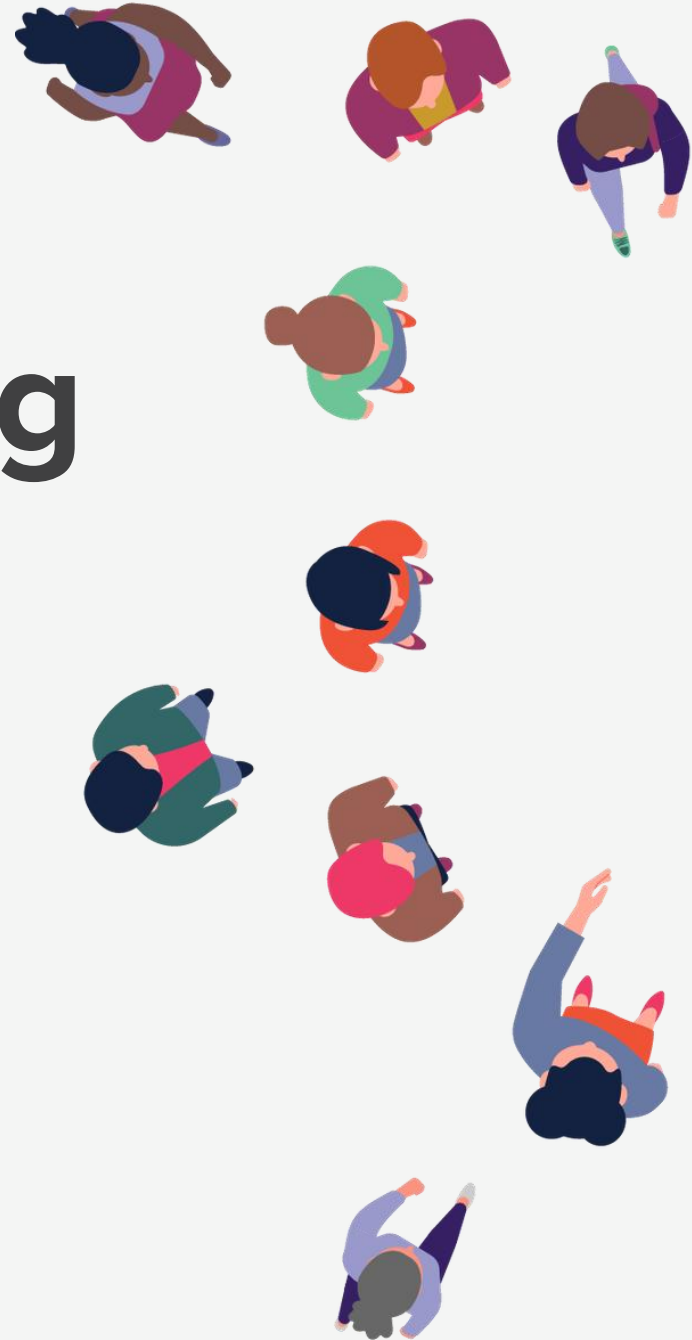


Understanding testing

Metastatic breast cancer

Speaker: Igor Makhlin, MD



Testing in Metastatic Breast Cancer

Igor Makhlin, MD

Hospital of the University of Pennsylvania

Abramson Cancer Center

10/3/26

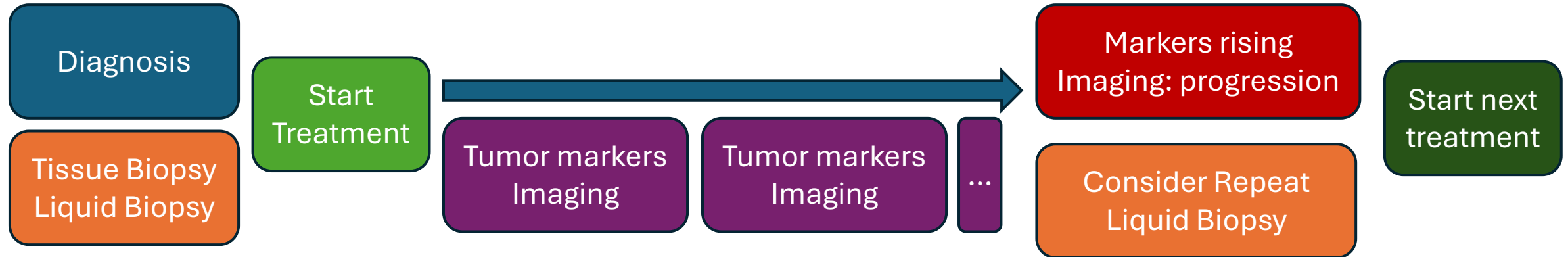
Agenda

- Principles of Testing – major categories
- The basics of diagnosis: Biopsy, histology, receptors
- Germline testing
- Next-generation sequencing, tissue and blood
- Circulating tumor DNA for treatment choice
- Monitoring treatment response
- Biomarker-based Imaging
 - FES PET/CT vs FDG PET/CT
- Real world cases

Principles in Testing

- Broad Categories for Testing
 - Diagnose the cancer
 - Histology, Receptors – **is this breast cancer, and what type?**
 - Germline testing
 - Genetic changes that are inherited from mom/dad
 - **Characterize the cancer** - Tumor-specific Testing
 - Source
 - Tissue-based
 - Blood-based
 - For informing *treatment choice*: circulating tumor DNA (ctDNA) panel testing
 - For monitoring treatment response: “MRD” ctDNA panels, tumor markers
 - Image-based testing
 - CT’s vs FDG PET/CT vs FES PET/CT

Major landmarks in testing

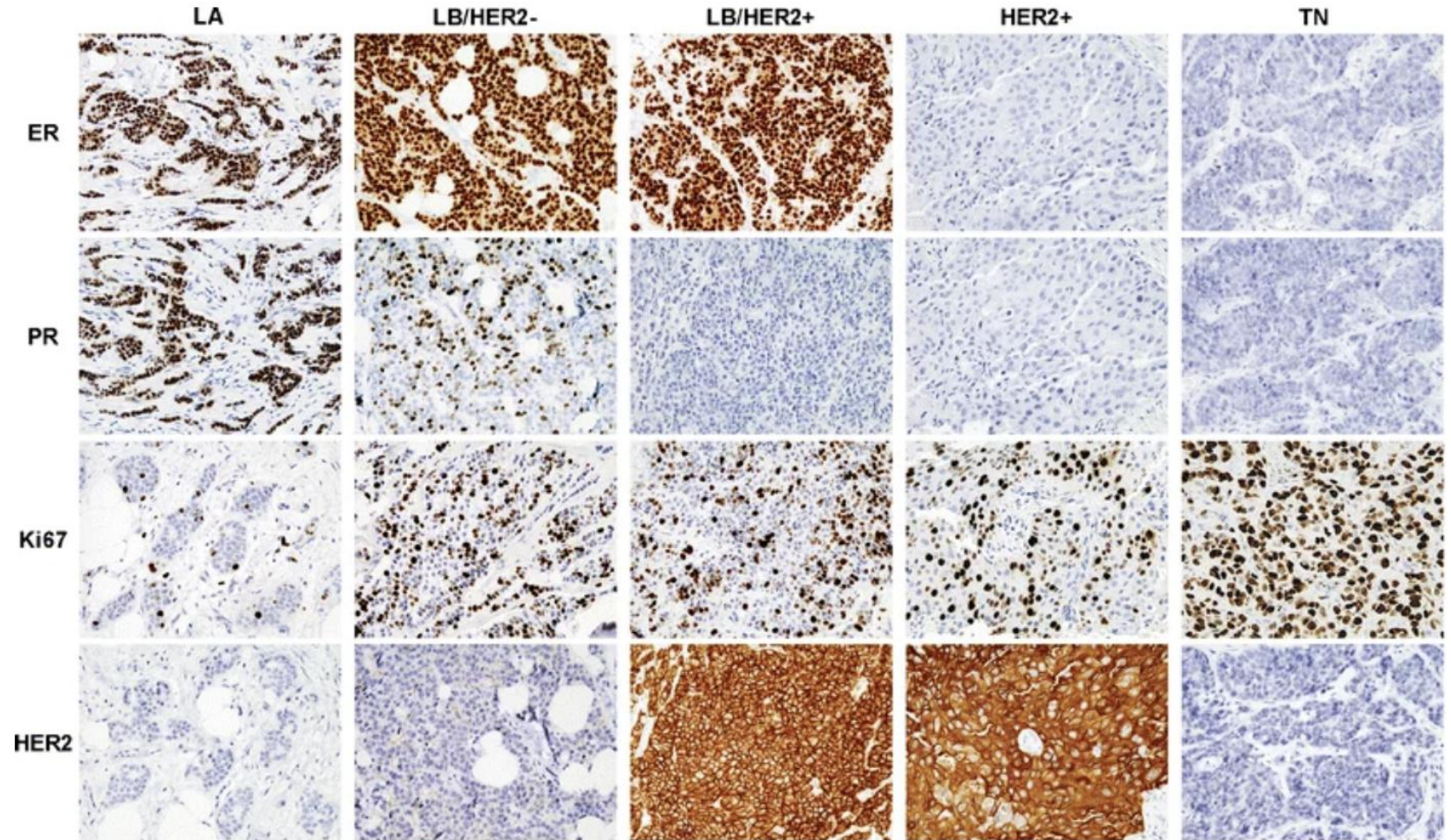


Diagnose the Cancer

- Upon a *new* presumed metastatic breast cancer, step #1 is to obtain a tissue biopsy
- Confirms that this is indeed breast cancer via **immunohistochemistry (IHC)**
 - Applies breast-cancer specific ‘stains’ to biopsy tissue
- For recurrent disease, can be helpful to understand if it looks the same as prior breast cancer (e.g. early stage lobular, if this is ductal, could there be a new primary?)
- Confirm receptors - ER, PR, and HER2 – **the most important biomarkers to guide initial and subsequent treatment**

Diagnose the Cancer – Tissue Biopsy & Receptors

Staining the tissue from biopsy with different markers confirms breast cancer and its subtype (e.g. ER+ HER2-).



