Com|Pl|i|t DX

SAMPLE INFORMATION

Name	-	**Date Sp. Extracted**	-
ID/Medical ID	-	**Req. Physician**	-
Date of Birth	-	**Report No**	26xxxxxxGR
Material #1	PARAFFIN EMBEDDED TISSUE-BLOCK	**Date Received**	-
Material #2	-	**Date of Report**	-
Sample #1 ID	-	**Tumor type**	BREAST CANCER

Com.Pl.i.t. Dx (23 genes, 7 fusions) | Comprehensive Panel for Individualized Treatment

1. Clinically Significant Biomarkers*

Biomarker	Result	Therapies with strong clinical significance		Therapies with potential significance	Therapies with potential resistance
		Approved/ Standard of care therapies (Level A)	Therapies in well powered studies (Level B)	Off label/ Investigational therapies (Level C)	
PIK3CA	Wild-Type	**Gedatolisib+Fulvestrant+Palbociclib Gedatolisib+Fulvestrant			-
ESR1	Exon 10 p.Y537S VAF: 18.3%	Elacestrant Imlunestrant Vepdegestrant	Fulvestrant	-	Aromatase Inhibitors
Microsatellite Instability (MSI)	Stable (MSS)	-	-	-	-

*Note: Variants' Level of Evidence (LoE) (e.g. A, B, C etc) are based on the Joint consensus recommendation of AMP, ACMG, ASCO and CAP for reporting genetic variants in cancer. For a detailed description of the recommendation please refer to Fig. 1

^ The analysis of selected biomarkers included in the present multigene panel is performed within the scope of accreditation according to ELOT EN ISO 15189:2022 (see «Methodology» section). Accreditation according to ELOT EN ISO 15189:2022 applies exclusively to the analytical determination of the selected biomarkers. Interpretations related to the prediction of therapeutic response are not included in the scope of accreditation.

** indicated for HR+/HER2− breast cancer with PIK3CA wild-type status (no detectable PIK3CA mutation).

52, Spaton Ave., 15344, Gerakas, Greece, G.E.MI. nr: 0007856001000 | info@genekor.com, www.genekor.com | Tel. (+30) 210 6032138 Fax. (+30) 210 6032148

A company certified with ISO 9001:2015 (Cert. No 041150049) and ISO/IEC 27001:2022(Cert. No 048190009)

-

George Nasioulas, PhD Molecular Biologist, Scientific Director, Medical ID:26025301255

Com|PI|i|t DX

Name	-	Report No	26xxxxxxGR

52, Spaton Ave., 15344, Gerakas, Greece, G.E.MI. nr: 0007856001000 | info@genekor.com, www.genekor.com | Tel. (+30) 210 6032138 Fax. (+30) 210 6032148
A company certified with ISO 9001:2015 (Cert. No 041150049) and ISO/IEC 27001:2022(Cert. No 048190009)

-

George Nasioulas, PhD Molecular Biologist, Scientific Director, Medical ID:26025301255

ComPlit DX

Name	-	Report No	26xxxxxxGR

2. Methodology

NGS analysis

DNA is extracted from the sample under investigation using the Qiamp DNA FFPE Kit (Qiagen). A capture based targeted next generation sequencing (NGS) analysis was performed, using the Oncology 188-Gene Variant Assay (GenePlus) which is a qualitative test which enables the simultaneous detection of single nucleotide variants (SNVs), insertions and deletions (InDels), fusions and copy number variations (CNVs) as well as microsatellite instability (MSI) in a single workflow.

Sequencing was carried out on the DNBSEQ-T7 NGS platform (MGI). The analysis of 23 genes includes the entire exon regions and CNVs of 15 genes, partial exon regions of 8 genes and introns/promoters/fusion breakpoint regions of 7 genes. The test also reports on Microsatellite Instability (MSI) status.

Sequencing data are analyzed through bioinformatics pipeline for variant calling and interpretation using the Gene+Box data analysis and management system. Alteration- and tumor type-specific therapeutic implications are classified using the OncoKB™ Levels of Evidence system, which assigns clinical actionability to individual mutational events. For additional details about the OncoKB™ curation process, please refer to the version-controlled OncoKB™ Curation Standard Operating Procedure.

Genes Analyzed

23 Gene Alterations									
AKT1	BRCA1*	BRCA2*	CDH1	CDK4*	CDK6*	CCND1*	EGFR*	ERBB2*(HER2)	ERBB3
ESR1	FBXW7	FGFR1	FGFR2*	FGFR3*	GATA3	KRAS*	NF1	PALB2*	PIK3CA*
PTEN*	RB1*	TP53*							
7 Fusion Genes									
FGFR1	FGFR2	FGFR3	NTRK1	NTRK2	NTRK3	RET			
MSI									

*CNV (amplification/deletion) analysis is included for these genes

Biomarkers within the scope of accreditation (ISO15189:2022)

-*BRCA1*, *BRCA2* (SNV/indel detection by NGS)
-MSI (NGS)

Sensitivity: Positive reference standards are tested with the assay, all corresponding mutation sites can be accurately detected, and the positive percent agreement (PPA) for all variants (SNVs, Indels, fusions and CNVs) assessed was 95%.

52, Spaton Ave., 15344, Gerakas, Greece, G.E.MI. nr: 0007856001000 | info@genekor.com, www.genekor.com | Tel. (+30) 210 6032138 Fax. (+30) 210 6032148
A company certified with ISO 9001:2015 (Cert. No 041150049) and ISO/IEC 27001:2022(Cert. No 048190009)

-

George Nasioulas, PhD Molecular Biologist, Scientific Director, Medical ID:26025301255

Com|PI|i|t DX

Name	-	Report No	26xxxxxxGR

Specificity: Negative reference standards are tested with the assay, and the negative percent agreement (NPA) of SNVs, Indels, fusions and CNVs was 99%.

Limit of Detection (LoD): The detection limit of the method is 1-2% of mutant allelic content, depending on the genomic region. DNA variations detected at frequencies of less than 5% are confirmed using NGS or an alternative method (Real-Time PCR). All fusions detected are confirmed with an alternative method (Real-Time PCR or FISH).

