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See the complete picture

of HR-positive, HER2-negative advanced Breast Cancer

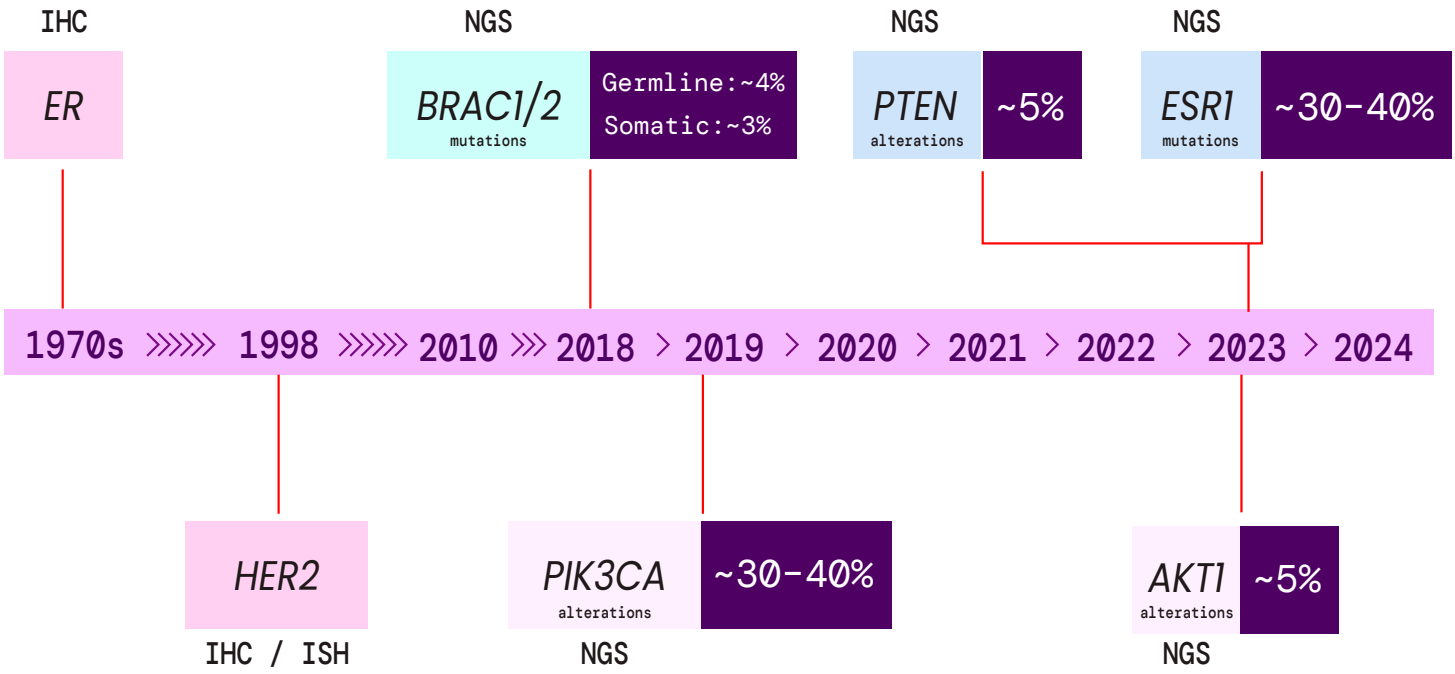
Biomarker testing in HR-positive, HER2-negative aBC.
A clinician's guide to report interpretation and consultation

GB-63597 | April 2025

HR-positive, HER2-negative aBC is not just one disease

HR-positive, HER2-negative aBC is a biomarker / genomic alteration-defined disease, characterised by molecular heterogeneity.[1,2] Individualised approaches to treatment are possible with targeted therapies for different molecular subtypes, offering eligible patients potentially better clinical outcomes.[2]

Timeline of the key actionable* biomarkers in HR-positive, HER2-negative aBC and their prevalences[3–11]



IHC[3,10]

- Detects cell surface protein over-expression e.g. ER and HER2
- Used to subclassify the tumour

Germline testing[10]

- Identifies inherited genomic alterations (e.g. *BRACA1/2*)
- Can guide screening and treatment

Tumour genomic testing[2,10]

