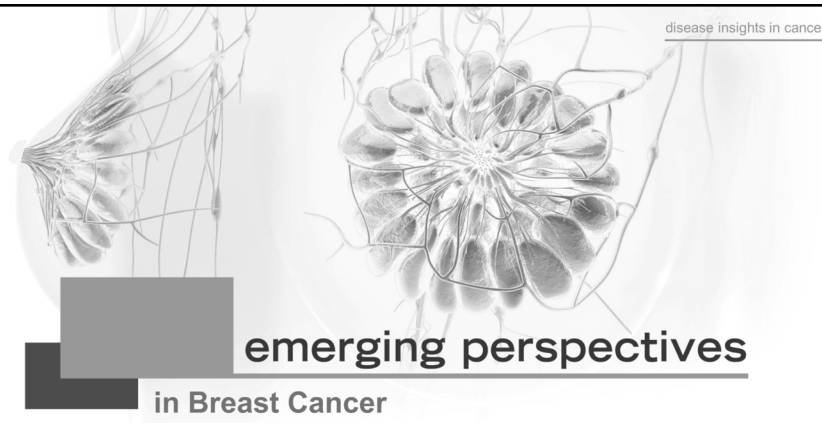

emerging perspectives

in Breast Cancer

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Disclosures

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Acknowledgments

This slide presentation was developed in collaboration with:

- **Rita Nanda, MD**, Associate Professor of Medicine, Co-Director of Breast Medical Oncology, University of Chicago, Chicago, IL
- **Ruta Rao, MD**, Associate Professor, Rush University Medical Center, Chicago, IL

Learning objectives

- **Review** established and emerging disease drivers/pathways in breast cancer (BC)
- **Describe** key biomarkers and their clinical relevance in the management of BC
- **Discuss** current challenges and future directions in the management of BC

Module 1

BREAST CANCER BACKGROUND

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emerging perspectives

BACKGROUND

Risk factors may increase the likelihood of developing BC

Ethnicity

- Historically, incidence among women ≥40 years old has been highest in white women
- Incidence rates are converging among white and African American women, particularly among women aged 50 to 59 years

Genetics/individual characteristics

- Family history of BC at a young age
- Genetic mutations (eg, *BRCA1/2* mutations)
- Increased mammographic breast density
- Benign proliferative breast disease

Age

- Late menopause
- Early menarche
- Older age at first live birth

Previous/current medical treatment

- Prolonged hormone replacement therapy
- Previous exposure to therapeutic chest wall irradiation

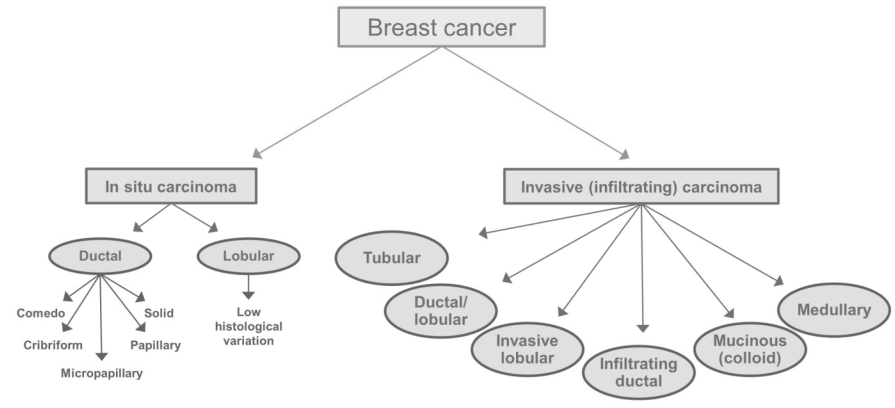
Lifestyle

- Alcoholism
- Lack of physical activity
- Smoking

BC=breast cancer; BRCA1/2=breast cancer susceptibility gene 1/2.
National Comprehensive Cancer Network. NCCN clinical practice guidelines in oncology; breast cancer. Version 1.2018. https://www.nccn.org/professionals/physician_gls/pdf/breast.pdf. Published March 20, 2018. Accessed April 11, 2018. National Comprehensive Cancer Network. NCCN clinical practice guidelines in oncology; breast cancer risk reduction. Version 1.2018. https://www.nccn.org/professionals/physician_gls/pdf/breast.pdf. Published February 2, 2018. Accessed April 13, 2018.

BACKGROUND

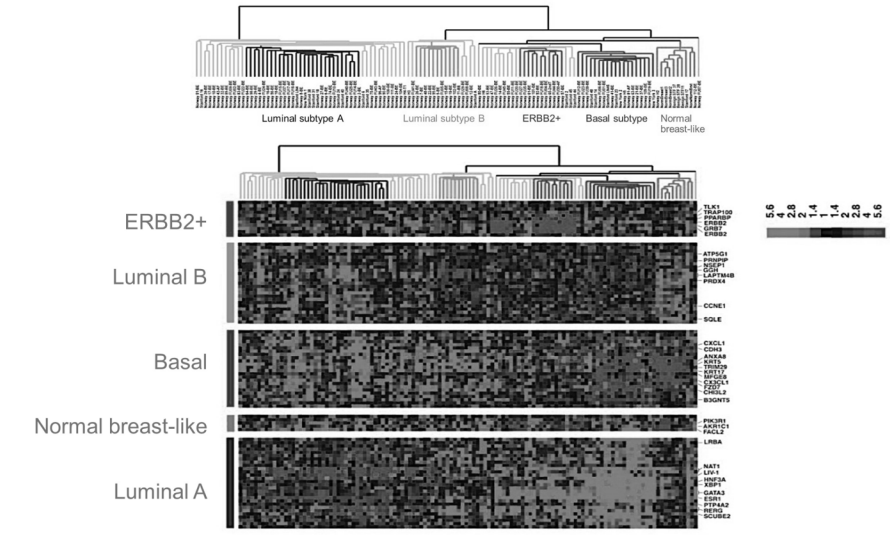
Traditional classification of BC based on histology



BC=breast cancer.
Malhotra GK, et al. *Cancer Biol Ther*. 2010;10:955-960.
Histological, molecular and functional subtypes of breast cancers, Malhotra GK, Zhao X, Band H, Band V, *Cancer Biology and Therapy*, 2010, Taylor & Francis Group, reprinted by permission of the publisher (Taylor & Francis Ltd, http://www.tandfonline.com).

BACKGROUND

Molecular classification of BC based on gene expression



BC=breast cancer.
Sørlie T, et al. *Proc Natl Acad Sci U S A*. 2003;100:8418-8423.
Republished with permission from Sørlie T, et al. *Proc Natl Acad Sci U S A*; Volume 100 Issue 14 p 8418-8423 copyright 2003 National Academy of Sciences, U.S.A.

BACKGROUND

The TNM classification system in breast cancer

The TNM staging system assigns each cancer a T, N, and M category

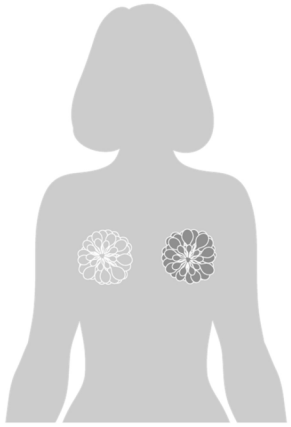
T (Tumor)	TX	Primary tumor cannot be measured
	T0	No evidence of primary tumor
	Tis	Carcinoma in situ (early cancer that has not spread to neighboring tissue)
	T1-4	Size and/or extent of the primary tumor
N (Node)	NX	Regional lymph nodes cannot be evaluated
	N0	No regional lymph node involvement (no cancer found in the lymph nodes)
	N1-3	Increasing involvement of regional lymph nodes (number and/or extent of spread)
M (Metastasis)	M0	No distant metastasis (cancer has not spread to other parts of the body)
	M1	Distant metastasis (cancer has spread to distant parts of the body)

In breast cancer, non-anatomic biological factors such as tumor grade, HR status, and HER2 status are also important for prognostic staging and modifying the assigned TNM stage group.

HR=hormone receptor; HER2=human epidermal growth factor receptor 2; TNM=tumor-node-metastasis.
American Joint Committee on Cancer. Cancer staging system. https://cancerstaging.org/references-tools/Pages/What-is-Cancer-Staging.aspx. Accessed April 11, 2018.
Giuliano AE, et al. *CA Cancer J Clin*. 2017;67:290-303.

Using biomarkers in the staging of breast cancer

- The expert panel in the 8th edition of the *AJCC Cancer Staging Manual* determined that all invasive carcinomas should have ER, PR, and HER2 status determined by appropriate assays whenever possible
- The TNM anatomic staging, without ER, PR, and HER2 status, can be assigned in settings and regions of the world where the biomarker status cannot be routinely obtained
- Multigene panels may provide prognostic and therapy-predictive information that has the ability to complement TNM and biomarker information. While the use of these multigene assays is not required for staging, tumor biomarkers and low multigene panel status can alter prognosis and stage



AJCC=American Joint Committee on Cancer; ER=estrogen receptor; HER2=human epidermal growth factor receptor 2; PR=progesterone receptor; TNM=tumor-node-metastasis.

Giuliano AE, et al. *CA Cancer J Clin.* 2017;67:290-303.

