Genomic Profiling to Personalize Care in HR+/HER2- Metastatic Breast Cancer

Soo-Chin LEE

Senior Consultant, Department of Haematology-Oncology, National University Cancer Institute, Singapore
Associate Director of Research, Department of Haematology-Oncology, National University Cancer Institute, Singapore
Director, Cancer Genetics Program, National University Cancer Institute, Singapore
Senior Principal Investigator, Cancer Science Institute, Singapore
Professor, Yong Loo Lin School of Medicine, National University of Singapore

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Genomic Profiling to Guide Therapeutic Decisions in Breast Cancer

Scope of Talk

Tumor Genomic Profiling (NGS)
Germline Genetic Testing (NGS)

Guiding Therapeutic Decisions in Breast Cancer

Breast Cancer Specific Predictive Biomarkers

Tier 1 Biomarkers: Tumor ER, PR, HER2 (**IHC**; routinely tested)

HER2+

HER2-

TNBC

HR+/HER2-

Tier 2 Biomarkers

HER2+

None!

TNBC	Biomarker-Drug
PD-L1 (IHC)	1st line **Pembrolizumab**
HER2 low (IHC)	≥2nd line **T-DXd**
Germline *BRCA1/2* mutations	**PARP inhibitor** *(olaparib, talazoparib)*

HR+/HER2- MBC has the **largest portfolio of Tier 2 biomarkers**

Testing Methodology	HR+/HER2-	Biomarker-Drug
IHC	HER2 low / ultralow	1st to ≥2nd line **T-DXd**
Tumor genomic profiling	Tumor PI3K mutations	1st line **inavolisib** *(early relapsed metastatic)*
		≥2nd line **alpelisib** or **capivasertib**
	Tumor AKT1/PTEN alterations	≥2nd line **capivasertib**
	Tumor ESR1 mutations	≥2nd line **oral SERD** *(elacestrant, imlunestrant)*
Germline genetic testing	Germline *BRCA1/2* mutations	**PARP inhibitor** *(olaparib, talazoparib)*

Tumor Agnostic Genetic Biomarkers Detectable on NGS

Relevant for any cancer type!

Tumor Agnostic Biomarker	Drug	Date approved by US FDA	Frequency of biomarker in breast cancer
MSI high	Pembrolizumab	May 2017	0-1.5%
High TMB (≥10 Mut/Mb)	Pembrolizumab	June 2020	~5%
NTRK fusion	Larotrectinib	Nov 2018	<1%
NTRK fusion	Entrectinib	Aug 2019	<1%

"Investigational" tumor genomic biomarkers for biomarker-directed early phase clinical trials

TP53 mutations

SMARCA4 mutations

….

Vidula et al. NPJ Breast Cancer Nov 2022
O'Meara et al. Oncotarget 2021; 12(5): 394-400
Manea et al. Ann Med Surg 2022; 79

"Gold Standard" Genetic Biomarkers Approved by FDA to Guide Therapy in HR+/HER2- Metastatic Breast Cancer

Biomarker	Drug	When approved	Test to detect biomarker	Frequency	Stability
Tumor PI3K mutations	Alpelisib Inavolisib Capivasertib	May 2019 Oct 2024 Nov 2023	**Tumor mutational analysis** *Specific mutations or NGS* *Tissue or ctDNA* *(**Tissue higher yield**)*	40-50% in HR+/HER2-	Truncal mutations that **occur early** *(informative to test primary tumor)*
Tumor AKT1/PTEN alterations (without PI3K mutations)	Capivasertib	Nov 2023		10-15% in HR+/HER2-	**Fairly stable**; do not change much with time
Tumor ESR1 mutations	Elacestrant Imlunestrant	Jan 2022 Sep 2025	**Tumor mutational analysis** *Specific mutations or NGS* *Tissue or ctDNA* *(**ctDNA preferred**)*	**<5-10% in treatment naïve HR+/HER2-** Up to 40-50% in ET treated patients	**Emerges with exposure to endocrine therapy**
Germline *BRCA* mutations	Olaparib Talazoparib	Jan 2018 Oct 2018	**Germline mutational analysis**	**5-10% in HR+/HER2-** ***(10-20% in TNBC)***	**Does not change with time** *(same from birth to death)*

Practical Considerations in Genomic Profiling in Breast Cancer to Guide Therapeutic Decisions in the Clinic