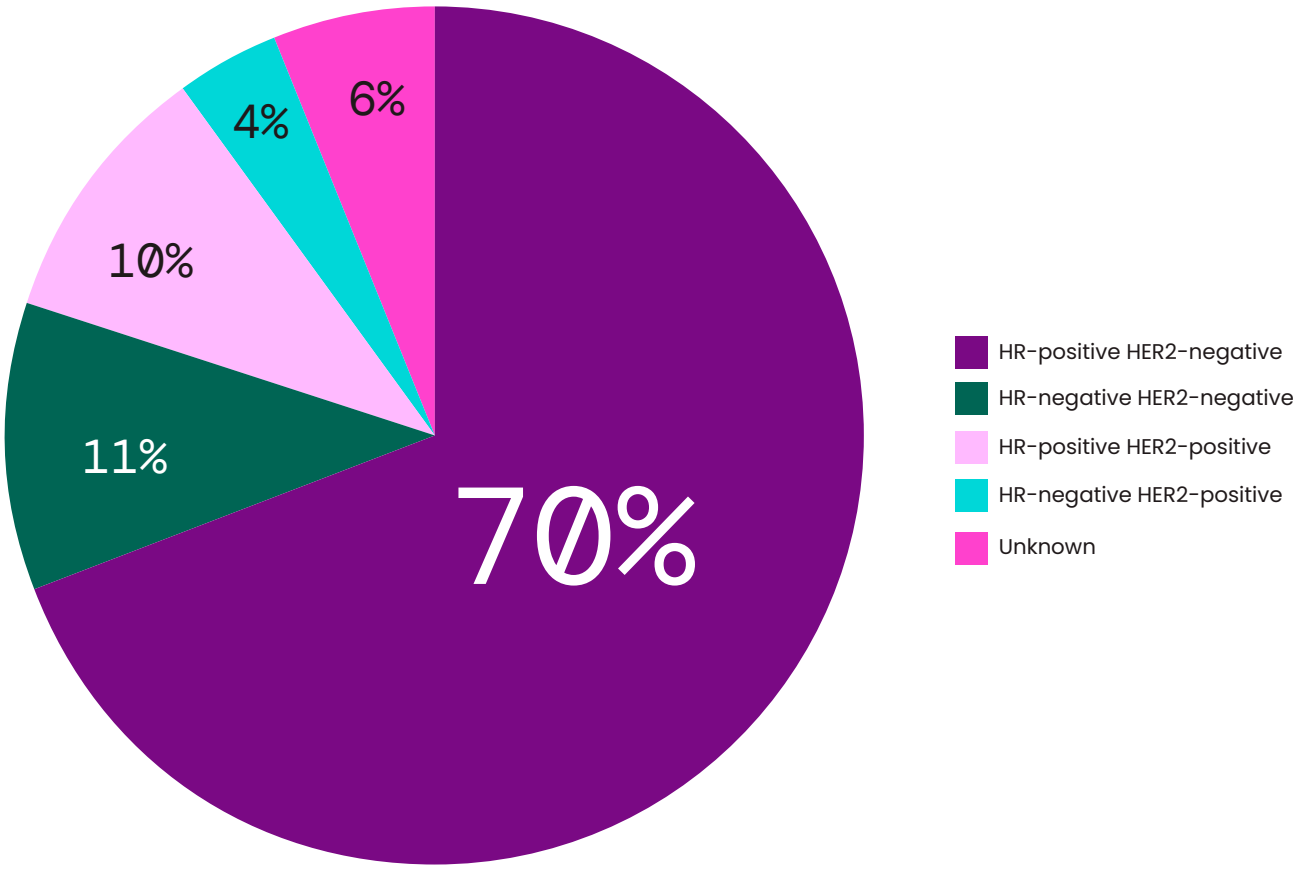
- Identifies genomic alterations in the tumour DNA / RNA e.g. NGS testing for *PIK3CA/AKT1/PTEN*
- Provides predictive / prognostic information and guides treatment

*Actionable denotes that there is an approved targeted therapeutic option for these biomarkers.

The most common traditional breast cancer subtype

HR-positive, HER2-negative breast cancer is one of the four traditional breast cancer subtypes identified by IHC.