Module 2

SUBTYPES OF BREAST CANCER

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ESTROGEN RECEPTOR/ PROGESTERONE RECEPTOR+ BREAST CANCER

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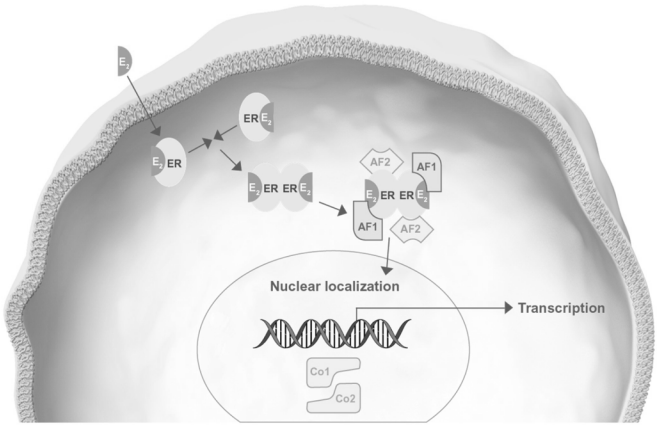
ER/PR+

Estrogen induces cell proliferation by activating transcription via the estrogen receptor

- ER and PR expression are observed in 75% and 55% of invasive BCs, respectively*
- ER and PR receptors directly bind to DNA-specific sequences or indirectly bind to other transcription factors†
- ER and PR expression help predict patients who may benefit from endocrine therapy



*Status of ER and PR in infiltrating mammary carcinoma (N=5497): 55% ER+/PR+, 20% ER+/PR–, 25% ER–/PR–, and 0% ER–/PR+.
†Involvement of ER/PR pathway in the development of hormone receptor–positive breast cancer is well-established.
AF1=activation function 1; AF2=activation function 2; BC=breast cancer; Co1=co-activator 1; Co2=co-activator 2; E2=17b-estradiol; ER=estrogen receptor; PR=progesterone receptor.

Michalides R, et al. *Cancer Cell*. 2004;5:597-605. Howell A, et al. *Cancer*. 2000;89:817-825. Matsumoto A, et al. *Jpn J Clin Oncol*. 2016;46:99-105. Nadji M, et al. *Am J Clin Pathol*. 2005;123:21-27. Osborne CK, Schiff R. *Annu Rev Med*. 2011;62:233-247.

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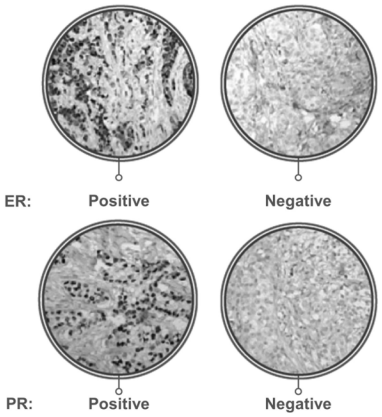
ER/PR+

Predictive biomarkers in BC: ER/PR

- ER and PR expression help predict patients who may benefit from endocrine therapy
- NCCN recommendation:
  - ER status should be determined for all samples of ductal carcinoma in situ (DCIS)
  - ER and PR should be determined for all samples of invasive breast cancer
- Detection method per NCCN: IHC
- ER/PR positivity per ASCO/CAP/NCCN:
  - ER/PR-positivity if ≥1% of cells stain positive for ER/PR by IHC
- ER/PR status can change from a primary tumor
  - Occurs in 15% to 30% of patients with recurrent disease

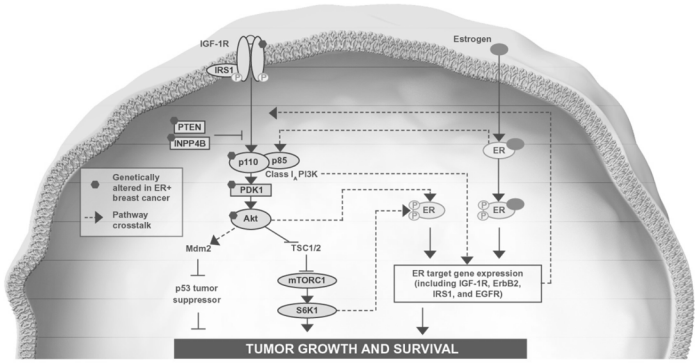


ASCO=American Society of Clinical Oncology; BC=breast cancer; CAP=College of American Pathologists; ER=estrogen receptor; IHC=immunohistochemistry; NCCN=National Comprehensive Cancer Network; PR=progesterone receptor.

National Comprehensive Cancer Network. NCCN clinical practice guidelines in oncology: breast cancer. Version 1.2018. https://www.nccn.org/professionals/physician_gls/pdf/breast.pdf. Published March 20, 2018. Accessed March 22, 2018. Matsumoto A, et al. *Jpn J Clin Oncol.* 2016;46:99-105. Li X, et al. *Oncol Lett.* 2015;9:1207-1212. Osborne KC, Schiff R. *Annu Rev Med.* 2011;62:233-247.

ER/PR+

Crosstalk between the established PI3K/Akt/mTOR and ER/PR pathways in BC



- PI3K/Akt/mTOR is a growth, survival, and proliferation pathway
- Hyperactivation of this pathway is implicated in tumorigenesis and in resistance to endocrine therapies targeted against ER+ breast cancer*
  - Preclinical evidence shows that PI3K/Akt/mTOR pathway inhibition can augment the benefit of targeting the ER pathway in hormone receptor–positive breast cancer

*Crosstalk between the PI3K/Akt/mTOR and ER/PR pathways as a mechanism of resistance is well-established in hormone receptor–positive breast cancer.

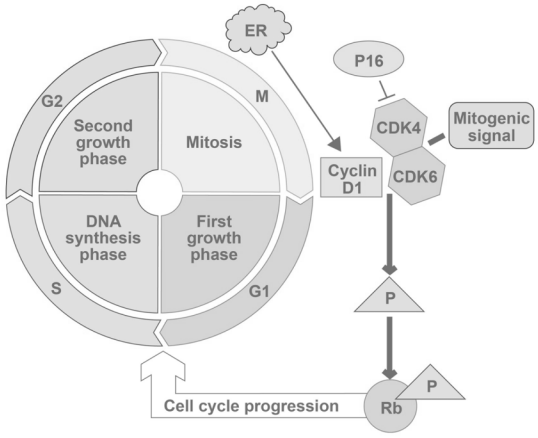
EGFR=epidermal growth factor receptor; ER=estrogen receptor; ErbB2=human epidermal growth factor receptor 2; IGF-1R=insulin-like growth factor 1 receptor; INPP4B=inositol polyphosphate 4-phosphatase type II; IRS-1=insulin receptor substrate 1; Mdm2=mouse double minute 2 homolog; mTORC1=mammalian target of rapamycin complex 1; p53=tumor suppressor 53; p85=Class IA PI3K catalytic subunit; p110=Class IA PI3K catalytic subunit; PDK1=phosphoinositide dependent kinase-1; PI3K=phosphatidylinositol 3-kinase; PR=progesterone receptor; PTEN=phosphatase and tensin homolog; S6K1=ribosomal protein S6 kinase beta-1.

Ciruelos Gil EM. *Cancer Treat Rev.* 2014;40:862-871.

ER/PR+

Crosstalk between ER/PR and CDK4/6 pathway in BC

- Imbalance of the cyclin D and CDK pathway in cancer cells may result in a more proliferative phenotype
- ER+ breast cancer may have features* suggesting particular dependence on the CDK4/cyclin-D1/Rb interaction
  - Aberrations leading to hyperactivation of cyclin D1-CDK4/6 are particularly common in ER+ BC†



*Examples of features would include alterations to cyclin D1, CDK4, and CDK6.

†Crosstalk between the the CDK4/6 and ER/PR pathways as a mechanism of resistance is well-established in hormone receptor–positive breast cancer.

BC=breast cancer; CDK4/6=cyclin-dependent kinase 4/6; ER=estrogen receptor; G1=gap1; G2=gap2; M=mitosis; P=phosphate; P16=cyclin-dependent kinase inhibitor 2A; PR=progesterone receptor; Rb=retinoblastoma protein; S=synthesis.

Murphy CG, Dickler MN. *Oncologist*. 2015;20:483-490. Mayer EL. *Curr Oncol Rep*. 2015;17:443.

HER2+ BREAST CANCER

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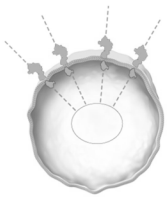
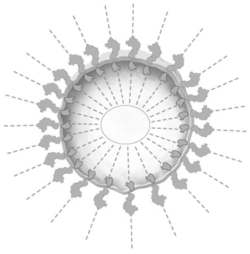
HER2+

HER2 is commonly overexpressed in a subset of BC

- The *HER2* oncogene is crucial in regulating cell growth and development, as it drives cell proliferation, migration, and invasion
- The *HER2* oncogene is amplified in 20% to 25% of invasive breast cancers



Normal HER2 expression:
20,000 receptors per cell



HER2 overexpression:
Up to 2,000,000 receptors per cell

BC=breast cancer; HER=human epidermal growth factor receptor.