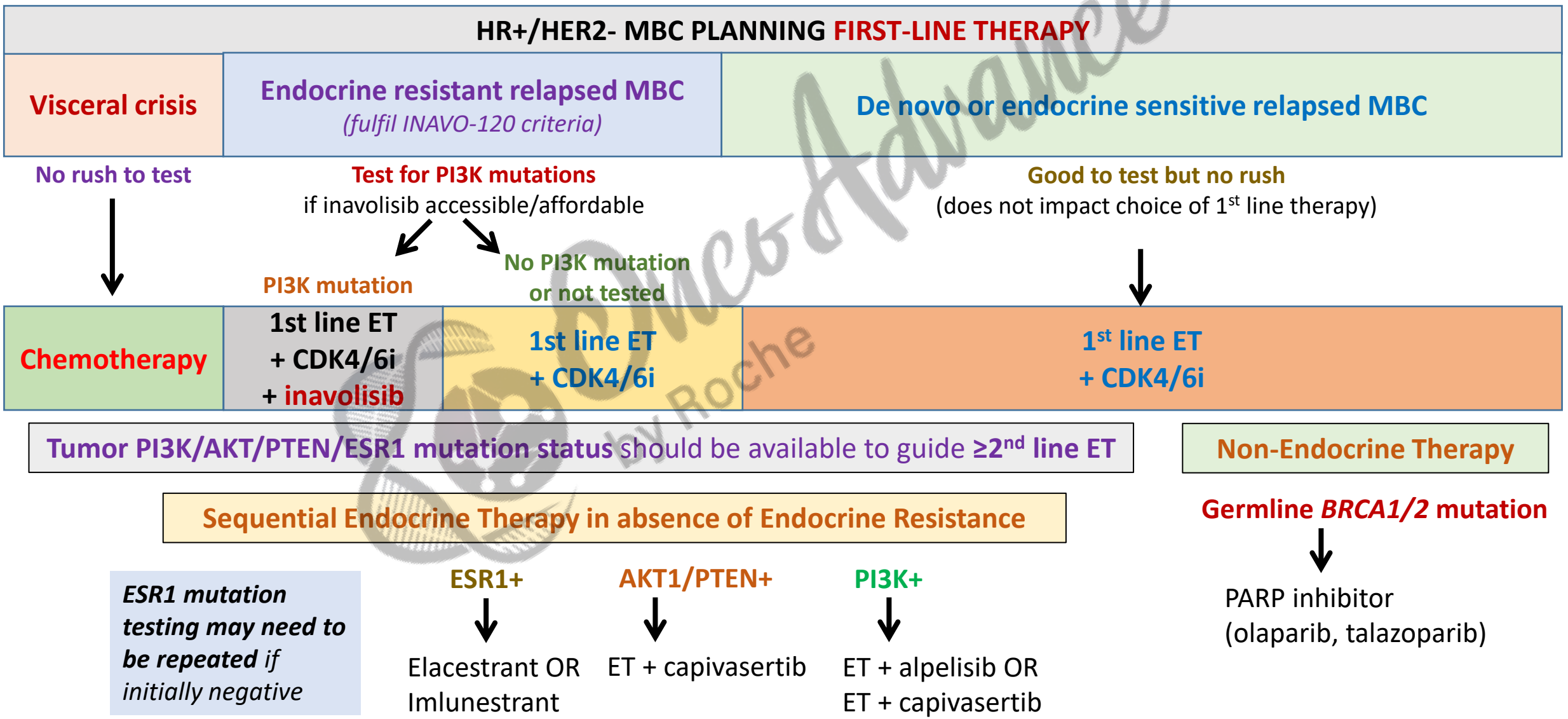
When to test?

What materials to send?

What test to order?

How to **interpret** the test?

When to Test for Gold Standard Breast Cancer Specific Genomic Biomarkers for HR+/HER2- Metastatic Breast Cancer?



Practical Considerations in Genomic Profiling in Breast Cancer to Guide Therapeutic Decisions in the Clinic

When to test?

What materials to send?

What test to order?

How to **interpret** the test?

What to look out for in a Genomic Profiling (NGS) report

FOUNDATIONONE®CDx

PATIENT

TUMOR TYPE
Breast carcinoma (NOS)

REPORT DATE
06 Apr 2023

COUNTRY CODE
SG

ORDERED TEST #
ORD-1597673-01

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ABOUT THE TEST FoundationOne®CDx is the first and only FDA-Approved comprehensive companion diagnostic for all solid tumors.

PATIENT
- **DISEASE** Breast carcinoma (NOS)
- **NAME** ST0098/IMAC1616A6, NUHS
- **DATE OF BIRTH**
- **SEX** Female
- **MEDICAL RECORD #**

PHYSICIAN
- **ORDERING PHYSICIAN**
- **MEDICAL FACILITY** National University Hospital - Dept. of Haemato-oncology
- **ADDITIONAL RECIPIENT** None
- **MEDICAL FACILITY ID** 312964
- **PATHOLOGIST** Not Provided

SPECIMEN
- **SPECIMEN SITE** Breast
- **SPECIMEN ID** IMAC1616A6
- **SPECIMEN TYPE** Slide Deck
- **DATE OF COLLECTION** 22 May 2019
- **SPECIMEN RECEIVED** 29 March 2023

What material was tested?

*Germline (usually blood **PBMC** in **EDTA** tubes)*

*Tumor (**tissue** vs **plasma ctDNA/cfDNA** in **Streck** tubes)*

When was the test done?

(in relation to therapeutics decision)

Contemporary or archival?

What genes were tested?

Did the test cover your genes of interest?