Distribution of traditional breast cancer subtypes identified by IHC[12]



Broad molecular profiling using Next-Generation Sequencing

Next-Generation Sequencing (NGS) is a type of 'high throughput' sequencing technology that uses parallel sequencing of multiple small fragments of DNA or RNA. It allows rapid sequencing of large amounts of genetic material.[16]

Broad molecular profiling using NGS can simultaneously detect all four classes of genomic alterations:

- Single nucleotide variants (also known as point mutations)
- Small duplications, insertions / deletions involving one or a few nucleotides, or more complex mutations
- Exon or gene copy number changes, including amplifications or deletions
- Structural variants or large structural anomalies of genetic material, which often result in fusion genes[17]

In addition, broad molecular profiling with NGS can detect molecular signatures (e.g. TMB, MSI), which is not possible with single-gene testing (e.g. PCR), or multigene hotspot NGS.[18,19]

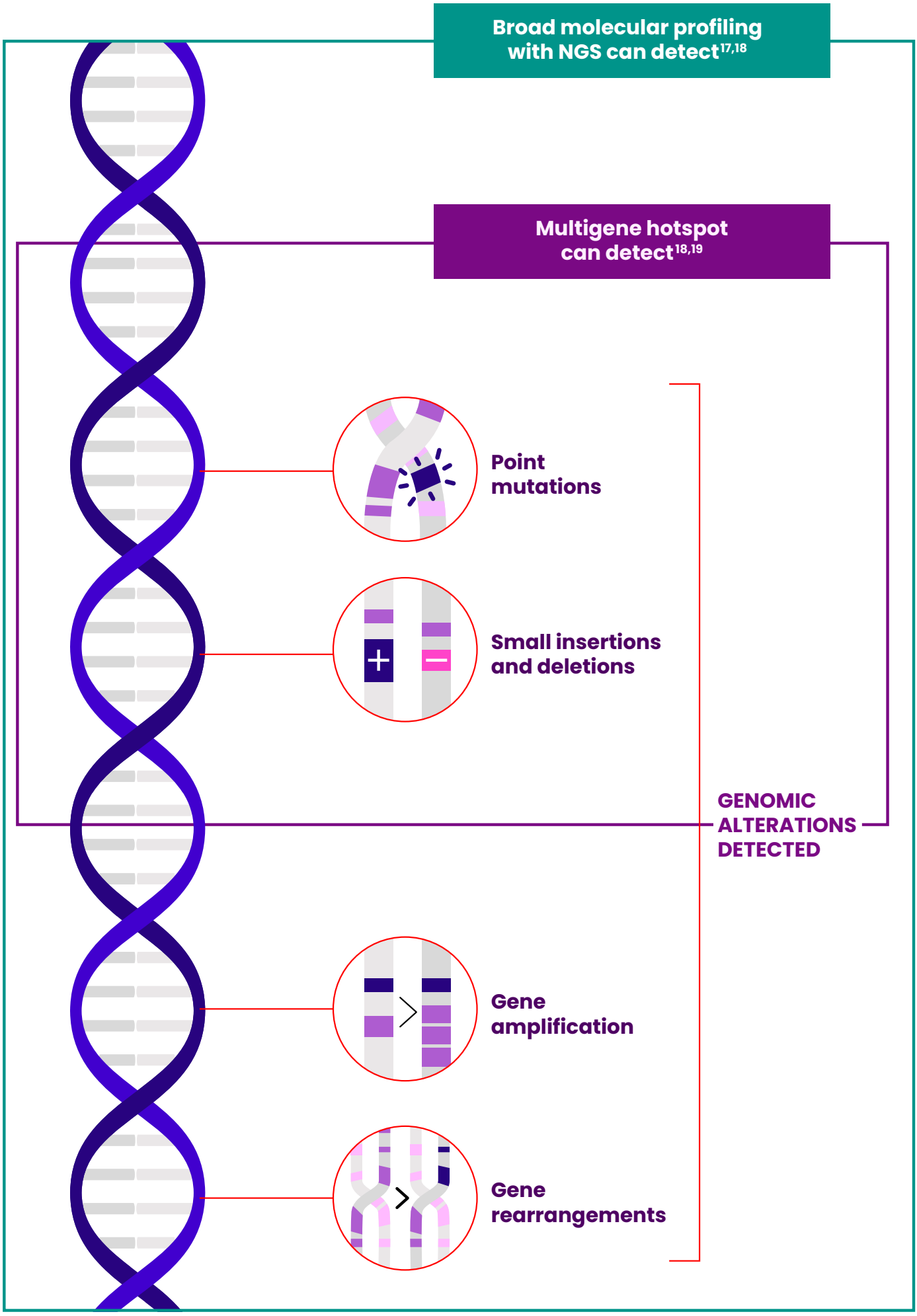
Several commercially available NGS-based assays are validated to detect key biomarkers in HR-positive, HER2-negative aBC (including FoundationOne® CDx [FDA-approved CDx], FoundationOne® Liquid CDx, or Guardant360® CDx).[18,20,21] Validated-in-house methods may also be used.[18,21]

