Canary Oncoceutics

# CANARY ACUITY™

Patient
**XXXXXXXXXXXXXXX**

Report Date: **15 October 2025**
Cancer: **Breast**
MRN/ID: **n/a**

| Patient | Specimen | Healthcare Provider |
|---|---|---|
| **XXXXXXXXXX**<br>Date of Birth: **69 years**<br>Gender: **Female**<br>Disease: **Breast Cancer (TNBC)**<br>Medical Record #: **n/a**<br>Order #: **XXXXXXXXXX** | ID: **XXXXXXXXXX**<br>Type: **FFPE**<br>Site: **Gastric biopsy**<br>Collection Date: **12 July 2025**<br>Receipt Date: **30 July 2025**<br>Country of Origin: **IN** | **Dr. XXXXXXXXXX**<br>**XXXXXXXXXX Hospital**<br>**Test:** Acuity<br>Comprehensive cancer gene panel by NGS<br>Constituents: **1091 genes**, **MSI**, **TMB**, **HRD**, **PGx**<br>Mean Coverage Achieved: **5,142x** |

## Executive Summary

The patient's tumor harbors an alteration in the ***PIK3CA*** and ***PTEN*** genes, with FDA-approved therapies available for the patient's tumor, including **Alpelisib + Fulvestrant**, **Capivasertib + Fulvestrant**, and **Inavolisib + Palbociclib + Fulvestrant**. Additionally, the tumor exhibits alterations in the ***CDH1***, ***MYC***, ***RAD21***, and ***TP53*** genes, for which there are no known FDA-approved treatments. The tumor also contains a mutation in the ***CCND1*** gene, which may have clinical significance based on preclinical and limited scientific studies. These studies suggest that the use of **Capivasertib + Paclitaxel** could provide clinical benefits. However, a preclinical study has demonstrated that this ***CCND1*** mutation confers *resistance* to **Capivasertib** when used alone. The tumor also has alterations of unknown significance in the ***CHEK2***, ***FANCA***, ***MSH6***, ***PMS2***, and ***RET*** genes. Finally, the patient's tumor expressed a ***high Tumor Mutation Burden***, suggesting that immunotherapies such as **Atezolizumab**, **Durvalumab**, **Nivolumab**, and **Pembrolizumab** could be effective.

## Primary Genomic Findings

### Tier I — Genomic Findings with Strong Clinical Implications

**Level A** – FDA-approved therapies for patient's tumor included in professional guidelines

| Alteration | Actionable Therapies |
|---|---|
| ***PIK3CA***:p.E545K (VAF 19%) | **Alpelisib + Fulvestrant, Capivasertib + Fulvestrant, Inavolisib + Palbociclib + Fulvestrant** |
| ***PTEN***:p.Y225* (VAF 29%) | **Capivasertib + Fulvestrant** |

**Level B** – Therapies based on well-powered scientific studies with consensus from experts

| Alteration | Actionable Therapies |
|---|---|
| None | None (refer to Clinical Trials Section) |

### Tier II — Genomic Findings with Potential Clinical Implications

**Level C** – FDA-approved therapies for tumors other than patient's tumor

| Alteration | Actionable Therapies |
|---|---|
| ***CDH1***:p.D367Pfs*25 (VAF 27%) | None (refer to Clinical Trials Section) |
| ***MYC***:Amplification (CN 16) | None (refer to Clinical Trials Section) |
| ***RAD21***:p.Q378* (VAF 20%) | None (refer to Clinical Trials Section) |
| ***TP53***:p.S183* (VAF 29%) | None (refer to Clinical Trials Section) |

**Level D** – Therapies based on preclinical studies or a few scientific studies with no consensus from experts

| Alteration | Actionable Therapies |
|---|---|
| ***CCND1***:Amplification (CN 14) | **Capivasertib + Paclitaxel; *Resistance*: Capivasertib** |

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**TIER III** — VARIANTS OF UNKNOWN SIGNIFICANCE (THESE VARIANTS ARE NOT TYPICALLY OBSERVED AT SIGNIFICANT ALLELE FREQUENCIES IN THE GENERAL POPULATION, AND NO CONVINCING STUDIES SHOW CANCER ASSOCIATION)

| | | | | |
|---|---|---|---|---|
| ***CHEK2***:p.Q20H (VAF 51%) | ***FANCA***:p.R914T (VAF 37%) | ***MSH6***:p.S24W (VAF 22%) | ***PMS2***:p.S523T (VAF 45%) | ***RET***:p.E884Q (VAF 20%) |

*Note: Tier III findings are genetic variations with uncertain clinical implications. These variants might not have been fully studied or documented in scientific research as of the date of this report. They are included here in case they prove to be of clinical relevance in upcoming studies or developments. Only potentially meaningful VUS are included in this report.*

## Primary Biomarker Findings

| BIOMARKER | RESULT | ACTIONABLE THERAPIES |
|---|---|---|
| **MSI** (Microsatellite Instability) | ***Stable*** | n/a |
| **TMB** (Tumor Mutation Burden) | ***High*** (15 Muts/Mb) | **Atezolizumab**, **Durvalumab**, **Nivolumab**, **Pembrolizumab** |
| **HRD** (Homologous Recombination Deficiency) | ***Negative*** (GSS 21) | n/a |
| LOH (Loss of Heterozygosity) | 6% | |
| TAI (Telomeric Allelic Instability) | 12% | |
| LST (Large State Transitions) | 3% | |