DNA GENE LIST: ENTIRE CODING SEQUENCE FOR THE DETECTION OF BASE SUBSTITUTIONS, INSERTION/DELETIONS, AND COPY NUMBER ALTERATIONS

ABL1	*ACVR1B*	*AKT1*	*AKT2*	*AKT3*	*ALK*	*ALOX12B*	*AMER1 (FAM123B or WTX)*	
APC	*AR*	*ARAF*	*ARFRP1*	*ARID1A*	*ASXL1*	*ATM*	*ATR*	*ATRX*
AURKA	*AURKB*	*AXIN1*	*AXL*	*BAP1*	*BARD1*	*BCL2*	*BCL2L1*	*BCL2L2*
BCL6	*BCOR*	*BCORL1*	*BRAF*	*BRCA1*	*BRCA2*	*BRD4*	*BRIP1*	*BTG1*
BTG2	*BTK*	*CALR*	*CARD11*	*CASP8*	*CBFB*	*CBL*	*CCND1*	*CCND2*
CCND3	*CCNE1*	*CD22*	*CD274 (PD-L1)*	*CD70*	*CD79A*	*CD79B*	*CDC73*	*CDH1*
CDK12	*CDK4*	*CDK6*	*CDK8*	*CDKN1A*	*CDKN1B*	*CDKN2A*	*CDKN2B*	*CDKN2C*
CEBPA	*CHEK1*	*CHEK2*	*CIC*	*CREBBP*	*CRKL*	*CSF1R*	*CSF3R*	*CTCF*
CTNNA1	*CTNNB1*	*CUL3*	*CUL4A*	*CXCR4*	*CYP17A1*	*DAXX*	*DDR1*	*DDR2*
DIS3	*DNMT3A*	*DOT1L*	*EED*	*EGFR*	*EMSY (C11orf30)*	*EP300*	*EPHA3*	*EPHB1*
EPHB4	*ERBB2*	*ERBB3*	*ERBB4*	*ERCC4*	*ERG*	*ERRFI1*	*ESR1*	*EZH2*
FANCA	*FANCC*	*FANCG*	*FANCL*	*FAS*	*FBXW7*	*FGF10*	*FGF12*	*FGF14*
FGF19	*FGF23*	*FGF3*	*FGF4*	*FGF6*	*FGFR1*	*FGFR2*	*FGFR3*	*FGFR4*
FH	*FLCN*	*FLT1*	*FLT3*	*FOXL2*	*FUBP1*	*GABRA6*	*GATA3*	*GATA4*
GATA6	*GID4 (C17orf39)*	*GNA11*	*GNA13*	*GNAQ*	*GNAS*	*GRM3*	*GSK3B*	*H3-3A (H3F3A)*
HDAC1	*HGF*	*HNF1A*	*HRAS*	*HSD3B1*	*ID3*	*IDH1*	*IDH2*	*IGF1R*
IKBKE	*IKZF1*	*INPP4B*	*IRF2*	*IRF4*	*IRS2*	*JAK1*	*JAK2*	*JAK3*
JUN	*KDM5A*	*KDM5C*	*KDM6A*	*KDR*	*KEAP1*	*KEL*	*KIT*	*KLHL6*
KMT2A (MLL)	*KMT2D (MLL2)*	*KRAS*	*LTK*	*LYN*	*MAF*	*MAP2K1 (MEK1)*	*MAP2K2 (MEK2)*	*MAP2K4*
MAP3K1	*MAP3K13*	*MAPK1*	*MCL1*	*MDM2*	*MDM4*	*MED12*	*MEF2B*	*MEN1*
MERTK	*MET*	*MITF*	*MKNK1*	*MLH1*	*MPL*	*MRE11 (MRE11A)*	*MSH2*	*MSH3*
MSH6	*MST1R*	*MTAP*	*MTOR*	*MUTYH*	*MYC*	*MYCL (MYCL1)*	*MYCN*	*MYD88*
NBN	*NF1*	*NF2*	*NFE2L2*	*NFKBIA*	*NKX2-1*	*NOTCH1*	*NOTCH2*	*NOTCH3*
NPM1	*NRAS*	*NSD2 (WHSC1 or MMSET)*		*NSD3 (WHSC1L1)*	*NT5C2*	*NTRK1*	*NTRK2*	*NTRK3*
P2RY8	*PALB2*	*PARP1*	*PARP2*	*PARP3*	*PAX5*	*PBRM1*	*PDCD1 (PD-1)*	*PDCD1LG2 (PD-L2)*
PDGFRA	*PDGFRB*	*PDK1*	*PIK3C2B*	*PIK3C2G*	*PIK3CA*	*PIK3CB*	*PIK3R1*	*PIM1*
PMS2	*POLD1*	*POLE*	*PPARG*	*PPP2R1A*	*PPP2R2A*	*PRDM1*	*PRKAR1A*	*PRKCI*

Are there actionable alterations?

(interpret in context of tumor type)

TUMOR TYPE: BREAST CARCINOMA (NOS) **HR+/HER2- MBC**

Genomic Alterations Identified†

PALB2 splice site 2748+1G>A
PIK3CA E110del, R88Q
ESR1 D538G
EPHB1 M447I

Additional Findings†

Microsatellite status MS-Stable
Tumor Mutation Burden TMB-Intermediate; 16 Muts/Mb

Additional Disease-relevant Genes with No Reportable Alterations Identified†

ERBB2

† For a complete list of the genes assayed and performance specifications, please refer to the Appendix

Alpelisib, capivasertib, elacestrant, Imlunestrant ***(breast cancer subtype specific)***