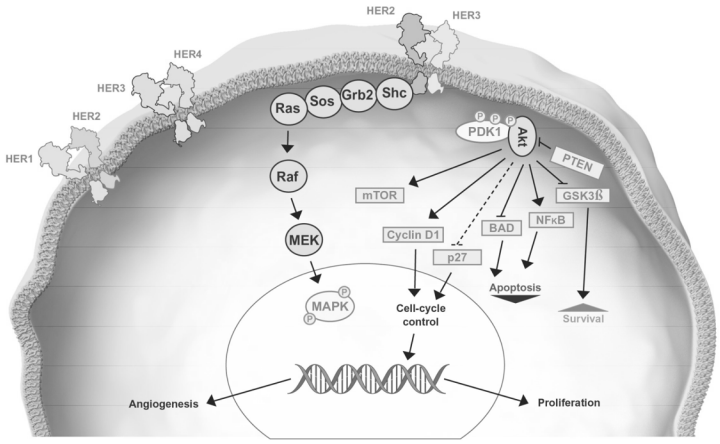
Moasser MM. *Oncogene*. 2007;26:6469-6487. Nahta R et al. *Nat Clin Pract Oncol*. 2006;3:269-280.

19

HER2+

HER2 pathway promotes growth and proliferation in BC*

- HER2:HER3 dimers have the strongest mitogenic signaling



*The HER2 signaling pathway in the development of HER2+ breast cancer is well-established.

BAD=Bcl-2–associated death promoter; BC=breast cancer; Grb2=growth factor receptor-bound 2; GSK=glycogen synthase kinase; HER=human epidermal growth factor receptor; MAPK=mitogen-activated protein kinase; MEK=mitogen-activated protein kinase kinase; mTOR=mammalian target of rapamycin; NFκB=nuclear factor kappa-light-chain-enhancer of activated B-cells; P=phosphorylation; p27=cyclin-dependent kinase inhibitor 1B; PDK=phosphoinositide-dependent kinase; PI3K=phosphatidylinositol 3-kinase; PTEN=phosphatase and tensin homolog; Raf=rapidly accelerating fibrosarcoma; Ras=rat sarcoma; Shc=SHC-transforming protein 1; Sos=son of sevenless.

Rowinsky EK. *Oncologist*. 2003;8(suppl 3):5-17.

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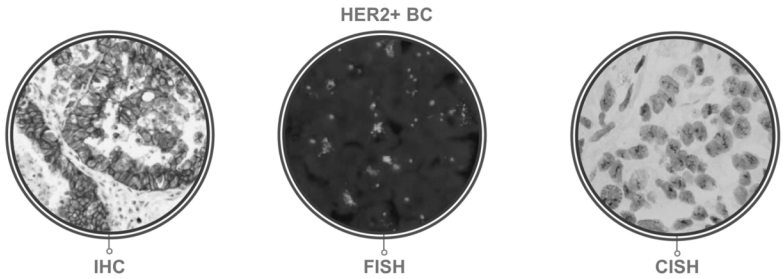
HER2+

Predictive biomarker in BC: HER2 amplification

- HER2 is amplified in 20% to 25% of invasive BCs
- HER2 status determination as recommended by **NCCN**
  - By IHC (quantity of HER2 cell surface receptors) or by a complementary method using ISH (number of *HER2* gene copies)
  - Consistent with the 2018 **ASCO/CAP** guidelines
    - Either IHC or ISH
    - The use of dual-probe instead of single-probe ISH assays is now recommended



ASCO=American Society of Clinical Oncology; BC=breast cancer; CAP=College of American Pathologists; CISH=chromogenic in situ hybridization; FISH=fluorescence in situ hybridization; HER2=human epidermal growth factor receptor 2; IHC=immunohistochemistry; ISH=in situ hybridization; mRNA=messenger RNA; NCCN=National Comprehensive Cancer Network.

Kohler BA, et al. *J Natl Cancer Inst.* 2015;107:djv048. National Comprehensive Cancer Network. NCCN clinical practice guidelines in oncology: breast cancer. Version 1.2018. https://www.nccn.org/professionals/physician_gls/pdf/breast.pdf. Published March 20, 2018. Accessed March 22, 2018. Tanner M, et al. *Am J Pathol.* 2000;157:1467-1472. Wolff AC, et al. *Arch Pathol Lab Med.* Published Online: May 20, 2018 (doi:10.5858/arpa.2018-0902-SA).

Adapted by permission from Springer Nature: *Modern Pathology*, Heterogeneity of ERBB2 amplification in adenocarcinoma, squamous cell carcinoma and large cell undifferentiated carcinoma of the lung, Grob TJ, Kannengiesser I, Tsourlakis MC, et al. © 2012.

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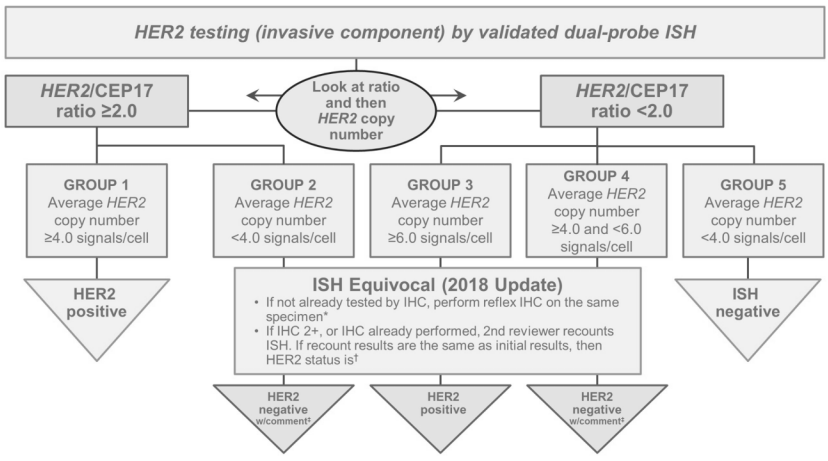
HER2+

ASCO/CAP guidelines for HER2 testing in breast cancer: ISH



* Refer to complete ASCO/CAP guidelines for full workup information. If the reflex IHC test results in a different category, then the HER2 status is assigned to that new category.
† If the ISH recount results in a different category, then the result should be adjudicated per internal procedures to define the final category.
‡ Refer to the updated ASCO/CAP guidelines for the specific comments associated with each recommendation.
ASCO=American Society of Clinical Oncology; CAP=College of American Pathologists; CEP17=chromosome enumeration probe 17; HER2=human epidermal growth factor receptor 2; IHC=immunohistochemistry; ISH=in situ hybridization.
Wolff AC, et al. Arch *Pathol Lab Med.* Published Online: May 20, 2018 (doi:10.5858/arpa.2018-0902-SA).

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ASCO/CAP guidelines on when to retest HER2 status due to histopathologic discordance

Initial HER2 test result	HER2+	HER2- (from core needle biopsy)	HER2-
	AND	AND	AND
Histopathologic findings	**Histologic grade 1 carcinoma of** • Infiltrating ductal or lobular carcinoma, ER and PR positive • Tubular (at least 90% pure) • Mucinous (at least 90% pure) • Cribriform (at least 90% pure) • Adenoid cystic carcinoma (90% pure) and often triple negative	**One of the following** • Tumor is grade 3 • Amount of invasive tumor in the core biopsy is small • Resection specimen contains high-grade carcinoma that is morphologically distinct from that in the core • Core biopsy result is equivocal for HER2 by both ISH and IHC • Questionable specimen handling of the core biopsy or the test is suspected to be negative on the basis of testing error	**Histologic grade 1 carcinoma of** • Infiltrating ductal or lobular carcinoma, ER and PR positive • Tubular (at least 90% pure) • Mucinous (at least 90% pure) • Cribriform (at least 90% pure) • Adenoid cystic carcinoma (90% pure) and often triple negative
	Should retest HER2 status	**May order a new test on the excision specimen**	**Should not order a new HER2 test**

Relationship between tumor heterogeneity and HER2 status determination.
ASCO=American Society of Clinical Oncology; CAP=College of American Pathologists; ER=estrogen receptor; IHC=immunohistochemistry; ISH=in situ hybridization; PR=progesterone receptor.
Wolff AC et al. *J Clin Oncol.* Published Online: May 30, 2018 (doi:JCO2018778738).

TRIPLE-NEGATIVE BREAST CANCER (TNBC)

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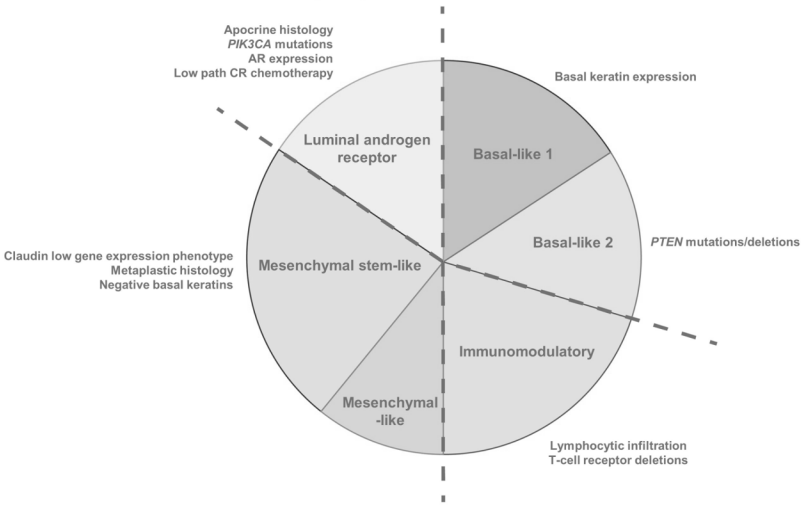
TNBC

TNBC: A diverse category of breast cancer



- Gene expression subtypes are associated with distinct molecular features

AR=androgen receptor; CR=complete response; PIK3CA=phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; PTEN=phosphatase and tensin homolog; TNBC=triple-negative breast cancer.
Turner NC, Reis-Filho JS. *Clin Cancer Res*. 2013;19:6380-6388.
Adapted from *Clinical Cancer Research*, 2013, 19/23, 6380-6388, Turner NC, Reis-Filho JS, Tackling the diversity of triple-negative breast cancer, with permission from AACR.