*Note: An MSI-High (MSI-H) status suggests a high mutation rate, often influencing treatment choices, especially pertaining to immune checkpoint inhibitors, and is vital for tailoring patient-specific therapeutic strategies[1]. A TMB value of ≥10 Muts/Mb is categorized as TMB High, while a value less than 10 Muts/Mb is deemed to be low[2]. A high HRD score is known to be associated with a positive response to PARP inhibitors. The HRD score is determined using LOH markers and HRD genes present in the Acuity comprehensive panel. A cut-off score of 42 is considered the threshold for HRD prediction, with a score greater than 42 being HRD-positive[3].*

*[1]Niu B., MSI-sensor 2 [Internet]. GitHub; 2024. Available from: https://github.com/niu-lab/msisensor; [2]https://www.fda.gov/drugs/drug-approvals-and-databases/fda-approves-pembrolizumab-adults-and-children-tmb-h-solid-tumors; [3]Sztupinszki Z, et al. Migrating the SNP array-based homologous recombination deficiency measures to next generation sequencing data of breast cancer. NPJ Breast Cancer. 2018;4:16.*

## Pharmacogenomic Findings

| BIOMARKER | GENE | EFFECT | DRUG |
|---|---|---|---|
| None | – | – | – |

## Clinical Trials

**SEE CLINICAL TRIALS SECTION BELOW**

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## Description of Genomic Findings

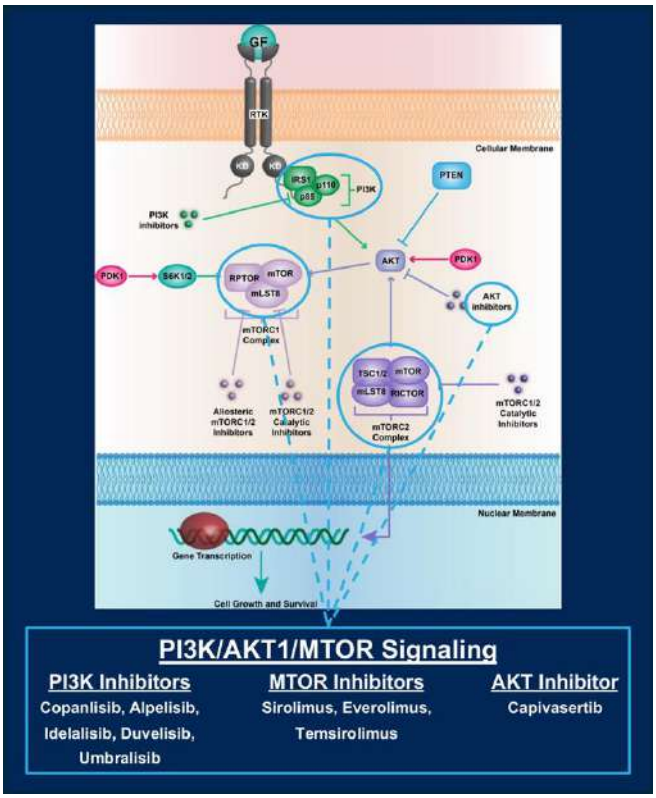
### Gene Altered: *PIK3CA*
### Alteration: p.E545K

PIK3CA (phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha) activates the PI3K/AKT/mTOR pathway to promote cell growth and division (Alqahtani, A. et al., 2019; Dbouk, H.A., 2013). Activating mutations of PIK3CA have been found in various tumor types, including breast cancer (Martínez-Sáez, O. et al., 2020; Mollon, L.E. et al., 2020), colon cancer (Ranjbar, R. et al., 2019), endometrial cancer, glioblastoma, skin cancer, ovarian cancer (Ishibashi, T. et al., 2019; Samuels, Y., 2010), and mammary angiosarcoma (Beca, F. et al., 2020). Additionally, PIK3CA amplification has been observed in esophageal adenocarcinoma (Essakly, A. et al., 2020).

Mutant PIK3CA has been linked to the development of various cancers, including colon cancer, glioma, gastric cancer, breast cancer, endometrial cancer, and lung cancer (Samuels, Y. et al., 2004). PIK3CA is mutated in 12.66% of all cancers, with the highest occurrence in invasive ductal carcinoma of the breast, colon adenocarcinoma, endometrial endometrioid adenocarcinoma, lung adenocarcinoma, and invasive lobular carcinoma of the breast. The most common mutations in PIK3CA are PIK3CA Mutation (12.16%), PIK3CA Codon 1047 Missense (3.28%), PIK3CA Codon 545 Missense (2.88%), PIK3CA H1047R (2.93%), and PIK3CA E545K (2.61%) (André, F. et al., 2017).

Phosphatidylinositol-3-kinase (PI3K) consists of a regulatory subunit (p85α) and a catalytic subunit (p110α), with the catalytic subunit encoded by the PIK3CA gene (Reinhardt, K. et al., 2022). PIK3CA is one of the most frequently mutated genes in cancer, and abnormal activation of PI3K is a transforming event (Gymnopoulos, M. et al., 2007). Several receptor tyrosine kinases, including EGFR, ERBB2 (HER2), RET, MET, and VEGFR, among others, translate extracellular signals into intracellular responses and recruit PI3K to the plasma membrane via scaffold proteins like IRS1 or by activating RAS. When stimulated, PI3K-110α converts its lipid substrate PIP2 (phosphatidylinositol-4,5-bisphosphate) into PIP3 (phosphatidylinositol-3,4,5-bisphosphate), activating multiple signaling pathways, notably the AKT-mTOR pathway. Once activated, AKT-mTOR signaling enhances cell survival, proliferation, growth, and motility (Bader, A.G. et al., 2005). Adding to this complexity, some PI3K/mTOR pathway-targeted drugs can diminish cancer cells' self-regulatory mechanisms, which are inherent in the PI3K-AKT-mTOR pathway, thereby increasing tumor resistance to these therapies (Chandarlapaty, S., 2012).