Pembrolizumab ***(tumor agnostic)***

CASE #1

48-year-old female; **Relapsed metastatic HR+/HER2- breast cancer**

Age 40:

pT2(2.5cm)N1(1/12)M0 grade 2 invasive ductal carcinoma left breast

ER 90%, PR 80%, HER2 1+

s/p left mastectomy + axillary clearance

s/p adjuvant ddAC x 4 followed by weekly paclitaxel x 12

s/p adjuvant radiotherapy

s/p adjuvant tamoxifen + medical ovarian suppression x 5 years

Age 48:

2 years after completing adjuvant endocrine therapy, presented with multiple bony metastases and 3 lung lesions

Biopsy of right lung lesion:

Metastatic breast cancer, Grade 2 IDC; **ER 70%, PR 70%, HER2 1+**

No visceral crisis

Biomarker information will not impact 1st line treatment decision for most patients with HR+/HER2- MBC

No visceral crisis: ET + CDK4/6i

Visceral crisis: Chemotherapy

Even in BRCA mutation carriers

Exception: PI3K mutation testing in **endocrine resistant relapsed** metastatic disease **if inavolisib is available** (INAVO120)

Will you test one or more of the predictive biomarkers *(tumor PI3K/AKT1/PTEN/ESR1 alterations, germline BRCA mutation)* **before initiating first-line palliative therapy?**

1. Yes
2. No

CASE #1

48-year-old female; **Relapsed metastatic HR+/HER2- breast cancer**

Age 40:

pT2(2.5cm)N1(1/12)M0 grade 2 invasive ductal carcinoma left breast

ER 90%, PR 80%, HER2 1+

s/p left mastectomy + axillary clearance

s/p adjuvant ddAC x 4 followed by weekly paclitaxel x 12

s/p adjuvant radiotherapy

s/p adjuvant tamoxifen + medical ovarian suppression x 3.5 years

Age 48:

Presented with multiple bony metastases and 3 lung lesions

Biopsy of right lung lesion:

Metastatic breast cancer

Grade 2 invasive ductal carcinoma; **ER 70%, PR 70%, HER2 1+**

No visceral crisis

Started on **1st line medical ovarian suppression + letrozole + ribociclib** **without** testing biomarkers upfront

In your practice, HOW OFTEN do you send tumor NGS testing for a patient like this to guide subsequent therapy?

1. Most of the time
2. Sometimes
3. Rarely

In your practice, WHEN do you send tumor NGS for a patient like this?

1. As soon as diagnosis of metastatic cancer is made
2. Within 6-18 months of starting 1st line therapy
3. After progression from 1st line treatment

Ideal to have the biomarker information **in time to guide ≥2nd line treatment decision**

CASE #1

48-year-old female
Relapsed metastatic HR+/HER2 Low breast cancer to bones and lungs
1st line OFS + letrozole + Ribociclib
About 1 year after starting 1st line OFS + letrozole + ribociclib, you feel that it is time to test for predictive biomarkers
Patient's disease remains well controlled at this point
Germline *BRCA1/2* testing: no pathogenic mutations

You decide to test for tumor PI3K/AKT1/PTEN/ESR1 mutations.

What test will you order for a patient like this in your practice?

1. Panel test *(restricted to PI3K/AKT1/PTEN/ESR1 mutations)*
2. NGS

Advantages of panel test:
Cheaper, requires less tissue, possibly faster turnaround
Advantages of NGS:
Broader coverage; include other actionable alterations

If you decide to use tumor NGS test, which material will you use for testing?

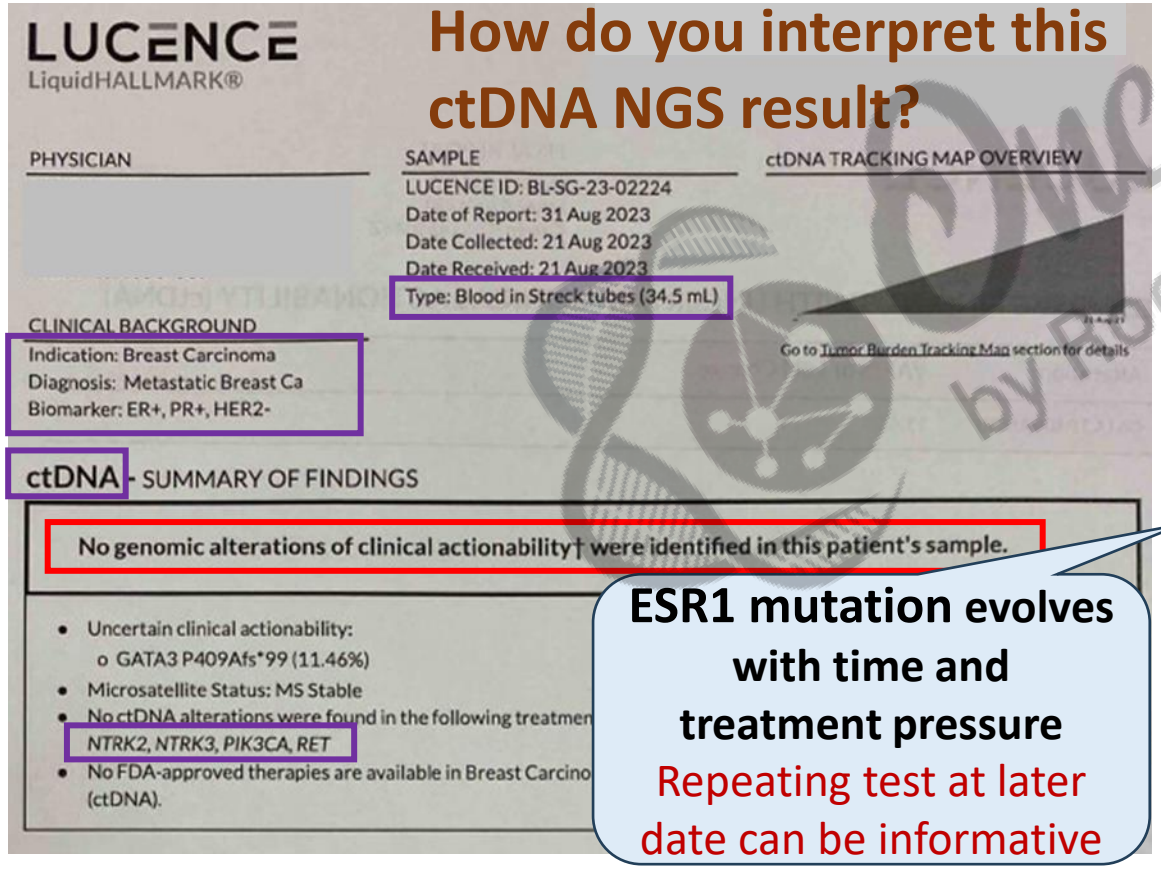
1. The mastectomy specimen 8 years ago
2. The lung biopsy 1 year ago
3. ctDNA from a fresh blood specimen

