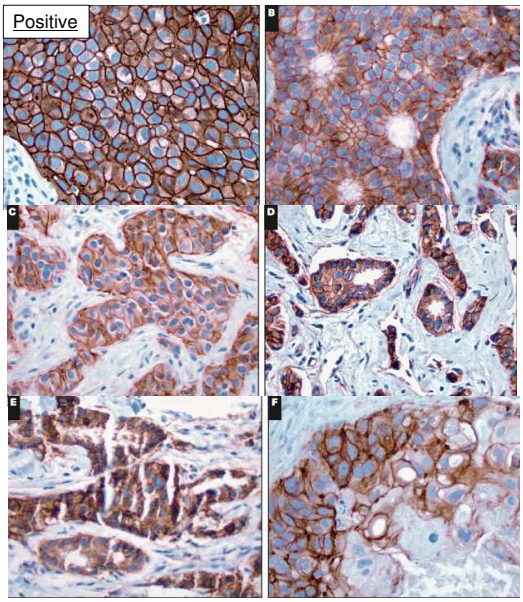
Edge artifact
lobular carcinomas can appear falsely positive in edges or between cells

Cytoplasmic positivity
only membrane positivity should be scored

Overinterpretation
moderate complete or granular membrane expression

13 of 19 IHC/FISH discordant cases were due to overinterpretation due to granular staining, crush artifact, and weak intensity.

Grimm EE et al. Am J Clin Pathol 134:284, 2010.

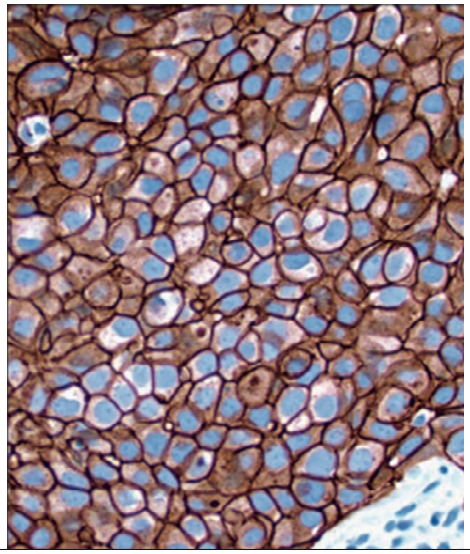


HER2 IHC: Avoiding false positive 3+

Have a very high threshold for interpreting a cancer as 3+.

Should have strong crisp complete membrane positivity throughout (>10% contiguous focus).

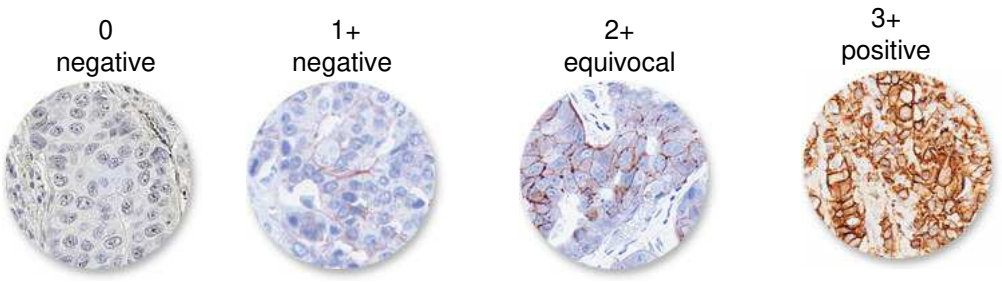
Have a low threshold for confirming by ISH in uncertain cases.

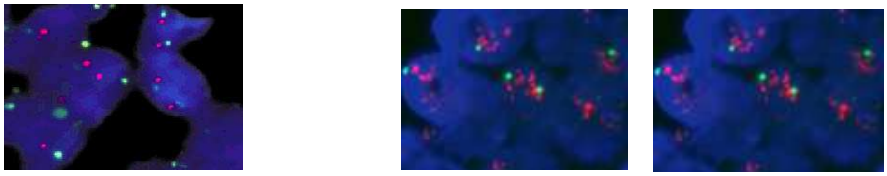


Grimm EE et al. Am J Clin Pathol 134:284, 2010.

Hybrid Testing Scheme: IHC with FISH

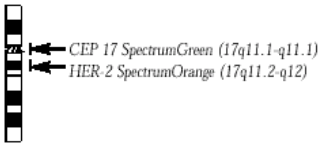
there is very high concordance between a IHC 3+ and amplification by FISH

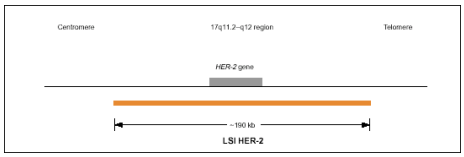




>95% 20-50% >95%

HER2 FISH





Two labelled DNA probes:

***HER2* probe**

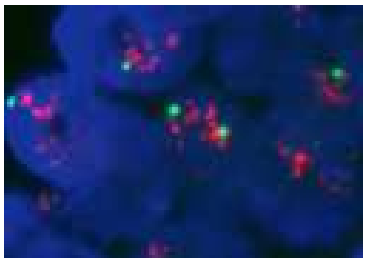
190 Kb; orange fluor

Hybridizes to entire length of HER2 gene

chromosome 17 probe (CEP17)

