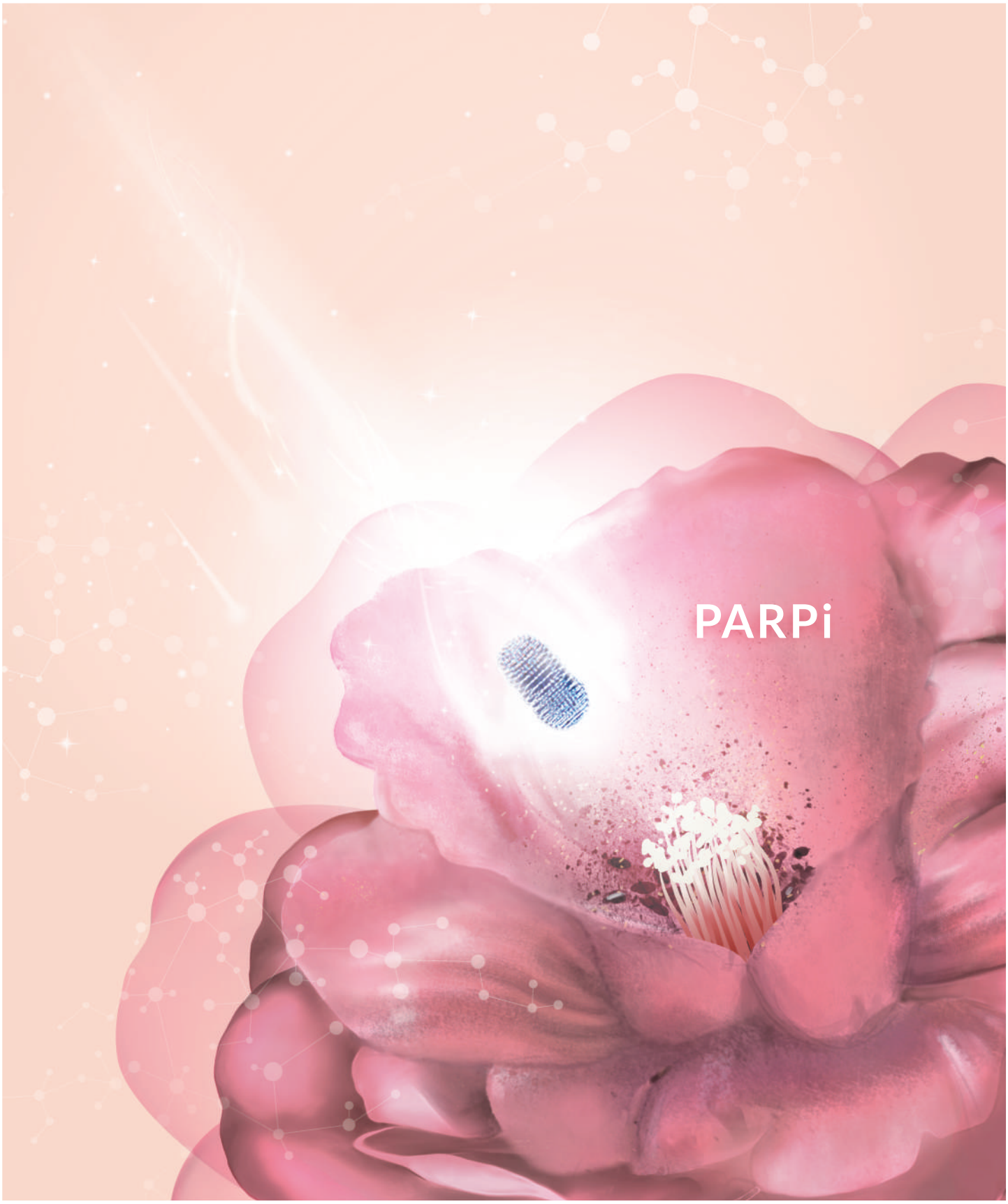


ACT HRD™

ACCURATELY IDENTIFY

PATIENTS ELIGIBLE FOR PARP INHIBITOR THERAPY

This material is intended for healthcare professionals only.