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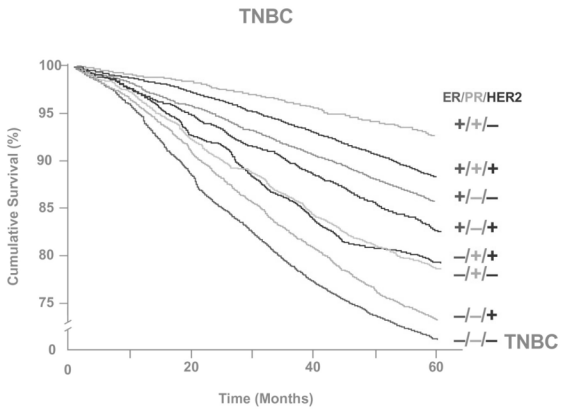
TNBC

TNBC is associated with a poor prognosis

- TNBC is characterized by shorter overall survival
- It has an aggressive clinical behavior, with a higher risk of both local and distant relapses



This graph illustrates the relative patient 5-year survival by tumor type according to time after treatment

ER=estrogen receptor; HER2=human epidermal growth factor receptor 2; PR=progesterone receptor; TNBC=triple-negative breast cancer.
Collignon J, et al. *Breast Cancer*. 2016;8:93-107. Carels N, et al. *Theor Biol Med Model*. 2016;13:7.

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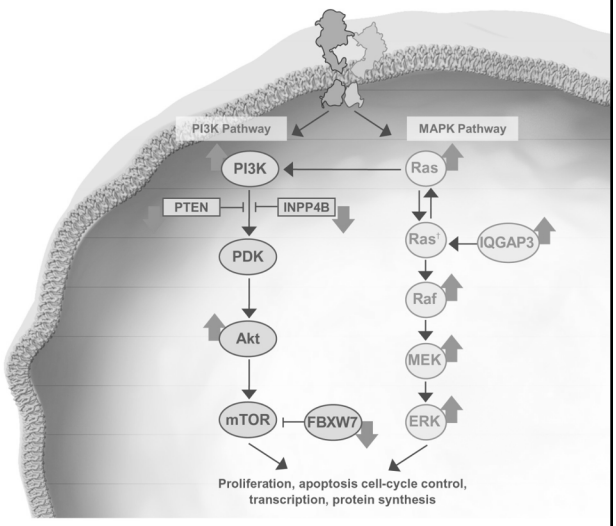
Exploring PI3K and MAPK signaling pathways in TNBC*

PI3K

- Genetic events in the PI3K pathway occur in 15% to 20% of TNBCs
  - Amplifications in PI3K
  - Deletion or underexpression of PTEN and INPP4B

MAPK

- Activation of the MAPK signaling pathway is more prevalent in TNBC than in other types of BC



*These emerging pathways are currently being investigated in the management of TNBC.

†Active GTP-bound Ras.

FBXW7=F-box and WD repeat domain containing 7; IQGAP3=IQ motif containing GTPase activating protein 3; INPP4B=inositol polyphosphate-4-phosphatase, type II; MAPK=mitogen-activated protein kinase; MEK=mitogen-activated protein kinase kinase; mTOR=mammalian target of rapamycin; PDK=phosphoinositide-dependent kinase; PI3K=phosphatidylinositol-3-kinase; PTEN=phosphatase and tensin homolog; Raf=rapidly accelerating fibrosarcoma; Ras=rat sarcoma; TNBC=triple-negative breast cancer.

Turner NC, Reis-Filho JS. *Clin Cancer Res*. 2013;19:6380-6388. Craig DW, et al. *Mol Cancer Ther*. 2013;12:104-116. Giltnane JM, Balko JM. *Discov Med*. 2014;17:275-283. Adapted from *Molecular Cancer Therapeutics*, 2013, 12/1, 104-116, Craig DW, O'Shaughnessy JA, Kiefer JA, et al, Genome and transcriptome sequencing in prospective metastatic triple-negative breast cancer uncovers therapeutic vulnerabilities, with permission from AACR.

Module 3

HALLMARKS OF CANCER

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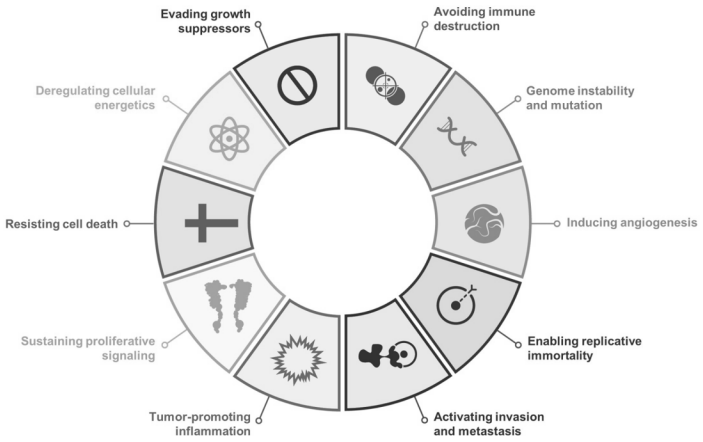
HALLMARKS

Hallmarks of cancer



Hanahan D, Weinberg RA. *Cell*. 2011;144:646-674.
Reprinted with permission from Elsevier.

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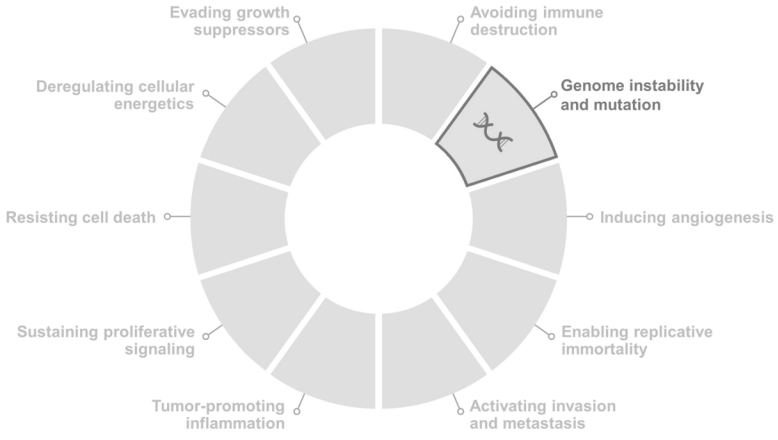
HALLMARKS

Hallmark of cancer: Genome instability and mutation



Hanahan D, Weinberg RA. *Cell*. 2011;144:646-674.
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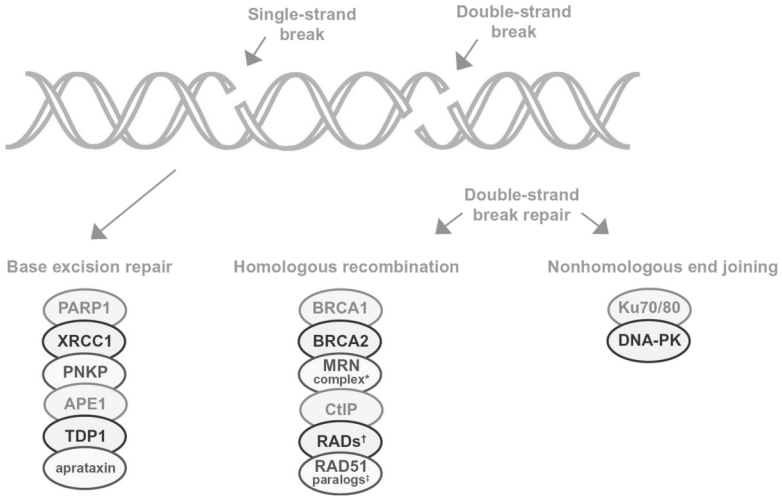
HALLMARKS

BRCA1 and *BRCA2* are key players in DNA repair



*MRN complex: MRE-RAD50-NBS1.
†RADs: RAD51, RAD52, RAD54, RAD54B.
‡RAD51 paralogs: RAD51B, RAD51C, RAD51D, XRCC2, XRCC3.