5.4 Kb; green fluor

pericentromere alpha satellite repeats

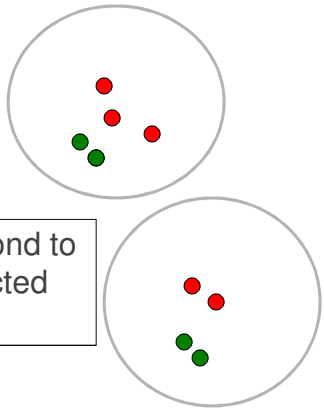


5 micron sections

Classical FISH Results

CEP17
HER2

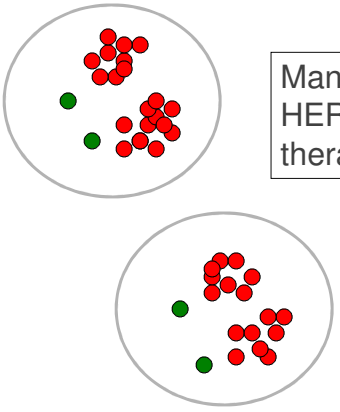
Not amplified



None respond to HER2 directed therapy

Group 5:
Ratio < 2.0 HER2 copy number < 4.0

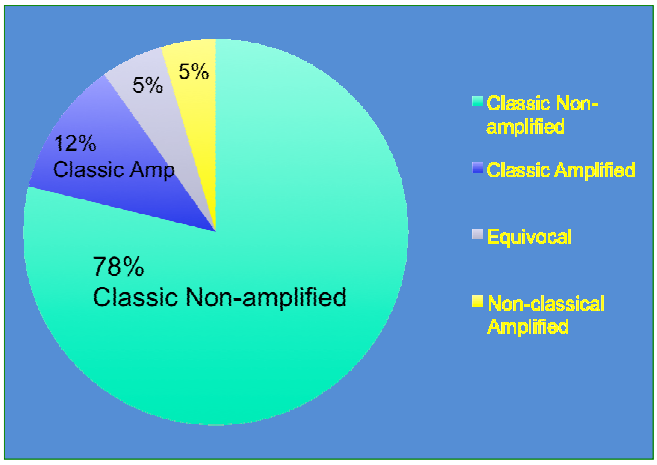
Amplified



Many respond to HER2 directed therapy

Group 1:
Ratio ≥ 2.0 HER2 copy number ≥ 4.0

~90% of all breast cancers have classical results



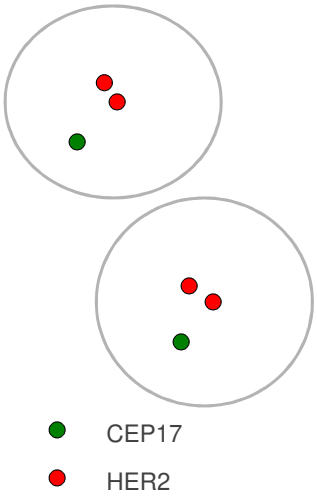
2018 update to ASCO/CAP HER2 guidelines addresses the remaining 10%

Equivocal and non-classical results
Groups 2, 3, 4

Ballard et al. Mod Pathol 30:227-235, 2017

Wolff A. et al. Archives of Path Lab Med. doi: 10.5858/arpa.2018-0902-SA, 2018.

Group 2: ratio ≥2.0 HER2 CN <4.0
previously classified as Positive (2013)



cases with loss of CEP17 ("monosomy")

→Ratio is ≥2 due to loss of CEP17

→no gain in HER2 CN

Now **NEGATIVE** (with IHC 0, 1+ or 2+)*

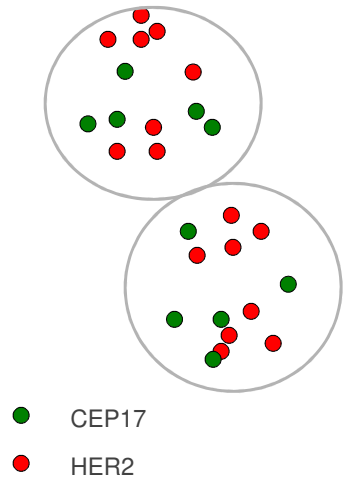
*Comment: evidence is limited on efficacy in this group

3+ IHC→ POS

>10,000 cases with central review testing for BCIRG there were NO Group 2 cases that were 3+

(Press MF et al, Archives Pathol and Lab Med, 2016)

Group 3: HER2 CN ≥6 but ratio <2
previously classified as Positive (2013)



CEP17 co-amplification ("polysomy")

Positive by HER2 CN ≥6

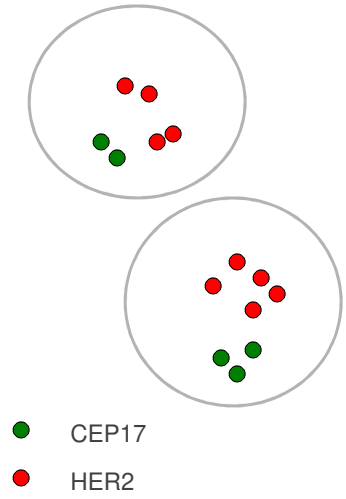
Ratio <2

Now **POSITIVE (with IHC 2+/3+)**

NEGATIVE (with IHC 0/1+)*

*Comment: There is insufficient data because these cases were not included in the initial clinical trials.

Group 4: ratio <2.0 with HER2 ≥4.0 and <6.0
previously classified as Equivocal (2013)



Slightly elevated HER2 CN (4-6)

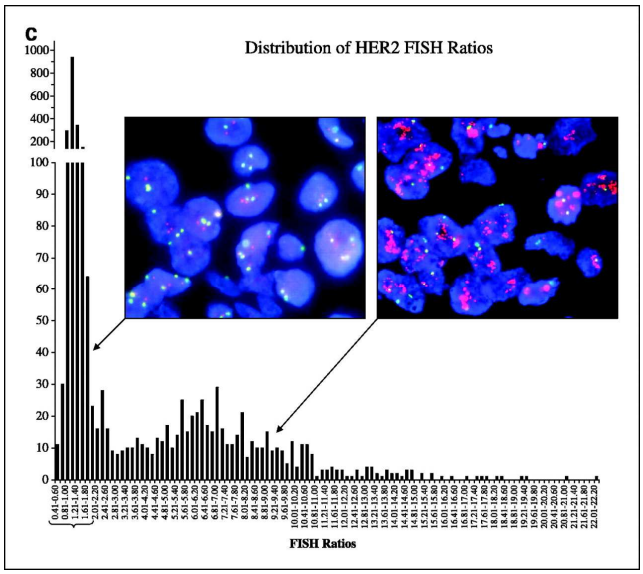
Ratio is <2

5% of all cases

Now POSITIVE (with IHC 3+)
NEGATIVE (with IHC 0/1+ or 2+)*

Comment: uncertain whether any benefit if not 3+.