ACT GENOMICS®

ACT HRD™ Technical Specifications

Number of Genes Analyzed	24 Genes
Types of Mutations Genes Analyzed[1,2]	• **HRD Status** (defined as *BRCA1/2* alterations and LOH Status) • **SNV, Small InDel, CNV, *BRCA* LGR, and LOH Status**
Sensitivity[3]	100.00% [95% CI: 85.69%, 100.00%]
Specificity[3]	90.91% [95% CI: 62.26%, 99.53%]
Accuracy[3]	97.06% [95% CI: 85.08%, 99.85%]
Precision[3]	95.83% [95% CI: 79.76%, 99.79%]
NGS Sequence Mean Depth	≥1000
Recommended Cancer Types	Ovarian Cancer, Prostate Cancer, Breast Cancer,...etc.
Sample Type	Tumor Tissue (FFPE) 5-20 unstained sections (5 μm/slide, surface area ≥ 125 mm^2) 1 H&E stained slide (5μm)
Turnaround Time	10 Working Days (* starting from the date of receipt of qualified samples at our CAP-accredited laboratory)

Note1. LOH status, CNV or LGR results may not be available if tumor purity is insufficient based on bioinformatics analysis. Tumor purity for LOH Status result should be more than 40% and CNV and LGR results should be more than 30%.

Note2. The test may not identify abnormal mutations in certain genes, or some results may be unavailable due to technical limitations or individual genetic variability. Please be assured that our laboratory follows strict protocols to ensure the highest quality in every test we perform.

Note3. Comparison Between ACTHRD and a FDA-Approved Assay

ACT HRD™ Gene List

BRCA1	*BRCA2*	*ARID1A*	*ATM*	*ATR*	*ATRX*	*BARD1*	*BRIP1*
CDK12	*CHEK1*	*CHEK2*	*FANCA*	*FANCL*	*FANCM*	*HDAC2*	*NBN*
PALB2	*PPP2R2A*	*PTEN*	*RAD51*	*RAD51B*	*RAD51C*	*RAD51D*	*RAD54L*

* Genes listed in blue tag include LGR testing

Hallmarks of ACT HRD™

Complete Information on HRD Status

The test covers *BRCA1/2* and HRR genes, including coding exons and common splicing regions. Using NGS technology, it detects single nucleotide variants (SNVs), small insertions/deletions (Small InDels), large genomic rearrangements (LGRs), and copy number variations (CNVs). Additionally, a proprietary algorithm is utilized to analyze and present LOH status information.

Bioinformatic Analysis based on International Databases

Up-to-date information extracted from international databases such as gnomAD, 1000 Genome, ClinVar, COSMIC, as well as ACT Genomics' proprietary genetic database based on Asian population, our analysis provides comprehensive variant interpretation and meaningful insights.

Clear and Detailed Medical Report

The report provides recommendations in accordance with the latest AMP Guidelines, as well as other relevant information such as disease risk based on the currently available clinical literature.

Quality Guaranteed

All tests carried out by ACT Genomics are conducted in a CAP accredited laboratory. Also garnered the LDTS (Laboratory Developed Tests and Services) certification by the Taiwan FDA (LDT0001).

Confirmatory Test Services in Family Members

If a deleterious or suspected deleterious *BRCA1/2* variant is found in an individual, we also provide confirmatory test services for family members.

Note : Genetic counseling is required prior to any follow-up confirmatory testing in other family members. Additional fees and charges may apply.

ACT HRD™

Identifies Patients Eligible for PARP Inhibitor Treatment

PARP inhibitors have made major breakthroughs in personalized cancer treatment over the past few years. With the recent U.S. FDA approvals, genetic testing can now be used in patients with ovarian, breast, prostate, and pancreatic cancer to identify individuals who would likely benefit from PARP inhibitor treatment.

Mutations in HRR Genes Lead to HRD

A functional homologous recombination repair (HRR) system is required for proper repair of DNA double-strand breaks (DSBs)[1,2,3] , which are detrimental to living cells if left unrepaired. Many genes are involved in the HRR mechanism, including the commonly known *BRCA1* and *BRCA2*, as well as other HRR genes such as *ATM*, *CHEK2*, *PALB2*, *and RAD51*. When any of these HRR genes undergoes mutation and unable to carry out its usual functions, homologous recombination deficiency (HRD) ensues. Cancer cells with HRD inevitably exhibit genome instability as a phenotype and loss of heterozygosity (LOH) is often observed in these cells[4]. LOH occurs when a cancer cell that is originally heterozygous at a locus loses one of its two alleles at that locus. Therefore, LOH can be used as an indicator to assess the HRD status in cancer cells.

PARP Inhibitor Treatment

PARP inhibitors are pharmacological agents that block the activity of a family of DNA damage repair (DDR) proteins called PARPs, which are responsible for repairing single-strand breaks before DNA replication and cell division. If the single-strand breaks remain unrepaired and persist through the DNA replication process, double-strand breaks are formed as a result. PARP inhibitors cause the formation of double-strand breaks by trapping at the sites of single-strand DNA breaks. In tumors with homologous recombination repair (HRR) deficiency (also known as HRD, which is caused by mutations in *BRCA1/2* or other HRR genes), these double-strand breaks cannot be properly repaired, ultimately leading to cell death in a phenomenon called synthetic lethality whereby the combination of two individually non-lethal defects (i.e., PARP inhibition and HRD) leads to a unique vulnerability[5,6].