Which materials to use for Tumor Genomic Profiling (NGS)?

Materials	Advantages	Disadvantages
Primary lumpectomy or mastectomy specimen	Plenty of tissue; unlikely to yield insufficient materials for tumor NGS	Older specimen May not reflect the tumor's most current status
Biopsy of the latest metastatic lesion	More contemporary tissue sample	Usually from a core or needle biopsy *(small specimen)* Sometimes unable to cut sufficient sections for NGS
ctDNA from a fresh blood sample	Most contemporary sample Most reflective of tumor's current status	May not be able to cover as many genes as tumor NGS Sensitivity generally lower Technically challenging for certain alterations, *e.g.*, gene fusions

CASE #1

48-year-old female; **Relapsed metastatic HR+/HER2 Low breast cancer to bones and lungs**
1 year after starting 1st line ET + CDK4/6i, you initiated tumor NGS testing
You requested to **retrieve the lung biopsy specimen for tumor NGS**
2 days later, you received a call from the Pathology department, informing you that the biopsy was too small with **insufficient materials** to cut enough slides for tumor NGS
You then decided to send a **fresh blood sample for ctDNA NGS**



ESR1 mutation evolves with time and treatment pressure
Repeating test at later date can be informative

Based on this result, the patient is <u>not</u> eligible to be treated with alpelisib, capivasertib or elacestrant.

Yes No

It could be informative to <u>repeat ctDNA NGS</u> at a later date (*e.g.*, when patient progresses).

Yes No

It could be informative to retrieve the <u>archival mastectomy specimen</u> for tumor NGS testing?

Yes No

CASE #1: Yield of Tumor PIK3CA Mutation

Primary Tumor vs Metastatic Tumor vs ctDNA

PI3K mutations are **truncal mutations**

Occur early; informative to test **primary tumor**

SOLAR1 Trial *(registration trial for alpelisib)*

Primary tumor (n=436): **60%** PI3K3 mutation

Metastatic tumor (n=125): **59%** PI3K mutation

ctDNA (n=561): **33%** PI3K mutation

(predictive of alpelisib benefit)

PI3K mutation can be tested on tumor or ctDNA

If ctDNA negative, should test tumor

Case #1: Tumor NGS on primary mastectomy specimen 8 years ago

Opens up **alpelisib** or **capivasertib** as subsequent therapy options

Biomarker Findings

Tumor Mutational Burden - 11 Muts/Mb

Microsatellite status - Cannot Be Determined [α]

Genomic Findings

For a complete list of the genes assayed, please refer to the Appendix.

- ***PIK3CA*** E545K
- ***FGFR1*** amplification
- ***MTAP*** loss
- ***BCL2*** amplification - equivocal[†]
- ***CDKN2A/B*** CDKN2A loss, CDKN2B loss
- ***CSF3R*** amplification - equivocal[†]
- ***GATA3*** splice site 925-3_925-2delCA
- ***MYCL (MYCL1)*** amplification - equivocal[†]
- ***NSD3 (WHSC1L1)*** amplification
- ***TP53*** R248Q
- ***VEGFA*** amplification - equivocal[†]
- ***ZNF703*** amplification

Andre et al. NEJM 2019; 380 (20): 1929-40

CASE #1

48-year-old female; **Relapsed metastatic HR+/HER2 Low breast cancer to bones and lungs**
Biopsy of metastatic lung lesion:
Grade 2 invasive ductal carcinoma; **ER 70%, PR 70%, HER2 1+**
1 year after starting 1st line ET + CDK4/6i, you initiated tumor NGS testing
Metastatic lung biopsy: insufficient materials for NGS testing
ctDNA NGS: no actionable alterations
Mastectomy specimen NGS: PI3K mutation
Germline *BRCA1/2* testing: no pathogenic mutations