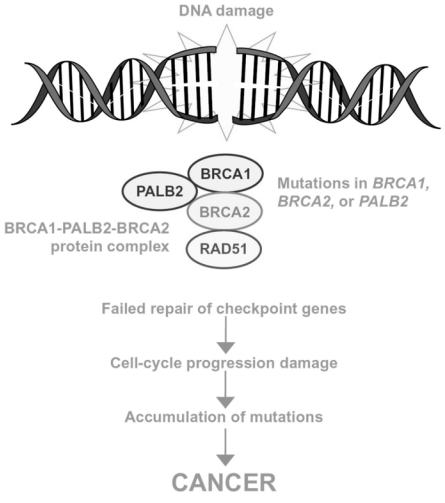
APE=apurinic/apyrimidinic endonuclease; BRCA1/2=breast cancer susceptibility protein 1/2; CtIP=CtBP-interacting protein; Ku70=Lupus Ku autoantigen protein p70; Ku80=Lupus Ku autoantigen protein p80; PARP=poly ADP–ribose polymerase 1; PK=protein kinase; PNKP=polynucleotide kinase 3′-phosphatase; TDP=tyrosyl-DNA phosphodiesterase; XRCC=x-ray repair cross-complementing protein.

Abbotts R, et al. *Cancer Manag Res*. 2014;6:77-92. Venkitaraman AR. *Science*. 2014;343:1470-1475.

HALLMARKS

Biomarkers that may increase the risk of developing BC: *BRCA1*, *BRCA2*, and *PALB2* mutations

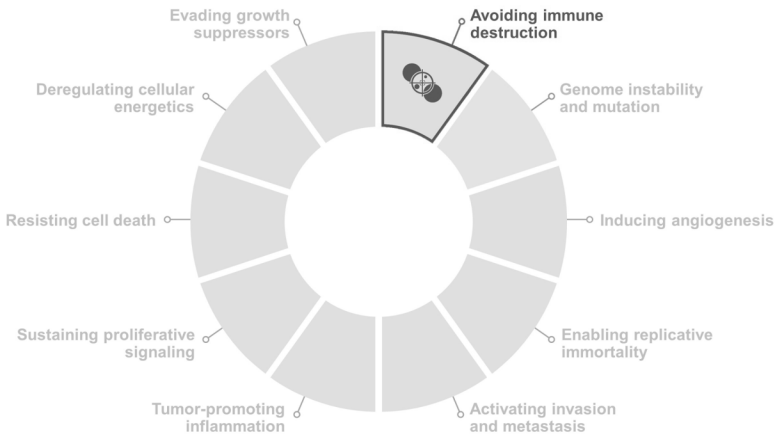
- The combination of *BRCA1* and *BRCA2* gene mutations was responsible for approximately 80% of the families with hereditary breast cancer
- Breast cancer has been reported in
  - 40% to 87% of *BRCA1* mutation carriers
  - 18% to 88% of *BRCA2* mutation carriers
- PALB2 functionally connects BRCA1 and BRCA2
- *PALB2* mutation carriers may have up to a 9-fold higher risk of breast cancer compared with the general population
- **Predisposition:** Germline mutations in *BRCA1/BRCA2* have shown to predispose individuals to breast and ovarian cancers



BC=breast cancer; BRCA1/2=breast cancer susceptibility protein 1/2; PALB2=partner and localizer of BRCA2; PARP=poly ADP–ribose polymerase 1.

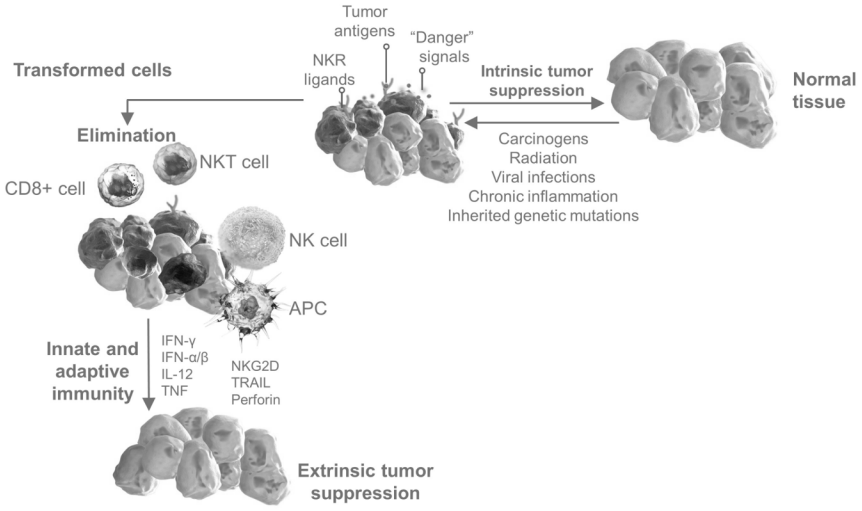
Antoniou AC, et al. *N Engl J Med*. 2014;371:497-506. de Jong MM, et al. *J Med Genet*. 2002;39:225-242. Fackenthal JD, Olopade OI. *Nat Rev Cancer*. 2007;7:937-948. Livraghi L, Garber JE. *BMC Med*. 2015;13:188-204. Mavaddat N, et al. *J Natl Cancer Inst*. 2013;105:812-822. Zhang F, et al. *Mol Cancer Res*. 2009;7:1110-1118.

Hallmarks of cancer: Avoiding immune destruction



Hanahan D, Weinberg RA. *Cell*. 2011;144:646-674.
Reprinted with permission from Elsevier.

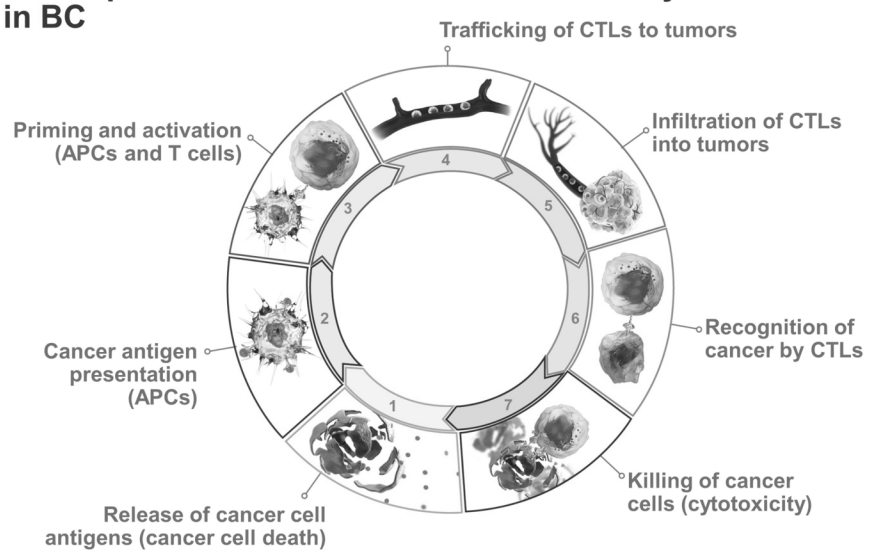
The immune system plays a critical role in protecting the body against cancer



APC=antigen-presenting cell; IFN-α/β=interferon alpha/beta; IFNγ=interferon gamma; IL-12=interleukin 12; NK=natural killer; NKG2D=natural killer group 2D; NKR=natural killer receptor; NKT=natural killer T; TNF=tumor necrosis factor; TRAIL=TNF-related apoptosis-inducing ligand.
Schreiber RD, et al. *Science*. 2011;331:1565-1570.
Modified with permission from the Annual Review of Science Volume 331 Issue 6024 © 2011 by Annual Reviews, http://www.annualreviews.org.

HALLMARKS

The importance of T cell–mediated immunity in BC



APC=antigen-presenting cell; BC=breast cancer; CTL=cytotoxic T lymphocytes.
Chen D, Mellman I. *Immunity*. 2013;39:1-10.
Reprinted with permission from Elsevier.

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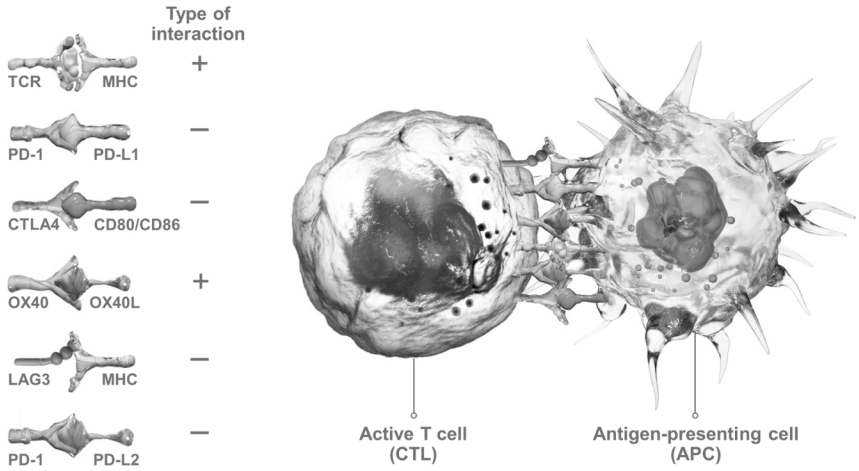
HALLMARKS