The performance characteristics of the assay are summarized in the following table:

Variant Type	Limit of Detection
Single nucleotide variations (SNV)	VAF ≥2-5%
Insertions/deletions (Indel)	VAF ≥2-5%
Fusion (or rearrangement)	VAF ≥2%
Copy number (CNV)	≥3 copies

Disclaimer

1. This test is mainly used to assist clinical decision-making, and the result does not represent clinical decision.
2. The test should be interpreted by combining the actual patient context. The medication information provided only on the basis of genetic test results, and the actual medication should follow the physician's instructions.
3. The clinical trials only present partial relevant clinical recruitment trials. For more comprehensive and updated information, please refer to the website: https://clinicaltrials.gov/.
4. As evidence on variants and drugs evolves, previous classifications may later be modified. The interpretation of a variant is based on current available evidence.
5. Sequence variants were reported using Human Genome Variation Society (HGVS) nomenclature. Classification and interpretation of variants follow guidelines of American College of Medical Genetics and Genomics (ACMG), Association of Molecular Pathology (AMP), American Society of Clinical Oncology (ASCO) and College of American Pathologists (CAP).
6. Database and references used: Reference genome (GRCh37), annotation using A Locus Reference Genomic (LRG), database referencing 1000G (phaseIII-ucsc), EXAC (0.3.1), dbSNP (147), PolyPhen2/SIFT (ensdb v73), PhyloP (2013-12-06), Clinvar (2018-8) and Cosmic(V80).

Limitations

1. The test is limited to test genomic variations on DNA level and does not involve RNA level or protein level.
2. Limited tissue detection may not represent the whole DNA variations of lesions because of tumor heterogeneity.
3. Scientific data show that not all patients carry genomic variations that are associated with targeted drug, therefore not all subjects can be matched with targeted therapies or clear resistance mechanism.
4. Genetic variation beyond the detection range of this test or some non-gene mutation related factors such as drug interactions may affect the clinical effects of drugs.

52, Spaton Ave., 15344, Gerakas, Greece, G.E.MI. nr: 0007856001000 | info@genekor.com, www.genekor.com | Tel. (+30) 210 6032138 Fax. (+30) 210 6032148
A company certified with ISO 9001:2015 (Cert. No 041150049) and ISO/IEC 27001:2022(Cert. No 048190009)

-

George Nasioulas, PhD Molecular Biologist, Scientific Director, Medical ID:26025301255

Com|PI|i|t DX

Name	-	Report No	26xxxxxxGR

5. The detection method cannot distinguish between somatic mutations and germline mutations effectively without control sample analysis.
6. Every molecular test has an internal 0.5-1% chance of failure. This is due to rare molecular events and factors related to the preparation and analysis of the samples.
7. Improper sample collection, transportation and storage may lead to compromised or invalid results.

52, Spaton Ave., 15344, Gerakas, Greece, G.E.MI. nr: 0007856001000 | info@genekor.com, www.genekor.com | Tel. (+30) 210 6032138 Fax. (+30) 210 6032148
A company certified with ISO 9001:2015 (Cert. No 041150049) and ISO/IEC 27001:2022(Cert. No 048190009)

-

George Nasioulas, PhD Molecular Biologist, Scientific Director, Medical ID:26025301255

Com|PI|i|t DX

Name	-	Report No	26xxxxxxGR

3. Quality Control Results

Quality Control Index	Result	Criterion
Average effective sequencing depth[1]	**6315**	≥ 500
Tumor cell content[2]	60%	≥20%
Overall Assessment[3]	PASS	

Note

1. Average effective sequencing depth: Average sequencing depth on target with duplicated reads.
2. An overall tumor cell content percentage of ≥ 20% is recommended for the efficient detection of somatic alterations in the sample analyzed.
3. Overall Assessment: The quality control overall assessment results are divided into two levels: "PASS" and "RISK". When the overall quality assessment result is "RISK", 94-96% of coverage was achieved in the genes analysed, hence there is a small range where clinical actionable variations could be undetected.

52, Spaton Ave., 15344, Gerakas, Greece, G.E.MI. nr: 0007856001000 | info@genekor.com, www.genekor.com | Tel. (+30) 210 6032138 Fax. (+30) 210 6032148
A company certified with ISO 9001:2015 (Cert. No 041150049) and ISO/IEC 27001:2022(Cert. No 048190009)

-

George Nasioulas, PhD Molecular Biologist, Scientific Director, Medical ID:26025301255

ComPlit DX

Name - **Report No** 26xxxxxxGR

4. Important biomarkers findings

Gene	Finding (VAF)	Gene	Finding (VAF)
AKT1	Not Detected	*PIK3CA*	**(%)**
BRCA1/2	Not Detected	*PTEN*	Not Detected
ERBB2	Not Detected	*NTRK1/2/3* rearrangement	Not Detected
ESR1	**p.Y537S (18.3%)**		

5. Immune Checkpoint inhibitors biomarkers

Biomarker/Variant	Result	Clinical Interpretation
Treatment effect - positive correlation		
TP53 mutation	Not Detected	-
KRAS mutation	Not Detected	-
BRCA1/2 mutation	Not Detected	-
Treatment effect - negative correlation		
PTEN inactivating mutation	Not Detected	-
EGFR mutation (L858R/EX19del)	Not Detected	-

Com|PI|i|t DX

Name	-	Report No	26xxxxxxGR

6. Interpretations for targeted therapies

Genetic Variation:	*PIK3CA*: Wild-Type		OncoKB

Treatment Information

The FDA has approved gedatolisib in combination with fulvestrant and palbociclib, or in combination with fulvestrant alone, for the treatment of adults with hormone receptor–positive (HR+), human epidermal growth factor receptor 2–negative (HER2−), PIK3CA wild-type locally advanced or metastatic breast cancer following progression on or after a CDK4/6 inhibitor and an aromatase inhibitor. The approval marks the first regulatory authorization of a multitarget PI3K/AKT/mTOR (PAM) pathway inhibitor for patients with PIK3CA wild-type disease, a population representing approximately 60% of patients with HR+/HER2− advanced breast cancer who lack the mutation targeted by currently approved PI3K isoform–specific inhibitors. The approval is supported by data from the PIK3CA wild-type cohort of the phase 3 VIKTORIA-1 trial (NCT05501886), a randomized, open-label study that met both primary end points of progression-free survival (PFS) improvement with the gedatolisib-based triplet and doublet regimens vs fulvestrant monotherapy.