The PIK3CA gene encodes a lipid kinase that, along with KRAS, regulates signaling pathways downstream of the EGFR. Additionally, the PI3K p110 subunit, encoded by PIK3CA, can be activated by RAS proteins. Normally, phosphatase and tensin homologs deleted on chromosome ten (PTEN) inhibits PI3K-initiated signaling (Sartore-Bianchi, A. et al., 2009). Several intracellular functions, including promoting cell transformation, tumor initiation, and growth, and resistance to apoptosis, are mediated by phosphoinositol-3-kinase (PI3K). Extracellular growth factors and hormones enhance its activity (Reinhardt, K. et al., 2022). In many cancers, deregulation of PI3K triggers the activity of the serine/threonine kinase AKT, which regulates multiple downstream proteins to promote uncontrolled cell and tumor growth (Vivanco, I., 2002).




***E545K***

E545K is a hotspot mutation located within the PIK helical domain of the PIK3CA protein. It leads to increased phosphorylation of AKT and MEK1/2, promotes growth factor-independent cell survival, and is transforming in culture (Dogruluk, T. et al., 2015, Ng, P.K. et al., 2018). The mutation has been identified in breast, colon cancer, and glioblastoma (Gymnopoulos, M. et al., 2007; Yu, K. et al., 2020).

Expression of this mutation in chicken embryonic fibroblasts, Ba/F3 cells, MCF10A breast cells, and in a transgenic mouse model showed that it is activating, as indicated by increased kinase activity, downstream pathway activation, factor-independent proliferation, anchorage-independent colony formation, in vivo tumor growth in xenograft models, and in vivo glioblastoma formation compared to wildtype PIK3CA (Gymnopoulos, M. et al., 2007; Dogruluk, T. et al., 2015; Bader, A.G. et al., 2006; Isakoff, S.J. et al., 2005; Yu, K. et al., 2020). Mutations at this position are predicted to disrupt p85-mediated inhibition of catalytic activity, likely leading to constitutive activation of PIK3CA enzyme activity (Gabelli, S.B. et al., 2010).

In a phase III trial for ER+, HER2− advanced breast cancer that had previously progressed on endocrine therapy, patients received either palbociclib (a CDK4/6 inhibitor) plus fulvestrant (an ESR1 antagonist) or a placebo plus fulvestrant. PIK3CA alterations, including the E545K mutation, were found at a significantly higher percentage in end-of-treatment samples compared to pre-treatment samples in patients from both treatment groups, suggesting that PIK3CA alterations may contribute to resistance to fulvestrant (O'Leary, B. et al., 2018).

*Alpelisib*

Alpelisib is a kinase inhibitor that primarily targets the α-isoform of class I phosphatidylinositol-3-kinase (PI3Kα). The combination of alpelisib and fulvestrant offers a favorable benefit-risk profile for treating postmenopausal women and men with HR-positive, HER2-negative, PIK3CA-mutated, advanced, or metastatic breast cancer. The safety profile of alpelisib combined with fulvestrant showed good tolerability in this group of individuals facing a life-threatening condition (Narayan, P. et al., 2021). Although alpelisib has the highest selectivity for PI3Kα, with 50-, 63-, and 260-fold selectivity over PI3Kγ, PI3Kδ, and PI3Kβ, respectively, some inhibition of PI3Kγ and PI3Kδ likely occurs during treatment, at least in some patients (Fritsch, C. et al., 2014; Vanhaesebroeck, B. et al., 2021). The PI3Kα selectivity of alpelisib and its pharmacokinetics facilitated successful breast cancer trials, leading to FDA approval in 2019 (Vanhaesebroeck, B. et al., 2021).

PIK3CA mutations activate the PI3K pathway, which is associated with poor prognosis in 28%-46% of hormone receptor-positive (HR+) and human epidermal growth factor receptor-2-negative (HER2-) advanced breast cancers (ABCs). The SOLAR-1 study found that adding alpelisib to fulvestrant therapy led to a statistically significant and clinically meaningful increase in progression-free survival (PFS) in PIK3CA-mutated, HR+, HER2- ABC patients (André, F. et al., 2021).