Key regulators of cytotoxic T lymphocytes

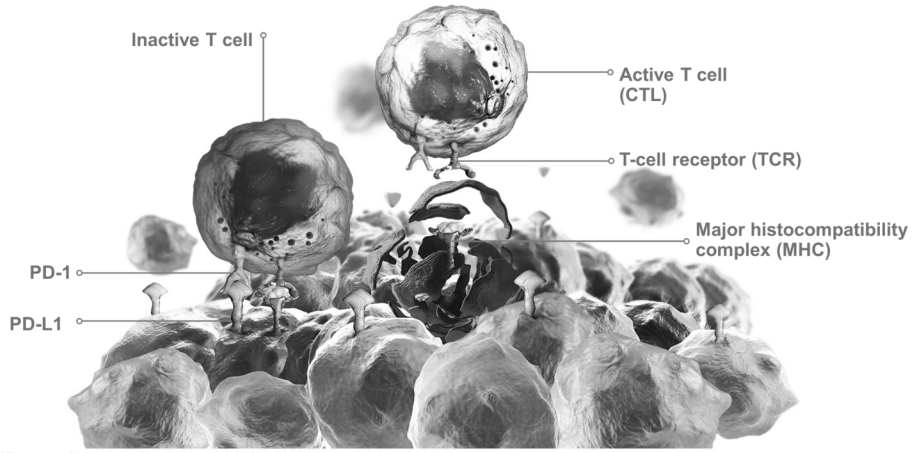


CTL=cytotoxic T lymphocyte; CTLA4=cytotoxic T-lymphocyte-associated protein 4; MHC=major histocompatibility complex; PD-1=programmed death-1; PD-L1=programmed death-ligand 1; PD-L2=programmed death-ligand 2; TCR=T-cell receptor.
Pardoll D, Drake C. *J Exp Med*. 2012;209:201-209. Pardoll DM. *Nat Rev Cancer*. 2012;12:252-264.
Adapted by permission from Springer Nature: *Nature Reviews Cancer*, The blockade of immune checkpoints in cancer immunotherapy, Pardoll DM, © 2012.

36

HALLMARKS

In the tumor microenvironment, the PD-L1/PD-1 pathway is one of several pathways in cancer cells that can modulate the activity of CTLs



CTL=cytotoxic T lymphocyte; PD-1=programmed death-1; PD-L1=programmed death-ligand 1.
Pardoll DM. *Nat Rev Cancer*. 2012;12:252-264.
Adapted by permission from Springer Nature: *Nature Reviews Cancer* The blockade of immune checkpoints in cancer immunotherapy, Pardoll DM, © 2012.

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HALLMARKS

Expression of PD-L1 is elevated in BC

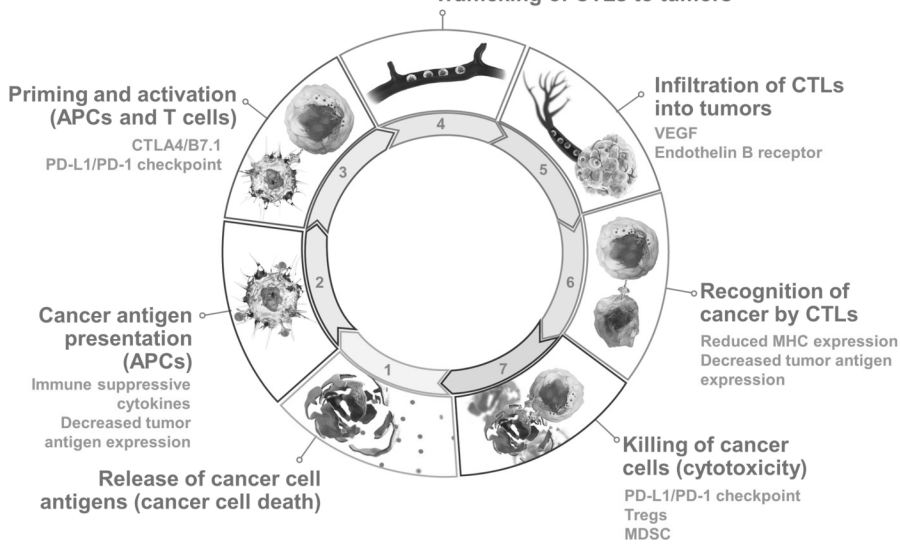
Tumor types (N=437)	PD-1 expression (range)	PD-L1 (% of tumor cells)	Concurrent PD-1 and PD-L1 expression
Carcinomas (n=380 total)			
Breast (n=116)	51% (1-20)	45%	29%
Colon (n=87)	50% (1- >20)	21%	12%
NSCLC (n=44)	75% (1-20)	50%	43%
Pancreas (n=23)	43% (1-16)	23%	9%
Prostate (n=20)	35% (1-6)	25%	5%
Merkel cell carcinoma (n=19)	17% (1-4)	0%	0%
Endometrium (n=16)	86% (1-13)	88%	79%
Ovary (n=14)	93% (1-16)	43%	36%
Liver (n=13)	38% (1-5)	8%	0%
Bladder (n=11)	73% (1-10)	55%	55%
Kidney (n=11)	36% (1-3)	67%	33%
CUP (n=6)	50% (1-4)	33%	33%
Sarcomas (n=33 total)	30% (1- >10)	97%	30%
Melanoma (n=24 total)	58% (1-15)	92%	58%

BC=breast cancer; CUP=cancers of unknown primary; NSCLC=non-small cell lung cancer; PD-1=programmed death-1; PD-L1=programmed death-ligand 1.
Gatalica Z, et al. *Cancer Epidemiol Biomarkers Prev*. 2014;23:2965-2970.
Adapted from *Cancer Epidemiology, Biomarkers and Prevention*, 2014, 23/12, 2965-2970, Gatalica Z, Snyder C, Maney T, et al, Programmed cell death 1 (PD-1) and its ligand (PD-L1) in common cancers and their correlation with molecular cancer type, with permission from AACR.

38

HALLMARKS

General mechanisms of evasion from immune surveillance



APC=antigen-presenting cell; CTL=cytotoxic T lymphocyte; CTLA4=cytotoxic T-lymphocyte-associated antigen 4; MDSC=myeloid-derived suppressor cell; MHC=major histocompatibility complex; PD-1=programmed death-1; PD-L1=programmed death-ligand 1; Treg=regulatory T cell; VEGF=vascular endothelial growth factor.

Chen DS, Mellman I. *Immunity*. 2013;39:1-10. Vinay DS, et al. *Semin Cancer Biol.* 2015;35(suppl):S185-S198.

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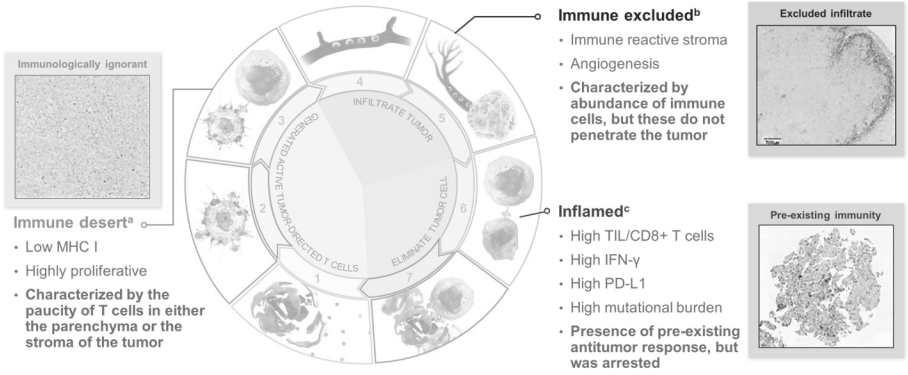
HALLMARKS

Overall, tumors can be classified into immune phenotypes based on their mechanisms of evading T cell–mediated antitumor immunity



Specific immune phenotypes are associated with different mechanisms of immune escape from immune surveillance and may have significant impact on the clinical development of immunotherapeutic agents.

[a]Immune desert: Failure can occur in step 1, 2, or 3 of the CIC.
[b]Immune excluded: Failure can occur in step 4 or 5 of the CIC.
[c]Inflamed: Failure can occur in step 6 or 7 of the CIC.

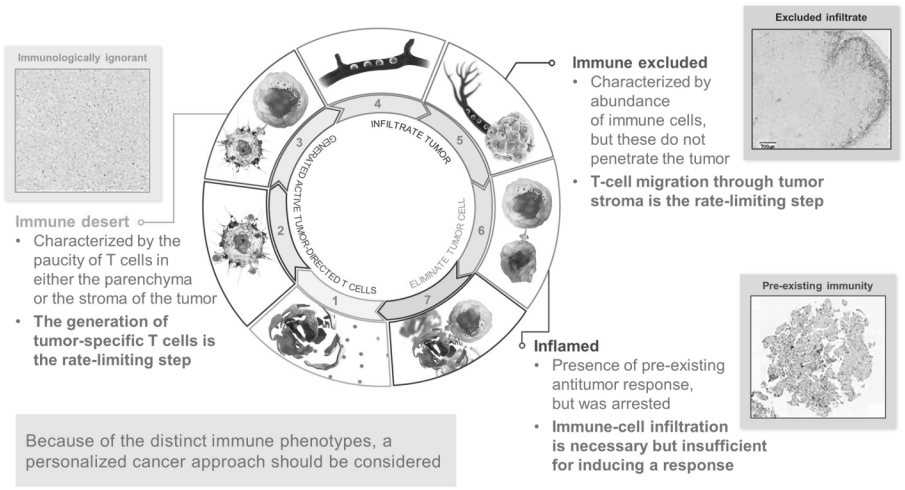
CIC=cancer-immunity cycle; IFN-γ=interferon-gamma; MHC I=major histocompatibility complex class I; PD-L1=programmed death-ligand 1; TIL=tumor-infiltrating lymphocytes.