Diagnose the Cancer – Case from Clinic

- 75 year old woman presented with a growing mass in the left breast
- Workup with mammogram, ultrasound, MRI and biopsy confirmed triple negative breast cancer
- PET Scan demonstrated an FDG-avid lesion in the liver
- Biopsy of the liver lesion – meant to confirm the metastatic breast cancer – showed a cancer of presumed *GI* origin
- A colonoscopy was done and confirmed a primary colon cancer
- **We thus never *assume* a metastatic breast cancer – we must biopsy and confirm!**

Diagnose the Cancer – Case from Clinic

- 32 year old woman felt a lump in her right breast and sought evaluation
- Mammogram and ultrasound showed two distinct masses about 2cm apart from each other as well as a lymph node in the right axilla
- PET scan showed multiple bone lesions
- Biopsy of **both** breast masses showed a triple negative breast cancer in #1 and a **HER2+** breast cancer in #2 – a rare situation but critical to our formulation of treatment plan!

Germline Testing

- Look for inherited genes that increase risk for breast cancer
 - E.g. BRCA1, BRCA2, PALB2
 - Contemporary panels have >30 genes
 - These do not change in your lifetime, but the testing can change (if new genes are discovered that need to be tested)
- **Every patient with new metastatic breast cancer diagnosis should get germline testing if not done previously**
- Has treatment implications, e.g. BRCA1/2 mutation = Olaparib pill, a chemo-free approach

Germline Testing

Reason for testing

Diagnostic test for both a personal and a family history of disease

Test performed

Sequence analysis and deletion/duplication testing of the 19 genes listed in the Genes Analyzed section.

- Invitae Hereditary Breast and Gyn Cancers Guidelines-Based Panel
- Invitae BRCA1 and BRCA2 Panel
- 18 individual genes with RNA analysis



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.658_659del (p.Val220Ilefs*4)	heterozygous	PATHOGENIC

About this test

This diagnostic test evaluates 19 gene(s) for variants (genetic changes) that are associated with genetic disorders. Diagnostic genetic testing, when combined with family history and other medical results, may provide information to clarify individual risk, support a clinical diagnosis, and assist with the development of a personalized treatment and management strategy.

Invitae Panel

BRCA1/2 Analyses with CustomNext-Cancer® +RNAinsight®

RESULTS

BRCA2 Pathogenic Mutation: c.6082_6086delGAAGA

BRIP1 Pathogenic Mutation: c.2990_2993delCAAA

SUMMARY

POSITIVE: Pathogenic Mutations Detected

INTERPRETATION

- This individual is heterozygous for the **c.6082_6086delGAAGA (p.E2028Kfs*19)** pathogenic mutation in the *BRCA2* gene.
 - This result is consistent with a diagnosis of hereditary breast and ovarian cancer (HBOC) syndrome.
 - **Risk estimate:** increased lifetime risks for female breast cancer (45-69%), ovarian cancer (13-29%), male breast cancer (1.8-7.1%), pancreatic cancer (5-10%) and prostate cancer (19-61%); increased risk for melanoma.
- This individual is heterozygous for the **c.2990_2993delCAAA (p.T997Rfs*61)** pathogenic mutation in the *BRIP1* gene.
 - **Risk estimate:** approximately a 5-15% cumulative risk for ovarian cancer.
- The expression and severity of disease for this individual cannot be predicted.
- The interactive effect and relative contribution of these alterations on clinical phenotype is unknown at this time.
- Genetic testing for pathogenic mutations in family members can be helpful in identifying at-risk individuals.
- Genetic counseling is a recommended option for all individuals undergoing genetic testing.

No additional pathogenic mutations, variants of unknown significance, or gross deletions or duplications were detected. Genes Analyzed (30 total): *APC, ATM, BARD1, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDKN2A, CHEK2, MLH1, MSH2, MSH6, MUTYH, NTHL1, PALB2, PMS2, PTEN, RAD51C, RAD51D, SMAD4, STK11* and *TP53* (sequencing and deletion/duplication); *AXIN2, HOXB13, MSH3, POLD1* and *POLE* (sequencing only); *EPCAM* and *GREM1* (deletion/duplication only). RNA data is routinely analyzed for use in variant interpretation for all genes.

Ambry Genetics Panel

Germline Testing

- **Variant of Uncertain Significance (VUS)** is common
- Means a mutation was found for which there are not enough evidence to suggest it is a **cancer causing mutation**
- Most commonly get's reclassified as benign, or remains VUS
- We do NOT act on these!

BRCA1/2 Analyses with CustomNext-Cancer® +RNAinsight®

RESULTS

MLH1

Variant, Unknown Significance: p.Y684S

SUMMARY

Variant of Unknown Significance Detected

INTERPRETATION

- No known clinically actionable alterations were detected.
- One variant of unknown significance was detected in the *MLH1* gene.
- **Risk Estimate:** should be based on clinical and family history, as the clinical significance of this result is unknown.
- Genetic counseling is a recommended option for all individuals undergoing genetic testing.

This individual is heterozygous for the p.Y684S (c.2051A>C) variant of unknown significance in the *MLH1* gene, which may or may not contribute to this individual's clinical history. Refer to the supplementary pages for additional information on this variant. No additional pathogenic mutations, variants of unknown significance, or gross deletions or duplications were detected. Genes Analyzed (32 total): **APC, ATM, BARD1, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDKN2A, CHEK2, MLH1, MSH2, MSH6, MUTYH, NF1, NTHL1, PALB2, PMS2, PTEN, RAD51C, RAD51D, SMAD4, STK11 and TP53** (sequencing and deletion/duplication); **AXIN2, HOXB13, MSH3, POLD1, POLE and RPS20** (sequencing only); **EPCAM and GREM1** (deletion/duplication only). RNA data is routinely analyzed for use in variant interpretation for all genes.

Comment(s): The *BRCA2* "c.7762_7764delinsTT (p.Ile2588Phefs*60)" variant, which was previously identified in this individual's relative(s) per provided document(s), was not detected in this individual's specimen.

Characterize the tumor

- Next-generation sequencing (NGS)
 - Looks for mutations in the DNA of the cancer (**somatic**)
 - **Driver mutation:** at least partly responsible for driving the growth of the cancer
 - E.g. PIK3CA, AKT1, PTEN
 - **Resistance mutation:** evolves out of selection pressure and predicts resistance to certain types of treatments
 - E.g. ESR1 predicts resistance to aromatase inhibitors
 - **Bystander mutations**
 - Often multiple mutations picked up that have no actionable role in breast cancer and may be unrelated – these are ‘bystanders’ and not relevant to treatment

Next Generation Testing - Sources

- Tissue-based
 - Involves collection of cancer cells via needle biopsy vs collection of pleural fluid or malignant fluid
 - **The only way to get receptors (cannot get through blood test)**
 - Invasive procedure hence risk for bleeding, infection, etc. (albeit low risk)
 - Always necessary at diagnosis but usually *not* necessary at subsequent progression events
- Liquid Biopsy
 - Blood test that collects circulating tumor DNA and then sequences for mutations

Next Generation Testing - Tissue Biopsy

Results with Therapy Associations

BIOMARKER	METHOD	ANALYTE	RESULT	THERAPY ASSOCIATION		BIOMARKER LEVEL*
ERBB2 (Her2/Neu)	IHC	Protein	HER2-Low Score 1+	BENEFIT	fam-trastuzumab deruxtecan-nxki	Level 1
PIK3CA	Seq	DNA-Tumor	Pathogenic Variant Exon 10 p.E545K	BENEFIT	alpelisib + fulvestrant	Level 1
					capivasertib + fulvestrant, inavolisib + fulvestrant + palbociclib	Level 2
ER	IHC	Protein	Positive 2+, 70%	BENEFIT	abemaciclib, palbociclib, ribociclib	Level 2
					endocrine therapy	Level 2
					everolimus	Level 2
ER/PR/Her2/Neu	IHC	Protein	HR-Positive HER2-Low	BENEFIT	datopotamab deruxtecan, sacituzumab govitecan	Level 2

Sample Caris Report

Cancer-Type Relevant Biomarkers

Biomarker	Method	Analyte	Result
AR	IHC	Protein	Positive 1+, 50%
BRAF	Seq	DNA-Tumor	Mutation Not Detected
MSI	Seq	DNA-Tumor	Stable
NTRK1/2/3	Seq	RNA-Tumor	Fusion Not Detected
RET	Seq	RNA-Tumor	Fusion Not Detected
Genomic LOH	Seq	DNA-Tumor	Low
Tumor Mutational Burden	Seq	DNA-Tumor	Low, 3 mut/Mb
AKT1	Seq	DNA-Tumor	Mutation Not Detected
BRCA1	Seq	DNA-Tumor	Mutation Not Detected
BRCA2	Seq	DNA-Tumor	Mutation Not Detected
ERBB2 (Her2/Neu)	CNA-Seq	DNA-Tumor	Amplification Not Detected
	Seq	DNA-Tumor	Mutation Not Detected