Broad molecular profiling using NGS is an efficient method for identifying key biomarkers in breast cancer.[9]

Clinical testing guidelines endorse NGS testing in HR-positive, HER2-negative aBC[2,9,19]

- National Comprehensive Cancer Network (NCCN) Clinical Guidelines in breast cancer recommend testing for *PIK3CA/AKT1/PTEN* alterations and *ESR1* mutations using NGS in HR-positive HER2-negative aBC.[2]
- ESMO Precision Working Group recommends carrying out tumour NGS as standard of care in HR-positive HER2-negative aBC.[9]
- ASCO somatic genomic testing guidelines recommend that multigene panel-based testing* should be considered part of the standard evaluation.[19]

* Multigene panel-based testing assays should be used if more than one biomarker-linked therapy is approved for the patient's disease.


Broad molecular profiling with NGS can detect[17,18]
Multigene hotspot can detect[18,19]
Point mutations
Small insertions and deletions
Gene amplification
Gene rearrangements
GENOMIC ALTERATIONS DETECTED

Benefits of NGS testing in breast cancer

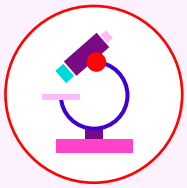
NGS is a powerful tool for comprehensive genomic analysis and is tissue-, time- and cost-efficient.[22] It can identify patients who harbour an actionable biomarker and are eligible for approved biomarker-associated therapies.[22] In addition, multigene panel-based testing with NGS can identify patients with genomic alterations who may benefit from a tumour agnostic precision medicine or be eligible for a clinical trial.[2,9,19,22]

Benefits

- Multiple genes can be tested simultaneously, potentially saving time, money, and tissue[24]
- Previously unknown disease markers may be discovered[24]
- The broad information collected may help accelerate research[24]
- Direct assessment of the tumour[25]

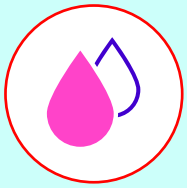
Limitations

- Large amount of complex data generated which may be difficult to interpret[24]
- Takes longer and is more expensive than testing for a single biomarker[24]
- Requires sufficient quality and quantity of tumour tissue[26]
- Not able to fully capture spatial and temporal tumour heterogeneity[23]



Tissue biopsy
Considered the 'gold standard' for diagnostic and biomarker testing.[23]

2–3 weeks turnaround time for NGS.[24]



Liquid biopsy
Liquid biopsy testing is also a potential option for testing that can be complementary to tumour tissue testing and is useful in certain circumstances, such as capturing tumour heterogeneity and clonal evolution (i.e. for *ESR1*m testing), and is guideline recommended (NCCN, ESMO, ASCO).[2,3,9,19]

NGS workflow from diagnosis to treatment recommendations[27,28]

Sample assessment, storage and processing

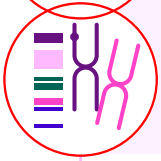
01 Following patient consent, biopsies are taken and processed in a certified laboratory using standardised protocols and storage methods.

Molecular profiling and bioinformatics

02 Pathological diagnosis and tumour cell content are validated by an independent pathologist. After DNA isolation and library preparation, NGS is performed using an appropriate platform.

