Breast Cancer

CME Prepared 2021/22

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Background

"Breast cancer arises in the lining cells (epithelium) of the ducts (85%) or lobules (15%) in the glandular tissue of the breast." [1]

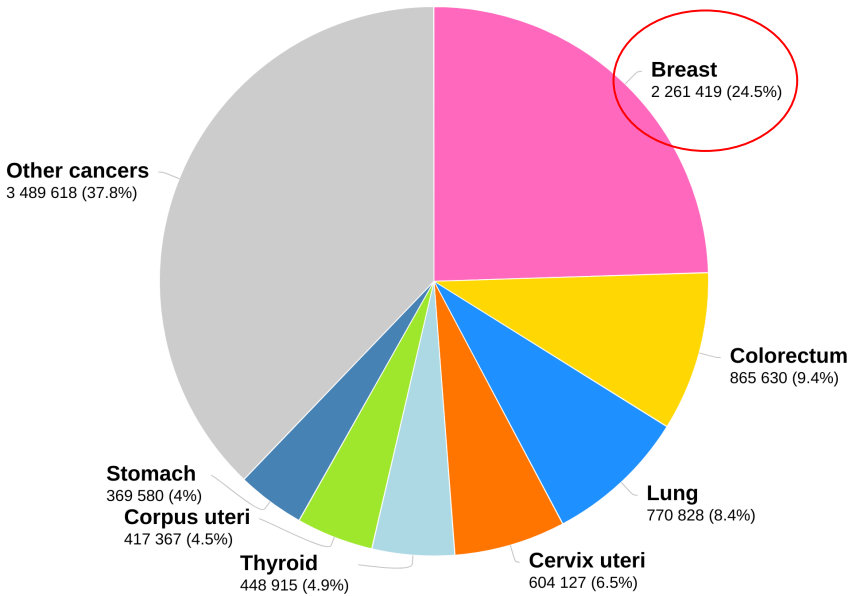
- The cancerous growth is initially confined to the duct/lobule and has minimal potential to metastasise [1]
- Leading cause of cancer in women worldwide [2]

Breast cancer in Malaysia

- Most common form of cancer affecting women in Malaysia
- 1 in 20 women are at risk (compared to 1 in 8 in Europe & the US) [3]

[1] World Health Organization Fact Sheet, Breast Cancer
[2] Feng Y et al. (2018), Genes Dis, 5(2):77-106
[3] Yip CH et al. (2006), Asian Pac J Cancer Prev, 7(3):369-74

Estimated number of new cases in 2020, worldwide, females, all ages



Total : 9 227 484

Data source: Globocan 2020
Graph production: Global Cancer Observatory (http://gco.iarc.fr)

International Agency for Research on Cancer
World Health Organization

Image from: Globocan 2020: New global cancer data (https://www.uicc.org/news/globocan-2020-new-global-cancer-data)

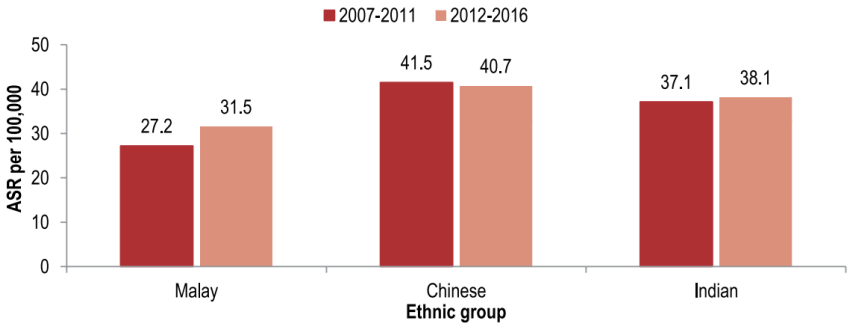


Figure 25. Female Breast: Comparison of age-standardised rate by year and major ethnic group, Malaysia

Race & Age-specific incidence rates in Malaysia

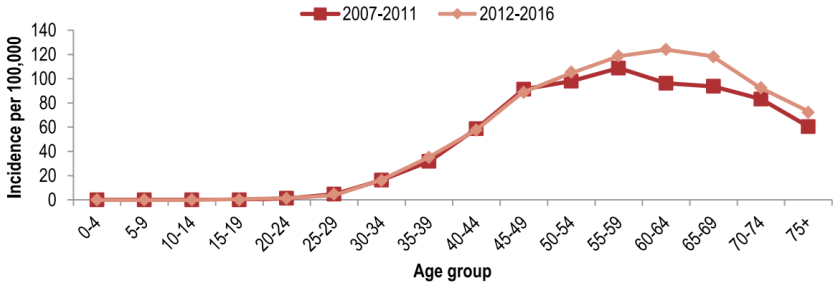


Figure 26. Female Breast: Comparison of age-specific incidence rate by year, Malaysia

Images from: Malaysia Cancer National Registry Report (2019) [4]

Risk factors

1. **Age & gender**
- Most breast cancers are diagnosed in women aged 55 or older [2]
2. **Genetic predispositions**
- About 5–10% of breast cancers are linked to gene mutations inherited from a parent [5]
3. **Positive family history**
- Having a first-degree relative (mother, sister, or daughter) with breast cancer almost doubles a woman's risk [6]

[2] Feng Y et al. (2018), Genes Dis, 5(2):77-106
[5] Veronesi U et al. (2005), Lancet, 365(9472):1727–1741
[6] Kaminska M et al. (2015),Prz Menopauzalny, 14(3):196–202

4. Being overweight or obese

5. Having a previous benign breast lump

6. Race and ethnicity

7. Radiation exposure [6]

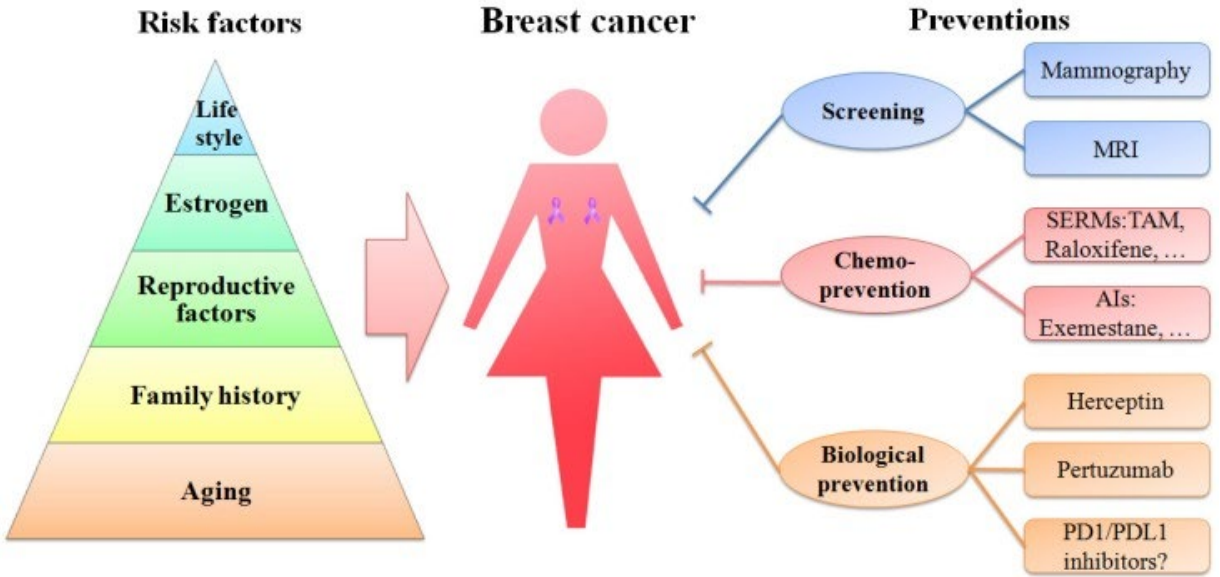


Image from: Sun YS et al. (2017) [22]

[6] Kaminska M et al. (2015),Prz Menopauzalny, 14(3):196–202
[22] Sun YS et al. (2017), Int J Biol Sci, 13(11):1387-1397