Gene information

Phosphatidylinositol-3-kinase (PI3K) is comprised of a regulatory subunit (p85α) as well as a catalytic subunit (p110α) and it is the catalytic subunit that is encoded by the PIK3CA gene. PIK3CA is among the most commonly mutated genes in cancer and aberrant activation of PI3K is a transforming event (PMID: 17376864). Multiple receptor tyrosine kinases, including EGFR, ERBB2 (HER2), RET, MET, and VEGFR, among others, convert extracellular cues into intracellular signals and recruit PI3K to the plasma membrane via scaffold proteins such as IRS1 or by activating RAS. Upon stimulation, PI3K-110α converts its lipid substrate PIP2 (phosphatidylinositol - 4,5 - bisphosphate) to PIP3 (phosphatidylinositol - 3,4,5 - bisphosphate), which activates several signaling cascades, including the well-characterized AKT-mTOR pathway. Once activated, AKT-mTOR downstream signaling promotes cell survival, proliferation, growth and motility (PMID: 16341083). Adding to this complexity, exposure to some PI3K/mTOR pathway-targeted drugs relieves cancer cells of self-regulatory properties inherent in the PI3K-AKT-mTOR pathway thereby promoting tumor resistance to these agents (PMID: 22576208).

Gedatolisib DrugBank

Gedatolisib is an investigational, potent pan-class I phosphatidylinositol 3-kinase (PI3K) and mammalian target of rapamycin (mTOR) inhibitor that targets the PI3K/AKT/mTOR signaling pathway, a key regulator of cell growth, proliferation, survival, and metabolism. By simultaneously inhibiting both PI3K and mTOR, gedatolisib may overcome resistance mechanisms associated with selective PI3K inhibitors. Clinical studies have demonstrated promising activity, particularly in hormone receptor-positive, HER2-negative advanced breast cancer, especially in combination with endocrine therapy and CDK4/6 inhibitors. It is currently under clinical investigation across multiple solid tumor types.

Com|PI|i|t DX

Name	-	Report No	26xxxxxxGR

Fulvestrant DrugBank

Fulvestrant is a selective estrogen receptor degrader (SERD) that binds to the estrogen receptor (ER), inhibits its activity, and promotes its degradation, leading to complete blockade of estrogen signaling. It is approved for the treatment of hormone receptor (HR)-positive, HER2-negative advanced or metastatic breast cancer, either as monotherapy or in combination with targeted agents such as CDK4/6, PI3K, or AKT inhibitors. Fulvestrant is administered as an intramuscular injection and is a standard endocrine therapy option for patients with endocrine-sensitive or endocrine-resistant disease.

Palbociclib DrugBank

Palbociclib is an oral, selective cyclin-dependent kinase 4 and 6 (CDK4/6) inhibitor that blocks cell cycle progression by preventing phosphorylation of the retinoblastoma (Rb) protein, thereby inducing G1 cell cycle arrest. It is approved in combination with endocrine therapy, such as an aromatase inhibitor or fulvestrant, for the treatment of hormone receptor (HR)-positive, HER2-negative advanced or metastatic breast cancer. Palbociclib has been shown to significantly improve progression-free survival and is a standard first-line and subsequent-line treatment option in patients with endocrine-sensitive or endocrine-resistant disease.

Genetic Variation:	*ESR1*: c.1610A>C (p.Y537S)	VAF*: 18.3%	OncoKB

Treatment Information

- On May 1, 2026, the Food and Drug Administration approved vepdegestrant, a heterobifunctional protein degrader, for adults with estrogen receptor (ER)-positive, human epidermal growth factor receptor 2 (HER2)-negative, ESR1-mutated advanced or metastatic breast cancer, as detected by an FDA-authorized test, with disease progression following at least one line of endocrine therapy.
- Recently, the Food and Drug Administration approved imlunestrant, an estrogen receptor antagonist, for adults with estrogen receptor (ER)-positive, human epidermal growth factor 2 (HER2)-negative, estrogen receptor-1 (ESR1)-mutated advanced or metastatic breast cancer with disease progression following at least one line of endocrine therapy. Efficacy was evaluated in EMBER-3 (NCT04975308), a randomized, open-label, active-controlled, multicenter trial that enrolled 874 patients with ER-positive, HER2-negative locally advanced or metastatic breast cancer previously treated with an aromatase inhibitor either alone or in combination with a CDK4/6 inhibitor. In the ESR1 mutated population (n=256), a statistically significant difference in investigator-assessed PFS for imlunestrant compared to investigator's choice of endocrine therapy was observed. The median PFS was 5.5 months in the imlunestrant arm and 3.8 months in the investigator's choice arm. The ORR was 14.3% in the imlunestrant arm and 7.7% in the investigator's choice arm.
- The FDA has granted an accelerated approval to elacestrant for the treatment of patients with estrogen receptor-positive, HER2-negative advanced or metastatic breast cancer following at least 1 prior lines of endocrine therapy. The approval is supported by data from the phase 3 EMERALD study (NCT03778931). The investigational oral selective estrogen receptor degrader (SERD) was evaluated among patients with ESR1-mutant disease (n = 115) vs standard-of-care (SOC) endocrine therapy (n = 113) of either fulvestrant or an aromatase inhibitor. The median progression-free survival was 3.8 months (95% CI, 2.2-7.3) vs 1.9 months (95% CI, 1.9-2.1) with the SERD and SOC, respectively (HR, 0.55; 95% CI, 0.39-0.77; P = .0005).2 The overall survival outcomes were not statically

Com|PI|i|t DX

Name	-	Report No	26xxxxxGR

significant (HR, 0.90; 95% CI, 0.63-1.30). Results presented during the 2022 SABCS showed that the duration of prior CDK4/6 inhibition played a role for patients who received elacestrant. Among those with ESR1-mutant disease who received at least 12 months of CDK4/6 inhibition (n = 78), the median PFS was 8.61 months vs 1.91 months with SOC (n = 81; HR, 0.410; 95% CI, 0.262-0.634). The 6-, 12-, and 18-month PFS rates were 55.81%, 35.81%, and 28.49% with elacestrant vs 22.66%, 8.39%, and 0% with SOC. Fulvestrant has been approved for women with breast cancer: as a standalone treatment for postmenopausal women with HR-positive metastatic breast cancer whose disease progressed after treatment with other antiestrogen therapy, in combination with the CDK4/6 inhibitor Palbociclib for the treatment of women with advanced or metastatic HR-positive, HER2-negative breast cancer that has progressed after endocrine therapy and as a standalone treatment, or monotherapy, for postmenopausal women with advanced hormone receptor (HR)-positive, HER2-negative breast cancer who have not undergone endocrine therapy previously. Turner et al (2020) have shown that the detection of ESR1 mutations in baseline ctDNA is associated with inferior progression free survival (PFS) and overall survival (OS) in patients treated with exemestane versus fulvestrant (PMID: 32546646).