Hegde PS, et al. *Clin Cancer Res*. 2016;22:1865-1874. Kim JM, Chen DS. *Ann Oncol.* 2016;27:1492-1504. Chen DS, Mellman I. *Nature*. 2017;541:321-330.

Reprinted with permission from Elsevier.

Adapted from *Clinical Cancer Research*, 2016, 22/8, 1865-1874, Hegde PS, Karanikas V, Evers S, The Where, the When, and the How of Immune Monitoring for Cancer Immunotherapies in the Era of Checkpoint Inhibition, with permission from AACR.

The need to consider a personalized approach to mobilizing T cell–mediated antitumor immunity



Because of the distinct immune phenotypes, a personalized cancer approach should be considered

Hegde PS, et al. *Clin Cancer Res*. 2016;22:1865-1874. Chen DS, Mellman I. *Nature*. 2017;541:321-330. Kim JM, Chen DS. *Ann Oncol*. 2016;27:1492-1504. Roche ASCO 2017 presentation. http://www.roche.com/dam/jcr:b76e8bae-f253-455b-81fe-d124660729e6/en/asco-ir2017.pdf. Accessed April 5, 2018.
Reprinted with permission from Elsevier.
Adapted from *Clinical Cancer Research*, 2016, 22/8, 1865-1874, Hegde PS, Karanikas V, Evers S, The Where, the When, and the How of Immune Monitoring for Cancer Immunotherapies in the Era of Checkpoint Inhibition, with permission from AACR.

Module 4

BIOMARKERS IN BREAST CANCER

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Biomarkers: Definition and types

What is a biomarker?

A characteristic that is objectively measured and evaluated as an indicator of:

- Normal biological processes
- Pathogenic processes
- Pharmacologic responses to a specific therapeutic intervention

Prognostic

Provides information about the patient's overall cancer outcome, regardless of therapy

Predictive

Provides information on the effect of a therapeutic intervention in a patient (ie, predicts response to a particular therapy)

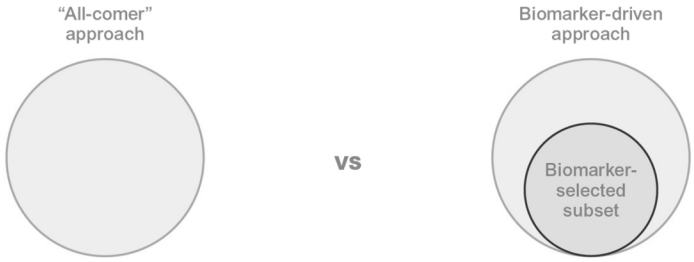
Pharmacogenomic

Provides data on interindividual differences in the PK, PD, and toxicity of therapy

PD=pharmacodynamics; PK=pharmacokinetics.

Khleif SN, et al. *Clin Cancer Res.* 2010;16:3299-3318. Oldenhuis CN, et al. *Eur J Cancer.* 2008;44:946-953. Ventola CL. *P T.* 2013;38:545-560.

The importance of biomarkers for improving patient care



Using a biomarker-driven approach may improve outcomes in individual patients

- Optimizing patient care by treating only the subset most likely to benefit (a target population) vs "all comers"
- For some types of cancer for which biomarkers have been identified, the extent of optimization is dependent on the level of knowledge surrounding the disease

Select NCCN and ASCO/CAP guidelines regarding biomarker testing in BC

NCCN	ASCO/CAP
ER/PR • *ALL* DCIS (ER only) • *ALL* invasive BC	**ER/PR** • *ALL* primary invasive BC • *ALL* recurrences of BC • *ALL* stage IV metastatic sites
HER2 • *ALL* newly diagnosed invasive BC • *FIRST* recurrences of BC, whenever possible	**HER2** • *ALL* primary invasive BC • *ALL* recurrences of BC • *ALL* stage IV metastatic sites
BRCA1/2 • Familial history of *BRCA1/2* mutations	***BRCA1/2* (ASCO)** • Familial history of *BRCA1/2* mutations • TNBC, especially at age <60 years

ASCO=American Society of Clinical Oncology; BC=breast cancer; BRCA1/2=breast cancer susceptibility gene 1/2; CAP=College of American Pathologists; DCIS=ductal carcinoma in situ; ER=estrogen receptor; HER2=human epidermal growth factor receptor 2; NCCN=National Comprehensive Cancer Network; PR=progesterone receptor; TNBC=triple-negative breast cancer.

National Comprehensive Cancer Network. NCCN clinical practice guidelines in oncology: breast cancer. Version 1.2018. https://www.nccn.org/professionals/physician_gls/pdf/breast.pdf. Published March 20, 2018. Accessed March 22, 2018. National Comprehensive Cancer Network. NCCN clinical practice guidelines in oncology: genetic/familial high-risk assessment: breast and ovarian. Version 1.2018. https://www.nccn.org/professionals/physician_gls/pdf/genetics_screening.pdf. Published October 3, 2017. Accessed March 22, 2018. Lu KH, et al. *J Clin Oncol*. 2014;32:833-841. Wolff AC, et al. *Arch Pathol Lab Med*. Published Online: May 20, 2018 (doi:10.5858/arpa.2018-0902-SA). Hammond MEH, et al. *J Oncol Pract*. 2010;6:195-197.

PROGNOSTIC MARKERS

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Prognostic biomarkers in BC: Gene expression signatures

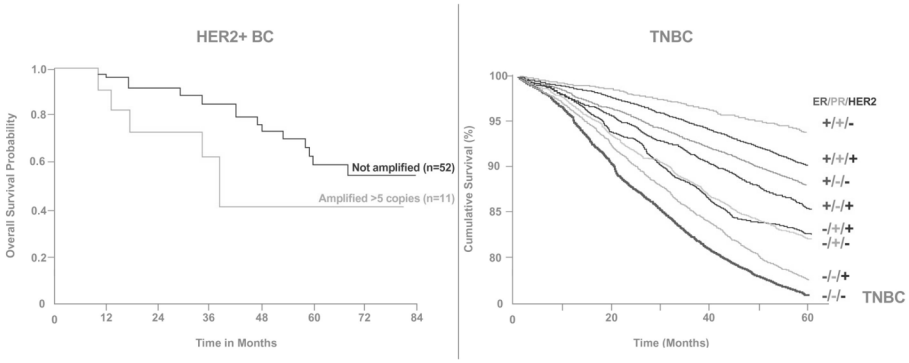
- A number of gene expression profiles as determined by microarray are commonly used as prognostic biomarkers in the adjuvant setting. These include the 21, 50, and 70 gene assays
- While all assays have been clinically validated, the NCCN panel believes that the 21-gene assay has been best validated for its use as a prognostic biomarker, based on the currently available data
- According to the 2018 NCCN guidelines, assignment of HER2 status based on mRNA assays or multigene arrays is not recommended
- The 8th edition of the AJCC Cancer Staging Manual breast cancer staging guidelines indicates multigene panels assays may alter prognosis

ASCO=American Society of Clinical Oncology; BC=breast cancer; CAP=College of American Pathologists; HER2=human epidermal growth factor receptor 2; mRNA=messenger RNA; NCCN=National Comprehensive Cancer Network; ROR-PT=risk of relapse score.

Jatoi I, et al. *J Clin Oncol.* 2011;29:2301-2304. Liu M, et al. *NPJ Breast Cancer.* 2016;2:15023. National Comprehensive Cancer Network. NCCN clinical practice guidelines in oncology: breast cancer. Version 1.2018. https://www.nccn.org/professionals/physician_gls/pdf/breast.pdf. Published March 20, 2018. Accessed July 13, 2018. Giuliano AE, et al. *CA Cancer J Clin.* 2017;67:290-303.

Prognostic biomarkers in BC: HER2+ and TNBC

- HER2+ and TNBC are associated with poor prognosis



This study was performed before the existence of targeted HER2+ therapy.

BC=breast cancer; ER=estrogen receptor; HER2=human epidermal growth factor receptor 2; PR=progesterone receptor; TNBC=triple-negative breast cancer.

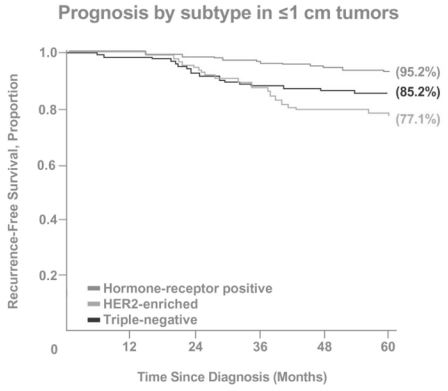
Slamon DJ, et al. *Science.* 1987;235:177-182. Perez EA, et al. *Cancer Treat Rev.* 2014;40:276-284. Carels N, et al. *Theor Biol Med Model.* 2016;13:7.