Clinical interpretation of molecular data

Validation of immediately actionable biomarkers



03 Bioinformatics analysis is followed by data curation and validation of putative molecular targets.

Molecular tumour board

Treatment

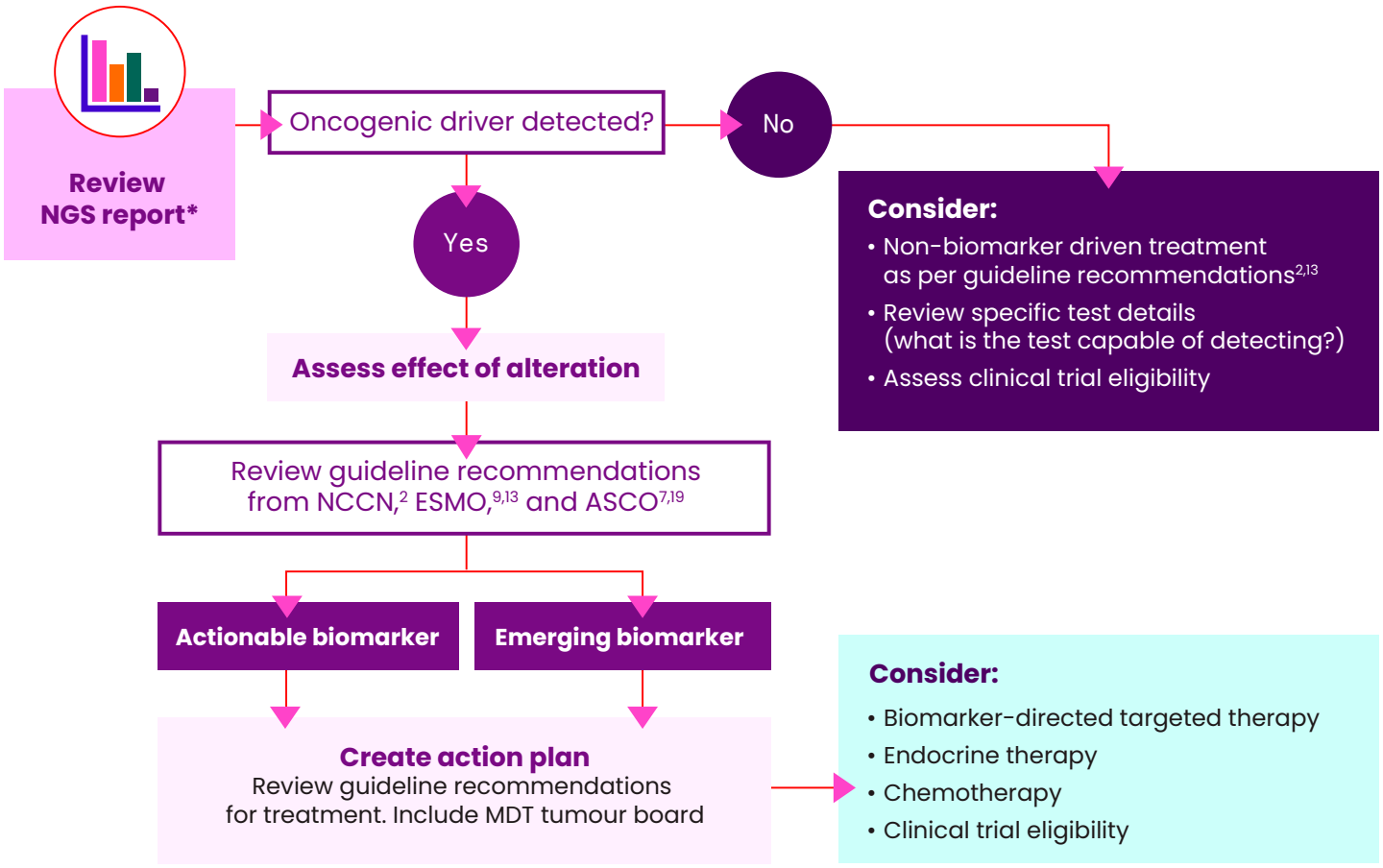
04 Following discussion in a molecular tumour board meeting, personalised treatment strategies are recommended.[27,28]

Biomarker testing should be performed on tumour tissue samples from the primary or metastatic tumour for all patients with HR-positive, HER2-negative aBC, ideally at metastatic diagnosis, to inform the treatment plan.[2,9]

Actioning an NGS report

The results of NGS testing are reported in a variety of templates, with large amounts of data that can be difficult to interpret.[29]

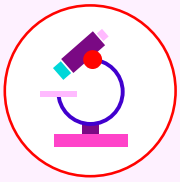
The approach presented in the flow chart below, provides simple guidance and a framework to help navigate the results of a typical NGS report.[30-32]



***The NGS report summary may extend beyond one page, and it is important it is read in its entirety**

Clinical NGS reports share common features and basic information about the test[31]

A high-level summary of the key findings



Potentially clinically relevant biomarkers, with any matched clinical trials

Identified biomarkers with established utility

ESMO and ASCO recommendations for genomic alteration testing in metastatic breast cancer[7,9,19,37]

Guideline recommendations for biomarker test reporting use evidence-based variant categorisation to rate biomarkers by clinical significance and level of confidence in clinical effect.[29,33-35,37] An NGS report summary contains important and actionable information for establishing a treatment plan.[31]