A first-in-human trial was conducted in which alpelisib was administered to adult patients with advanced solid tumors that harbored a PIK3CA gene alteration and whose disease progressed despite standard therapy or for whom no standard treatment was available (NCT01219699). During the dose-escalation phase, patients with PIK3CA-altered advanced solid tumors received oral alpelisib once or twice daily on a continuous schedule. In the dose-expansion phase, patients with PIK3CA-altered tumors and those with PIK3CA-wildtype, ER+/HER2- breast cancer received 400 mg of alpelisib once daily (Juric, D. et al., 2018). A total of 134 patients received treatment. The maximum tolerated doses of alpelisib were identified as 400 mg once daily and 150 mg twice daily. Nine patients (13. 2%) in the dose-escalation phase experienced dose-limiting toxicities, including hyperglycemia (n = 6), nausea (n = 2), and both hyperglycemia and hypophosphatemia (n = 1). Common treatment-related adverse events across all grades included hyperglycemia (51.5%), nausea (50.0%), decreased appetite (41.8%), diarrhea (40.3%), and vomiting (31.3%). Alpelisib was rapidly absorbed, with a half-life of 7.6 hours at a dose of 400 mg once daily, and minimal accumulation was observed. Objective tumor responses were noted at doses of 270 mg or higher once daily; the overall response rate was 6. 6.0% (n = 8), with one patient with endometrial cancer achieving a complete response, and seven patients with cervical, breast, endometrial, colon, and rectal cancers achieving partial responses. Stable disease was achieved in 70 patients (52. 2%) and lasted longer than 24 weeks in 13 patients (9.9%). The disease control rate, including complete and partial responses, as well as stable disease, was 58%. 2%. In patients with estrogen receptor-positive/human epidermal growth factor receptor 2-negative breast cancer, median progression-free survival was 5. 5.5 months. Frequently mutated genes (present in at least 10% of tumors) included TP 53 (51. 3%), APC (23. 7%), KRAS (22. 4%), ARID 1 A (13. 2%), and FBXW 7 (10. 5%) (Juric, D. et al., 2019).

The SOLAR-1 trial (NCT02437318) compared the effect of the ER antagonist fulvestrant with or without alpelisib, finding that the combination treatment improves progression-free survival (PFS) in patients with PIK3CA-mutated, ER-positive, HER2-negative (HR+/HER2-) advanced breast cancer who had previously received endocrine therapy. Importantly, alpelisib had no effect on PFS in individuals without a PIK3CA mutation (Vanhaesebroeck, B. et al., 2021). In the SOLAR-1 trial, men and postmenopausal women with HR+, HER2- ABC whose disease progressed on or after aromatase inhibitor (AI) therapy were randomized 1:1 to receive alpelisib (300 mg/day) plus fulvestrant (500 mg every 28 days with an additional dose on day 15) or placebo plus fulvestrant.

Overall survival (OS) in the PIK3CA-mutant group was assessed using Kaplan-Meier analysis, and a one-sided stratified log-rank test was performed with an O'Brien-Fleming efficacy boundary of P ≤ 0.0161. In the PIK3CA-mutated group (n = 341), the median OS [95% confidence

interval (CI)] was 39.3 months (34.1-44.9) for alpelisib-fulvestrant and 31.4 months (26.8-41.3) for placebo-fulvestrant [hazard ratio (HR) = 0.86 (95% CI, 0.64-1.15; P = 0.15)]. OS results did not cross the predefined efficacy boundary. The median OS (95% CI) for patients with lung and/or liver metastases was 37.2 months (28.7-43.6) with alpelisib-fulvestrant and 22.8 months (19.0-26.8) with placebo-fulvestrant [HR = 0.68 (0.46-1.00)]. Median times to chemotherapy (95% CI) were 23.3 months (15.2-28.4) for alpelisib-fulvestrant and 14.8 months (10.5-22.6) for placebo-fulvestrant [HR = 0.72 (0.54-0.95)]. No new safety signals emerged with extended follow-up. In both groups, the median OS (95% CI) was 37.2 months (28.7-43.6) for alpelisib-fulvestrant and 22.8 months (19.0-26.8) for placebo-fulvestrant [HR = 0.68 (0.46-1.00)]. The median time to chemotherapy was 23.3 months (15.2-28.4) for the alpelisib-fulvestrant group and 14.8 months (10.5-22.6) for the placebo-fulvestrant group [HR = 0.72 (0.54-0.95)]. Longer follow-up showed no new safety concerns (André, F. et al., 2021).

*Capivasertib*

Capivasertib reduces PI3K/AKT/mTOR signaling by inhibiting AKT1, AKT2, and AKT3, potentially leading to decreased cell proliferation (Davies, B.R. et al., 2012; Smyth, L.M. et al., 2020; Yap, T.A. et al., 2020). Capivasertib combined with fulvestrant is FDA-approved for treating patients with hormone receptor-positive, ERBB2 (HER2)-negative, locally advanced or metastatic breast cancer with one or more PIK3CA/AKT1/PTEN alterations.

Capivasertib is an orally available inhibitor of the serine/threonine protein kinase AKT (protein kinase B) with potential antineoplastic activity. It binds to and inhibits all AKT isoforms. Inhibition of AKT prevents the phosphorylation of AKT substrates that mediate cellular processes such as cell division, apoptosis, and glucose and fatty acid metabolism. In vivo, capivasertib proved to be effective in HER2-positive, PIK3CA-mutated breast cancer xenografts, and it also synergized with anti-HER2 agents trastuzumab and lapatinib, as well as with treatment using docetaxel. Additionally, as monotherapy, it was tested in vitro on 182 cell lines derived from solid and hematologic tumors (Davies, B.R. et al., 2015). Furthermore, capivasertib induced a dose-dependent inhibition of growth and survival in invasive lobular carcinoma (ILC) human and mouse breast cancer cell lines (Teo, K. et al., 2018). Based on these preclinical results, the AKT inhibitor capivasertib has entered a Phase II study to assess its efficacy. In this study, 35 patients with prevalent cancer types, including breast cancer (18 [51%]) and gynecologic cancers (11 [31%]), are being evaluated. This group includes 7 patients with endometrioid adenocarcinoma (n = 4) or ovary (n = 3). The clinical outcome showed an overall response rate (ORR) of 28.6% (95% CI, 15%-46%). One patient with endometrioid endometrial adenocarcinoma achieved a complete response (CR) and remained on therapy for 35.6 months. The median follow-up period was 28.4 months, and the overall 6-month progression-free survival (PFS) rate was 50% (95% CI, 35%-71%). This non-randomized trial demonstrated the clinical significance of capivasertib (Kalinsky, K. et al., 2021).