Cutoff point for HER2 Positive FISH



Biology is a continuum.

Some breast cancers are at the threshold between clearly positive and clearly negative.

2018 Update is closing the gap

More tx response data needed for Groups 2-4

How should we define and report HER2 heterogeneity?

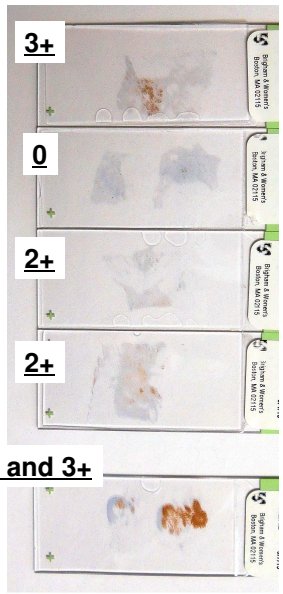
Want to identify cases with distinct clustered subpopulations with different HER2 gene status.

Carcinomas are classified as HER2 positive if >10% of the cancer is positive (IHC/ISH).

The cells must be “observed in a **homogeneous and contiguous** population” (i.e. not scattered)

breast

Lymph node



Discrete contiguous second population of cells with IHC 3+

Four blocks of primary carcinoma

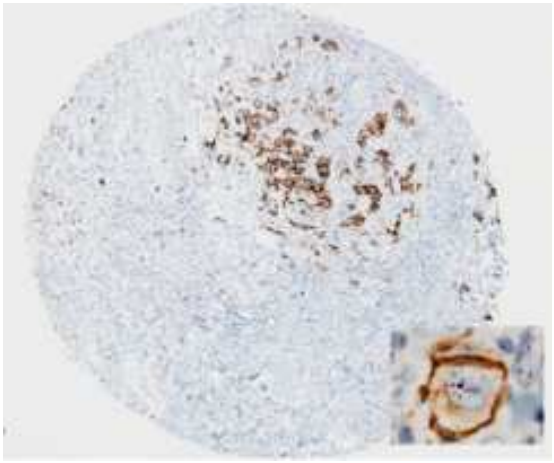
Lymph node metastases

Cancers with discrete identifiable subpopulations of HER2 positive cells are rare, <5% of total

Discrete second population may be a source of “resistant” disease

Oncologists may wish to tailor therapy to include both the positive and negative areas, especially if triple negative.

→Rare: we report HER2 status for both populations with % tumor.

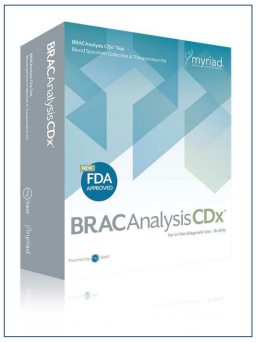


Nitta et al. A gene-protein assay for HER2. Diag Pathol 7:60, 2012

Beyond ER/PR/HER2

Newer Breast Biomarkers

New FDA approved targeted therapies in breast cancer



www.myriad.com

olaparib
g***BRCA***mut
Jan 2018

VENTANA PD-L1 (SP142) Assay

www.ventana.com

atezolizumab
PD-L1+ TNBC
Mar 2019

www.qiagen.com

www.foundationone.com

alpelisib
PIK3CA-mutated HR+
May 2019

Concurrent approval for a companion diagnostic

Tissue agnostic approvals with or without a companion diagnostic

Local lab developed PRC for MSI or IHC for dMMR

pembrolizumab
MMR-D (MSI-H)
Advanced solid tumors
May 2017

Local lab developed NGS or FISH for NTRK fusion

larotrectinib/entrectinib
***NTRK* fusions**
Advanced solid tumors
Nov 2018/Aug 2019

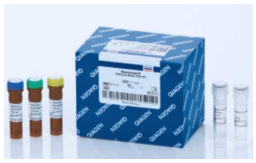
www.foundationone.com

pembrolizumab
TMB-high
Advanced solid tumors
June 2020

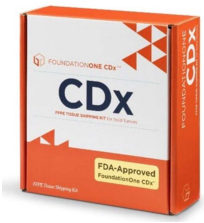
New FDA approved targeted therapies have led to newer predictive biomarkers

VENTANA PD-L1 (SP142) Assay

www.ventana.com



www.qiagen.com



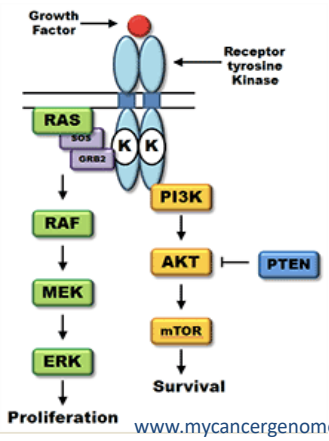
www.foundationone.com

- *PIK3CA* mutations
- *gBRCA1/2* mutations
- PD-L1 expression
- MMR-D (MSI-H)
- TMB-H
- *NTRK* fusions

How they are measured

Technical and reporting challenges for pathologists

PIK3CA

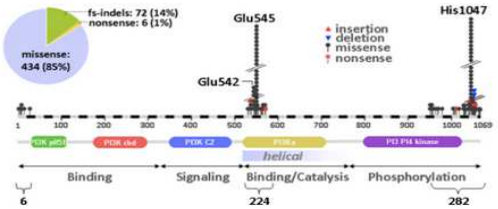


www.mycancergenome.org

- PI3K is a heterodimer with 2 subunits
- *PIK3CA* gene encodes one subunit, p110α
- When growth factor binds to PI3K it activates signaling:
  - MAPK pathway (RAS-RAF-MEK-ERK)
  - PI3K pathway (PI3K-AKT-mTOR)

PIK3CA

- Missense mutations
- Hotspots
  - exon 9 (helical)
  - exon 20 (kinase)

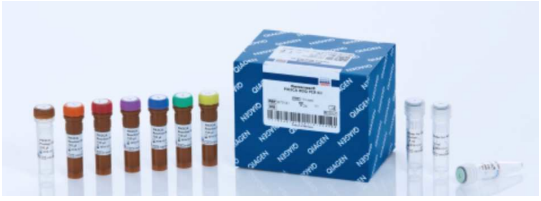


35-40% of ER positive/HER2 negative cancers
22% of HER2 positive cancers
18% of TN cancers