Gene information

ESR1 is a nuclear receptor that encodes the estrogen receptor alpha (ERα) protein. ERα plays a major role in sexual reproductive development and maintenance, specifically in the female reproductive organs (PMID: 10368776). Binding of the hormone estrogen, the ERα ligand, induces conformational changes to ERα, which allows the receptor to dissociate from HSP90 and dimerize with itself or ERβ. The dimer then translocates to the nucleus where it binds to promoters and enhancers of target genes either directly, via Estrogen Responsive Elements (ERE) in the DNA, or via other transcription factor complexes such as FOS/JUN/SP-1 (PMID: 11162939, 10681512). Once bound, the estrogen receptor recruits co-regulators that modulate the transcription of ESR1 target genes, resulting in changes in proliferation, migration and differentiation (PMID: 21779010). Lack of ERα delays the development of WNT1- and ERBB2 mutant-induced mouse mammary tumors (PMID: 10213494, 12019156) and reduces the occurrence of estrogen and carcinogen-induced mouse mammary tumors (PMID: 20181624, 11156389), indicating the involvement of ERα in tumorigenesis. While mutations in ESR1 are generally very rare in primary cancers (<5%), a number of mutations occurring in the ligand-binding domain (LBD) of the receptor were identified in ~12-25% of hormone-resistant metastatic breast cancer patients (PMID: 24398047, 24185512, 24185510, 24217577). The most recurrent mutations, Y537S and D538G, result in a constitutively active receptor, which is shown to confer acquired resistance to estrogen deprivation therapies.

Variant Information

The ESR1 Y537S mutation is located in the ligand-binding domain of the protein. This mutation has been found in breast cancer (PMID: 24185512). Expression of this mutation in breast cancer cell lines demonstrated that it is activating as measured by increased protein activity, cell proliferation and tumor growth in a xenograft model compared to wildtype (PMID: 8923465, 9398213, 11914066, 26836308, 24185512, 30733228). In vitro and in vivo studies demonstrated that this mutation is resistant to the ESR1 inhibitors tamoxifen and fulvestrant, but is sensitive to AZD9496 when expressed in embryonic kidney and breast cancer cell lines as measured by protein activity, cell proliferation, and tumor growth upon drug treatment (PMID: 24185512, 24185510, 27986707). Among 195 patients with estrogen-receptor-positive breast cancer who were treated with the CDK4/6 inhibitor palbociclib plus fulvestrant, the ESR1 Y537S mutation was found at a significantly higher percentage in post-treatment samples compared to pre-treatment samples (PMID: 30206110). The ESR1 Y537S mutation is known to be pathogenic.

ComPlit DX

Name	-	Report No	26xxxxxxGR

Elacestrant

DrugBank

Elacestrant is an oral, estrogen receptor antagonist that is FDA-approved for the treatment of postmenopausal adult patients with ER+/HER2-, ESR1-mutated advanced or metastatic breast cancer. Oncogenic ESR1 ligand-binding domain missense mutations for treatment with elacesterant were detected with the Guardant360 CDx assay. FDA approval was based on the results of the Phase III EMERALD (NCT03778931) trial of elacestrant versus standard-of-care endocrine monotherapy in 477 patients with ER+/HER2- advanced breast cancer who had received one or two lines of endocrine therapy (n=228, patients with ESR1 missense mutations in codons 310-547).

In the Phase III EMERALD (NCT03778931) trial, the ESR1-mutated cohort had a six-month progression-free survival (PFS) of 40.8% (95% CI=30.1-51.4) versus 19.1% (95% CI=10.5-27.8) and a twelve-month progression-free survival of 26.8% (95% CI=16.2-37.4) versus 8.2% (95% CI=1.3-15.1) for the elacestrant versus standard-of-care arm, respectively (PMID: 35584336). In the updated results from the Phase III EMERALD (NCT03778931) trial based on the duration of prior CDK4/6i treatment, the ESR1-mutated cohort previously treated with CDK4/6i for ≥6.0 months (n=103 elacestrant arm; n=102 stand-of-care arm), ≥12.0 months (n=78 elacestrant arm; n=81 stand-of-care arm) or ≥18.0 months (n=55 elacestrant arm; n=56 stand-of-care arm) demonstrated a median PFS of 4.1 months, 8.6 months and 8.6 months with elacestrant versus 1.9 months (HR=0.52 [95% CI=0.36-0.74]), 1.9 months (HR=0.41 [95% CI=0.26-0.63]) and 2.1 months (HR=0.47 [95% CI=0.20-0.79]) with standard-of-care treatment (PMID: 39087959).

Imlunestrant

DrugBank

Imlunestrant is an orally available, small-molecule estrogen receptor (ER) antagonist that is FDA-approved for the treatment of adults with ER-positive, HER2-negative, ESR1-mutated advanced or metastatic breast cancer with disease progression following at least one line of endocrine therapy. ESR1 mutations for treatment with imlunestrant were detected by the Guardant360 CDx assay. FDA approval was based on the results of the Phase III EMBER-3 (NCT04975308) trial of imlunestrant versus standard-of-care (SOC) treatment in 256 patients with ER-positive, HER2-negative locally advanced or metastatic breast cancer previously treated with an aromatase inhibitor either alone or in combination with a CDK4/6 inhibitor. Patients were excluded from the trial if they were eligible to receive a PARP inhibitor.

In the Phase III EMBER-3 (NCT04975308) trial, the imlunestrant monotherapy cohort (n=138) demonstrated a clinical benefit rate of 43.5% (n=60), an objective response rate (ORR) of 14.3% (95% CI=7.8-20.8), with a 0.9% complete response (CR) rate and 13.4% partial response (PR) rate, a median duration of response (DOR) of thirteen months (range=2-20), an estimated restricted mean survival time at 19.4 months of 7.9 months (95% CI=6.8-9.1) and a median progression-free survival (PFS) of 5.5 months (95% CI=3.9-7.4) (PMID: 39660834). The SOC monotherapy (fulvestrant or exemestane) (n=118) cohort demonstrated a clinical benefit rate of 33.1% (n=39), an ORR of 7.7% (95% CI=2.2-13.2), with a 7.7% PR rate, a median DOR of seven months (range=4-16), an estimated restricted mean survival time at 19.4 months of 5.4 months (95% CI=4.6-6.2) and a median PFS of 3.8 months (95% CI=3.7-5.5) (HR=0.62 [95% CI=0.46-0.82]; p=0.0008) (PMID: 39660834).