Another investigational study was conducted to assess the efficacy and toxicity of capivasertib in a sample of 63 patients (20 on monotherapy, 43 in combination with fulvestrant) diagnosed with metastatic breast cancer. The overall response rate (ORR) observed was 20% for monotherapy. In the combination group, the response was 36% in fulvestrant-pretreated patients and 20% in fulvestrant-naïve patients, although the latter group may have had more aggressive disease at baseline. Improved progression-free survival (PFS) was also observed with a ≥50% decrease in AKT1 E17K at cycle two, day 1 (Smyth, L.M. et al., 2020).

Furthermore, it has been evaluated for treating triple-negative breast cancer patients as a first-line treatment. In the double-blind, placebo-controlled, randomized Phase II trial named PAKT, a total of 140 patients were randomly assigned (1:1) to paclitaxel 90 mg/m2 (days 1, 8, 15) with either capivasertib (400 mg twice daily) or placebo (days 2-5, 9-12, 16-19) for every 28 days until disease progression or unacceptable toxicity. The PIK3CA/AKT1/PTEN mutational status was assessed for a patient sample size of 70. The clinical outcome has shown a median progression-free survival (PFS) of 5.9 months with capivasertib plus paclitaxel and 4.2 months with placebo plus paclitaxel (hazard ratio [HR], 0.74; 95% CI, 0.50 to 1.08; one-sided P = 0.06 [predefined significance level, one-sided P = 0.10]). The median overall survival (OS) was 19.1 months with capivasertib plus paclitaxel and 12.6 months with placebo plus paclitaxel (HR, 0.61; 95% CI, 0.37 to 0.99; 2-sided P=0.04). In patients with PIK3CA/AKT1/PTEN-altered tumors (n = 28), median PFS was 9.3 months with capivasertib plus paclitaxel and 3.7 months with placebo plus paclitaxel (HR, 0.30; 95% CI, 0.11 to 0.79; 2-sided P=0.01). Thus, the overall conclusion of this study has indicated significantly longer PFS and OS after adding the AKT inhibitor capivasertib to first-line paclitaxel therapy (Schmid, P. et al., 2020).

Finally, capivasertib combined with olaparib, a PARP inhibitor, was studied in a Phase I clinical trial for solid tumors. The study involved 41 individuals with breast or ovarian cancer linked to the E17K mutation. The results showed that two participants had a complete or partial response to the treatment, one of whom had ovarian cancer. That patient had been in partial response (PR) for over two years and remains in response at the time of writing. Additionally, the tumor size decreased significantly from 43.4 mm to 22.3 mm at baseline to 42.3 mm to 8.1 mm (day

433) compared to the start of therapy (Davies, B.R. et al., 2015).

*Fulvestrant*

Fulvestrant, a new estrogen receptor (ER) downregulator, is a pure anti-estrogen that completely blocks the trophic actions of estrogens without causing any partial agonist effects. It decreases the expression of the estrogen receptor, progesterone receptor, and markers of proliferation and cell turnover, and it inhibits growth in cultured estrogen-sensitive breast cancer cells. The drug is well-tolerated with minimal systemic side effects (Cheung, K.L., 2002). It is FDA-approved for use in patients with hormone receptor-positive breast cancer.

Fulvestrant binds competitively and reversibly to estrogen receptors in cancer cells, exerting its anti-estrogen effects through two separate mechanisms. First, it binds to the receptors and downregulates them, preventing estrogen from binding. Second, it degrades the estrogen receptors to which it is bound. Both mechanisms inhibit the growth of tamoxifen-resistant and estrogen-sensitive human breast cancer cell lines (Carlson, R.W., 2005).

Fulvestrant is used to manage and treat advanced breast cancer. It is a selective estrogen receptor degrader. It works by binding to, inhibiting, and degrading estrogen receptors, leading to the complete shutdown of estrogen signaling through the body's estrogen receptors. Compared to earlier drugs like tamoxifen, which act as partial agonists at the estrogen receptor, fulvestrant functions as a full antagonist at the receptor. It inhibits both AF-1 and AF-2 transcriptional activities, preventing estrogen from exerting its effects. As a result, it acts as a full antagonist at the estrogen receptor (Nathan, M.R., 2017).

Fulvestrant has been investigated for endometrial cancer in a Phase II study. This study aimed to assess the activity and toxicity of fulvestrant, a pure estrogen receptor antagonist, in patients with advanced or recurrent endometrial cancer who express estrogen and/or progesterone receptors (ER/PR). Thirty-five patients participated in the study and received at least one dose of fulvestrant (intention-to-treat population, ITT). Twenty-six patients received three doses of fulvestrant (per-protocol population, PP). Although there were no complete responses, four patients showed partial responses (11.4% ITT), and there were 8 cases of stable disease. The median time to progression was 2.3 months (ITT). Overall survival was 13.2 months (ITT). The treatment was well tolerated. Fulvestrant at a dose of 250 mg IM every four weeks demonstrates limited activity but good tolerability in patients with ER- and/or PR-positive advanced or recurrent endometrial cancer. An initial loading dose strategy and the use of 500 mg/week might enhance the treatment's effectiveness (Emons, G. et al., 2013).