Fountzilas et al. Oncotarget 2016, Osborne CK, Schiff R. Annu Rev Med. 2011;62:233-247,Zardavas D et al (Loi). J Clin Oncol. 2018 Apr1;36(10):981-990

Predicting Response to *PIK3CA* Inhibition

In May 2019, FDA approved alpelisib (p110a inhibitor) for the treatment of ***PIK3CA*** mutated advanced HR+/HER2- postmenopausal breast cancer



www.qiagen.com

Simultaneously, FDA approved companion diagnostic test: *therascreen PIK3CA* (real-time PCR kit from Qiagen):

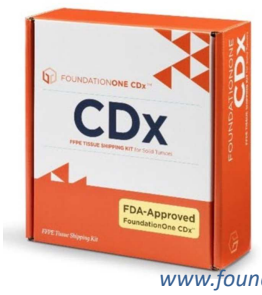
Tissue or liquid biopsy (blood)

Can bring this in house as single gene test or send out

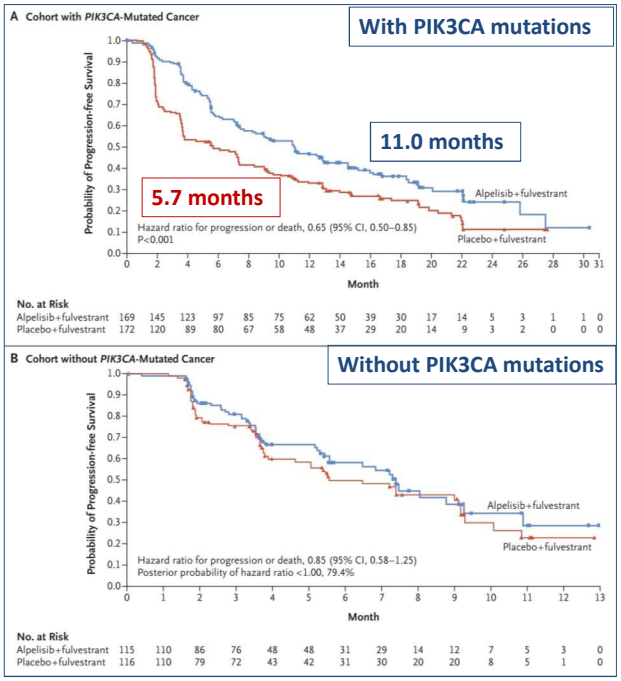
Dec 2019 FDA approved FoundationOne companion dx

NGS assay

Tissue and blood



www.foundationone.com



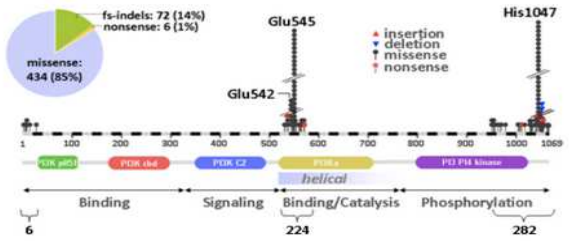
Andre et al. NEJM 380:1929-40. 2019.

SOLAR-1 Trial

phase III trial in HR+/HER2- advanced BC with *PIK3CA* mutations

PFS nearly doubled with the addition of the PI3K inhibitor alpelisib in cohort with *PIK3CA* mutations compared to endocrine therapy alone

Challenges



PIK3CA mutated cancers in SOLAR-1 included 11 mutations detected by PCR

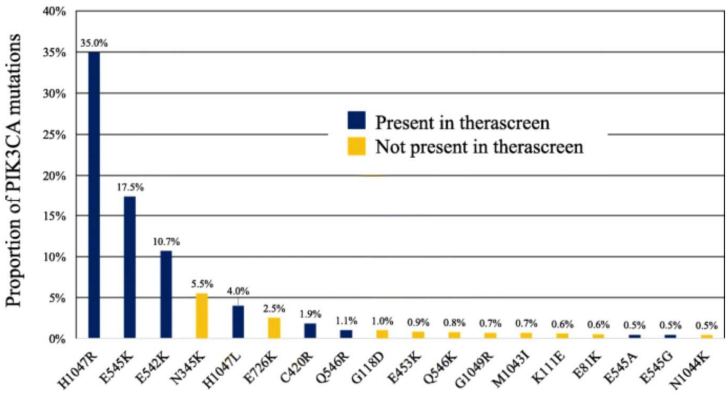
Most academic sites are using NGS assays → other less common (activating) mutations:

-How should these events be reported?

-Do these patients also respond?

Fountzilas et al. Oncotarget 2016, Osborne CK, Schiff R. Annu Rev Med. 2011;62:233-247,Zardavas D et al (Loi). J Clin Oncol. 2018 Apr1;36(10):981-990

PIK3CA somatic mutations in breast cancer



>6000 breast cancers in publicly available databases

Therascreen would have picked up 80% pts

Significant % at other sites would be missed.

12% of mutated tumors had double PIK3CA mutations

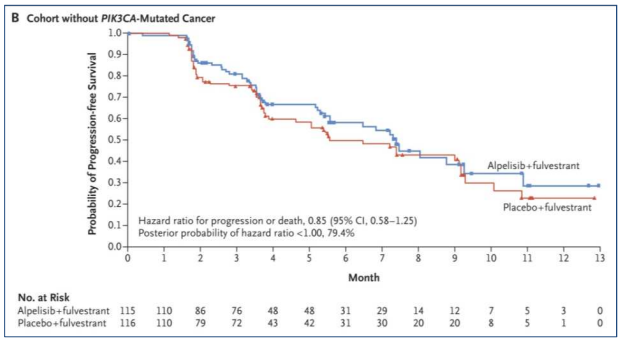
Clinical utility of other mutations at other sites needs further study