Vepdegestrant

DrugBank

Vepdegestrant is an orally bioavailable, proteolysis-targeting chimera (PROTAC) estrogen receptor (ER) degrader that is FDA approved for the treatment of adults with ER-positive, HER2-negative, ESR1-mutated advanced or metastatic breast cancer, as detected

Com|PI|i|t DX

Name	-	Report No	26xxxxxxGR

by an FDA-authorized test, with disease progression following at least one line of endocrine therapy. Select ESR1 mutations for treatment with vepdegestrant are detected by the Guardant360 CDx. FDA approval was based on the results of the Phase III VERITAC-2 (NCT05654623) trial of vepdegestrant versus fulvestrant in patients with previously treated, ESR1-mutated advanced or metastatic breast cancer.

In the Phase III VERITAC-2 (NCT05654623) trial, the vepdegestrant cohort harboring ESR1 mutations (n=136) demonstrated a median progression-free survival of 5.0 months (95% CI=3.7-7.4) and an overall response rate (ORR) of 19% (95% CI=12-27) (PMID: 40454645). The fulvestrant cohort harboring ESR1 mutations (n=134) demonstrated a median PFS of 2.1 months (95% CI=1.9-3.5) (HR=0.58 [95% CI=0.43-0.78]; p<0.001) and an ORR of 4% (95% CI=1.6-10) (PMID: 40454645).

Fulvestrant DrugBank

Fulvestrant is an intramuscularly injected, steroidal selective estrogen receptor degrader (SERD) that is an estrogen receptor (ER) antagonist and results in ER degradation. There are promising clinical data of response to fulvestrant in patients with ESR1-mutant breast cancer.

In a retrospective analysis of the Phase III SoFEA (NCT00253422) trial of fulvestrant or exemestane in patients with hormone receptor-positive breast cancer, patients who had ER mutations had a significantly longer median progression-free survival (PFS) when treated with fulvestrant (5.7 months) versus exemestane (2.6 months) (HR, 0.52; P = 0.02), while patients without ER mutations had no significant difference in median PFS between the two treatment arms (P = 0.77) (PMID: 27269946). The addition of abemaciclib to fulvestrant in patients with hormone receptor-positive breast cancer (abemaciclib plus fulvestrant, n=446; placebo plus fulvestrant, n=223) further increased overall survival in these patients (46.7 months versus 37.3 months; HR= 0.757; 95% CI [0.606-0.945]; P = .01) (PMID: 31563959). In the Phase III PADA-1 (NCT03079011) trial of combination fulvestrant and palbociclib in 172 patients with estrogen receptor-positive, HER2-negative advanced breast cancer with rising ESR1 mutation in blood, median PFS was 11.9 months (95% CI= 9.1-13.6) in the fulvestrant and palbociclib experimental group versus 5.7 months (95% CI= 3.9-7.5) in the aromatase inhibitor and palbociclib control group (PMID: 36183733).

*VAF: Variant Allele Frequency

Com|PI|i|t DX

Name	-	Report No	26xxxxxxGR

7. Clinical Trials to consider

ESR1 associated clinical trials

NCT07062965	PHASE3
Title	A Study to Learn About the Study Medicine Called PF-07248144 in Combination With Fulvestrant in People With HR-positive, HER2-negative Advanced or Metastatic Breast Cancer Who Progressed After a Prior Line of Treatment.
Treatment	PF-07248144 | Fulvestrant | Everolimus | Exemestane
Location	Australia, Belgium, Brazil, Bulgaria, Canada, China, Czechia, Finland, France, Germany, Greece, Hungary, India, Israel, Italy, Japan, Mexico, Netherlands, Poland, Slovakia, South Korea, Spain, Sweden, Taiwan, Turkey (Türkiye), United Kingdom, United States

NCT06380751	PHASE3
Title	Saruparib (AZD5305) Plus Camizestrant or Plus Endocrine Therapy, Compared With CDK4/6 Inhibitor Plus Endocrine Therapy or Plus Camizestrant in HR-Positive, HER2-Negative (IHC 0, 1+, 2+/ ISH Non-amplified), BRCA1, BRCA2, or PALB2m Advanced Breast Cancer
Treatment	Saruparib (AZD5305) | Camizestrant | Abemaciclib | Ribociclib | Palbociclib | Fulvestrant | Letrozole | Anastrozole | Exemestane
Location	Argentina, Australia, Austria, Brazil, Bulgaria, Canada, Chile, China, Czechia, France, Germany, Hong Kong, Hungary, India, Israel, Italy, Japan, Malaysia, Peru, Poland, Portugal, Puerto Rico, South Korea, Spain, Taiwan, Thailand, Turkey (Türkiye), United Kingdom, United States

NCT06065748	PHASE3
Title	A Study to Evaluate Efficacy and Safety of Giredestrant Compared With Fulvestrant (Plus a CDK4/6 Inhibitor), in Participants With ER-Positive, HER2-Negative Advanced Breast Cancer Resistant to Adjuvant Endocrine Therapy (pionERA Breast Cancer)
Treatment	Giredestrant | Fulvestrant | Abemaciclib | Palbociclib | Ribociclib | LHRH Agonist | FoundationOne Liquid CDx Assay (F1LCDx)
Location	Argentina, Australia, Austria, Belgium, Brazil, Canada, Chile, China, Colombia, Costa Rica, Finland, France, Germany, Greece, Guatemala, Hong Kong, Hungary, India, Israel, Italy, Kenya, Mexico, New Zealand, Peru, Poland, Portugal, Puerto Rico, Romania, Singapore, Slovenia, South Africa, South Korea, Spain, Taiwan, Thailand, Turkey (Türkiye), United States