Phase I and II studies on selective estrogen receptor down-regulators used for endometrial cancer treatment have been reviewed. The pharmacokinetic and pharmacodynamic features of selective receptor down-regulators have also been investigated. Expert opinion: Selective estrogen receptor down-regulators may show clinical efficacy in treating gynecological malignancies because of their pure estrogen receptor antagonist properties. However, data are still limited, and some unresolved questions remain. Fulvestrant has poor oral bioavailability and low pharmacodynamic properties. Further trials are needed to evaluate new selective estrogen receptor down-regulator agents with better pharmacodynamic and pharmacokinetic profiles (Bogliolo, S. et al., 2017).

Fulvestrant is the only SERD approved by the FDA for metastatic or advanced HR+ breast cancer. It was first licensed in 2002 as a second-line endocrine therapy for patients with disease progression after initial antiestrogen treatment, based on two Phase-III trials comparing its effectiveness and safety to anastrozole (Wang, G., 2020). FDA approval was granted after the Phase III MONARCH 2 trial, which met its primary goal of improving progression-free survival (PFS). In this trial, 669 women with advanced HR+ and HER2- breast cancer participated. Those treated with fulvestrant 500 mg and abemaciclib 150 mg showed a statistically significant increase in median PFS, from 7.1 months to 16.4 months, with a hazard ratio of 0.553 (95 percent CI 0.449-0.681; p<0.0001) (Sledge, G.W. et al., 2020; Wang, G., 2020).

In two randomized, controlled clinical studies involving postmenopausal women with locally advanced or metastatic breast cancer (a North American double-blind study and a European open-label study), intramuscular fulvestrant was compared to oral anastrozole. Participants had previously received adjuvant endocrine therapy or had metastatic malignancies that initially responded to endocrine therapy. A total of 851 individuals were enrolled, with 428 receiving fulvestrant 250 mg monthly via intramuscular injection and 423 receiving anastrozole 1 mg daily (Bross, P.F. et al., 2002).

In the North American and European trials, fulvestrant response rates were 17% and 20%, respectively, compared to 17% and 15% in the anastrozole therapy arms. There were no statistically significant differences. Using two-sided 95.4% confidence intervals, analysis of the response rates in each trial ruled out a difference in response >10% with respect to anastrozole, thus satisfying criteria used by the FDA as the basis for approval of previous hormonal treatments for breast cancer. In either trial, there were no statistically significant differences in time to progression or survival between the two treatment groups (Bross, P.F. et al., 2002).

ESR1 mutations frequently occur in hormone receptor-positive metastatic breast cancer after previous aromatase inhibitor treatment. The Phase III EFECT and SoFEA trials

randomized patients with hormone receptor-positive metastatic breast cancer who had progressed after nonsteroidal aromatase inhibitor therapy, assigning them to fulvestrant 250 mg or exemestane. ESR1 mutations were found in 30% (151/383) of baseline samples. For patients with ESR1 mutations, PFS was 2.4 months (95% CI, 2.0-2.6) on exemestane and 3.9 months (95% CI, 3.0-6.0) on fulvestrant (hazard ratio [HR], 0.59; 95% CI, 0.39-0.89; P = 0.01). In patients without ESR1 mutations, PFS was 4.8 months (95% CI, 3.7-6.2) on exemestane and 4.1 months (95% CI, 3.6-5.5) on fulvestrant (HR, 1.05; 95% CI, 0.81-1.37; P = 0.69). There was a significant interaction between ESR1 mutation status and treatment (P = 0.02). Patients with ESR1 mutations had a 1-year OS of 62% (95% CI, 45%-75%) on exemestane and 80% (95% CI, 68%-87%) on fulvestrant (P = 0.04; restricted mean survival analysis). Those without ESR1 mutations showed a 1-year OS of 79% (95% CI, 71%-85%) on exemestane and 81% (95% CI, 74%-87%) on fulvestrant (P = 0.69) (Turner, N.C. et al., 2020).

Preclinical investigations show that Y537S has the highest transactivation activity and the greatest relative resistance to tamoxifen, fulvestrant, and several new selective estrogen receptor degraders (SERDs) and selective estrogen receptor modulators (SERMs) among all ESR1 LBD variants (the SERCA H3B-6545 is currently under study). PALOMA-3 and SoFEA investigations in patients revealed that Y537S had the lowest PFS on fulvestrant and, unlike other ESR1 mutations, was enriched in patients receiving fulvestrant and fulvestrant + palbociclib. Therefore, while all ESR1 LBD mutations are chosen for AI monotherapy patients and predict poor response to AI, Y537S is the mutation responsible for resistance to ER-targeted treatments, and trials and analyses should stratify by mutation types present in each patient's heterogeneous tumor (Brett, J.O. et al., 2021).

*Alpelisib + Fulvestrant*

Alpelisib is a selective inhibitor of the alpha form of PI3K (PI3Kα) that is FDA-approved in combination with the selective estrogen receptor (ER) degrader fulvestrant for treating postmenopausal patients with ER+ HER2- PIK3CA-mutated advanced or metastatic breast cancer. FDA approval is based on data from the Phase III randomized, double-blind, placebo-controlled SOLAR-1 trial, which compared alpelisib plus fulvestrant to placebo plus fulvestrant in 572 breast cancer patients. In patients with PIK3CA mutations (n=341), progression-free survival was eleven months (95% CI= 7.5-14.5) with alpelisib plus fulvestrant versus 5.7 months (95% CI= 3.7-7.4) with placebo plus fulvestrant (HR= 0.65; 95% CI= 0.5-0.85; p= 0.0013). In contrast, PIK3CA-wildtype patients had a hazard ratio of 0.85 (95% CI= 0.58-1.25). Overall response in the PIK3CA-mutant group was 35.7 months (95% CI= 27.4-44.7; n=126) with alpelisib plus fulvestrant versus 16.2 months (95% CI= 10.4-23.5; n=136) with placebo plus fulvestrant (André, F. et al., 2019). Previous studies, including in vivo xenograft studies and a Phase I trial, also showed that treating ER+ PIK3CA-mutant breast cancer with fulvestrant and alpelisib leads to greater tumor shrinkage than either drug alone, as fulvestrant inhibits ER-dependent gene transcription induced by PI3Kα inhibition with alpelisib (Bosch, A. et al., 2015; Juric, D. et al., 2019).