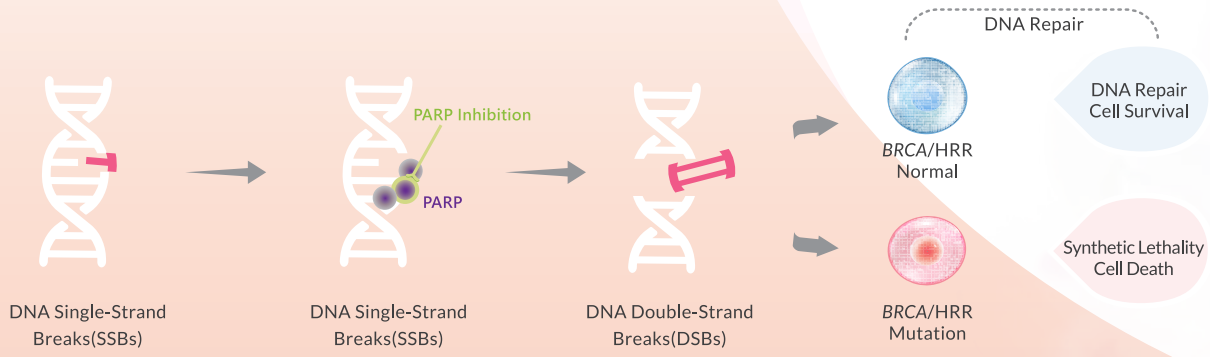


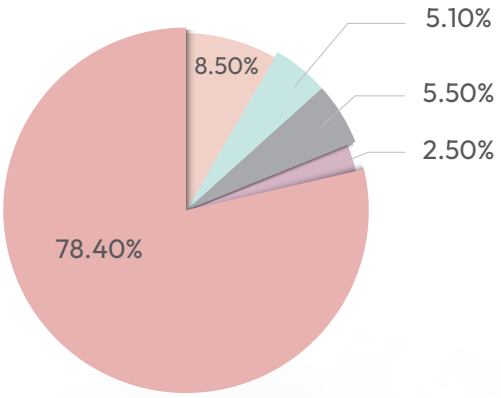
Figure1：Mechanism of Synthetic Lethality in *BRCA*/HRD Cancer[7,8,9,10]

1.Venkitaraman AR. Annu Rev Pathol 2009; 4: 461-487.
2.Li X, Heyer WD. Cell Res. 2008;18:99-113.
3.Lord CJ, Ashworth A. Nat Rev Cancer. 2016;16:110-120.
4.Watkins JA, Irshad S, Grigoriadis A, Tutt AN. Breast Cancer Res. 2014;16:211
5.Hartwell LH, Szankasi P, Roberts CJ, et al. Science 1997; 278: 1064-1068.
6.Turner, N., Tutt, A. & Ashworth, A. Nat. Rev. Cancer 4, 814–819 (2004).
7.Venkitaraman AR, . Science 2014; 343: 1470-1475
8.Livraghi L, Garber JE. BMC Med 2015; 13:188.
9.Farmer H, McCabe N, Lord CJ, et al.Nature 2005; 434: 917-921
10.Bryant HE, Schultz N, Thomas HD, et al. Nature 2005; 434: 913-917.

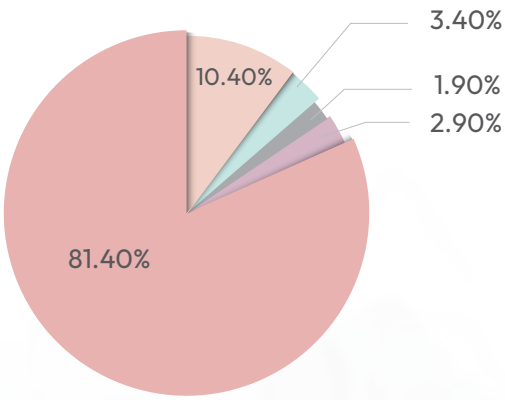
Mutations in non-BRCA HRR Genes and Cancer

Besides the well-studied *BRCA1* and *BRCA2* genes, there is a horde of other HRR genes involved in the DNA repair process. Mutations in any of these non-BRCA HRR genes may also give rise to HRD and dysfunctional HRR system. Therefore, genetic testing of *BRCA1/2* alone is not adequate to select patients who may benefit from PARP inhibitor treatment.