Biomarker	Method	Analyte	Result
ESR1	Seq	DNA-Tumor	Mutation Not Detected
		RNA-Tumor	Fusion Not Detected
FGFR1	Seq	RNA-Tumor	Fusion Not Detected
FGFR2	Seq	RNA-Tumor	Fusion Not Detected
FGFR3	Seq	RNA-Tumor	Fusion Not Detected
MTAP	CNA-Seq	DNA-Tumor	Deletion Not Detected
NF1	Seq	DNA-Tumor	Mutation Not Detected
PALB2	Seq	DNA-Tumor	Mutation Not Detected
PD-L1 (22c3)	IHC	Protein	Negative, CPS: 0
PR	IHC	Protein	Negative 0
PTEN	CNA-Seq	DNA-Tumor	Deletion Not Detected
	IHC	Protein	Positive 1+, 100%
	Seq	DNA-Tumor	Mutation Not Detected

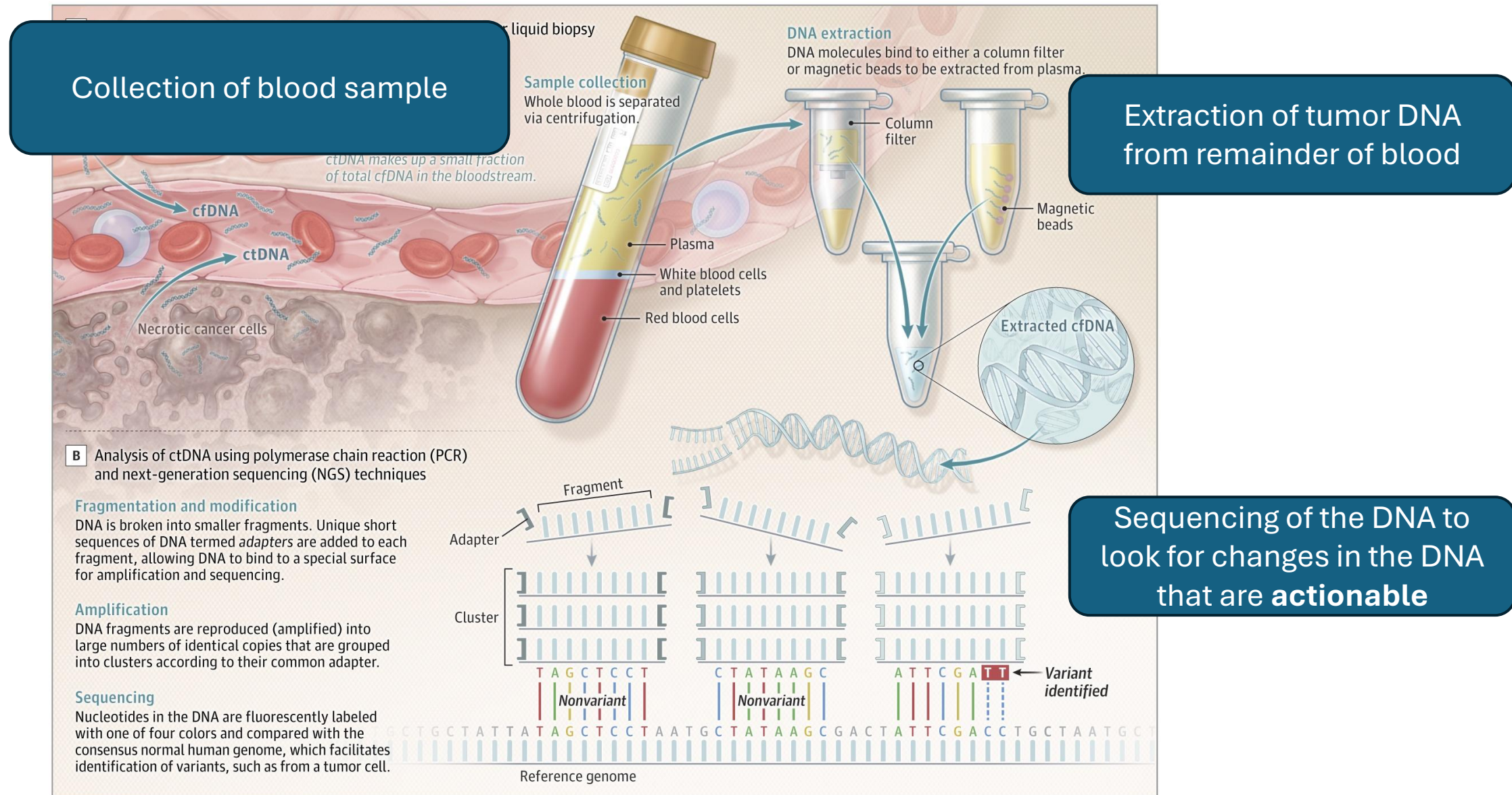
Genomic Signatures

Biomarker	Method	Analyte	Result
Microsatellite Instability (MSI)	Seq	DNA-Tumor	Stable
Tumor Mutational Burden (TMB)	Seq	DNA-Tumor	Result: Low 3 Low 10 High
Genomic Loss of Heterozygosity (LOH)	Seq	DNA-Tumor	Low - 0% of tested genomic segments exhibited LOH (assay threshold is ≥ 16%)

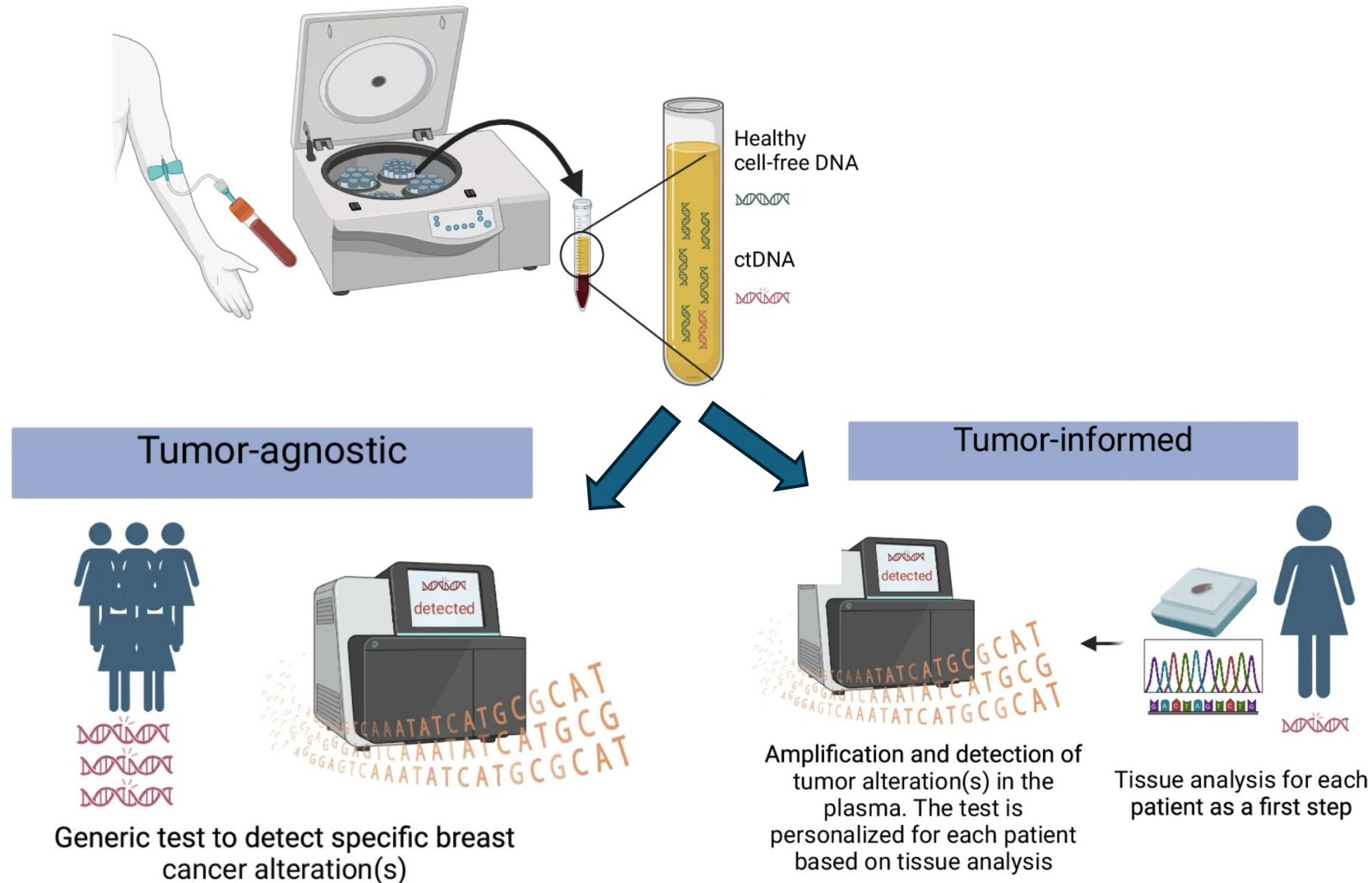
Genes Tested with Pathogenic or Likely Pathogenic Alterations

Gene	Method	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %
PMS2	Seq	DNA-Tumor	Pathogenic Variant	p.S46I	2	c.137G>T	45

Next Generation Testing – Liquid Biopsy



Liquid Biopsy – Tumor Agnostic vs Tissue Informed



Next Generation Testing – Liquid Biopsy

Sample Guardant360 Report

Potentially
actionable!