*Capivasertib + Fulvestrant*

Capivasertib is an orally available, ATP-competitive pan-AKT small molecule inhibitor that is FDA-approved with fulvestrant for adult patients with ER+/HER2- locally advanced or metastatic breast cancer who have one or more PIK3CA/AKT1/PTEN alterations, as detected by an FDA-approved test. This is for patients who have experienced progression on at least one endocrine-based regimen in the metastatic setting or recurrence within twelve months of completing adjuvant therapy. Eligible PIK3CA/AKT1/PTEN alterations (including PIK3CA R88Q, N345K through G1049R; AKT1 E17K; and all PTEN oncogenic mutations) were identified using the FoundationOne CDx assay. FDA approval was based on the results of the Phase III CAPItello-291 (NCT04305496) trial, which compared capivasertib plus fulvestrant to placebo plus fulvestrant in 708 adult patients with ER+/HER2- locally advanced or metastatic breast cancer, including 289 patients with eligible PIK3CA/AKT1/PTEN alterations. In this trial, the cohort with PIK3CA/AKT1/PTEN-altered tumors receiving capivasertib plus fulvestrant (n=155) showed an objective response rate (ORR) of 26% (95% CI=19-34), with a 2.3% complete response (CR) rate, 23% partial response (PR) rate, and a median progression-free survival (PFS) of 7.3 months (95% CI=5.5-9.0) (Turner, N.C. et al., 2023). The placebo plus fulvestrant cohort with PIK3CA/AKT1/PTEN-altered tumors (n=134) had an ORR of 8% (95% CI=4-14), including an 8% PR rate, and a median PFS of 3.1 months (95% CI=2.0-3.7) (HR=0.50 [95% CI=0.38-0.65]; p<0.0001) (Turner, N.C. et al., 2023).

*Inavolisib + Palbociclib + Fulvestrant*

Inavolisib is an oral, small-molecule PI3Kα inhibitor approved by the FDA for use with palbociclib and fulvestrant in treating adults with endocrine-resistant, PIK3CA-mutated, HR-positive, HER2-negative, locally advanced or metastatic breast cancer. This is for cases detected by an FDA-approved test after recurrence following adjuvant endocrine therapy. PIK3CA oncogenic mutations for treatment with inavolisib plus palbociclib and fulvestrant were identified using the FoundationOne Liquid CDx.

FDA approval was based on the results of the Phase III INAVO120 (NCT04191499) trial of inavolisib plus palbociclib and fulvestrant versus palbociclib plus fulvestrant in 325 patients with PIK3CA-mutated, HR+/HER2- breast cancer. In

the Phase III INAVO120 trial, the inavolisib cohort (n=161) showed an overall response rate (ORR) of 58.4%, including seven complete responses (CR) and 87 partial responses (PR). The median progression-free survival (PFS) was 15.0 months (95% CI=11.3-20.5). Median overall survival (OS) was not evaluable (95% CI=0.43-0.97). The median duration of response (DOR) was 18.4 months (95% CI=10.4-22.2) (Turner, N.C. et al., 2024). In contrast, the palbociclib plus fulvestrant-only cohort (n=164) demonstrated an ORR of 25.0%, with one CR and 40 PRs. The median PFS was 7.3 months (95% CI=5.6-9.3) (HR=0.43 [95% CI=0.32-0.59]; p<0.0001). The median OS was 31.1 months (95% CI=22.3-NE) (HR=0.64 [95% CI=0.43-0.97]; p=0.0338). The median DOR was 9.6 months (95% CI=7.4-16.6) (Turner, N.C. et al., 2024).