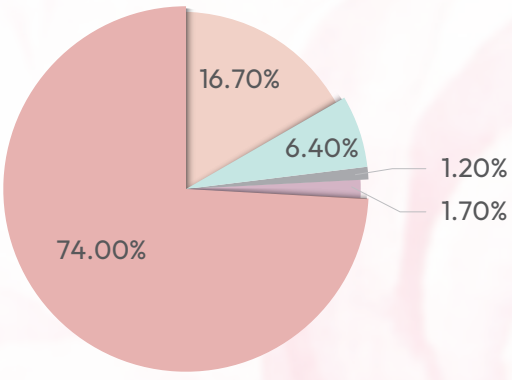
Ovarian Cancer



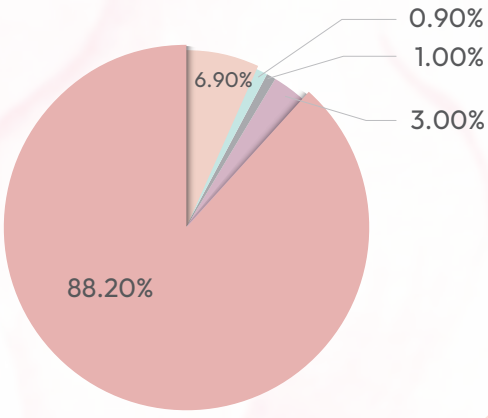
Breast Cancer



Prostate Cancer



Pancreatic Cancer



non-*BRCA* HRR Genes Mutation | unknow n *BRCA* Mutation | No *HRR* Mutation | s*BRCA* Mutation | g*BRCA* Mutation

Source: Cai C, Dettman EJ, Zhou W, et al. Prevalence of homologous recombination biomarkers in multiple tumor types: an observational study. Future Oncology. 2024. doi:10.1080/14796694.2024.2367957.

ACTHRD™ Report

ACTHRD™ Report

Identifier
Project ID:
Report No.:
Report Date:

Subject		
Identifier:		Subject ID:
Date of Birth:		Gender:
Diagnosis: Ovarian Cancer		
Ordering Physician		
Referral Doctor:		Tel:
Referral Institution:		
Address:		
Specimen		
Specimen ID:	Collection Site:	Specimen Type:
Date Received:	Sample ID:	D/ID:

ABOUT ACTHRD™

The test is a next-generation sequencing (NGS) based assay to detect single nucleotide variants (SNVs), small insertions and deletions (InDels), copy number alterations (loss) (CNA-loss), large genomic rearrangements (LGRs) in the *BRCA1* and *BRCA2* genes, and analyze the genomic loss of heterozygosity (LOH) status using DNA isolated from formalin-fixed, paraffin-embedded (FFPE) tumor tissue specimens. Results of the test are used as an aid in identifying cancer patients with positive homologous recombination deficiency (HRD) status for treatment with PARP inhibitors. Additionally, the test is intended to provide tumor profiling on SNVs, InDels, and CNA-loss of 22 homologous recombination repair (HRR) genes for use by qualified health care professionals in accordance with professional guidelines, and is not conclusive or prescriptive for labeled use of any specific therapeutic products

Report Summary for Actionable Variants/Biomarkers

Homologous Recombination Deficiency (HRD) Status

HRD Status	Gene/Biomarker	Result
Positive	*BRCA1*	Negative
	BRCA2	Positive
	Loss of heterozygosity (LOH)	Positive (0.57)

* Homologous Recombination Deficiency (HRD) Positive= Either BRCA1/2 mutant or LOH positive

S Sensitive R Resistant

Variants/Biomarkers with Clinical Significance (Target Therapy)

Genomic Alterations	Evidence Level 1, 2 (FDA-approved, NCCN guideline)	Evidence Level 3A, 3B, 4 (Others)
LOH positive	S Niraparib, Olaparib	S Rucaparib
BRCA2 Y1655*	S Niraparib, Olaparib, Rucaparib	S Talazoparib
BRCA2 Heterozygous deletion	S Niraparib, Olaparib, Rucaparib	S Talazoparib

Note:
- The therapeutic agents and possible effects to a given drug are based on mapping the variants/biomarkers with ACT Genomics clinical knowledge database. The mapping results only provide information for reference, but not a medical recommendation.
- Please refer to the corresponding sections for more detailed information about genomic alteration and clinical relevance listed above.