Com|PI|i|t DX

Name	-	Report No	26xxxxxxGR

NCT05816655	PHASE2
Title	Comparison of Clinical Efficacy Between Letrozole + Ribociclib and Fulvestrant + Letrozole + Ribociclib in Hormone Receptor Positive, HER2 Negative Metastatic Breast Cancer
Treatment	Fulvestrant plus AI plus ribociclib | AI plus ribociclib
Location	South Korea

NCT06062498	PHASE2
Title	Elacestrant vs Elacestrant Plus a CDK4/6 Inhibitor in Patients With ERpositive/HER2-negative Advanced or Metastatic Breast Cancer
Treatment	elacestrant, palbociclib, abemaciclib, ribociclib
Location	United States

NCT06067503	PHASE2
Title	Biomarkers to Detect Endocrine Therapy Resistance
Treatment	18F-fluorofuranylnorprogesterone | Liquid Biopsy | Positron Emission Tomography/Computed Tomography
Location	United States

NCT06195306	PHASE2
Title	Low Dose Tamoxifen With or Without Omega-3 Fatty Acids for Breast Cancer Risk Reduction
Treatment	Biospecimen Collection | Mammography | Omega-3-Acid Ethyl Esters | Questionnaire Administration | Random Periareolar Fine-Needle Aspiration | Tamoxifen
Location	United States

NCT06064812	PHASE1 | PHASE2
Title	A Phase I/II Study of FWD1802 in Patients With ER+/HER2- Advanced BC.
Treatment	FWD1802
Location	China

Com|PI|i|t DX

Name	-	Report No	26xxxxxxGR

NCT04174352	EARLY_PHASE1
Title	FES Imaging to Optimize Tamoxifen for Metastatic Breast Cancer
Treatment	Tamoxifen | FES PET/CT
Location	United States

Press here for a live search of clinical trials for ESR1

PIK3CA associated clinical trials

NCT06757634	PHASE3
Title	Phase 3 Study of Gedatolisib as First-Line Treatment for Patients With HR-Positive, HER2-Negative Advanced Breast Cancer (VIKTORIA-2)
Treatment	Arm A: Gedatolisib + Palbociclib + Fulvestrant | Arm B: Ribociclib + Fulvestrant | Arm C: Gedatolisib + Palbociclib + Letrozole | Arm D: Ribociclib + Letrozole
Location	Argentina, Australia, Belgium, Brazil, Bulgaria, Czechia, France, Germany, Greece, Hungary, Italy, Malaysia, Mexico, Poland, Portugal, Romania, South Korea, Spain, Taiwan, Thailand, Turkey (Türkiye), United States

Press here for a live search of clinical trials for PIK3CA

Com|PI|i|t DX

Name	-	Report No	26xxxxxxGR

8. Appendix

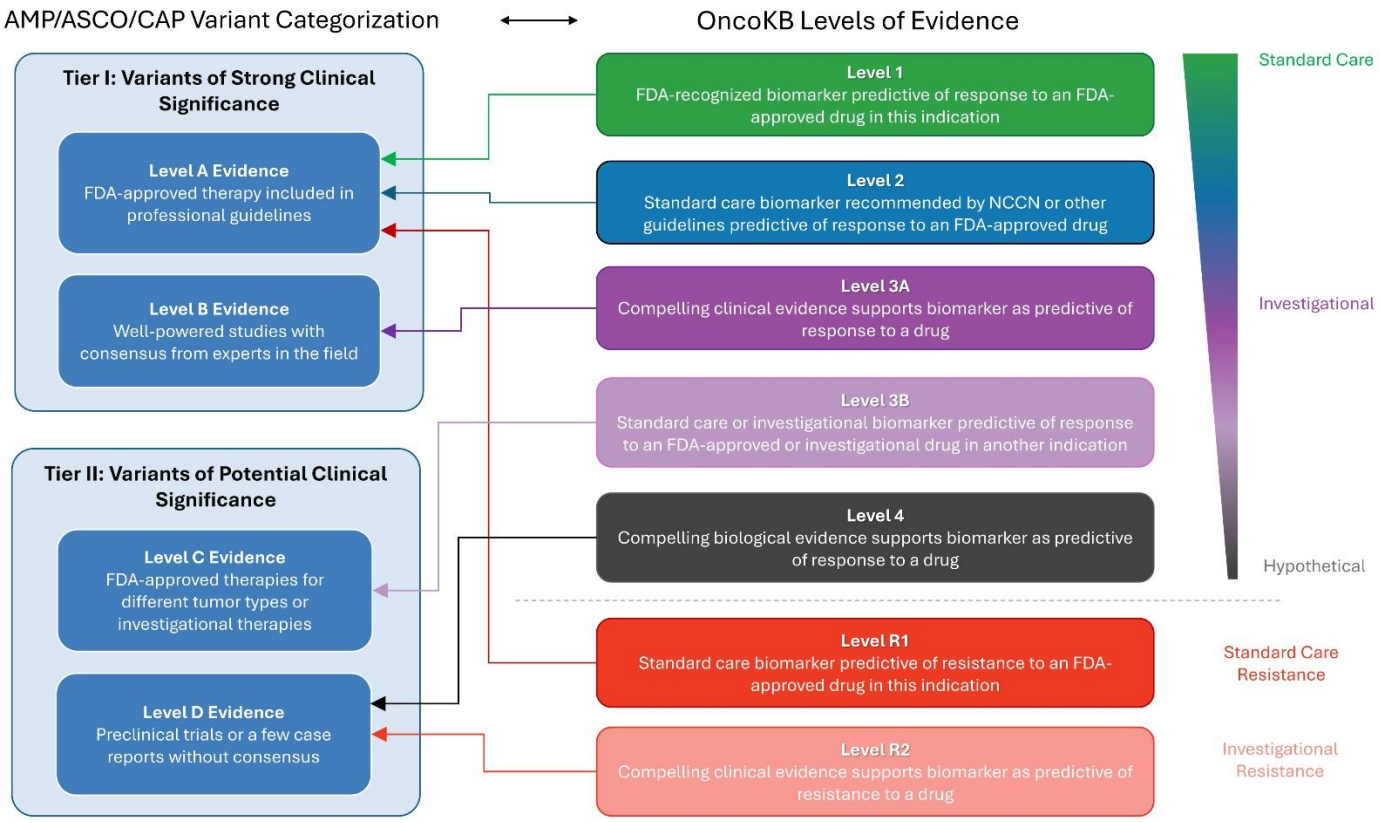

Figure 1. Joint consensus recommendation of AMP, ACMG, ASCO and CAP for reporting genetic variants in cancer. [1-2]

1. Li MM, Datto M, Duncavage EJ, et al. J Mol Diagn. 2017 Jan;19(1):4-23.
2. Leichsenring J, Horak P, Kreutzfeldt S, et al. Int J Cancer. 2019 Dec 1;145(11):2996-3010.