-some are activating in vitro/pre-clinical studies

We are currently reporting as Tier 2

Martinez-Saez…A Prat. **Breast Cancer Research** 2020 May 13;22(1):45

Interesting Question

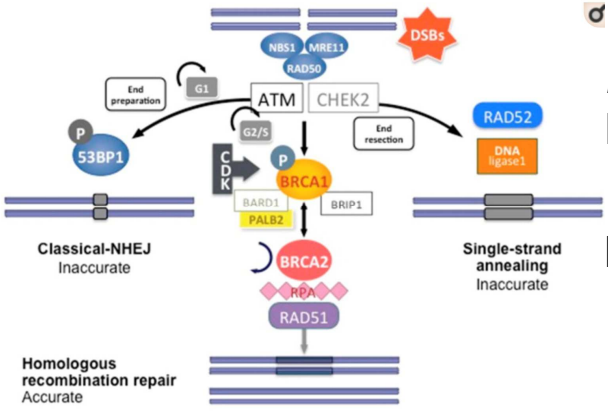


Is the inadvertent inclusion of less common activating mutations responsible for slight benefit seen in alpelisib arm in *PIK3CA* "negative" group

Andre et al. NEJM 380:1929-40. 2019.

BRCA1/2

BRCA1/2 are tumor suppressor genes



BRCA1/2 proteins involved in DNA ds break repair

Mutations in these genes cause

- 75% to 80% of hereditary breast cancer
- 5% to 10% of all breast cancer

Dos Santos et al. Int J Mol Sci. 2020 Jun; 21(11): 3850.

Predicting Response to PARP Inhibition

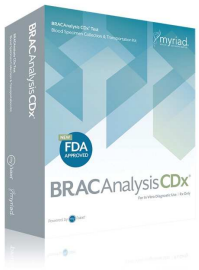
In January 2018, FDA approved olaparib (PARP inhibitor) for the treatment of patients with **germline** *BRCA*-mutated (g*BRCA*m), *HER2* negative metastatic BC

-failed prior chemotherapy

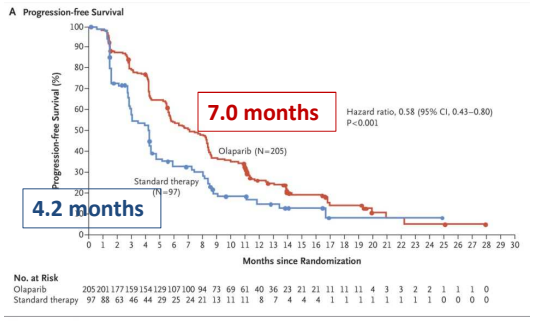
FDA approved Companion Dx (Myriad)

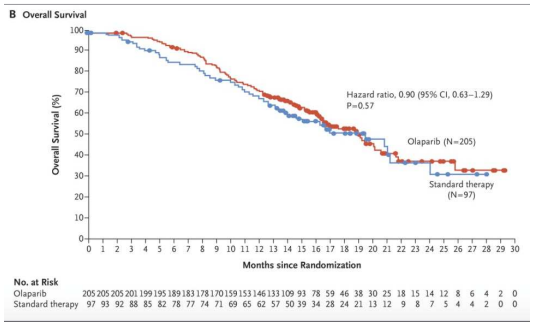
--Single-nucleotide variants and small insertions and deletions Identified by PCR and Sanger sequencing

--Large deletions and duplications detected using multiplex PCR



www.myriad.com





Robson et al, N Engl J Med 2017; 377:523-533

OlympiAD Trial

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Olaparib for Metastatic Breast Cancer in Patients with a Germline BRCA Mutation

Mark Robson, M.D., Seock-Ah Im, M.D., Ph.D., Elżbieta Senkus, M.D., Ph.D., Binghe Xu, M.D., Ph.D., Susan M. Domchek, M.D., Norikazu Masuda, M.D., Ph.D., Suzette Delaloge, M.D., Wei Li, M.D., Nadine Tung, M.D., Anne Armstrong, M.D., Ph.D., Wenting Wu, Ph.D., Carsten Goessl, M.D., et al.

Phase III trial that randomized 302 pts with **g*BRCA*m**, *HER2*- met BC to olaparib vs physician's choice of chemotx

median PFS was significantly longer with olaparib monotherapy than with standard chemotherapy (7.0 vs. 4.2 months)

Challenges in *BRCA* mutation testing

Strong family history
Known high-risk

Genetic counseling
Consent
Blood sample
Germline testing

Variant classification

Pathogenic
Likely pathogenic

VUS

Benign
Likely benign

NGS Testing of tumor tissue to detect targetable somatic variants

Possible germline variant is flagged

Pathogenic
Likely pathogenic

VUS

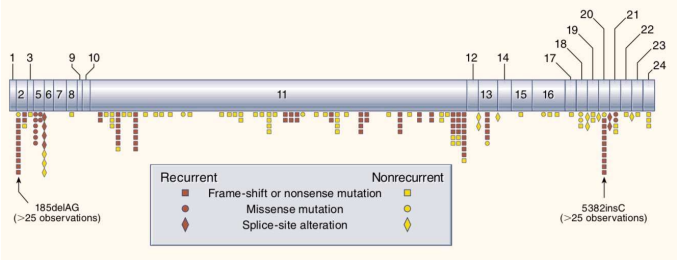
Benign
Likely benign

Many patients are tested initially because of family hx/high-risk

Others are not tested until a possible germline variant is identified by somatic mutation testing

Large number of Variants of Unknown Significance
Classifications change over time

Challenges in *BRCA* mutation testing



BRCA mutation types and technologies

3 broad classes of sequence changes:

- SNVS
- insertions/deletions
- large rearrangements

Many labs are transitioning to NGS

- advantages: increased throughput, multiplexing and lower costs
- disadvantage: lower sensitivity for large deletions/rearrangements (10%)

Collins FS. BRCA1 – Lots of Mutations, Lots of Dilemmas. NEJM 1996.

Interesting Question
What about patients with somatic mutations?