Gold Standard Predictive Biomarker Checklist

HER2 low? YES (T-DXd is an option)
Germline *BRCA* mutation? No *(PARPi is not an option)*
Tumor PI3K mutation? YES (Alpelisib and Capivasertib are options)
Tumor AKT1/PTEN alterations? No
Tumor ESR1 mutation? No *(Elacestrant/Imlunestrant is not an option now); ESR1 mutations may emerge later (repeating test may be informative)*

CASE #2

55-year-old female
De novo metastatic HR+/HER2- breast cancer to lungs and liver
1st line AI + CDK4/6i: 9 months PFS
Metastatic liver lesion sent for **tumor NGS**
2nd line fulvestrant + capivasertib: 5 months PFS

Tumor NGS on metastatic liver biopsy

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PATIENT TUMOR TYPE REPORT DA

QRF#

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PATIENT
DISEASE
NAME
DATE OF BIRTH
SEX
MEDICAL RECORD #

PHYSICIAN
ORDERING PHYSICIAN
MEDICAL FACILITY National University Hospital Singapore
ADDITIONAL RECIPIENT None
MEDICAL FACILITY ID 204111
PATHOLOGIST Not Provided

SPECIMEN
SPECIMEN SITE
SPECIMEN ID
SPECIMEN TYPE Slide
DATE OF COLLECTION
SPECIMEN RECEIVED

Biomarker Findings
Microsatellite Status - MS-Stable
Tumor Mutational Burden - TMB-Intermediate (6 Muts/Mb)

Genomic Findings
For a complete list of the genes assayed, please refer to the Appendix.

BRCA2 N2135fs*3
PTEN H64fs*35
ACVR1B deletion exon 2
NOTCH3 rearrangement exon 33

6 Therapies with Clinical Benefit
0 Therapies with Lack of Response

Based on this result, will you treat this patient with a PARP inhibitor?

Yes **No**

Based on this result, will you recommend the patient's 28-year old daughter to undergo *BRCA* testing?

Yes **No**

This is a **tumor NGS** test.
There should be a **confirmed germline *BRCA1/2* pathogenic mutation** for **PARPi treatment** and for **cascade testing** of family members.

CASE #2:

Can a Tumor Pathogenic BRCA mutation be an Incidental Germline Finding?

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PATIENT | TUMOR TYPE | REPORT D/

ORF#

Tumor NGS on metastatic liver biopsy

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PATIENT

DISEASE
NAME
DATE OF BIRTH
SEX
MEDICAL RECORD #

PHYSICIAN

ORDERING PHYSICIAN
MEDICAL FACILITY National University Hospital Singapore
ADDITIONAL RECIPIENT None
MEDICAL FACILITY ID 204111
PATHOLOGIST Not Provided

SPECIMEN

SPECIMEN SITE
SPECIMEN ID
SPECIMEN TYPE Slide
DATE OF COLLECTION
SPECIMEN RECEIVED

Biomarker Findings

Microsatellite Status - MS-Stable
Tumor Mutational Burden - TMB-Intermediate (6 Muts/Mb)

Genomic Findings

For a complete list of the genes assayed, please refer to the Appendix.

BRCA2 N2135fs*3
PTEN H64fs*35
ACVR1B deletion exon 2
NOTCH3 rearrangement exon 33

6 Therapies with Clinical Benefit
0 Therapies with Lack of Response
13 Clinical Trials

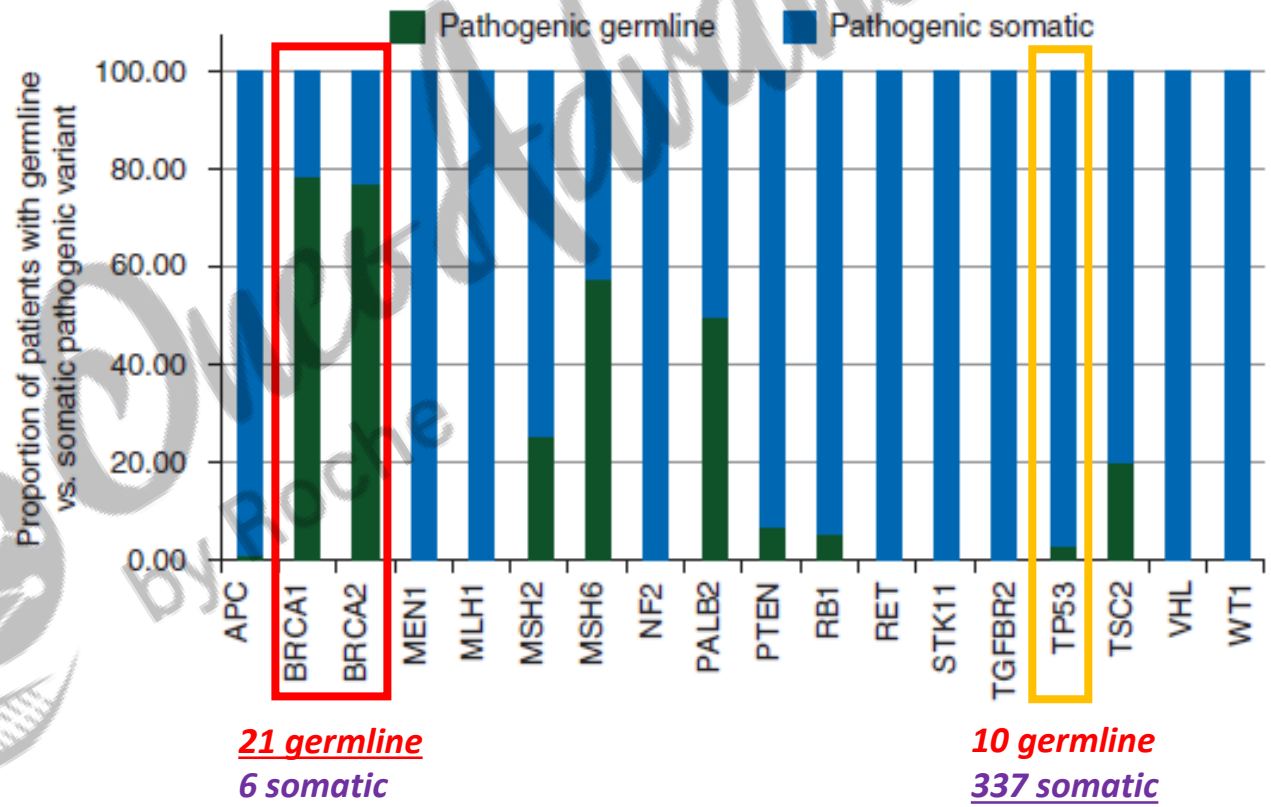
What is the likelihood that the **tumor *BRCA2* mutation** is an **incidental germline finding**?