Prognostic/Predictive Factors

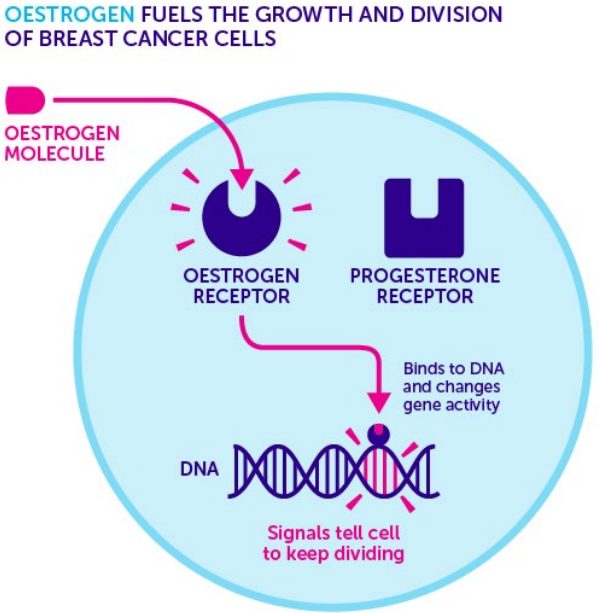
- Patient age
- Axillary lymph node status
- Tumor size
- Lymphatic invasion
- Histologic grade
- Response to neoadjuvant therapy
- Estrogen receptor/progesterone receptor (ER/PR) status
- *HER2* gene amplification or overexpression

[According to CAP (College of American Pathologists) guidelines] [7]

[7] Chalasani P et al. (2021), Breast Cancer, Medscape

ER/PR/HER2 status

- Presence of the **estrogen** (ER) & **progesterone** receptors (PR) = a component of routine evaluation of breast cancer specimens
- Necessary for determining prognosis and treatment
- Helps determine risk of recurrence
- Hormone receptor-positive tumours = responsive to hormone therapy [8]



Credit: Cancer Research UK

[8] Mohammed, H et al. (2015), Nature, 523, 313–317

HER2

- Prompts cancer to grow & metastasise more rapidly if present in high amounts/overexpressed
- HER2/ERBB2 gene amplification: occurs in 10–40% of primary tumors [9]
- HER2 protein overexpression: found in almost 25% of breast cancers [9]
- In the past, HER2 overexpression was associated with a worse prognosis [7]
- Routine use of HER2-targeted therapies (e.g. monoclonal antibodies: trastuzumab, pertuzumab) = improved prognosis

[9] Paik S et al. (1990), J. Clin. Oncol, 8(1):103–112
[7] Chalasani P et al. (2021), Breast Cancer, Medscape

Types of Breast Cancer

- Classified based on *pathology*, *invasiveness*, *expression of tumour markers* etc.

1. **<u>Non-invasive/carcinoma in situ</u>**
   - Confined to the milk ducts/lobules in the breast - do not grow into normal tissues within the breast
   - Most common: Ductal carcinoma in situ (DCIS) ~90%
   - Less common: Lobular carcinoma in situ (LCIS) [1]

2. **<u>Invasive</u>**
   - Grow into/Invade normal healthy tissues
   - Most breast cancers are invasive

[1] Sharma GN et al. (2010), J Adv Pharm Technol Res. 1(2):109-126

Non-Invasive

Ductal carcinoma in situ (DCIS)

- Forms in cells lining milk duct
- 15-20% of all breast cancers [10]
- May become invasive if left untreated
- Usually develops in one breast
- Usually treated with lumpectomy → radiation therapy [2]

Lobular carcinoma in situ (LCIS)

- Forms in cells lining lobule
- Marker of increased risk of developing invasive cancer [2]
- Tends to develop in both breasts

[2] Feng Y et al. (2018), Genes Dis, 5(2):77-106
[10] SEER Training Modules, U. S. National Institutes of Health, National Cancer Institute

Invasive/Infiltrating ductal carcinoma (IDC)

- Forms in cells lining milk duct & spreads into nearby tissue
- Most common type of breast cancer (70-80%) [5]

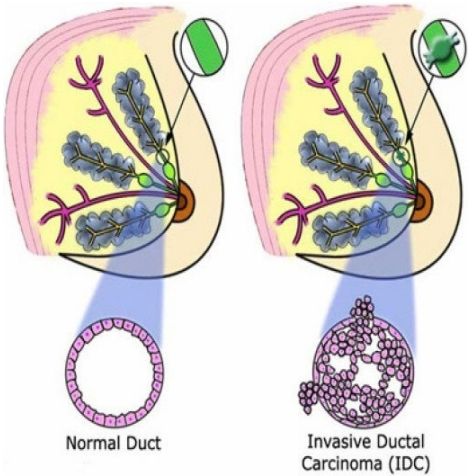


Image from: Sharma GN et al. (2010), J Adv Pharm Technol Res. 1(2):109-126

Invasive/Infiltrating lobular carcinoma (ILC)

- Forms in cells lining lobule & spreads into nearby tissue
- Second most common type of breast cancer (10-15%) [10]
- Slow-growing → may not respond to chemotherapy

[5] Veronesi U et al. (2005), Lancet, 365(9472):1727–1741
[10] SEER Training Modules, U. S. National Institutes of Health, National Cancer Institute

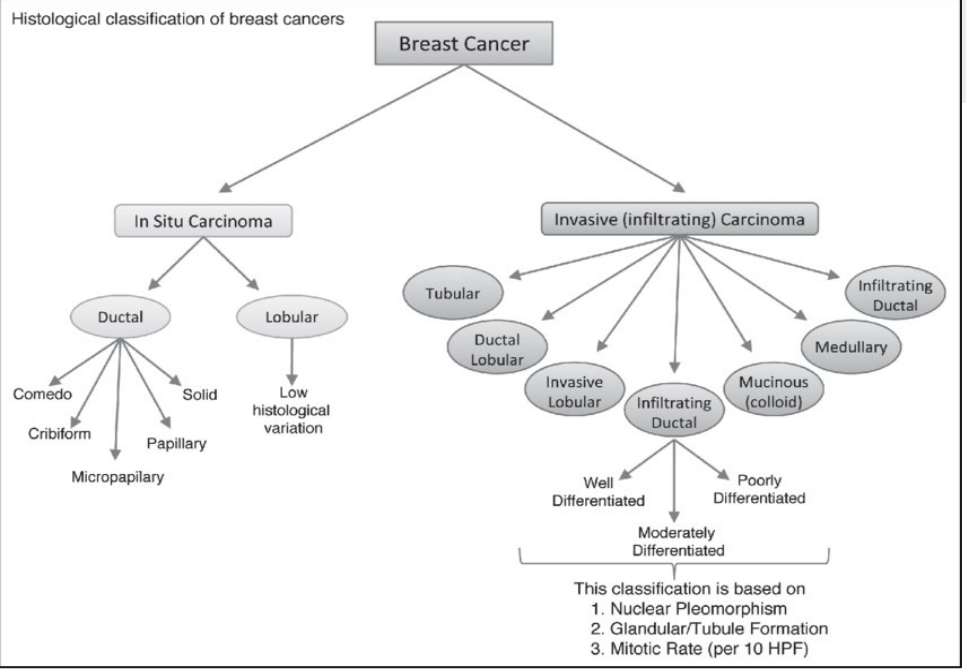


Figure: Histological classification of breast cancers.[11]