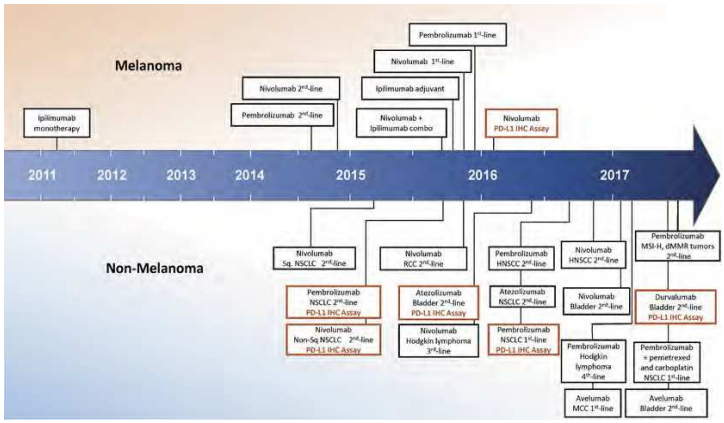
- Does a somatic mutation in *BRCA1/2* also predict response to PARP inhibition?
  - Prior studies in Ovarian Cancer showed response to PARPi in both germline and somatic *BRCA*-mutated cancers
- somatic *BRCA*1/2 mutations are present in ~3% of breast cancers
- Recent phase II study has shown that PARP inhibition is an effective treatment for patients with metastatic BC and **somatic** *BRCA1/2* mutations

Tung et al. TBCRC 048. J Clin Oncol 38: 4274-82, 2020.

Tissue-agnostic Markers

What we know about these markers in breast cancer

FDA approvals: immune checkpoint inhibitors



Recent FDA approvals for immune checkpoint inhibitors

Three new biomarkers:
- PD-L1
- MMR-D (MSI-H)
- High TMB

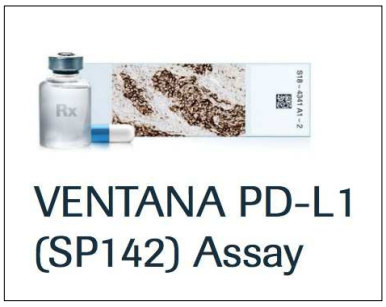
The number of new checkpoint inhibitors is large and continues to grow.
Best way to predict response has been area of intense focus in past several years.

Taube J, et al. Mod Path. 2018 Feb 3; 21:214-234

FDA Approvals for PD-L1 positive TNBC

atezolizumab for PD-L1 positive advanced **TNBC** (Mar 2019)

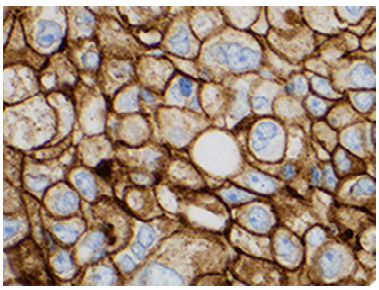
CDx:



www.Ventana.com

pembrolizumab for PDL1 positive advanced **TNBC** (Nov 2020)

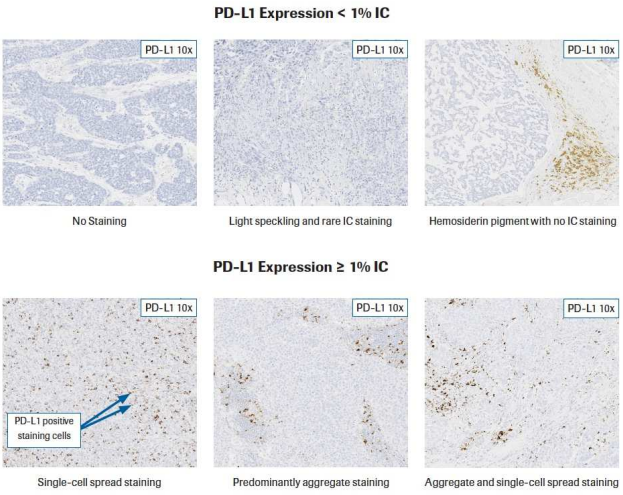
CDx:



www.dako.com

223C

What is “PD-L1 positive breast cancer?”



Ventana SP142 Interpretation Guide

www.Ventana.com

For atezolizumab (SP142):

A positive result for PD-L1 is defined as expression in **immune cells in ≥1% of tumor area**.

For pembrolizumab (22C3):

A positive result for PD-L1 is defined as a Combined Positive Score (CPS) ≥10

Major challenge

Each checkpoint inhibitor has its own IHC test for PD-L1 expression

	Ab	Melanoma	NSCLC	Urothelial	HNSCC	Gastric
Anti-PD-1						
Nivolumab (BMS)	28-8 Dako	1% (TC)	1%, 5%, 10% (TC)	1% (TC)	1% (TC)	
Pembrolizumab[a] (Merck)	22C3 Dako		1%[b], 50%[c] (TC)			1% (TC/IC)
Anti-PD-L1						
Durvalumab (AstraZeneca)	SP263 Ventana			25% (TC/IC)		
Atezolizumab (Roche/Genentech)	SP142 Ventana		50% (TC)/10% (IC)	5% (IC)		

-different abs for each agent

-different cutoff points for each tumor type

-in different cell populations

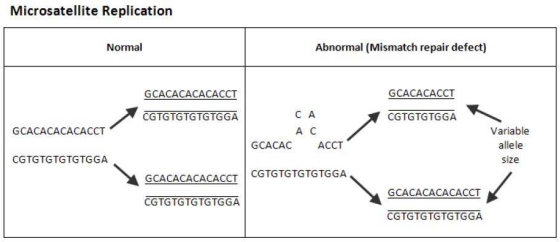
Need more harmonization work in this area

Mismatch-repair Deficiency (MMR-D) and Microsatellite Instability (MSI)

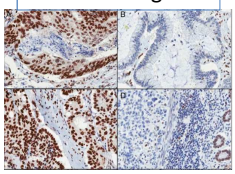
MMR genes (*MLH1/PMS2*, *MSH2/MSH6*) repair errors that occur during replication of DNA repeat sequences

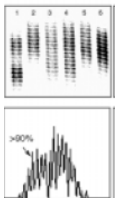
defective DNA mismatch repair leads to hypermutation and instability of DNA repeat regions (MSI-H)

MMR-D can be detected either by IHC or PCR
- -Loss of MMR gene expression on IHC
- -MSI (different sized products on PCR)