Summary of Genomic & Epigenetic Biomarkers with Associated Treatment Options			
✓ Approved in indication ~ Approved in other indication ✕ Lack of response			
DETECTED ALTERATION(S) / BIOMARKER(S)	ASSOCIATED FDA-APPROVED THERAPIES	CLINICAL TRIALS (SEE PAGE 14)	% CFDNA OR COPY NUMBER
ESR1 E380Q	✓ Elacestrant, Imlunestrant ✕ Anastrozole, Exemestane, Letrozole	Yes	0.2%
PIK3CA H1047R	✓ Alpelisib+fulvestrant, Capivasertib+fulvestrant, Inavolisib+palbociclib+fulvestrant	Yes	3.6%
PIK3CA K111N	✓ Inavolisib+palbociclib+fulvestrant ~ Alpelisib+fulvestrant, Capivasertib+fulvestrant	Yes	0.3%
NF1 S1766*	~ Mirdametinib, Selumetinib	Yes	0.4%
PIK3CA E81K	~ Alpelisib+fulvestrant, Capivasertib+fulvestrant, Inavolisib+palbociclib+fulvestrant	Yes	0.1%
NF1 S979Tfs*5	~ Mirdametinib, Selumetinib	Yes	0.9%
FGFR2 Rearrangement	~ Erdafitinib, Futibatinib, Pemigatinib	Yes	2.7%
APC Q1928*	None	Yes	0.8%
SETD2 S1658*	None	Yes	0.6%
RIT1 M90I	None	Yes	0.3%
PTCH1 Q156*	None	Yes	0.3%
TP53 V272M	None	Yes	0.2%
TP53 R175H	None	No	2.6%
TP53 R273H	None	No	0.3%
GATA3 Splice Site SNV	None	No	0.2%

Next Generation Testing – More than just mutations!

- Pharmacogenomics
 - Tests key enzymes involved in processing certain drugs
 - Slow metabolizers may need dose adjustment of certain chemotherapies
 - E.g. DPYD for Capecitabine, UGT1A1*28 for Sacituzumab
- Tumor Mutational Burden – predictor of response to immunotherapy
- Tumor fraction % - quantifies tumor DNA that can be trended across time / samples
- Can now offer prediction for type of cancer based on DNA profile and extensive database on prior cancers tested

Next Generation Testing – Liquid Biopsy

Additional Biomarkers

Tumor Mutational Burden (TMB)	157.63 mut/Mb
MSI-High	Not detected
Homologous Recombination Deficiency (HRD)	Genomic Instability Status Not detected
	HRR mutations Not detected

Predicts immunotherapy response

Tumor Fraction [‡]	2.4% - Based on 1706 patient-specific biomarkers
-----------------------------	--

Can trend tumor fraction across samples

[‡] Tumor fraction is defined as the proportion of tumor molecules present in the cfDNA within the submitted specimen and is based on epigenomic signals.

Patient Biomarkers

Pharmacogenomics[‡]

CYP2D6 *1/*3 Intermediate metabolizer

This genotype results in intermediate CYP2D6 enzyme activity. The oncology drugs tamoxifen and gefitinib are expected to be metabolized at a slower rate than a normal metabolizer, but more rapidly than a poor metabolizer. See guidelines (pharmgkb.org) for additional information. Activity score: 1.0

UGT1A1 *1/*28 Intermediate metabolizer

This genotype results in intermediate UGT1A1 enzyme activity. Irinotecan is expected to be metabolized at a level between normal and poor metabolizers leading to increased toxicity, such as neutropenia or hyperbilirubinemia. See guidelines (pharmgkb.org) for additional information.

DPYD, HLA-B, TPMT No abnormal variant detected in reportable alleles (see Interpretations)

Human Leukocyte Antigen (HLA) Genotyping

HLA-A: A*03:01, A*11:01

HLA-B: B*07:02, B*35:01

HLA-C: C*04:01, C*07:02

Results reflect evaluable alleles in *HLA-A*, *-B*, and *-C*. HLA Class I results are intended as an aid for oncology therapy selection and/or clinical trial matching and are not validated for transplant allele typing.

Predicts possible increased toxicity from drugs like Sacituzumab Govitecan

[‡] Results reflect evaluable alleles in CYP2D6, DPYD, TPMT, UGT1A1, and HLA-B*57:01 (See Interpretation Section).

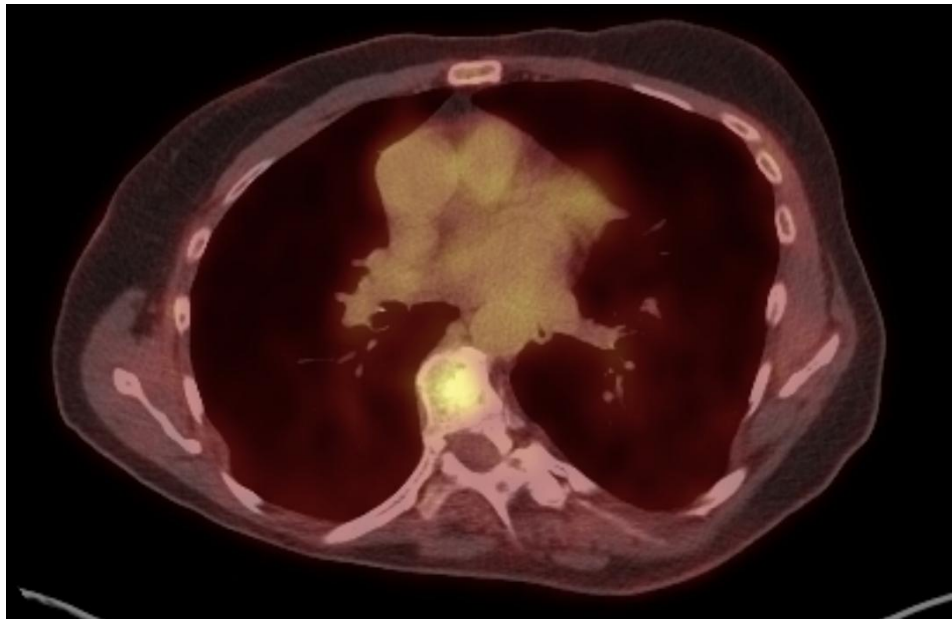
Next Generation Testing – Case from Clinic

- 70 year old woman with metastatic ER+ HER2- breast cancer
- Had received letrozole and Ribociclib for 3 years, and then had progression in the bones
- A liquid biopsy was performed

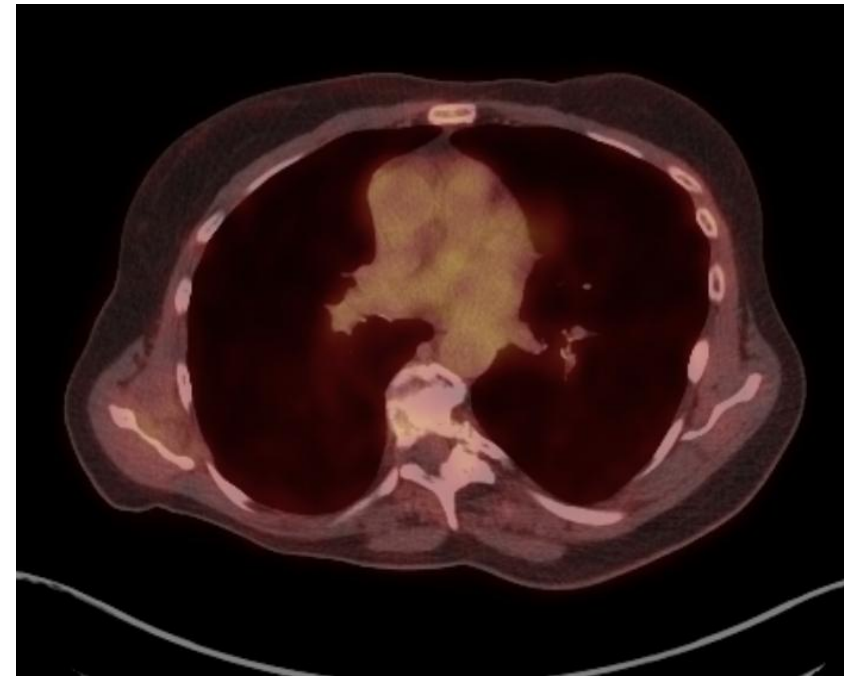
☒ Approved in indication ☐ Approved in other indication ☒ Lack of response			
DETECTED ALTERATION(S) / BIOMARKER(S)	ASSOCIATED FDA-APPROVED THERAPIES	CLINICAL TRIALS (SEE PAGE 6)	% CFDNA OR COPY NUMBER
ESR1 D538G	☒ Elacestrant ☒ Anastrozole, Exemestane, Letrozole	Yes	0.05%
PIK3CA H1047L	☒ Alpelisib+fulvestrant, Capivasertib+fulvestrant, Inavolisib+palbociclib+fulvestrant	Yes	0.05%
BRCA1 L1229fs	☐ Niraparib, Olaparib, Rucaparib, Talzaparib	Yes	0.2%
NF1 T36fs	☐ Selumetinib	Yes	0.06%
ARID1A Splice Site SNV	None	Yes	0.5%
KIT N822I	None	Yes	0.4%
CDH1 V17fs	None	No	0.3%

Next Generation Testing – Case from Clinic, Cont'd

- She was thus initiated on Elecestrant, an oral targeted therapy, due to the ESR1 mutation