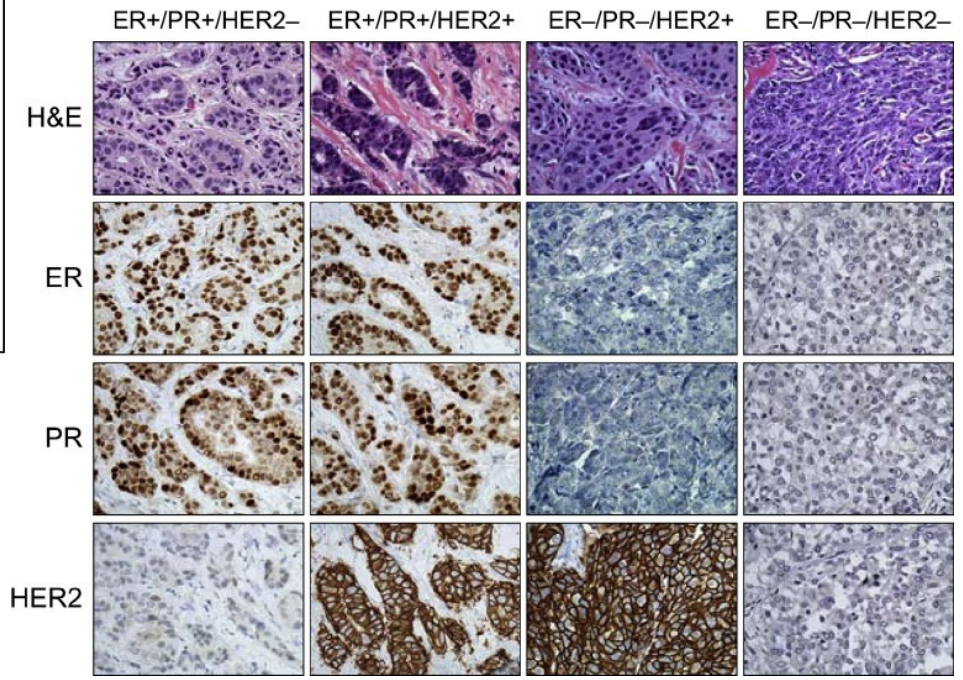
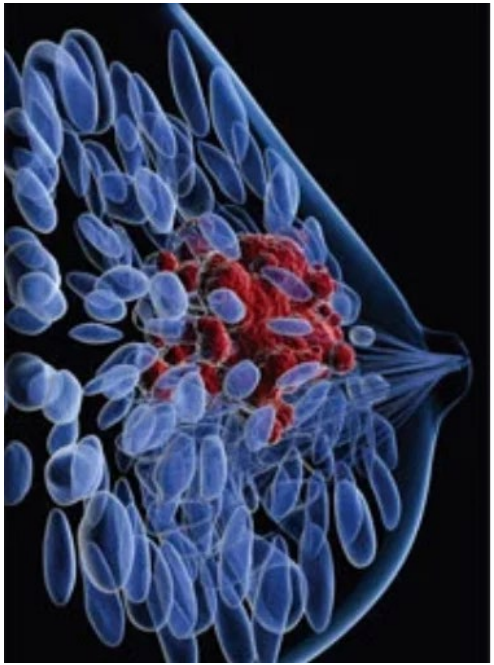


Figure: Histological classification of invasive breast cancer based on ER, PR & HER2 expression.[34]

[11] Malhotra GK et al. (2010), Cancer Biol Ther, 10(10):955-60
[34] Rivenbark A et al. (2013), The American Journal of Pathology, 183(4), 1113-1124



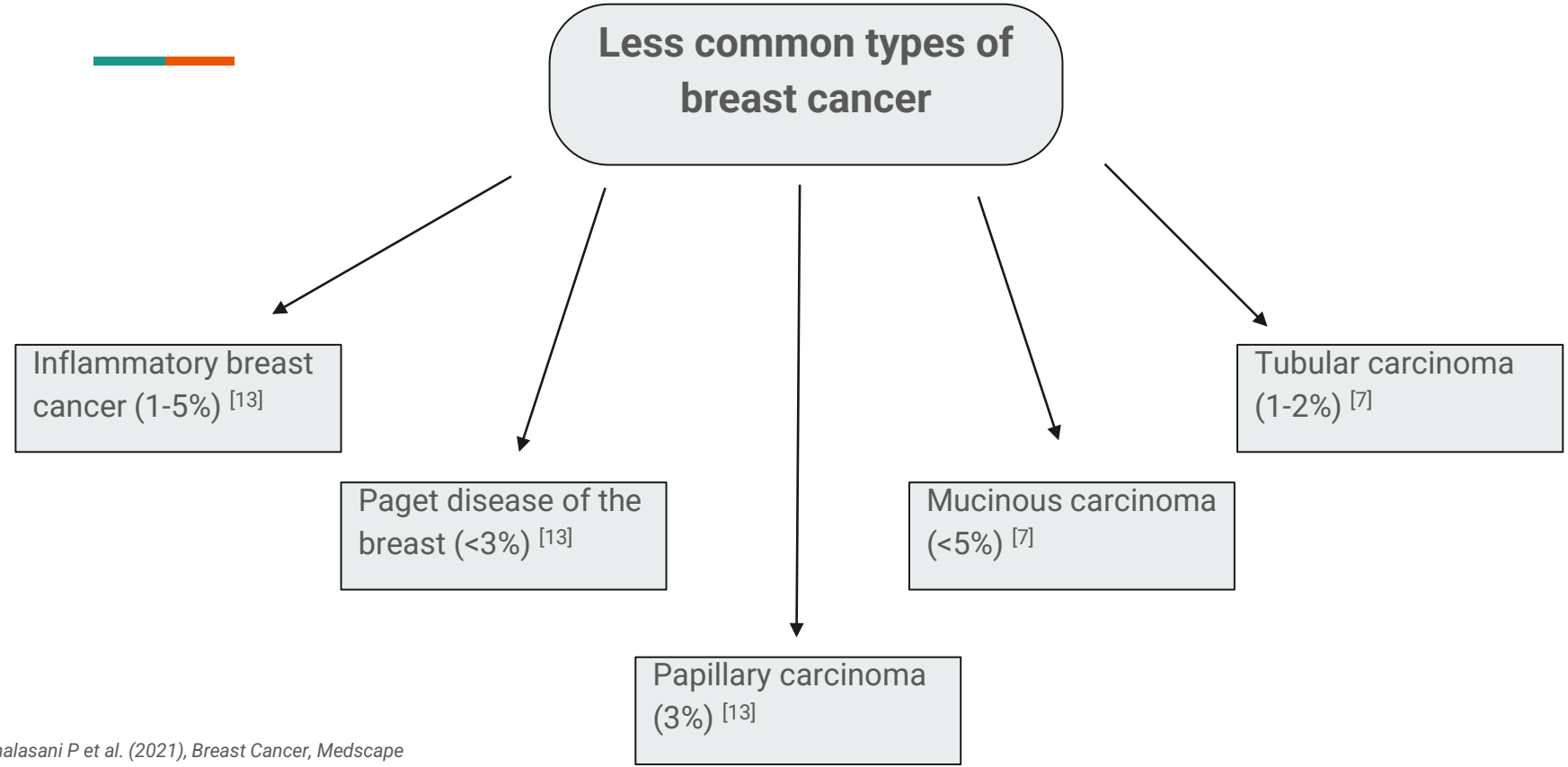
Credit:https://www.onclive.com/view/assessing-the-changing-treatment-landscape-in-triple-negative-breast-cancer

Triple negative breast cancers (TNBCs), defined by lack of expression of ER, PR or HER2 receptors, represent approximately 15-20% of all breast cancers [12]

- Limited response to hormone therapy & immunotherapy → Difficult treatment [12]
- Most common histology seen in TNBCs = infiltrating ductal carcinoma [2]
- More common in women with BRCA1 mutation [2]