IHC of MMR genes





PCR products

Lynch Syndrome GeneReview: Microsatellite Instability (MSI) Testing. Kohlmann W, Gruber S. 2018
Richman et al. J Clin Pathol. 2016 Jan;69(1):35-41.
Bacher et al. Disease Markers 20 (2004) 237–250

Mismatch-repair Deficiency (MMR-D) and Microsatellite Instability (MSI)

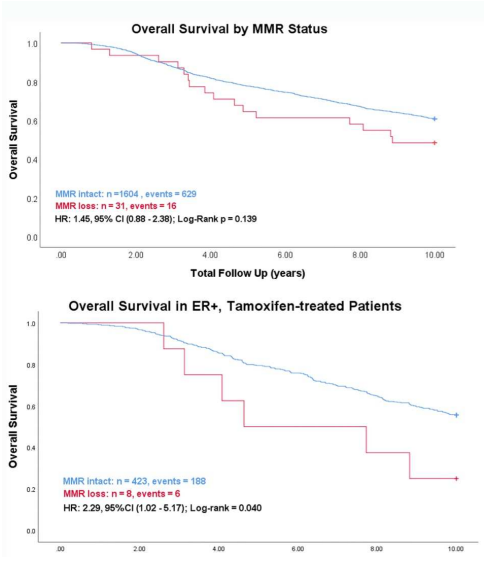
--Typically think of MMR-D (MSI-H) cancers as those associated with Lynch Syndrome

--1-2% of breast cancers are MMR-D

--present in all major breast cancer subtypes

--associated with:
- -high-grade features
- -low PR expression
- -high TILs

--associated with worse overall survival, especially in ER+, Tam treated patients



*Cheng et al. Breast Cancer Research and Treatment **volume 179**, pages3–10(2020)*

MMR-deficient (MSI-H) solid tumors

- In May 2017, the FDA approved pembrolizumab for advanced **MMR-D (MSI-H)** solid tumors that have progressed following prior treatment and who have no satisfactory alternative treatment
- The approval was based on data from five single-arm KEYNOTE trials
- Included **two patients** with **MMR-D breast cancer,** both had partial response
- No official companion diagnostic/local testing can be used

High Tumor Mutational Burden (TMB)

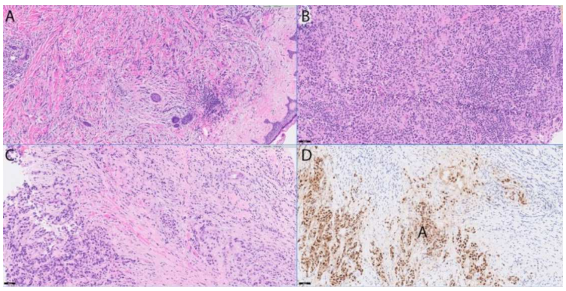
TMB = number of somatic mutations per megabase

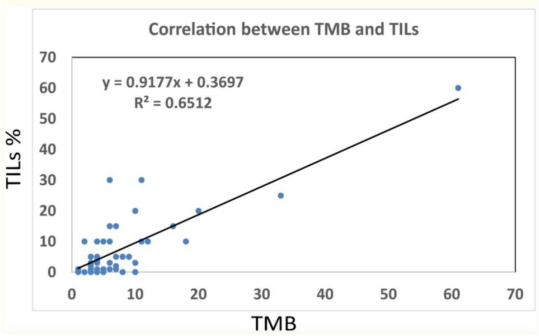
~5% of breast cancers

Associated with high TILs and mutations in DNA damage repair genes

Associated with longer PFS when treated with checkpoint inhibitors

Barroso-Sousa R. Clin Cancer Res 2020;26:2565–72





Mei P et al. Diagn Pathol. 2020; 15: 50

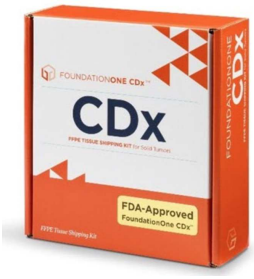
High Tumor Mutational Burden (TMB)

In June 2020, the FDA approved pembrolizumab for advanced solid tumors with high TMB using CDx

Approval based on KEYNOTE-158 which looked at response to pembro in 10 cohorts of patients

Response rate of 29%

- versus 6% in patients with a TMB <10
- complete in 4%, partial in 25%



Keynote 158 did not include breast cancer patients

www.foundationone.com

MPRIP	TPM3	TPR
TFG	ARHGEF2	LMNA
SQSTM1	TRIM63	PPL
TRIM24	PAN3	SQSTM1
TPM4	TFG	MYO5A

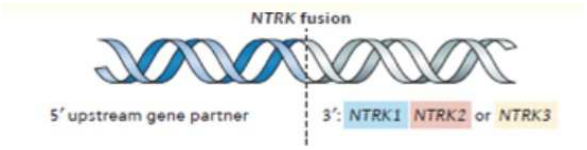
IRF2BP2	
TRAF2	

RFWD2	
STRN	
EML4	

CD74	QKI	ETV6
NFASC	ETV6	BTBD1
BCAN	NACC2	
TP53	BCR	
CTRC	TLE4	

RABGAP1L	CHTOP	AFAP1	IGFBP7
GRIPAP1	LRRC71	[illegible]	[illegible]PL24
PLEKHA6	PDE4DI	IRS04F1	[illegible]YL3
DAB2IP	VCL	AGBL4	AFAP1
LYN	RBPMS	UBE2R2	HNRNPA2B1

NTRK Fusions

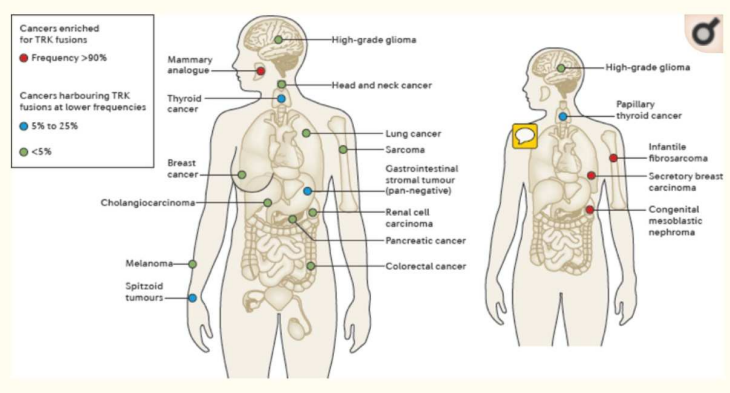


NTRK genes are a family of RTKs

Can be constitutively activated by fusion to a number of different 5' partners

Cocco E et al. Nat Rev Clin Oncol. 2018 Dec; 15(12): 731–747.