ACT GENOMICS

ACT Genomics' laboratory is accredited by CAP (CAP number: 9028096).
ACT Genomics only provides a technical report of the test; please consult a specialist physician to determine the appropriate clinical solution and follow the instructions of the physician. The results are only valid for the tested sample(s).
ACT Genomics Laboratory HQ: 3F., No. 345, Xinhu 2nd Rd., Neihu Dist., Taipei, Taiwan 11494
Email: service@actgenomics.com T: +886-2-2795-3660 F: +886-2-2795-5016

HRD Status

The HRD Status is defined as deleterious or suspected deleterious alterations of *BRCA1/2*, and LOH Status positive. Results of the HRD Status is used as an aid in identifying cancer patients eligible for treatment with PARP inhibitors.

SNV & Small InDel

Small-scale DNA variations such as single nucleotide variants (SNVs) and small insertions and deletions (InDels) are two common types of genetic mutations where sequences of DNA are changed, inserted, or deleted in an individual.

CNV

Copy number variation refers to heterozygous (one-copy loss)/homozygous (two-copy loss) deletion that leads to loss of gene function.

ACTHRD™ Report

Identifier
Project ID:
Report No.:
Report Date:

Testing Results of Variants/Biomarkers with Clinical Relevance

Homologous Recombination Deficiency (HRD) Status

Positive

* Homologous Recombination Deficiency (HRD) Positive= Either BRCA1/2 mutant or LOH positive

Single Nucleotide and Small InDel Variants

Gene Alterations	Allele Frequency	Classification
BRCA2 c.4965del (Y1655*)	68.7%	Deleterious

Copy Number Alterations

Gene	Chromosome	Variation	Copy Number
BRCA2	Chr13	Heterozygous deletion	1

Large Genomic Rearrangements

Gene	Alteration
Not detected	

Loss of Heterozygosity Status

LOH Status	LOH Score
Positive	0.57

Note:
- CNA, LGR, and LOH status in the tumor were determined based on 55% tumor purity (calculated by NGS/estimated by the pathologist).
- Samples with tumor purity lower than 40% are unable to be analyzed for the LOH status.
- Samples with tumor purity lower than 30% are unable to be analyzed for CNA and LGR.
- The homologous recombination deficiency (HRD) status is defined as deleterious or suspected deleterious alterations of *BRCA1* and *BRCA2*, and/or LOH status positive. The threshold for LOH status positive is set at a score ≥ 0.4. Of note, the HRD definition is in accordance to the approved therapeutic product labeling in ovarian cancer and not in other cancer types.
- Only deleterious or suspected deleterious variants are listed in this section. For variants of unknown significance (VUS), please refer to Supplementary Information on Testing Results. Variants classified as benign, likely benign, and synonymous variants are not reported.

Supplementary Information for Therapeutic Implications

Targeted Therapies

Genomic Alterations	Therapies	Evidence Level
LOH positive	S Niraparib, Olaparib	1
BRCA2 Y1655*	S Niraparib, Olaparib, Rucaparib	1
BRCA2 Heterozygous deletion	S Niraparib, Olaparib, Rucaparib	1
BRCA2 Y1655*	S Talazoparib	3A
BRCA2 Heterozygous deletion	S Talazoparib	3A
LOH positive	S Rucaparib	3B

Therapies associated with benefit or lack of benefit are based on biomarkers detected in this tumor and published evidence in professional guidelines or peer-reviewed journals.

Level	Description
1	FDA-recognized biomarkers predictive of response or resistance to FDA-approved drugs in this indication
2	Standard care biomarkers (recommended by the NCCN guideline) are predictive of response or resistance to FDA-approved drugs in this indication
3A	Biomarkers predictive of response or resistance to therapies approved by the FDA or NCCN guideline in a different cancer type
3B	Biomarkers that serve as inclusion criteria for clinical trials (minimal supportive data required)
4	Biomarkers that show plausible therapeutic significance based on small studies, few case reports, or preclinical studies

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BRCA LGR

Large genomic rearrangements (LGRs) are large-scale DNA structural variations that constitute a significant proportion of *BRCA1/2* mutations in cancer patients. LGR testing enables a more complete investigation on *BRCA1/2* mutations.

LOH Status

An analysis of almost 9,000 SNPs is performed by a proprietary algorithm to obtain a LOH Status which reflects the HRD Status of tumor tissues.

Therapeutic Implication

Based on the specimen used, the test report includes *BRCA1/2* variant interpretation and classification according to the international standards so as to support treatment recommendations.

Note: All tests carried out by ACT Genomics are conducted in a CAP-accredited laboratory. Please consult your physician for any enquiries related to clinical interpretation of the test results. The images used are for illustrative purposes only. The actual report may differ.

CAP ACCREDITED
COLLEGE of AMERICAN PATHOLOGISTS

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