PROGNOSTIC MARKERS

Patient prognosis by breast cancer subtype in small tumors

Risk of recurrence in small ≤1-cm breast tumors by molecular subtype

- A retrospective analysis reviewed data for 965 patients with node-negative invasive breast cancer (tumors ≤1 cm) diagnosed at MDACC between 1990 and 2002
  - 77% were hormone-receptor positive
  - 13% were triple-negative
  - 10% were HER2-positive
- The risk of relapse-free survival and distant relapse-free survival associated with molecular subtype was evaluated at a median follow-up of 74 months

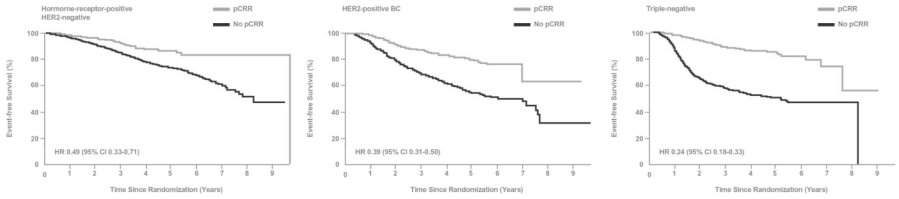


HER2=human epidermal growth factor receptor 2; MDACC=MD Anderson Cancer Center.
Gonzalez-Angulo AM, et al. *J Clin Oncol.* 2009;27:5700-5706.

PROGNOSTIC MARKERS

Achievement of pCR is a potential prognostic marker in BC

- This pooled analysis conducted by the FDA involved 11,955 patients treated with chemotherapy followed by surgery (neoadjuvant) from 12 international trials



- Achieving pCR in aggressive diseases such as HER2+ and triple-negative BC was associated with improved clinical outcome
- pCR in HER2+ BC was also associated with improved clinical outcomes in an updated meta-analysis by Broglio et al

BC=breast cancer; CI=confidence interval; HER2=human epidermal growth factor receptor 2; HR=hazard ratio; pCR=pathological complete response.
Cortazar P, et al. *Lancet.* 2014;384:164-172. Broglio KR, et al. *JAMA Oncol.* 2016;2:751-760.

Module 5

CURRENT CHALLENGES AND FUTURE DIRECTIONS

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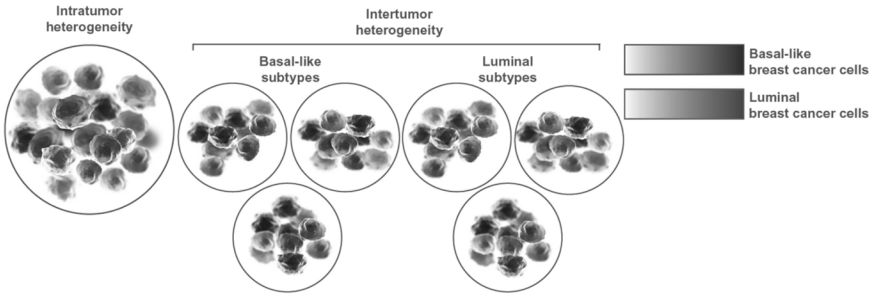
emerging perspectives

Histologic and molecular heterogeneity characterize the basis of breast cancer

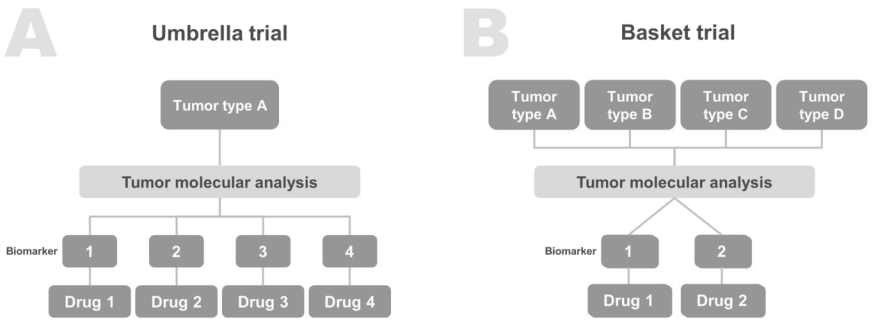


Clinical implications of genetic heterogeneity

- Development of resistance
  - Heterogeneity leads to a high degree of diversity between and within tumors
    - Could be one of the main reasons for therapeutic resistance

Polyak K. *J Clin Invest*. 2011;121:3786-3788.
Republished with permission of The American Society for Clinical Investigation, from Heterogeneity in Breast Cancer, Polyak K, Volume 121, Issue 10, 2011; permission conveyed through Copyright Clearance Center, Inc.

The use of biomarkers in designing clinical trials has the potential to expedite clinical development



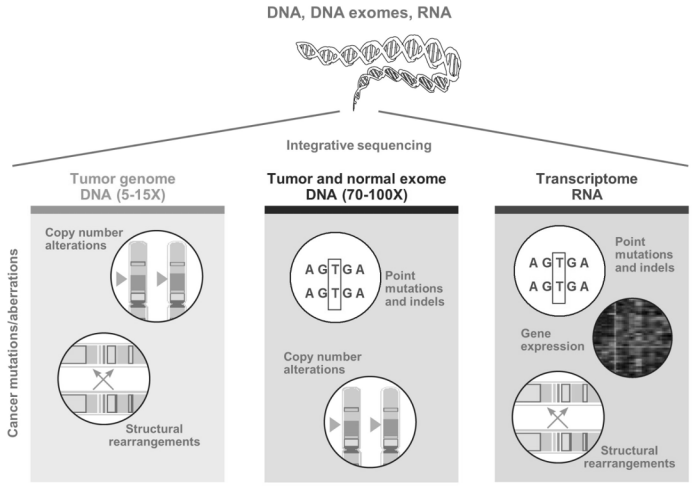
Biankin AV, et al. *Nature*. 2015;526:361-370.
Adapted by permission from Springer Nature: *Nature*, Patient-centric trials for therapeutic development in precision oncology, Biankin AV, Piantadosi S, Hollingsworth SJ, © 2015.

Key challenges with biomarker development/testing in BC: The need for adequate tumor tissues/samples

- **Tumor tissue:** Given our improved understanding of key disease drivers, an increasing number of tests are needed
- **Access/quantity:** Adequate amount of tissue is crucial for proper biomarker assessment
- **Tissue quality:** There is a need for standardizing pre-analytic variables, with the goal of developing standardized methods of tissue procurement and processing, as these variables affect the quality of tissue for biomarker testing
- **Tumor heterogeneity:** It is necessary to study multiple spatially separated biopsy samples as genomic, transcriptomic, and proteomic profiles of tumor cells in 1 region or at 1 time may be divergent from tumor cells in different regions

BC=breast cancer.
Meyerson M, et al. *Nat Rev Gen*. 2010;11:685-696. Sherman ME, et al. *Cancer Epidemiol Biomarkers Prev*. 2010;19:966-972.
Levy BP, et al. *Oncologist*. 2015;20:1175-1181. Hicks DG, et al. *J Natl Cancer Inst Monogr*. 2011;2011:43-45.

With the emerging importance of understanding the relationship between genetic mutations and tumor development, next-generation sequencing is becoming a more valuable tool



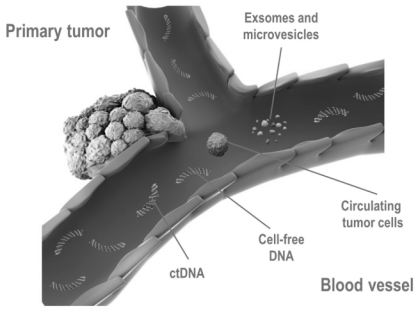
Simon R, et al. *Nat Rev Drug Discov*. 2013;12:358-369.

Liquid biopsies may provide valuable biomarker data through less invasive methods

- Liquid biopsy is a minimally invasive diagnostic technique that measures biomarkers from bodily fluids such as blood, urine, and saliva

Liquid biopsy approaches

Circulating tumor DNA (ctDNA)	Detects mutations from small fragments of tumor DNA released into the bloodstream
Circulating tumor cells	Correlates disease activity with number of cells released from primary tumor mass into the bloodstream
Tumor exosomes	Extracts molecular information from actively released vesicles carrying RNA, DNA, and protein derived from tumor cells



- The FDA has cleared a blood-based test using circulating tumor cells to assess the prognosis of some metastatic carcinomas, including BC
- Liquid biopsies, while a promising technology, have not yet been validated in diagnosis of breast cancers

BC=breast cancer; EGFR=epidermal growth factor receptor.

Diaz LA Jr, et al. *J Clin Oncol*. 2014;32:579-586. Elshimali YI, et al. *Int J Mol Sci*. 2013;14:18925-18958. Gold B, et al. *J Mol Diagn*. 2015;17:209-224. Alix-Panabières C, et al. *Nat Med Biomed Eng*. 2017;1:0065. Andree KC, et al. *Mol Oncol*. 2016;10:395-407.

Summary

- Advances in molecular biology and sequencing technologies have resulted in a better understanding of key disease drivers involved in the pathogenesis of breast cancer and have also generated insights for biomarker identification and evaluation
- Because breast cancer is a group of heterogeneous diseases associated with distinct genetic abnormalities or disease drivers, a more personalized or targeted approach may be needed to optimize patient care
- Despite recent advances, a number of challenges and unmet needs still remain in the management of patients with breast cancer