NTRK Fusions



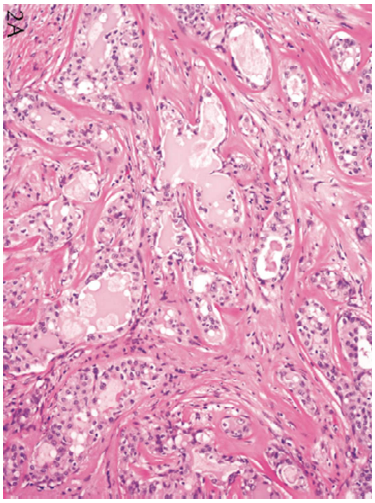
Present in >90% :

infantile fibrosarcomas
congenital mesoblastic nephromas
secretory breast carcinomas

Also present at much lower frequencies in many adult tumors:

<1% in cancer types including lung, colorectal, pancreatic, breast cancers, melanoma
up to 25% of thyroid tumors and GISTs

Cocco E et al. Nat Rev Clin Oncol. 2018 Dec; 15(12): 731–747.



Pathognomonic for secretory cancer
t(12;15) translocation
ETV6-NTRK3 fusion gene

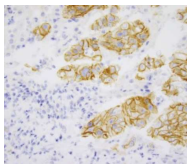
Vasudev et al. Arch Pathol Lab Med. 2011;135:1606

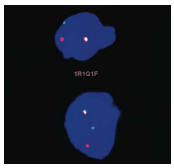
NTRK Fusions in Breast Cancer

Also seen in a very small subset (0.1%) of other breast cancers (ductal, lobular, metaplastic…)

Wide variety of fusion partners
NTRK1: CGN, GATAD2B, LMNA, MDM4, PEAR1, TPM3
NTRK3: all ETV6

Detection by IHC/FISH ok for cases with high prior probability
Sequencing best for cases with low prior probability



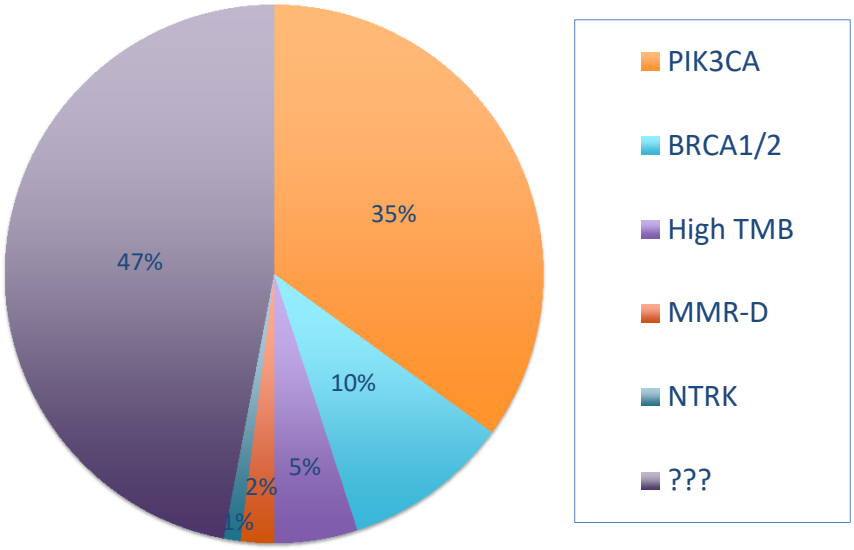


Number of rows returned: 10

Row #	Count	TS	cBio	chr	Gene	DNA Change	Alt frq	Coverage	Tier
1	7	0	cBio	chr19	CRTC1	c.1277del (p.P426Qfs*85), exon 10	8.5%	82	3
2	1	0	cBio	chr3	POLQ	c.6533del (p.S2178Tfs*7), exon 21	86%	200	3
3	2	0	cBio	chr13	RB1	c.1585del (p.Y529Tfs*3), exon 17	59%	56	3
4	24	0	cBio	chr17	TP53	c.847C>T (p.R283C), exon 8	86%	109	3
5	37	0	cBio	chr17	TP53	c.638G>A (p.R213Q), exon 6	69%	89	3
6	1	0	cBio	chr19	CRTC1	c.1102C>A (p.Q368K), exon 10	7.1%	56	4
7	1	0	cBio	chr13	FLT3	c.836A>G (p.H279R), exon 7	77%	142	4

Ross et al. Cancer Res 2018;78(4 Suppl):Abstract nr P2-09-15

New Predictive Markers in Breast Cancer



Summary and Recommendations

Breast cancers can be classified into gene-expression based subtypes

ER positive, HER2 negative cancers ("luminal") often require multigene classifier testing to determine risk of recurrence/sensitivity to chemotx

-results can vary for individual pts between testing platforms

Determination of HER2 status often requires FISH testing

-be sure you are working from the latest guidelines (2018) for classification of cases in groups 2, 3 and 4

-be appropriately cautious about HER2 IHC results that don't fit with your FISH results –>communicate with your breast pathologists

Summary and Recommendations

There are two recent FDA approvals impacting breast cancer:

→olaparib for g***BRCA1/2*** mutated advanced cancers

→alpelisib for advanced ER+/HER2- cancers with ***PIK3CA*** mutations

Be aware of the need to update BRCA1/2 VUSs regularly

Be cautious about reporting PIK3CA mutations that were not included in the SOLAR1 trial, especially with regard to evidence for activation

Recent tissue-agnostic approvals also impact breast cancer:

→MMR-D and TMB-H status will impact 2-5% of breast cancers

→*NTRK*-fusions will be seen in <1%