1. Low (<10%)
2. Moderate (40-50%)
3. High (>70%)

Likelihood of a Tumor Pathogenic Mutation being Germline in nature varies with the Gene

N=1000 tumor/germline pairs with NGS; 19 cancer genes (MD Anderson)
Total ~800 pathogenic variants in the tumor (~5% germline)

If a pathogenic *BRCA1/2* mutation is identified on tumor NGS, confirmatory germline *BRCA1/2* genetic testing is strongly recommended



Most tumor TP53 mutations are true acquired somatic mutations

But most tumor *BRCA1/2* mutations are incidental germline findings *(≥70% probability)*

Meric-Bernstam et al. Ann Oncol 2016: 27(5): 795-800

CASE #2

The patient returns 4 weeks later and informed you that she sought a second opinion with another oncologist. A blood NGS test was done as you have advised.

How do you interpret this blood NGS result?

PATIENT | **PHYSICIAN** | **SAMPLE**

LUCENCE ID: BL-SG-21-00978
Date of Report: 12 Nov 2021
Date Collected: 03 Nov 2021
Date Received: 03 Nov 2021
Type: Blood in Streck tubes (27 mL)

TEST PERFORMED

Targeted next-generation sequencing on plasma cfDNA was performed. See Test Details for more information.

SUMMARY OF GENOMIC FINDINGS

GENOMIC FINDINGS WITH *STRONG* CLINICAL ACTIONABILITY

BRCA2 Q2345Tfs*15: 47.99%, Frameshift indel

GENOMIC FINDINGS WITH *POTENTIAL* CLINICAL ACTIONABILITY

BRCA2 Q2345Tfs*15: 47.99%, Frameshift indel

GENOMIC FINDINGS WITH UNCERTAIN CLINICAL ACTIONABILITY

RB1 L569I (0.39%)

NO ALTERATIONS FOUND IN THE FOLLOWING DISEASE-RELEVANT GENES

BRCA1

This is a test on **plasma cfDNA** collected in **Streck tubes** (***still a tumor test!***).
This is **not** a germline test.

Another clue that this is a **tumor** test: **Allele Frequency** reported

This result confirms the *BRCA2* mutation previously identified in the tissue to be an incidental germline finding.

Yes No

Based on this result, the patient is eligible to be treated with a PARP inhibitor.

Yes No

CASE #3

61-year-old female
Metastatic HR+/HER2- breast cancer
Failed 2 lines endocrine based therapy in 6 months
Now on **oral capecitabine**
Tumor NGS: no actionable alterations
You ordered a **blood NGS test**

Blood NGS test

How do you interpret this blood NGS result?

Ambry Genetics

FINAL REPORT - 04/04/2024

Ordered By
Medical Professional:
Client:
Additional Authorized Recipient:
Genomics, Lifestrands N/A

Patient Name:
Accession #:
AP2 Order #: 2546938
Specimen #:
Specimen: Blood EDTA (Purple top)
Birthdate: 11/17/1973
MRN #: N/A
Indication: Diagnostic
Sex at Birth: F
Collected: 03/11/2024
Received: 03/14/2024
Test Started: 03/14/2024

BRCANext™: Analyses of 19 High-Risk Hereditary Breast & Gynecologic Cancer Genes

RESULT: POSITIVE

One Pathogenic variant identified in BRCA2. BRCA2 is associated with autosomal dominant hereditary breast and ovarian cancer syndrome and autosomal recessive Fanconi anemia.

GENE	VARIANT	ZYGOSITY	VARIANT CLASSIFICATION
BRCA2	c.7032dup (p.Gln2345Thrfs*15)	heterozygous	PATHOGENIC

Yes, this is a germline test!
(blood collected into EDTA tube)

Based on this result, the patient is eligible to be treated with a PARP inhibitor.