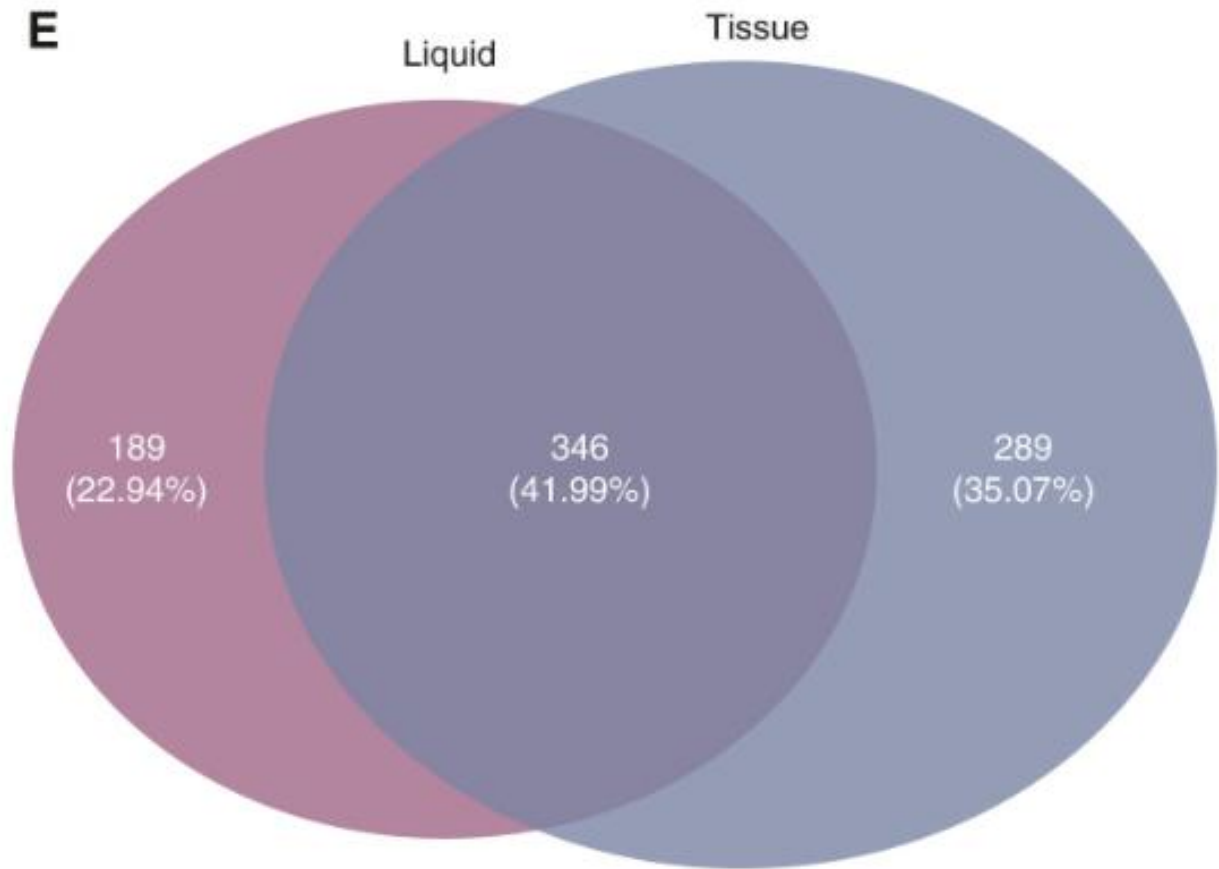
Progression in Bones



First follow up scan after starting Elecestrant

Liquid vs Tissue – what's better?

- Helpful to get both at diagnosis with tissue biopsy
- Liquid and Tissue biopsy each able to pick up exclusive mutations
- Subsequent time points, liquid is more practical and non-invasive



Tumor Markers

- Proteins that may be produced by breast cancers and measured
 - **Cancer Antigen 15-3**
 - **Cancer Antigen 27.29**
- Helpful if elevated at baseline – may be collected at regular intervals and tends to track with response or progression of the breast cancer on current treatment
 - Can trigger moving up a scan sooner if markers are rising fast
- Some cancers do not produce enough levels to measure and therefore not useful to check
- We do NOT generally change treatment based on tumor marker rise alone; it aids but does not supersede imaging

Interval summary

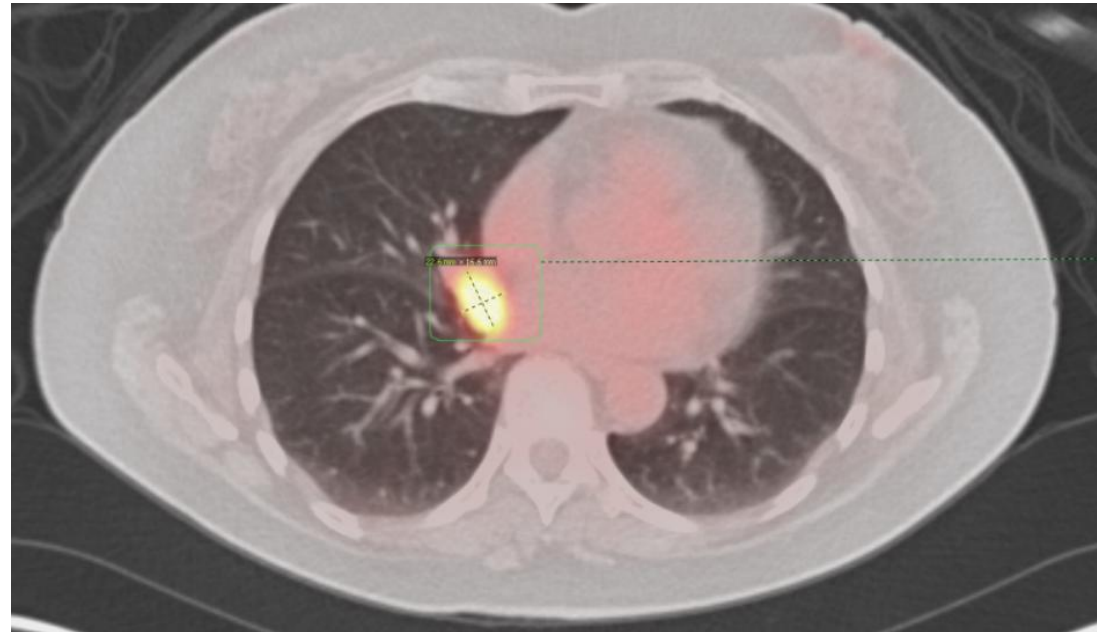
- At diagnosis
 - **Biopsy a tumor site** to confirm breast cancer and receptors
 - **Send NGS on tumor tissue and liquid biopsy**
 - **Get germline testing (if not done before, or done remotely e.g. before 2015 when panels were more limited)**
 - **Check baseline CA 15-3 and CA 27.29 tumor markers for future tracking**

Imaging as a biomarker

- Standard imaging
 - CT Chest Abdomen Pelvis + Bone Scan (**Anatomic**)
 - FDG PET/CT – uses modified glucose radiotracer, which accumulates more in cancer cells than healthy cells (**Functional**)
 - FES PET/CT – **Fluoroestradiol PET**
 - Radio-labeled estradiol binds to estrogen receptor – *specific for ER+ breast cancer*
 - Useful for diagnostic trouble shooting
 - Can be useful in predicting likelihood for response to anti-estrogen-based treatment
 - Cannot be done if patient taking fulvestrant or tamoxifen

Imaging as a biomarker – Comparison of Imaging

- 70 year old woman diagnosed with an ER+ HER2+ LEFT breast cancer involving the left axillary lymph nodes
- A staging CT scan showed a solitary lung nodule, but location of the nodule made biopsy difficult
- Given that the cancer expressed ER, an FES PET/CT was done to further work this up
- Nodule was FES positive
- Confirmed metastatic diagnosis



Putting it all together – Real World Cases

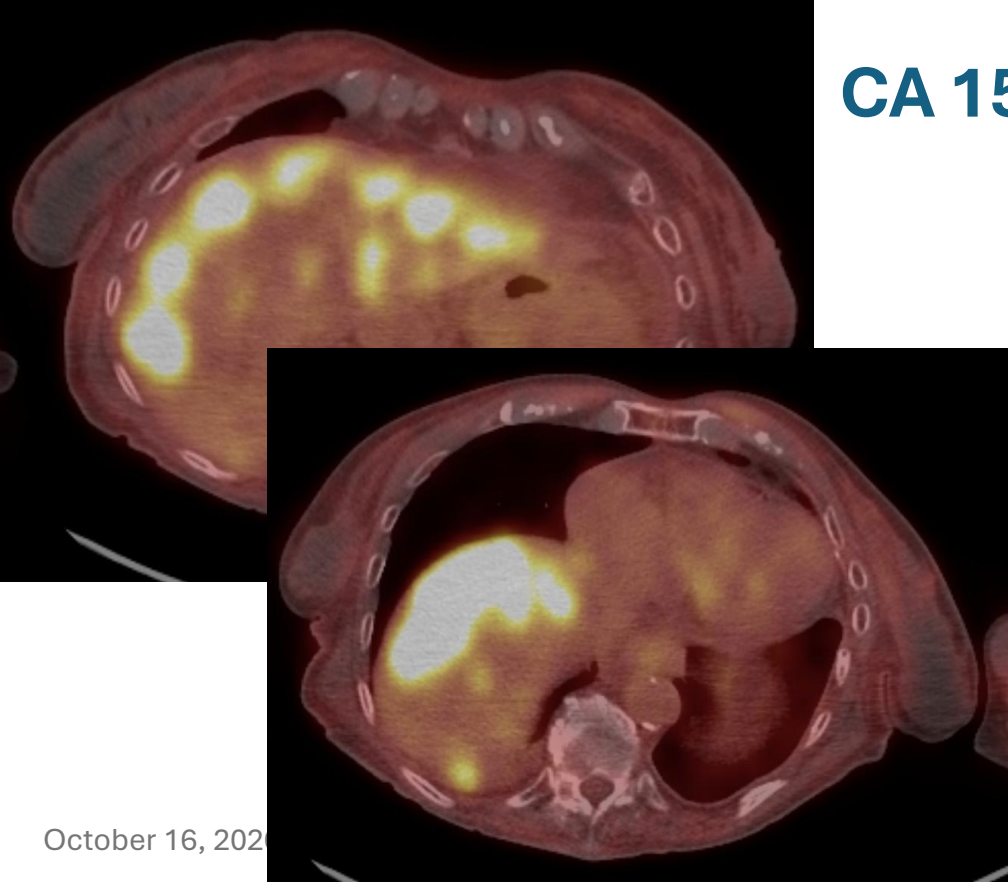
Case #1 - Utilizing Liquid Biopsy for Treatment Decisions