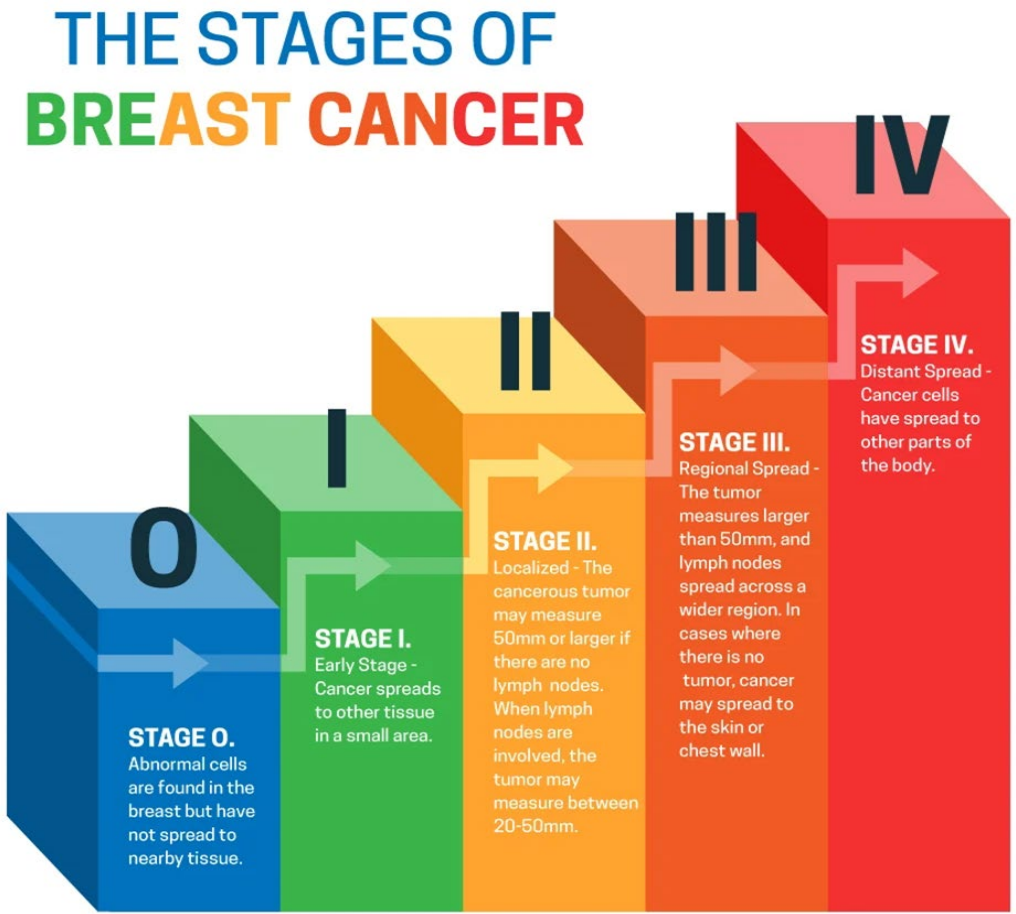
[2] Feng Y et al. (2018), Genes Dis, 5(2):77-106
[12] Yao H et al. (2017), Oncotarget, 8(1):1913-1924

Less common types of breast cancer



[7] Chalasani P et al. (2021), Breast Cancer, Medscape
[13] Hulka B.S. (1996), Prog Clin Biol Res, 395:159–174

Stages of Breast Cancer



Credit: https://blog.westsuburbanmc.com/the-5-stages-of-breast-cancer-and-prognosis

TNM staging [14]

T Category	T Criteria
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Tis (DCIS)	Ductal carcinoma in situ
Tis (Paget)	Paget disease not associated with invasive carcinoma or DCIS
T1	Tumor size ≤ 20 mm
T1mi	Tumor size ≤ 1 mm
T1a	Tumor size > 1 mm but ≤ 5 mm
T1b	Tumor size > 5 mm but ≤ 10 mm
T1c	Tumor size > 10 mm but ≤ 20 mm
T2	Tumor size > 20 mm but ≤ 50 mm
T3	Tumor size > 50 mm
T4	Tumor with direct extension to the chest wall and/or the skin with macroscopic changes
T4a	Tumor with chest wall invasion
T4b	Tumor with macroscopic skin changes including ulceration and/or satellite skin nodules and/or edema
T4c	Tumor with criteria of both T4a and T4b
T4d	Inflammatory carcinoma

cN Category	cN Criteria
cNX	Regional nodes cannot be assessed (previously removed)
cN0	No regional nodal metastases
cN1	Metastases to movable ipsilateral level I and/or level II axillary nodes
cN1mi	Micrometastases
cN2	Metastases to fixed or matted ipsilateral level I and/or level II axillary nodes; or metastases to ipsilateral internal mammary nodes without axillary metastases
cN2a	Metastases to fixed or matted ipsilateral level I and/or level II axillary nodes
cN2b	Metastases to ipsilateral internal mammary nodes without axillary metastases
cN3	Metastases to ipsilateral level III axillary nodes with or without level I and/or level II axillary metastases; or metastases to ipsilateral internal mammary nodes with level I and/or level II axillary metastases; or metastases to ipsilateral supraclavicular nodes
cN3a	Metastases to ipsilateral level III axillary nodes with or without level I and/or level II axillary metastases
cN3b	Metastases to ipsilateral internal mammary nodes with level I and/or level II axillary metastases
cN3c	Metastases to ipsilateral supraclavicular nodes

M Category	M Criteria
M0	No clinical or imaging evidence of distant metastases
cM0(i+)	No clinical or imaging evidence of distant metastases, but with tumor cells or deposits measuring ≤0.2 mm detected in circulating blood, bone marrow, or other nonregional nodal tissue in the absence of clinical signs and symptoms of metastases
cM1	Distant metastases on the basis of clinical or imaging findings
pM1	Histologically proven distant metastases in solid organs; or, if in nonregional nodes, metastases measuring >0.2 mm

[14] Hortobagyi GN et al. (2017), American Joint Committee on Cancer, AJCC cancer staging manual, 8th ed. New York, NY: Springer, 589–636

Numbered staging: 5 stages → 0-IV

- Determined by:
- TNM staging
- Tumour grade
- ER/PR/HER2 status [14]

Stage	TNM
Stage 0	Tis, N0, M0
Stage IA	T1, N0, M0
Stage IB	T0, N1mi, M0
	T1, N1mi, M0
Stage IIA	T0, N1, M0
	T1, N1, M0
	T2, N0, M0
Stage IIB	T2, N1, M0
	T3, N0, M0
Stage IIIA	T0, N2, M0
	T1, N2, M0
	T2, N2, M0
	T3, N1, M0
	T3, N2, M0
Stage IIIB	T4, N0, M0
	T4, N1, M0
	T4, N2, M0
Stage IIIC	Any T, N3, M0
Stage IV	Any T, Any N, M1

Image from: Hortobagyi GN et al. (2017) [14]

[14] Hortobagyi GN et al. (2017), American Joint Committee on Cancer, AJCC cancer staging manual, 8th ed. New York, NY: Springer, 589–636

Common mutations in Breast Cancer [15]

- **BRCA1**
- **BRCA2**
- **ERBB2**
- **PIK3CA**
- **TP53**
- CDH1
- AR
- ATM
- PTEN
- BARD1
- BRIP1
- CHEK2
- PALB2
- RAD50
- RAD51
- STK11/LKB1

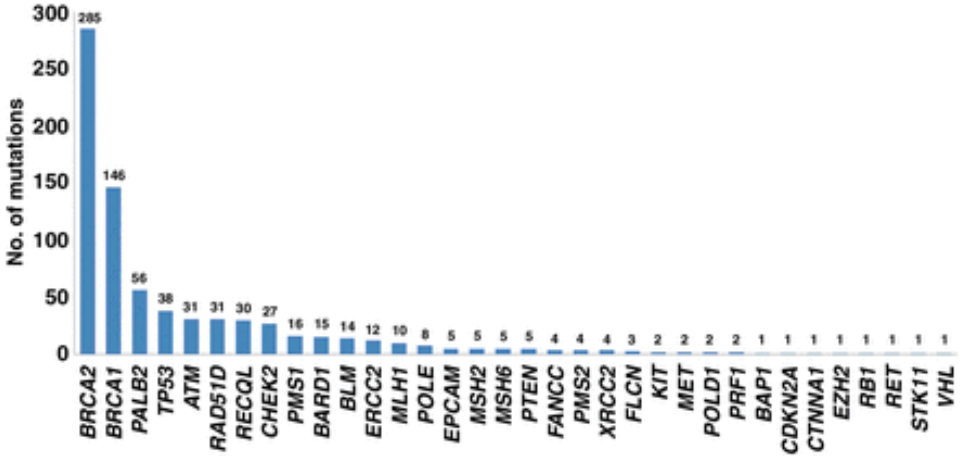
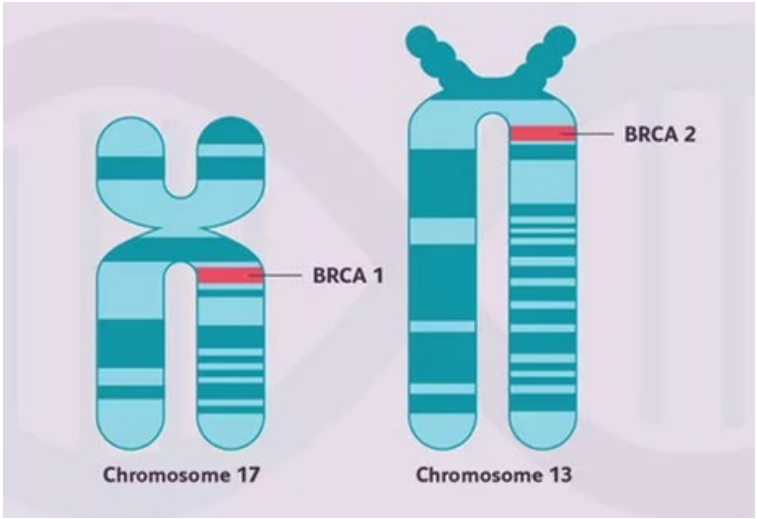