Com|PI|i|t DX

Name	-	Report No	26xxxxxxGR

Microsatellite Instability (MSI)

Microsatellite instability (MSI) is a condition of genetic hypermutability resulting from impaired DNA mismatch repair (MMR) mechanisms. It is characterized by the accumulation of mutations in short repetitive DNA sequences known as microsatellites. MSI-H is found across multiple cancer types, most notably in colorectal, endometrial, gastric, and ovarian cancer. It can arise sporadically or as part of Lynch syndrome, a hereditary condition caused by germline mutations in MMR genes (MLH1, MSH2, MSH6, PMS2).

The results of MSI are divided into three types: MSI-H, which means microsatellites are highly unstable; MSS, which means microsatellites are stable; MSI-U, which means that the sample does not meet the MSI evaluation conditions (tissues or pleural fluid samples may not have passed the MSI indicator calculation quality control due to the low DNA and/or content of tumor cells).

Pembrolizumab has received full FDA approval for adult and pediatric patients with unresectable or metastatic MSI-H or dMMR solid tumors (PMID: 35680043, 33264544) — representing the first full approval for an immunotherapy based on a predictive biomarker, regardless of solid tumor type. This approval applies to patients who have progressed after prior treatment and have no satisfactory alternative options. Beyond the tumor-agnostic indication, specific approvals have been granted for tumor types such as endometrial and colorectal cancer.

52, Spaton Ave., 15344, Gerakas, Greece, G.E.MI. nr: 0007856001000 | info@genekor.com, www.genekor.com | Tel. (+30) 210 6032138 Fax. (+30) 210 6032148
A company certified with ISO 9001:2015 (Cert. No 041150049) and ISO/IEC 27001:2022(Cert. No 048190009)

-

George Nasioulas, PhD Molecular Biologist, Scientific Director, Medical ID:26025301255

Com|PI|i|t DX

Name - **Report No** 26xxxxxxGR

9. Biomarker findings across all Levels of evidence (A-D)

Biomarker	Result	Therapies with strong clinical significance		Therapies with potential significance		Therapies with potential resistance
		Approved/ Standard of care therapies (Level A)	Therapies in well powered studies (Level B)	Off label/ Investigational therapies (Level C)	Therapies in pre-clinical trials (Level D)	
PIK3CA	Wild-Type	**Gedatolisib+Fulvestrant+Palbociclib Gedatolisib+Fulvestrant			-	-
ESR1	Exon 10 c.1610A>C (p.Y537S) VAF: 18.3%	Elacestrant Imlunestrant Vepdegestrant	Fulvestrant	-	-	Aromatase Inhibitors

Com|PI|i|t DX

Name	-	Report No	26xxxxxGR

10. References

1. Tops BB, Normanno N, Kurth H, Amato E, Mafficini A, Rieber N, Le Corre D, Rachiglio AM, Reiman A, Sheils O, Noppen C, Lacroix L, Cree IA, Scarpa A, Ligtenberg MJ, Laurent-Puig P. Development of a semi-conductor sequencing-based panel for genotyping of colon and lung cancer by the Onconetwork consortium. BMC Cancer. 2015 Jan 31;15:26. doi: 10.1186/s12885-015-1015-5. (PMID: 25637035)
2. https://civic.genome.wustl.edu/
3. http://cancer.sanger.ac.uk/
4. https://www.clinicaltrials.gov
5. http://atlasgeneticsoncology.org
6. https://www.oncokb.org/
7. https://www.mycancergenome.org/
8. https://pmkb.org//
9. Carlson KE, et al. **Altered ligand binding properties and enhanced stability of a constitutively active estrogen receptor: evidence that an open pocket conformation is required for ligand interaction.** Biochemistry. 1997; 36:14897-905. doi: 10.1021/bi971746l (PMID: 9398213)
10. Weis KE, et al. **Constitutively active human estrogen receptors containing amino acid substitutions for tyrosine 537 in the receptor protein.** Mol Endocrinol. 1996; 10:1388-98. doi: 10.1210/mend.10.11.8923465 (PMID: 8923465)
11. Campone M, et al. **Vepdegestrant, a PROTAC Estrogen Receptor Degrader, in Advanced Breast Cancer.** N Engl J Med. 2025; 393:556-568. doi: 10.1056/NEJMoa2505725 (PMID: 40454645)
12. Yoshidome K, et al. **Estrogen promotes mammary tumor development in C3(1)/SV40 large T-antigen transgenic mice: paradoxical loss of estrogen receptoralpha expression during tumor progression.** Cancer Res. 2000; 60:6901-10. (PMID: 11156389)
13. Gymnopoulos M, et al. **Rare cancer-specific mutations in PIK3CA show gain of function.** Proc Natl Acad Sci U S A. 2007; 104:5569-74. doi: 10.1073/pnas.0701005104 (PMID: 17376864)
14. Jeselsohn R, et al. **Emergence of constitutively active estrogen receptor-α mutations in pretreated advanced estrogen receptor-positive breast cancer.** Clin Cancer Res. 2014; 20:1757-1767. doi: 10.1158/1078-0432.CCR-13-2332 (PMID: 24398047)
15. Zinger L, et al. **Ligand-binding Domain-activating Mutations of ESR1 Rewire Cellular Metabolism of Breast Cancer Cells.** Clin Cancer Res. 2019; 25:2900-2914. doi: 10.1158/1078-0432.CCR-18-1505 (PMID: 30733228)
16. Sledge GW, et al. **The Effect of Abemaciclib Plus Fulvestrant on Overall Survival in Hormone Receptor-Positive, ERBB2-Negative Breast Cancer That Progressed on Endocrine Therapy-MONARCH 2: A Randomized Clinical Trial.** JAMA Oncol. 2020; 6:116-124. doi: 10.1001/jamaoncol.2019.4782 (PMID: 31563959)
17. Toy W, et al. **ESR1 ligand-binding domain mutations in hormone-resistant breast cancer.** Nat Genet. 2013; 45:1439-45. doi: 10.1038/ng.2822 (PMID: 24185512)
18. Bardia A, et al. **Elacestrant in ER+, HER2- Metastatic Breast Cancer with ESR1-Mutated Tumors: Subgroup Analyses from the Phase III EMERALD Trial by Prior Duration of Endocrine Therapy plus CDK4/6 Inhibitor and in Clinical Subgroups.** Clin Cancer Res. 2024; 30:4299-4309. doi: 10.1158/1078-0432.CCR-24-1073 (PMID: 39087959)
19. Miermont AM, et al. **Role of ERalpha in the differential response of Stat5a loss in susceptibility to mammary preneoplasia and DMBA-induced carcinogenesis.** Carcinogenesis. 2010; 31:1124-31. doi: 10.1093/carcin/bgq048 (PMID: 20181624)