Gene	Alteration	Biomarker test recommended by guidelines	
		ESMO (ESCAT score)	ASCO (quality of evidence)
ERBB2	Amplifications	Yes (IA)	No
	Hotspot mutations	Yes (IIB)	No
PIK3CA	Hotspot mutations	Yes (IA)	Yes (high)
ESR1	Mutations	Yes (IA)	Yes (high)
BRCA1/2	Germline pathogenic/ likely pathogenic variants	Yes (IA)	Yes (high)
	Somatic mutations	Yes (IIB)	No
PTEN	Mutations/deletion	Yes (I/II)	Yes (high)
AKT1	Mutations	Yes (I/II)	Yes (high)
PALB2	Germline pathogenic/ likely pathogenic variants	Yes (IIB)	No

ESCAT: ESMO Scale of Clinical Actionability for molecular Targets[36]

ESCAT evidence tier			Required level of evidence	Clinical implication
Ready for routine use	I Alteration-drug match is associated with improved outcome in clinical trials	I-A	Prospective, randomised clinical trials show the alteration drug match in a specific tumour type results in a clinically meaningful improvement of a survival end point	Access to the treatment should be considered standard of care
		I-B	Prospective, non-randomised clinical trials show that the alteration-drug match in a specific tumour type, results in clinically meaningful benefit as defined by ESMO MCBS 1.1	
		I-C	Clinical trials across tumour types or basket clinical trials show clinical benefit associated with the alteration-drug match, with similar benefit observed across tumour types	
Investigational	II Alteration-drug match is associated with antitumour activity, but magnitude of benefit is unknown	II-A	Retrospective studies show patients with the specific alteration in a specific tumour type experience clinically meaningful benefit with matched drug compared with alteration-negative patients	Treatment to be considered "preferable" in the context of evidence collection either as a prospective registry or as a prospective clinical trial
		II-B	Prospective clinical trial(s) show the alteration-drug match in a specific tumour type results in increased responsiveness when treated with a matched drug, however, no data currently available on survival end points	

* This is not an exhaustive list. Please refer to the ESMO ESCAT guidance for more information.

A prep tool for discussing biomarker results

As you prepare to communicate biomarker results with your patients, use this template as a preparation tool to help you summarise key information from the comprehensive report to discuss with your patients, as part of a shared decision-making process.

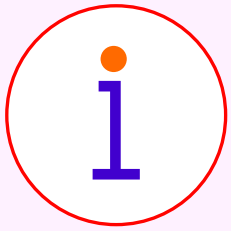
Key results from NGS report			
Biomarker[2]	✓	Specific alteration	Associated therapy
PIK3CA alteration			
AKT alteration			
PTEN alterations			
ESR1 mutation			
Germline *BRCA1/2* mutation			
Germline *PALB2* mutation			
Plan of action			

Consider recommendations on approved treatment or clinical trial eligibility.

Begin the biomarker conversation

It is important to discuss biomarker testing and its treatment implications with your patients. Below are some questions you should be prepared to answer

- Who will order my test, and how long will it take to get the results?
- Why is biomarker testing important?
- Which biomarkers will be tested?
- What does ‘broad molecular profiling’ mean?
- What will it tell me about my cancer and treatment options?
- What if an actionable biomarker is not detected?
- What sample(s) will be tested?
- Is there enough tumour tissue to obtain results from the biomarker testing?



When discussing testing, patients may be more familiar with the terms ‘biomarker testing’ or ‘comprehensive biomarker testing’.

Abbreviations

aBC=advanced breast cancer; *AKT1*=protein kinase B (gene); ASCO=American Society for Clinical Oncology; CDx=companion diagnostic; ER=oestrogen receptor; *ERBB2*=erb-B2 receptor tyrosine kinase 2 (gene); ESCAT=ESMO Scale for Actionability of Targets; ESMO=European Society for Medical Oncology; *ESR1*=oestrogen receptor 1 (gene); *ESR1*m=oestrogen receptor 1 (gene) mutated; FDA=United States Food and Drug Administration; FISH=fluorescence *in situ* hybridisation; HER2=human epidermal growth factor receptor 2; HR=hormone receptor; IHC=immunohistochemistry; ISH=*in situ* hybridisation; MDT=multidisciplinary team; NCCN=National Comprehensive Cancer Network; NGS=next generation sequencing; *PALB2*=partner and localiser of BRCA2 (gene); PCR=polymerase chain reaction; *PIK3CA*=p110α catalytic subunit of phosphatidylinositol 3-kinase (gene); *PTEN*=phosphatase and tensin homolog (gene); VUS=variant of uncertain significance.

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