Figure: Pathogenic germline mutations identified in 46 cancer susceptibility genes in 8085 breast cancer patients.[35]

[15] Sheikh A et al. (2015), Asian Pac J Cancer Prev, 16(6):2177-85
[35] Sun J et al. (2017), Clin Cancer Res, (23) (20) 6113-6119

BRCA1 & BRCA2

- Tumour suppressor genes
- Encode proteins responsible for DNA mismatch repair → repair damaged DNA[17]
- Confers increased risk of breast (and other) cancers
- Both proteins work at different stages of the DNA damage response & DNA repair[16]
- Both function in homologous recombination repair



Credit: https://www.verywellhealth.com/non-brca-gene-mutations-4173768

[16] Rohini Roy et al. (2016), Nat Rev Cancer,12(1): 68–78
[17] Stratton MR et al. (2008), Nat Genet, 40: 17–22

TP53

- Found in vast majority of triple negative breast cancers → target of particular interest [18]
- Found in breast carcinomas in 20-40% of all cases (depending on tumor size and stage of the disease) [19]

PIK3CA

- Most commonly mutated gene in HR-positive/HER2-negative breast cancer - affects about 40% of people with this subtype [20]
- Occurs in 20–30% of patients with breast cancer [21]

[18] Natalie Turner et al. (2013), Cancer Treatment Reviews, 39(5), 541-550
[19] Børresen-Dale AL. (2003), Hum Mutat, 21(3):292-300
[20] Cizkova M et al. (2012), Breast Cancer Res, 14(1):R28
[21] The Cancer Genome Atlas Network (2012), Nature, 490:61-70

Diagnostic methods

Next Generation Sequencing (NGS)

• A high-throughput methodology that enables rapid sequencing of the base pairs in DNA or RNA samples.

• Enables the amplification of hundreds to thousands of genes at one time in multiple samples, as well as discovery and analysis of different types of genomic features in a single sequencing run including single nucleotide variants (SNVs), copy number variation (CNV), structural variants and RNA fusions.

Advantages of NGS:

- Higher sensitivity to detect low frequency variants
- Faster turnaround time for high sample volumes
- Comprehensive genomic coverage
- Lower limit of detection
- Higher capacity with sample multiplexing
- Ability to sequence hundreds to thousands of genes or gene regions simultaneously
- Requires a single input of relatively low-quantity DNA or RNA

Targeted sequencing: Target-specific NGS panels are the most flexible option, it can be designed to sequence any gene or region of interest in a genome and can include structural and copy number variation, as well as RNA transcript analysis. Targeted panels generate smaller and more manageable data sets, which reduces the data analysis burden and can enrich the specific genetic sequences.

Figure: NGS workflow. Image credit: Thermofisher Scientific.

[23] R.Luthra et al. (2015), Cancers (Basel). 2015 Dec; 7(4): 2023–2036.

Real-time PCR

• A laboratory technique of molecular biology based on real-time polymerase chain reaction (PCR)

• Based on sequence-specific DNA probes consisting of oligonucleotides that are labelled with a fluorescent reporter, which permits detection after hybridization of the probe with its complementary sequence.

ARMS

• Allele-specific amplification is achieved by ARMS, which exploits the ability of Taq DNA polymerase to distinguish between a matched and a mismatched base at the 3' end of a PCR primer.

• When the primer is fully matched, the amplification proceeds with full efficiency. When the 3' base is mismatched, only low-level background amplification may occur. Therefore, a mutated sequence is selectively amplified even in samples where the majority of the DNA does not carry the mutation [24]

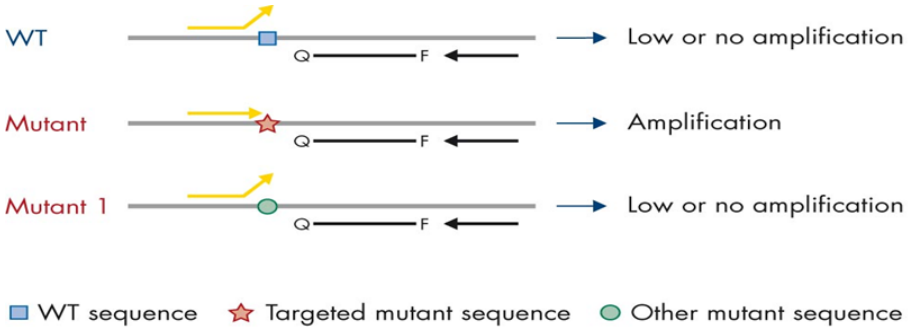


Figure: Identification of specific mutation by ARMS PCR.Adapted from Qiagen therascreen manual kit.

[24] Qiagen therascreen PIK3CA RGQ PCR manual kit (2013), version 1

MLPA – Multiplex Ligation dependent Probe Amplification

• A multiplex assay that detects copy number variations of genomic DNA sequences

• Each probe is composed of two half-probes (5' and 3' half-probes), consisting of a target-specific sequence and a universal primer sequence allowing the simultaneous multiplex PCR amplification of all probes

• Due to the large number of genes that can be analysed by a single technique, MLPA assay represents the gold standard for molecular analysis of all pathologies derived from the presence of gene copy number variation [26]. eg: BRCA1 and BRCA2 gene

• However, this MLPA assay will not detect other pathogenic variants such as point mutations or small insertions/deletions which comprise approximately 80 to 90% cancer-predisposition mutations in BRCA1 and BRCA2 gene

• This test should be used in combination with NGS analysis of BRCA1 and BRCA2 coding sequence for detection all classes of known inherited mutations

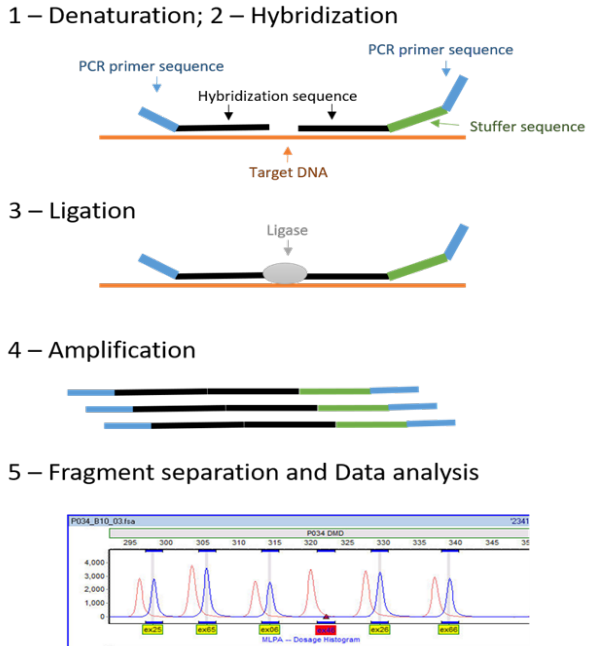


Figure: MPLA workflow.Adapted from Schouten J et al (2002) [25]

[25] Schouten J et al. (2002), Nucleic Acids Res. 2002;30 doi: 10.1093
[26] Liborio Stuppia et al. (2012), Int J Mol Sci. 2012; 13(3): 3245–3276

Immunohistochemistry (IHC)

• Is a method for detecting antigens in cells of a tissue section by exploiting the principle of antibodies binding specifically to antigens in biological tissues

• Is used to help diagnose diseases, such as cancer and may also be used to help tell the difference between different types of cancer.