74 YEAR OLD WOMAN WITH THE FOLLOWING TREATMENT HISTORY FOR ER+ HER2- MBC

09/2009-07/2011: letrozole + zoledronic acid (22 months)
01/2010-07/2013: capecitabine (43 months)
07/2013-10/2013 Fulvestrant (3 months)
10/2013- 12/2016: Exemestane + Everolimus (38 months)
12/2016-05/2017: Letrozole + Palbociclib (6 months)
05/2017-11/2020: Abraxane (42 months)
11/2020-02/2021: Gemcitabine (3 months)
02/2021-07/2021: Eribulin (5 months)
07/2021-02/2023: Tamoxifen (18 months)
02/2023-04/2023: Fulvestrant (2 months)
04/2023-07/2023: Elecestrant (3 months)
08/2023-10/2023: Sacituzumab (3 months)
10/2023-01/2024: Adriamycin weekly (3 months)
01/2024-10/2024: Abemaciclib (10 months)

Case #1 - Utilizing Liquid Biopsy for Treatment Decisions

PET/CT in 10/2024 with progression in Liver



CA 15-3 1,275

Guardant360 10/2019

Summary of Somatic Alterations & Association

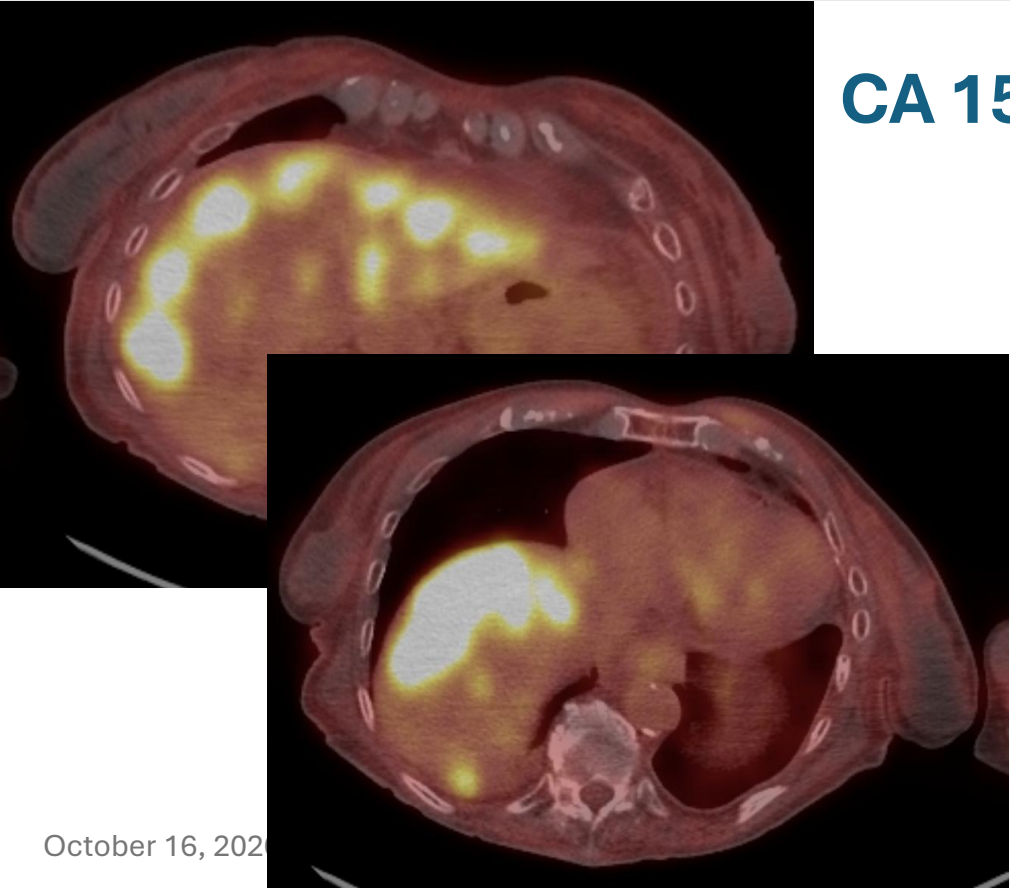
KEY ☒ Approved in indication ☒ Approved in other indication

Alteration	% cfDNA or Amplification
GNAS R201H	0.2%

October 16, 2024

Case #1 - Utilizing Liquid Biopsy for Treatment Decisions

PET/CT in 10/2024 with progression in Liver



CA 15-3 1,275

Updated Guardant360 10/2024

DETECTED ALTERATION(S) / BIOMARKER(S)	% CFDNA OR COPY NUMBER
<i>PTEN</i> R161fs	8.0%
<i>FGFR2</i> K659M	3.8%
<i>GNAS</i> R201H	0.4%

October 16, 2024

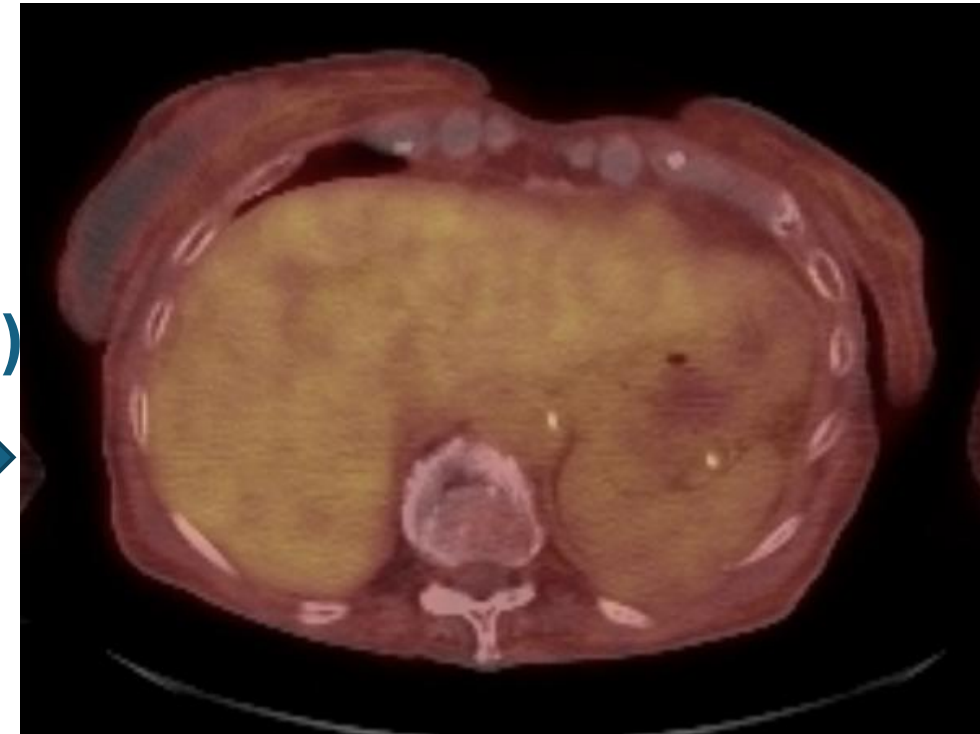
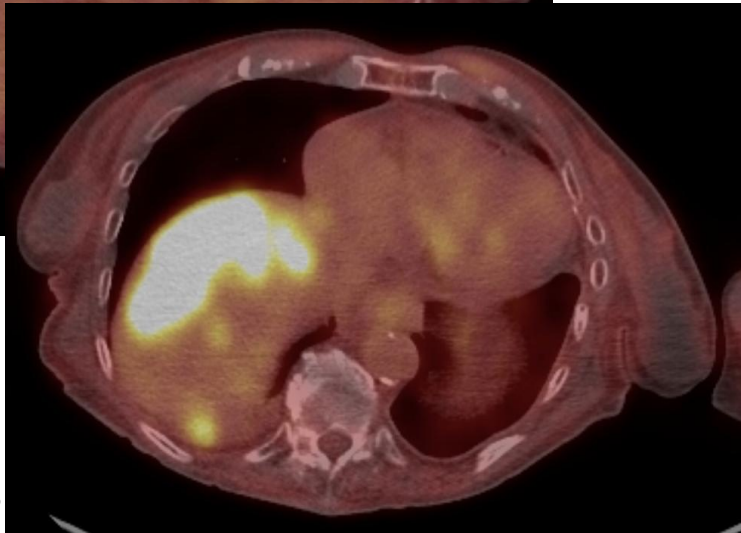
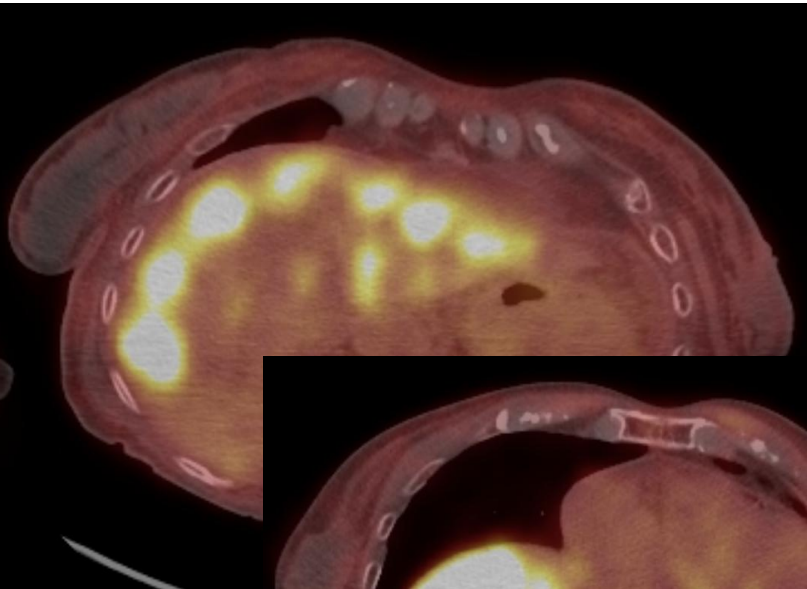
Case #1 - Utilizing Liquid Biopsy for Treatment Decisions