Com|PI|i|t DX

Name	-	Report No	26xxxxxxGR

20. Bidard FC, et al. **Switch to fulvestrant and palbociclib versus no switch in advanced breast cancer with rising ESR1 mutation during aromatase inhibitor and palbociclib therapy (PADA-1): a randomised, open-label, multicentre, phase 3 trial.** Lancet Oncol. 2022; 23:1367-1377. doi: 10.1016/S1470-2045(22)00555-1 (PMID: 36183733)

21. Zhong L and Skafar DF. **Mutations of tyrosine 537 in the human estrogen receptor-alpha selectively alter the receptor's affinity for estradiol and the kinetics of the interaction.** Biochemistry. 2002; 41:4209-17. doi: 10.1021/bi0121095 (PMID: 11914066)

22. Thomas C and Gustafsson JÅ. **The different roles of ER subtypes in cancer biology and therapy.** Nat Rev Cancer. 2011; 11:597-608. doi: 10.1038/nrc3093 (PMID: 21779010)

23. Robinson DR, et al. **Activating ESR1 mutations in hormone-resistant metastatic breast cancer.** Nat Genet. 2013; 45:1446-51. doi: 10.1038/ng.2823 (PMID: 24185510)

24. Toy W, et al. **Activating ESR1 Mutations Differentially Affect the Efficacy of ER Antagonists.** Cancer Discov. 2017; 7:277-287. doi: 10.1158/2159-8290.CD-15-1523 (PMID: 27986707)

25. Fribbens C, et al. **Plasma ESR1 Mutations and the Treatment of Estrogen Receptor-Positive Advanced Breast Cancer.** J Clin Oncol. 2016; 34:2961-8. doi: 10.1200/JCO.2016.67.3061 (PMID: 27269946)

26. Kushner PJ, et al. **Estrogen receptor pathways to AP-1.** J Steroid Biochem Mol Biol. 2000; 74:311-7. doi: 10.1016/s0960-0760(00)00108-4 (PMID: 11162939)

27. Merenbakh-Lamin K, et al. **D538G mutation in estrogen receptor-α: A novel mechanism for acquired endocrine resistance in breast cancer.** Cancer Res. 2013; 73:6856-64. doi: 10.1158/0008-5472.CAN-13-1197 (PMID: 24217577)

28. Hewitt SC, et al. **Lack of ductal development in the absence of functional estrogen receptor alpha delays mammary tumor formation induced by transgenic expression of ErbB2/neu.** Cancer Res. 2002; 62:2798-805. (PMID: 12019156)

29. Couse JF and Korach KS. **Estrogen receptor null mice: what have we learned and where will they lead us?.** Endocr Rev. 1999; 20:358-417. doi: 10.1210/edrv.20.3.0370 (PMID: 10368776)

30. Bocchinfuso WP, et al. **A mouse mammary tumor virus-Wnt-1 transgene induces mammary gland hyperplasia and tumorigenesis in mice lacking estrogen receptor-alpha.** Cancer Res. 1999; 59:1869-76. (PMID: 10213494)

31. Jhaveri KL, et al. **Imlunestrant with or without Abemaciclib in Advanced Breast Cancer.** N Engl J Med. 2025; 392:1189-1202. doi: 10.1056/NEJMoa2410858 (PMID: 39660834)

32. O'Leary B, et al. **The Genetic Landscape and Clonal Evolution of Breast Cancer Resistance to Palbociclib plus Fulvestrant in the PALOMA-3 Trial.** Cancer Discov. 2018; 8:1390-1403. doi: 10.1158/2159-8290.CD-18-0264 (PMID: 30206110)

33. Saville B, et al. **Ligand-, cell-, and estrogen receptor subtype (alpha/beta)-dependent activation at GC-rich (Sp1) promoter elements.** J Biol Chem. 2000; 275:5379-87. doi: 10.1074/jbc.275.8.5379 (PMID: 10681512)

34. Fanning SW, et al. **Estrogen receptor alpha somatic mutations Y537S and D538G confer breast cancer endocrine resistance by stabilizing the activating function-2 binding conformation.** Elife. 2016; 5:(unknown pages). doi: 10.7554/eLife.12792 (PMID: 26836308)

35. Chandarlapaty S. **Negative feedback and adaptive resistance to the targeted therapy of cancer.** Cancer Discov. 2012; 2:311-9. doi: 10.1158/2159-8290.CD-12-0018 (PMID: 22576208)

36. Turner NC, et al. **ESR1 Mutations and Overall Survival on Fulvestrant versus Exemestane in Advanced Hormone Receptor-Positive Breast Cancer: A Combined Analysis of the Phase III SoFEA and EFECT Trials.** Clin Cancer Res. 2020; 26:5172-5177. doi: 10.1158/1078-0432.CCR-20-0224 (PMID: 32546646)

Com|PI|i|t DX

Name	-	Report No	26xxxxxxGR

37. Bidard FC, et al. **Elacestrant (oral selective estrogen receptor degrader) Versus Standard Endocrine Therapy for Estrogen Receptor-Positive, Human Epidermal Growth Factor Receptor 2-Negative Advanced Breast Cancer: Results From the Randomized Phase III EMERALD Trial.** J Clin Oncol. 2022; 40:3246-3256. doi: 10.1200/JCO.22.00338 (PMID: 35584336)

38. Bader AG, et al. **Oncogenic PI3K deregulates transcription and translation.** Nat Rev Cancer. 2005; 5:921-9. doi: 10.1038/nrc1753 (PMID: 16341083)