• The antibodies are usually linked to an enzyme or a fluorescent dye. After the antibodies bind to the antigen in the tissue sample, the enzyme or dye is activated, and the antigen can then be seen under a microscope

• IHC is widely used in many research and clinical laboratories, it possible to visualize the distribution and localization of specific cellular components within cells

• A unique feature that makes IHC stand out among many other laboratory tests is that it is performed without destruction of histologic architecture

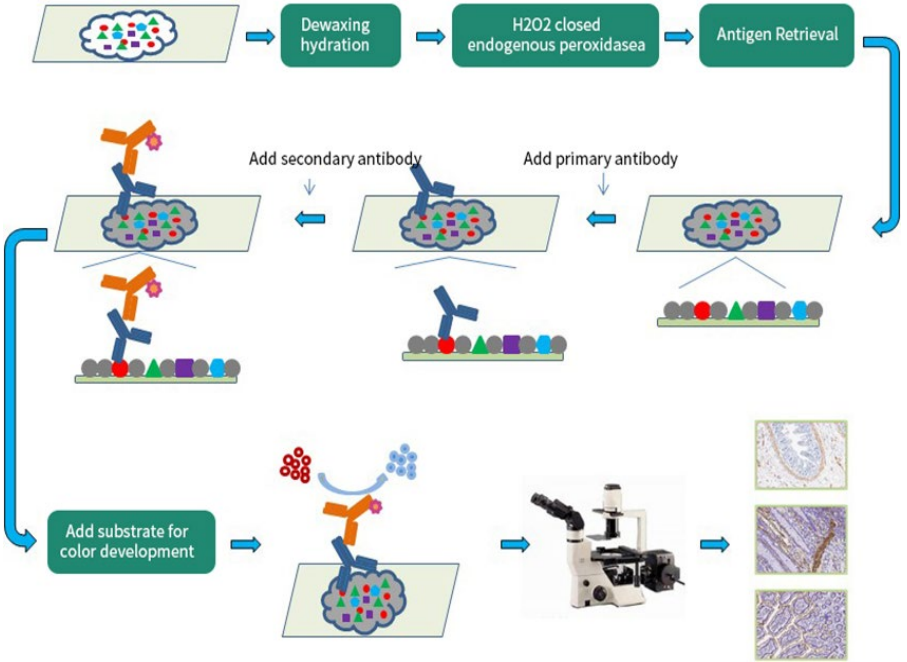


Figure: IHC workflow. Image credit: https://cn.sinobiological.com/category/ihc-protocol

[27] So-Woon K et.al (2016), J Pathol Transl Med. 50(6): 411–418.

Fluorescence in situ hybridization (FISH)

• A laboratory technique for detecting and locating a specific DNA sequence on a chromosome

• Can be used to visualize specific genes or portions of genes.

• The technique relies on exposing chromosomes to a small DNA sequence called a probe that has a fluorescent molecule attached to it.

• The probe sequence binds to its corresponding sequence on the chromosome- this process is called hybridization

• The probes, marked with a fluorescent dye, attach to those corresponding cells. Once the probes attach to the chromosomes in the tumour cells, they glow when hit with fluorescent light, clearly showing where the probes match to the cancer cells, and where any chromosomal differences are.

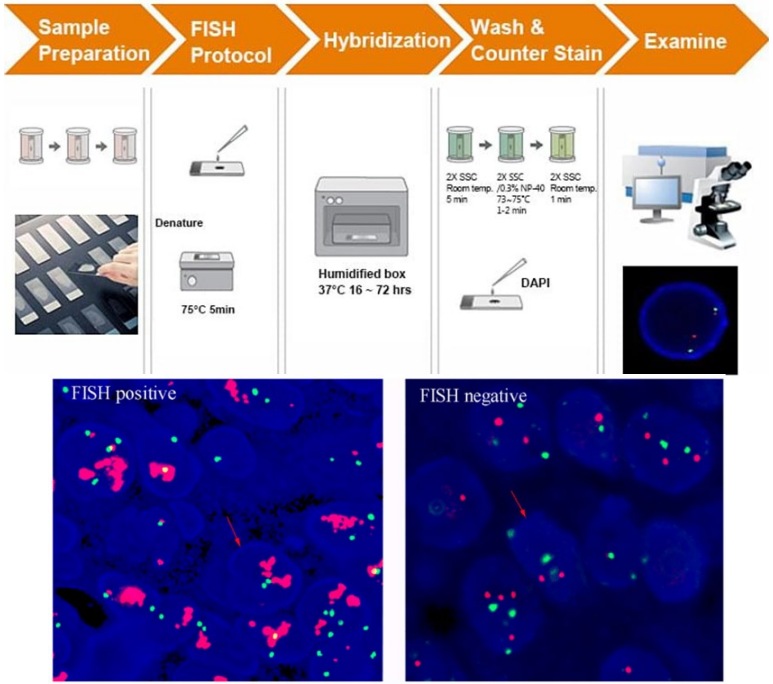


Figure: FISH assay workflow and result interpretation. Example of FISH HER result interpretation: HER2 (Red signal), CEP17 (Green signal) [28]

[28] Weiguo Sui et al. (2009), World Journal of Surgical Oncology volume 7, Article number: 83

Diagnostic methods available at SJMC

Next Generation Sequencing (NGS)

NGS BRCA testing (Tumor & Germline)

• Ion Torrent Oncomine BRCA Assay (Thermo Fisher Scientific) is a targeted next generation sequencing test for the detection of BRCA somatic and germline mutations.

• Genomic DNA was extracted from FFPE tissue sections, while for germline testing DNA was extracted from whole blood followed by library preparations by Ion Ampliseq Library Kit Plus and DNA Oncomine BRCA Assay kit. Ion GeneStudio S5 Prime sequencer sequenced the library and the data was analysed with Ion Reporter™ , then report is generated with the Oncomine™ Reporter software

• Specimen requirement: 10% formalin fixed paraffin embedded (FFPE) tissue block with >30% of tumour cells or 4-5 unstained slides with FFPE sections at 10uM thickness, tissue sections must have >30% of tumour cellularity for tumour testing. 4 x 3ml peripheral blood in EDTA for germline testing.

• Gene tested: BRCA1 and BRCA 2

• TAT: 2 to 3 weeks

Figure: Oncomine BRCA Assay kit

Figure: Ion chef system. Image credit: Thermofisher Scientific

NGS BRCA - HRR testing (Tumor & Germline)

• Ion Torrent Oncomine BRCA-HRR Assay (Thermo Fisher Scientific) is a targeted next generation sequencing test for comprising of thousands of variants across 28 types relevant of solid tumours in somatic and germline mutations related to homologous recombination repair (HRR) genes.

• Genomic DNA is extracted from FFPE tissue sections, while for germline testing, DNA is extracted from whole blood followed by library preparations by Ion Ampliseq Library Kit Plus and DNA Oncomine HRR Assay kit.

• Ion GeneStudio S5 Prime sequencer sequence the library and the data is analyse with Ion Reporter™ . The final report is generated with the Oncomine™ Reporter software

• Specimen requirements: 10% formalin fixed paraffin embedded (FFPE) tissue block with >30% of tumour cells or 4-5 unstained slides with FFPE sections at 10uM thickness, tissue sections must have >30% of tumour cellularity for tumour testing. 4 x 3ml peripheral blood in EDTA for germline testing.