PET/CT in 10/2024 with progression in Liver

CA 15-3 1,275

**Capivasertib
Fulvestrant
(CAPItello-291)**

PET/CT in 01/2025 CA 15-3 122



Case #2 – Liquid Biopsy Can Pickup Germline

- 67-year-old woman with ER+ HER2- metastatic breast cancer who transferred her care to Penn after moving from Jamaica
- Was on first line Palbociclib + Letrozole for 2 years and then developed progression in the lungs with pleural effusion
- A liquid biopsy was performed

Case #2 – Liquid Biopsy Can Pickup Germline

Summary of Detected Somatic Alterations, Immunotherapy Biomarkers & Associated Treatment Options

KEY  Approved in indication  Approved in other indication  Lack of response

Detected Alteration(s) / Biomarker(s)	Associated FDA-approved therapies	Clinical trial availability (see page 3)	% cfDNA or Amplification
BRCA2 E2028fs	 Olaparib, Rucaparib, Talazoparib	Yes	49.5%

Variants of Uncertain Clinical Significance

PIK3CA L37V (0.2%)

The functional consequences and/or clinical significance of alterations are unknown. Relevance of therapies targeting these alterations is uncertain.

- Notice the "% cfDNA" is 49.5%, which is ~50%, consistent with a presumed *germline* mutation
- **Confirmatory germline testing showed:**

BRCA1/2 Analyses with CustomNext-Cancer® +RNAinsight®

RESULTS

BRCA2 Pathogenic Mutation: c.6082_6086delGAAGA

BRIP1 Pathogenic Mutation: c.2990_2993delCAAA

SUMMARY

POSITIVE: Pathogenic Mutations Detected

INTERPRETATION

Case #2 – Liquid Biopsy Can Pickup Germline

Picking up a germline mutation in BRCA2 then opened up the option of an oral targeted treatment called Olaparib which is specifically designed to target cancers with a BRCA1 or BRCA2 *germline* mutation

Case #3 – Incorporating both liquid biopsy and FES imaging for treatment decision

- 67 year old woman with ER+ HER2-low metastatic breast cancer
- Has received several lines of therapy including:
 - Letrozole + Palbociclib
 - Fulvestrant
 - Everolimus + Fulvestrant
 - Capecitabine
 - Enhertu
 - Abraxane
 - Gemcitabine + Carboplatin

Case #3 – Incorporating both liquid biopsy and FES imaging for treatment decision

- Unfortunately the cancer had not responded to last 4 lines of chemotherapy (Xeloda, Enhertu, Abraxane, Gemcitabine/Carboplatin)
- A repeat liquid biopsy was performed

Biomarker(s)	Level	Associated FDA-Approved Therapies
ESR1 Y537S	21.7%	☒ Elacestrant, Imlunestrant, Vepdegestrant ☐ Anastrozole, Exemestane, Letrozole
AKT1 E17K	1.3%	☒ Capivasertib+fulvestrant
Sample Summary	Tumor Fraction : 37.1% - Based on 2866 patient-specific epigenomic biomarkers.	

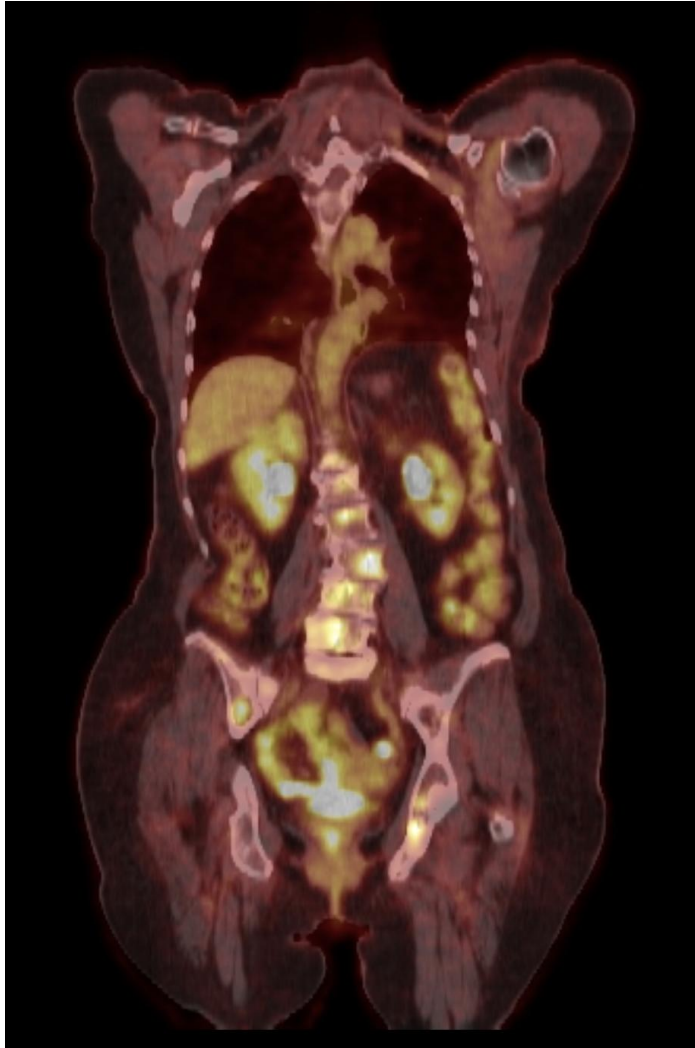
TWO targetable mutations

Case #3 – Incorporating both liquid biopsy and FES imaging for treatment decision

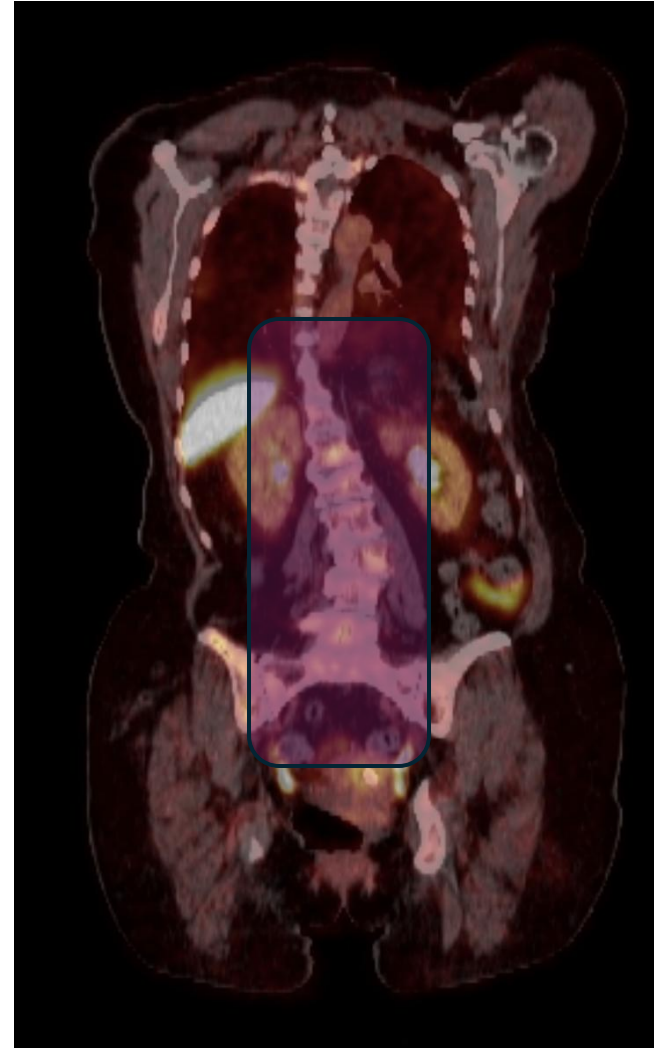
- Given that this patient had already received several lines of anti-estrogen based treatments, we wanted to gain more supporting evidence that this cancer could still respond to further anti-estrogen approaches
- **There is building evidence that if FES PET/CT is negative, the likelihood of response to further anti-estrogen approach is $\leq 11\%$**
- We thus performed FES PET/CT

Case #3 – Incorporating both liquid biopsy and FES imaging for treatment decision