Yes No

Based on this result, the patient's two adult children are recommended to undergo testing for the *BRCA2* mutation.

Yes No

Another clue that this is a **germline** test:
"Heterozygous" for the mutation
(terminology for germline result)

CASE #3

The patient returns 4 weeks later
She has been very distraught with the *BRCA* test results because of the implications on her family.
Her husband advised her to seek another opinion and send the test to another lab "just to be sure".
A second blood NGS test was done and was negative!

How do you interpret this result and advise the patient?

1. Send the test to a **third lab**
2. Test the patient's **tumor** for the *BRCA2* mutation
3. Test the patient's **parents** for the *BRCA2* mutation
4. Tell the patient that the second test is the **wrong test**

Methodology and Results of the 2nd blood NGS test

LucenceINSIGHT™ Test Details

The LucenceINSIGHT™ test is a next-generation sequencing (NGS) based test that identifies targeted cancer associated alterations including single nucleotide variations, indels and cancer associated viruses such as Epstein-Barr virus (EBV) and Human Papillomavirus (HPV) in circulating tumor DNA (ctDNA). LucenceINSIGHT™ also reports the Predicted Signal Localization sites through integrating clinical and laboratory parameters to inform diagnostic evaluation.

Alterations Tested By LucenceINSIGHT™

ACVR2A	*BMPR2*	*CDKN1B*	*ESR1*	*IDH2*	*MTOR*	*PPP2R1A*	*SPOP*
ADGRG6	*BRAF*	*CDKN2A*	*FBXW7*	*JAK1*	*MYC*	*PTEN*	*STK11*
AKT1	*BRCA1*	*CREBBP*	*FGFR1*	*KEAP1*	*NF1*	*PTPN11*	*TCF7L2*
ALK	*BRCA2*	*CTCF*	*FGFR2*	*KIT*	*NFE2L2*	*RET*	*TERT*
AMER1	*CASP8*	*CTNNB1*	*FGFR3*	*KRAS*	*NOTCH1*	*RHOA*	*TGFBR2*
APC	*CBFB*	*DICER1*	*FGFR4*	*MAP2K1*	*NRAS*	*RIT1*	*TP53*
AR	*CCND1*	*EGFR*	*FOXA1*	*MAPK1*	*PCBP1*	*RNF43*	*U2AF1*
ARID1A	*CCR4*	*EP300*	*GATA3*	*MED12*	*PDGFRA*	*RPL22*	
ATM	*CDC27*	*ERBB2*	*GNAS*	*MEN1*	*PIK3CA*	*SMAD4*	
B2M	*CDH1*	*ERBB3*	*HRAS*	*MET*	*PIK3R1*	*SMARCB1*	
BCOR	*CDKN1A*	*ERCC2*	*IDH1*	*MSH6*	*POLE*	*SMO*	

This is a test on **ctDNA** collected in **Streck tubes**
(ctDNA-based NGS test for early cancer detection in cancer-free individuals)
This is **not** a germline test.

Your LucenceINSIGHT™ Result

SAMPLE
LUCENCE ID: BL
Date of Report:
Date Collected:
Date Received:
Type: Blood in Streck tubes (20 mL)

INSIGHTS

Cancer Signal: Not Detected

No Cancer-Associated Alterations/Virus detected.
In a clinical study[1], less than 1% of individuals with no cancer signal detected had cancer.

Confused??

Blood NGS testing is **traditionally associated with germline testing**
Advancements in ctDNA/cfDNA NGS technology have resulted in more **"blood-based" tumor NGS tests** in the clinic
- *ctDNA/cfDNA NGS test for **cancer patients (actionable alterations)** in Case #2*
- *ctDNA/cfDNA NGS test for **non-cancer patients (early cancer detection)** in Case #3*

Clues for Tumor Test
ctDNA, cfDNA, plasma
Collected into **Streck tubes**
Allele frequency reported

Clues for Germline Test
Peripheral blood mononuclear cells
Collected into **EDTA tubes**
Heterozygous for pathogenic mutation

Not all Blood NGS Tests are Germline Tests!

Important to distinguish blood-germline from blood-tumor NGS tests
***Do not order the wrong test or interpret the test wrongly*!**

Molecular Profiling in Breast Cancer for Therapeutic Decisions

Conclusions

A small panel of **predictive biomarkers based on molecular alterations** detectable on NGS are now available to **guide treatment** choice in breast cancer.

Understanding the **characteristics of predictive biomarkers** and **limitations of testing** is important for proper interpretation of test results

- ***repeating the test*** *may be informative as some mutations evolve with time (e.g., ESR1 mutation)*
- ***testing a different specimen type*** *may be helpful as mutation detection rate may differ between different sample types (e.g., tissue vs ctDNA for PI3K mutations)*

***BRCA1/2* pathogenic mutation occur in ~2-3% of tumor NGS results**

- **highly likely to be incidental germline findings; confirmatory germline testing** strongly recommended

There is now an **increasing portfolio of blood-based tumor NGS tests** *(ctDNA, cfDNA)* in the clinic

- ***Should not be confused with a blood-based germline test!***

Thank you for your attention!