• Genes tested: RAD54L, FANCL, BARD1, FANCD2, PIK3CA, RAD50, XRCC2,PPP2R2A,NBN,PTEN, MRE11, ATM, CHEK1, RAD52, KRAS, POLE, BRCA1, BRCA2, RAD51B, RAD51, PALB2, TP53, CDK12, RAD15C, BRIP1, POLD1, CHEK2, RAD51D

• TAT: 2 to 3 weeks

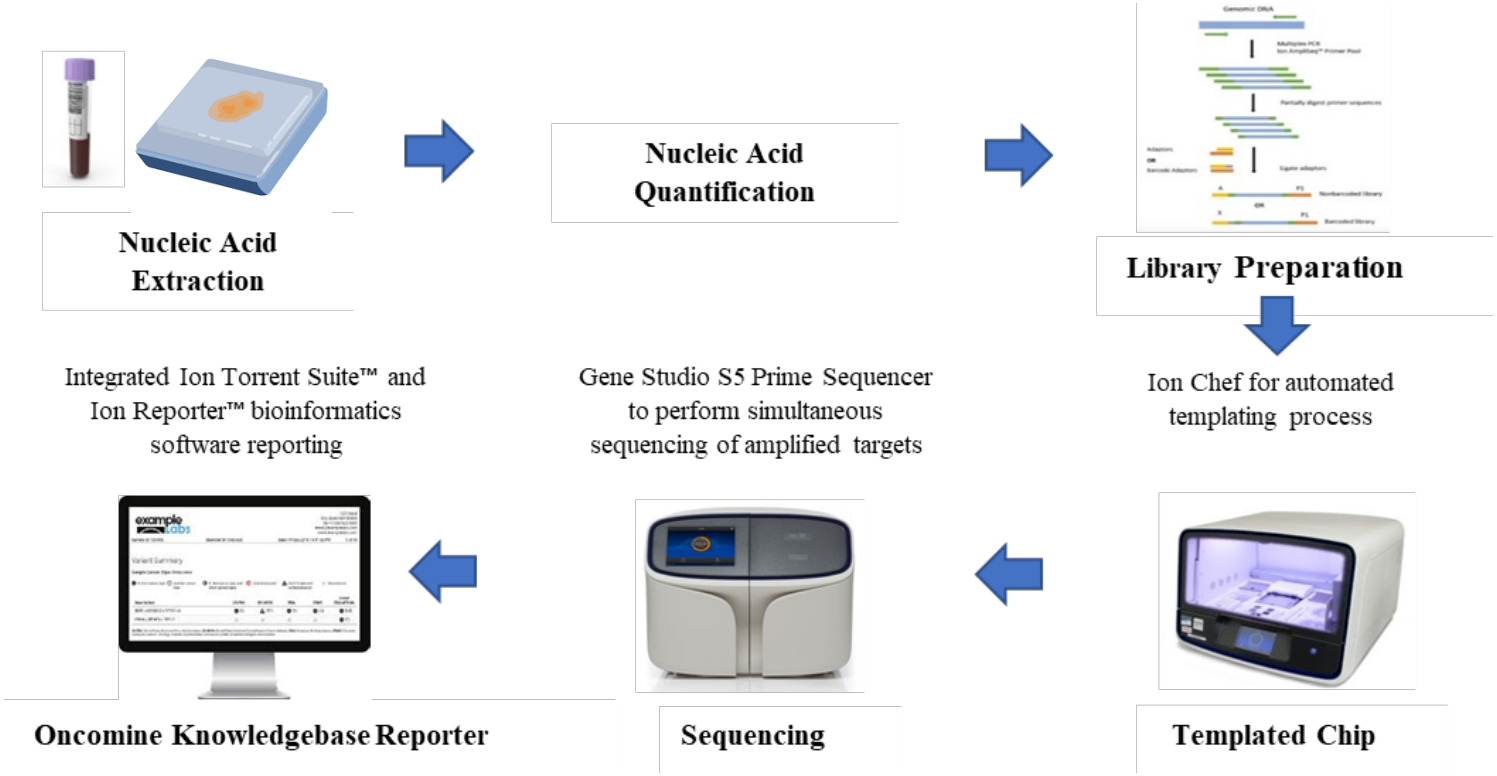


Figure : Oncomine BRCA and BRCA-HRR Next Generation Sequencing Workflow

Real time PCR
PIK3CA testing

• PIK3CA mutation testing by Qiagen therascreen PIK3CA RGQ PCR kit. This kit is a real-time qualitative PCR test to detect 11 mutations in PIK3CA using either FFPE or plasma

• This kit is the first companion diagnostic test approved by FDA to aid in the selection of patient who may be eligible for treatment with PIK3CA inhibitor, alpelisib

• Specimen requirements: 10% formalin fixed paraffin embedded (FFPE) tissue block with at least 20% tumour cells or 8-10 unstained slides with FFPE sections at 5uM thickness or 2x of 10ml whole blood in Streck tube for plasma testing

• Mutations tested:

(i) Exon 7 - C420R mutation

(ii) Exon 9 - E542K, E545A, E545D, E545G, E545K, Q546E and Q546R mutation

(iii) Exon 20 - H1047L, H1047R and H1047Y mutation

• TAT: 7 working days

Figure: Qiagen therascreen PIK3CA RGQ PCR kit

Figure: Rotor-Gene Q PCR machine

Immunohistochemistry (IHC)

HER2 scoring

• HercepTest™ is a semi-quantitative immunohistochemical assay to determine HER2 protein overexpression in breast cancer

• A 3-micrometre thick section from FFPE block is cut and mounted onto coated slides for HER2 protein using antibody (Ventana anti-HER2/neu rabbit monoclonal antibody, clone 4B5)

HER2 result scoring:

• 0 (negative) - No staining is observed, or membrane staining is observed in <10% of the tumour cells.

• 1+ (negative) - A faint/barely perceptible membrane staining is detected in >10% of tumour cells. The cells exhibit incomplete membrane staining.

• 2+ (equivocal) - A weak to moderate complete membrane staining is observed in >10% of tumour cells

• 3+ (positive) - A strong complete membrane staining is observed in > 10% of tumour cells

Note: Equivocal 2+ samples require confirmation using another analysis system, ideally FISH

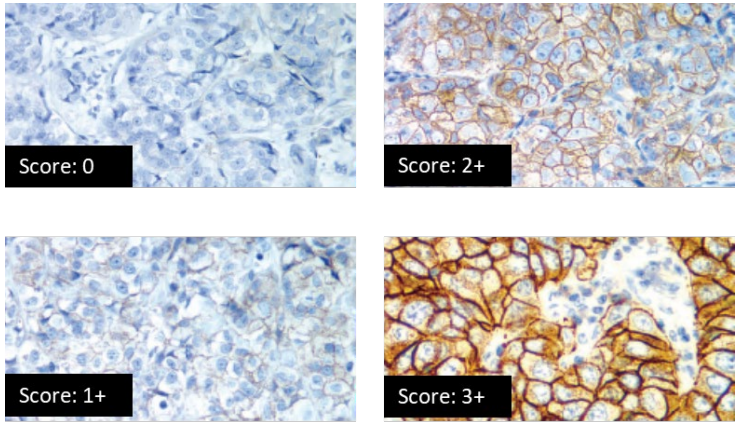


Figure: HER2 staining scoring patterns for tissue at 40x magnification. Adapted from Dako HercepTest kit interpretation manual breast cancer [29]

[29] HercepTest kit interpretation manual - Breast cancer

Fluorescence in situ hybridization (FISH)

HER2 amplification

• FISH testing is done to see if the cancer cells have extra copies of the HER2/neu gene

• Shows the mean number of HER2 copies using a DNA probe hybridizing to the HER2 gene alone or in association with centromeric probe as control for chromosome 17 copy number expressed as HER2/CEP17 ratio

• The HER2/neu DNA probe kit is used to detect amplification of ERBB2 (HER2/neu) gene in 10% neutral buffered formalin fixed, paraffin-embedded human tissue specimen

• HER2 result status

(i) Negative: HER2/CEP17 ratio of <2.0, average HER2 copy number <4.0 signals/cells

(ii) Positive: HER2/CEP17 ratio of > 2.0, average HER2 copy number >4.0 signals/cells