FDG PET/CT



FES PET/CT

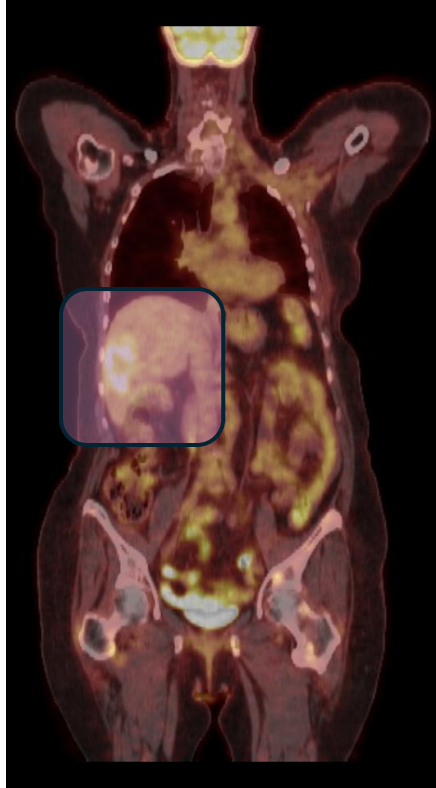


FES PET/CT shows that the bone are “FES AVID” i.e. express the estrogen receptor

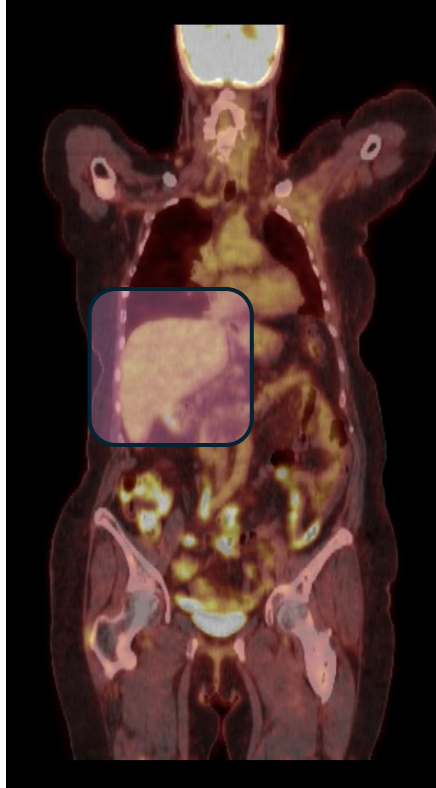
Case #3 – Incorporating both liquid biopsy and FES imaging for treatment decision

Treatment was thus initiated with Imlunestrant (an oral SERD approved for ESR1 mutation) + Abemaciclib

Liver Response

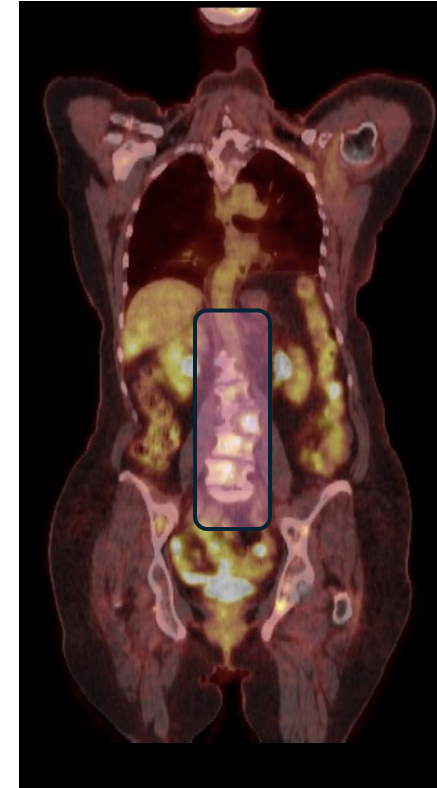


Baseline

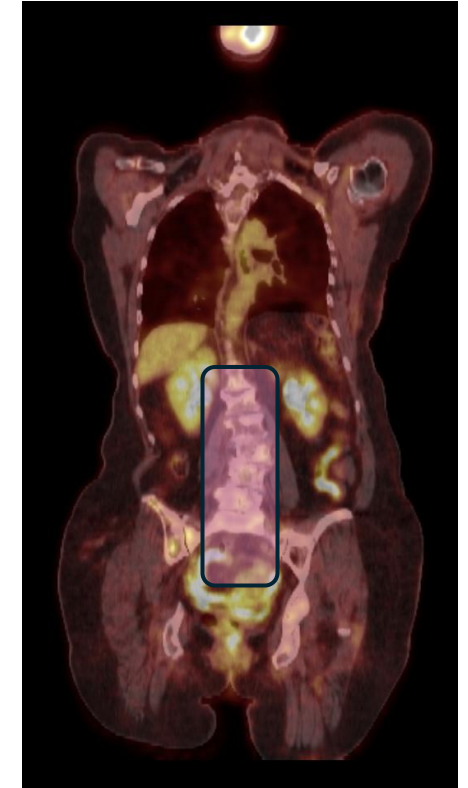


3 month follow up

Bone Response



Baseline



3 month follow up

Summary

- Molecular Testing is now **essential** in the treatment of metastatic breast cancer
- **Always** confirm initial diagnosis with a tissue biopsy if possible, with receptors and next-generation sequencing to guide initial and subsequent therapy
- **Molecular testing can be repeated at major decision-branches for treatment selection, with liquid biopsy preferred as a non-invasive approach**
- Pace of research and improvements in technologies advancing rapidly => **increasingly tailored and specific plan for patients!**

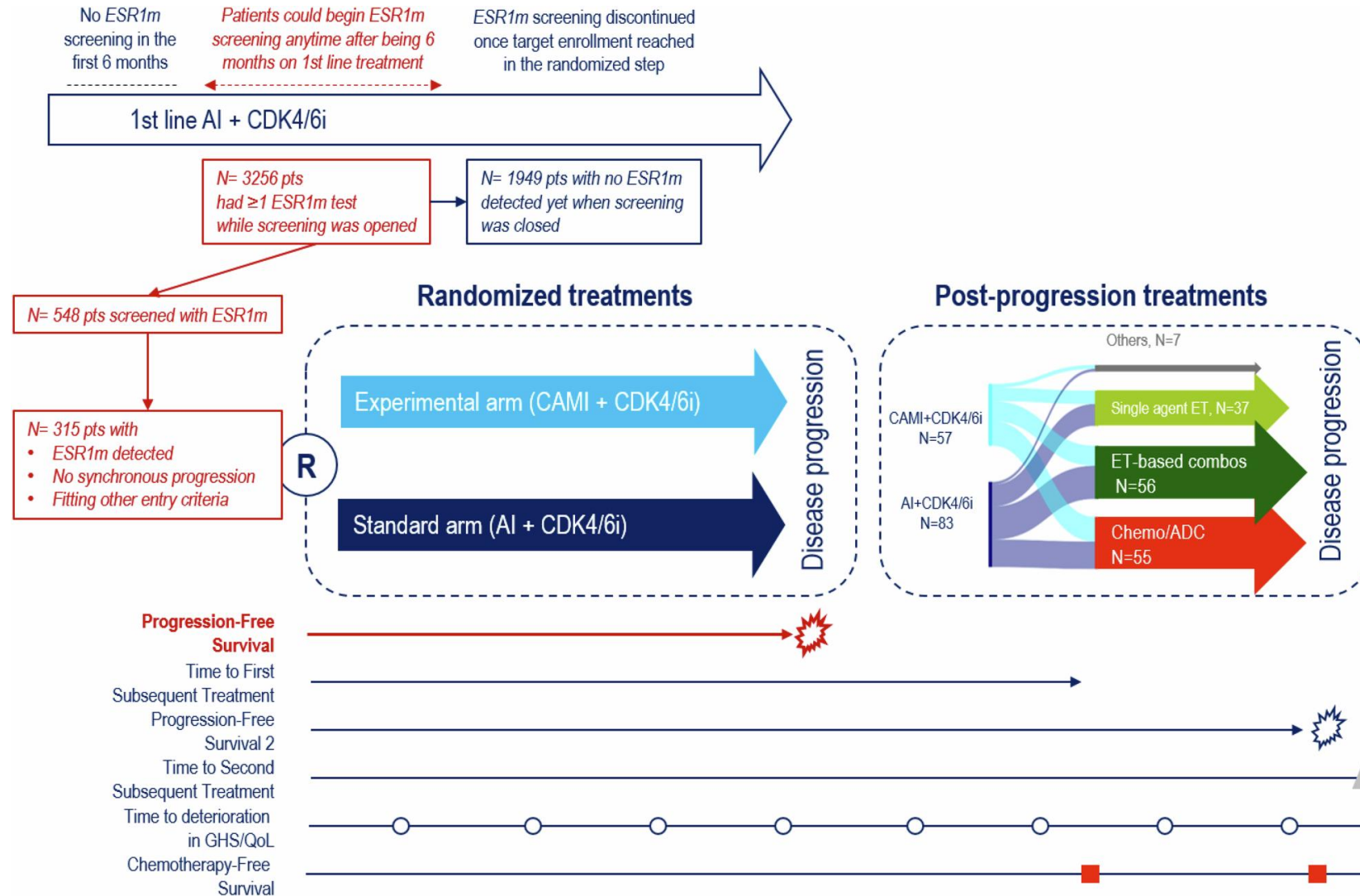
Questions?

Appendix

Evolving paradigms...

- SERENA 6 - Serial testing of ESR1 for preemptive switch
- Using ctDNA to measure response to therapy

Serial ctDNA monitoring – SERENA 6



Serial ctDNA monitoring – SERENA 6

- Clinical trial met its endpoint of prolonging time on ‘first-line’ treatment before progression on scans
- Demonstrated improved quality of life in those who switched to camizestrant upon testing positive for ESR1
- Represents a more targeted approach to tailoring treatment
- *Still not clear if sequencing oral SERD in later line is just as good or even better*
- *Later line oral SERD with CDK4/6i combinations were not approved at time of trial – these are now approved and can be highly effective*
- *Cost of serial testing could be a barrier*
- *‘Scanxiety’*
- *Reasonable to discuss with oncologist but guidelines are NOT clear on this as the right approach for everybody*

Monitoring ctDNA for Treatment Response

- Tests such as Signatera use tissue biopsy to generate a panel of mutations **specific to the patient's cancer** rather than generic 'off the shelf' panel of mutations
- More sensitive for detecting 'level' of tumor-specific DNA
- The 'clearance' or lack thereof is *prognostic* – i.e. clearance of ctDNA with new treatment predicts a longer period of treatment response

Monitoring ctDNA for Treatment Response

- The *trend* of quantitative circulating tumor DNA may provide insight into treatment response, complementary to standard periodic imaging
- Is it **actionable**? Do not yet have evidence to support early switch
- **May be helpful where cancer is difficult to image e.g. Lobular breast cancer**