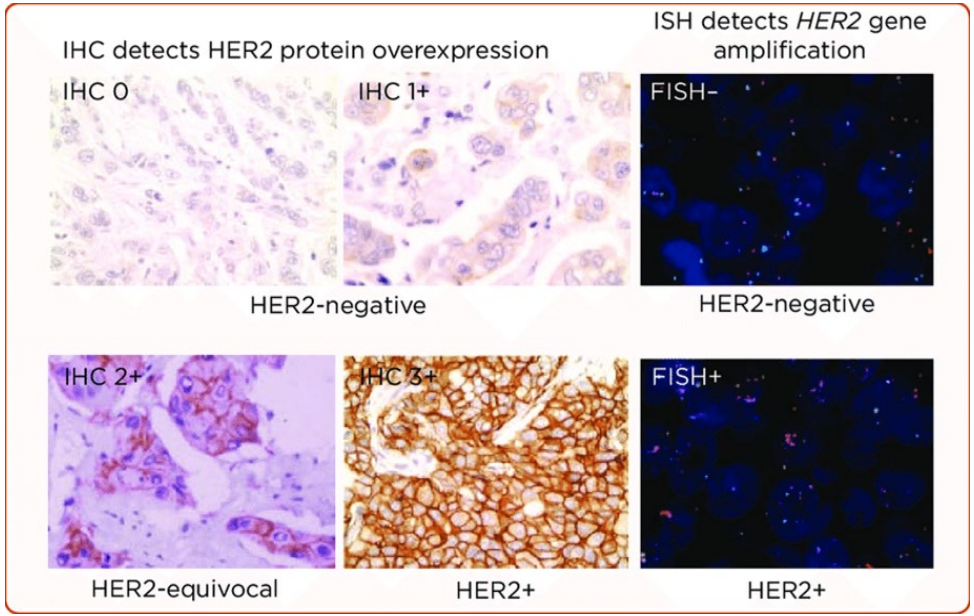


Figure: HER2 result status of breast cancer cells determined by HER2 testing (IHC scoring vs FISH). FISH HER2 amplification: HER2 (Red signal), CEP17 (Green signal) [28]

[28] Weiguo Sui et al. (2009), World Journal of Surgical Oncology volume 7, Article number: 83

Biomarkers associated with FDA- approved targeted therapies

BRCA1 and BRCA2 mutations

Lynparza (Olaparib), Talzenna (Talazoparib) - PARP inhibitor therapy

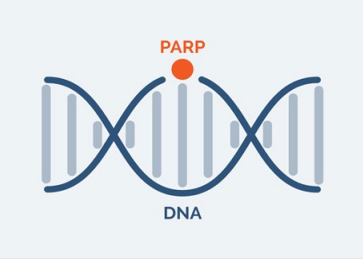
• PARP (poly ADP-ribose polymerase) – a type of protein that involved in several cellular processes including transcription, replication and DNA damaged repair

• PARP inhibitor – a targeted therapy that stop PARP from repairing damaged DNA

• To treat metastatic HER2-negative breast cancer with inherited BRCA1 and BRCA2 mutation that has been treated with chemotherapy

• Lowered the risk of disease progression or death by 42% [30]

• Common side effects: Nausea or vomiting, diarrhoea, headache, cough, tiredness or weakness, dizziness, shortness of breath



PARP is a protein that repairs DNA damage in cells so they can survive. In cancer cells, to stop this repair so they can't survive.

LYNPARZA helps kill cancer cells by preventing PARP from repairing them.

Figure: Lynparza mechanism of action. Adapted from https://www.lynparza.com/what-is-lynparza.html

[30] Mark Robson et al. (2017), N Engli J Med 2017 Aug 10;377(6):523-533

PIK3CA mutation

Alpelisib + fulvestrant (HR positive/Her-2 negative)

Piqray (alpelisib)

• Indicated in combination with fulvestrant for the treatment of postmenopausal women and men, HR - positive, HER2-negative, PIK3CA mutated, advanced or metastatic breast cancer as detected by an FDA-approved test following progression on or after an endocrine-based regimen

• Is a targeted therapy medicine called a PI3K inhibitor that inhibit the activation of the PI3K signalling pathway, this may result in inhibition of tumour cell growth and survival in susceptible tumour cell populations [31]

• Common side effects: rash, nausea, fatigue, diarrhoea, vomiting, high blood sugar level

[31] Jing Yang et al. (2019), Molecular Cancer volume 18, Article number: 26 (2019)

HER2 positive

Herceptin (trastuzumab)

• Herceptin was the first HER2-targeted therapy for breast cancer.

• It is a monoclonal antibody that binds to HER2 receptors present on the surface of HER2-positive tumour cells, blocking them from receiving growth signals and flagging them for destruction by the immune system.

• Used to treat HER2-positive breast cancer either early-stage or advanced-stage/metastatic to stop the cancer from growing by attaching itself to the HER2 receptors on the surface of breast cancer cells and blocking them from receiving growth signals and can slow or stop the growth of the breast cancer [32]

• Common side effects: headache, diarrhoea, nausea, fever, cough, rash, insomnia

Other HER2-targeted therapy available : Perjeta (Pertuzumab) - HER2 monoclonal antibody often used in combination with Herceptin

[32] Devika Gajria et al. (2011), Expert Rev Anticancer Ther. 2011 Feb; 11(2): 263–275.

ADDITIONAL TARGETED THERAPIES AND ASSOCIATED BIOMARKER TESTING FOR RECURRENT UNRESECTABLE (LOCAL OR REGIONAL) OR STAGE IV (M1) DISEASE

Biomarkers Associated with FDA-Approved Therapies

Breast Cancer Subtype	Biomarker	Detection	FDA-Approved Agents	NCCN Category of Evidence	NCCN Category of Preference
Any[a]	*BRCA1* mutation *BRCA2* mutation	Germline sequencing	Olaparib Talazoparib	Category 1 Category 1	Preferred
HR-positive/ HER2-negative[b]	*PIK3CA* activating mutation	PCR (blood or tissue block if blood negative), molecular panel testing	Alpelisib + fulvestrant[d]	Category 1	Preferred second-line therapy
HR-negative/ HER2-negative[c]	PD-L1 expression • Threshold for positivity: ≥1% on tumor-infiltrating immune cells PD-L1 expression • Threshold for positivity combined positive score ≥10	IHC	Atezolizumab + albumin-bound paclitaxel[e] Pembrolizumab + chemotherapy (albumin-bound paclitaxel, paclitaxel, or gemcitabine and carboplatin)[e]	Category 1 Category 1	Preferred first-line therapy[h]
Any	*NTRK* fusion	FISH, NGS, PCR (tissue block)	Larotrectinib[f] Entrectinib[f]	Category 2A Category 2A	Useful in certain circumstances
Any	MSI-H/dMMR TMB-H (≥10 muts/mb)	IHC, PCR (tissue block) NGS	Pembrolizumab[e,g]	Category 2A	Useful in certain circumstances

[a] Assess for germline *BRCA1/2* mutations in all patients with recurrent or metastatic breast cancer to identify candidates for PARP inhibitor therapy. While olaparib and talazoparib are FDA indicated in HER2-negative disease, the panel supports use in any breast cancer subtype associated with a germline *BRCA1* or *BRCA2* mutation.

[b] For HR-positive/HER2-negative breast cancer, assess for *PIK3CA* mutations with tumor or liquid biopsy to identify candidates for alpelisib plus fulvestrant. *PIK3CA* mutation testing can be done on tumor tissue or ctDNA in peripheral blood (liquid biopsy). If liquid biopsy is negative, tumor tissue testing is recommended.

[c] For TNBC, assess PD-L1 expression biomarker status on tumor-infiltrating immune cells to identify candidates for atezolizumab plus albumin-bound paclitaxel.

[d] The safety of alpelisib in patients with Type 1 or uncontrolled Type 2 diabetes has not been established.

[e] See NCCN Guidelines for Management of Immuotherapy-Related Toxicities.

[f] Larotrectinib and entrectinib are indicated for the treatment of solid tumors that have an *NTRK* gene fusion without a known acquired resistance mutation and have no satisfactory alternative treatments or that have progressed following treatment.

[g] Pembrolizumab is indicated for the treatment of patients with unresectable or metastatic, microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR) solid tumors, or TMB-H tumors that have progressed following prior treatment and who have no satisfactory alternative treatment options.

[h] While available data are in the first-line setting, these regimens can be used for second and subsequent lines of therapy if PD-1/PD-L1 inhibitor therapy has not been previously used. If there is disease progression while on a PD-1/PD-L1 inhibitor, there are no data to support an additional line of therapy with another PD-1/PD-L1 inhibitor.

[33] NCCN Clinical Practise Guidelines in Oncology. Breast cancer (2021), version 7, August 23, 2021.

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