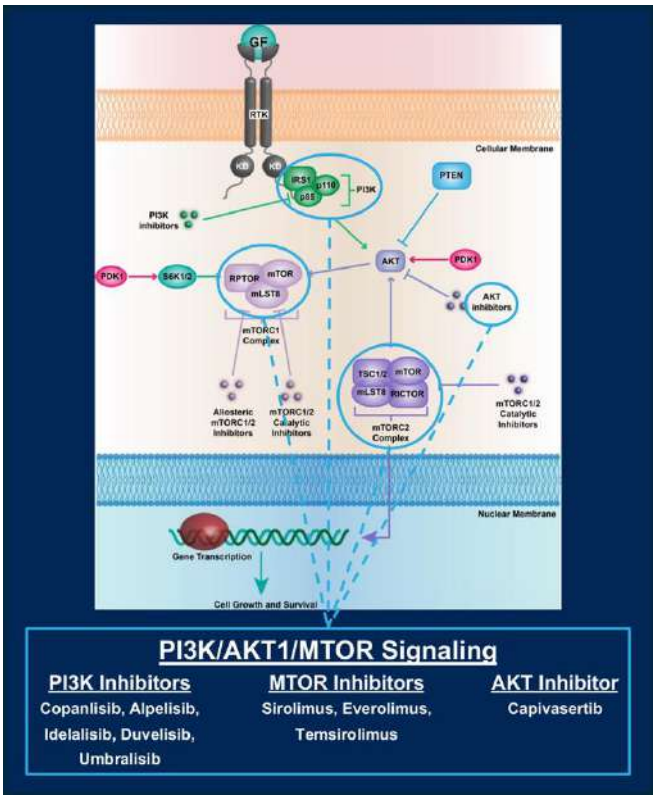
## Description of Genomic Findings

### Gene Altered: *PTEN*
### Alteration:p.Y225*

PTEN (phosphatase and tensin homolog) is a gene that encodes phosphatidylinositol 3,4,5-trisphosphate 3-phosphatase and dual-specificity protein phosphatase PTEN. This protein is a lipid/protein phosphatase that is known as a tumor suppressor (Wang, L. et al., 2018) with roles in the cell cycle, growth, DNA repair, cell survival, and regulation of the PI3K/AKT/mTOR signaling pathway (Hopkins, B.D. et al., 2014; Naderali, E. et al., 2018). Somatic mutations of PTEN occur in multiple malignancies, including glioma, melanoma (Giles, K.M. et al., 2019), prostate (Chalhoub, N., 2009; Gao, S. et al., 2018), endometrial (Liang, X. et al., 2018), breast, ovarian, renal, and lung cancers. Germline mutations of PTEN can lead to inherited hamartoma (Miller, D.T. et al., 2021) and Cowden syndrome (Chalhoub, N., 2009; Maehama, T., 2007).

PTEN is altered in 7.24% of all cancers, with endometrial endometrioid adenocarcinoma, conventional glioblastoma multiforme, prostate adenocarcinoma, breast invasive ductal carcinoma, and colon adenocarcinoma showing the highest prevalence of alterations. The most common alterations in PTEN include PTEN Mutation (5.50%), PTEN Frameshift (1.70%), PTEN Nonsense (1.66%), PTEN Loss (1.72%), and PTEN R130G (0.31%) (André, F. et al. 2017).

PTEN is a tumor suppressor gene that is among the most frequently mutated in human cancer (Li, J. et al., 1997; Song, M.S. et al., 2012; Steck, P.A. et al., 1997). It has various physiological roles, most notably functioning as a phosphatase that converts phosphatidylinositol (3,4,5)-trisphosphate (PIP3) into phosphatidylinositol (4,5)-diphosphate (PIP2) at the cell membrane (Chalhoub, N., 2009). When PTEN function is impaired through multiple mechanisms, including non-synonymous mutations, PIP3 accumulates, leading to constant activation of downstream AKT/mTOR signaling pathways. Consequently, PTEN inactivation encourages cell growth, proliferation, and survival (Cantley, L.C., 2002). Additionally, nuclear PTEN is believed to regulate RAD51 expression, thereby contributing to homologous recombination and DNA strand break repair (Bassi, C. et al., 2013; Shen, W.H. et al., 2007). Loss of PTEN can also increase genomic instability and create conditions for the buildup of other harmful mutations. PTEN mutations are common in many human cancers (Sansal, I., 2004). Germline loss-of-function mutations in PTEN are present in roughly 80% of individuals with Cowden disease, a cancer predisposition syndrome associated with high-penetrance breast and thyroid cancers (Marsh, D.J. et al., 1998; Pilarski, R. et al., 2013; Hollander, M.C. et al., 2011).




***p.Y225****

The Y225* variant causes a frameshift mutation, resulting in a premature stop codon at amino acid position 225. This produces a truncated PTEN protein that is likely non-functional due to the loss of critical *C*-terminal regions necessary for its normal activity (Lin, E.I. et al., 2015). The Y225* variant has been shown to decrease phosphatase activity in a yeast assay (Mighell, T.L. et al., 2018) and is therefore predicted to cause a loss of PTEN protein function.

PTEN truncating mutations can lead to various forms of C-terminally truncated PTEN proteins. Mutations closer to the *N*-terminus result in the loss of PTEN's phosphatase activity and its inability to negatively regulate the PI3K/AKT pathway (Simpson, L., 2001). Expression of a PTEN truncation mutation in mouse embryonic fibroblasts showed that these mutations are oncogenic and increase genome instability because PTEN cannot associate with chromosomal centromeres like the full-length protein does (Shen, W.H. et al., 2007).

*Capivasertib + Fulvestrant*

Capivasertib, an orally available, ATP-competitive pan-AKT inhibitor, is FDA-approved with fulvestrant for treating

patients with PTEN-mutant ER+/HER2– metastatic breast cancer. FDA approval was based on the results of the Phase III CAPItello-291 trial of capivasertib plus fulvestrant in adult patients with or without AKT pathway-altered (PIK3CA, AKT1, or PTEN) ER+/HER2– advanced breast cancer. Among the patients with only PIK3CA, AKT1, or PTEN-mutant tumors (n=289), the capivasertib plus fulvestrant group (n=155) showed an objective response rate of 26% (95% CI=19, 34), with a 2.3% complete response rate and 23% partial response rate, and a median progression-free survival of 7.3 months (95% CI=5.5, 9.0). In contrast, the placebo plus fulvestrant group (n=134) had an objective response rate of 8% (95% CI=4, 14), with an 8% partial response rate, and a median progression-free survival of 3.1 months (95% CI=2.0, 3.7) (HR=0.50 [95% CI=0.38, 0.65]; P<0.0001) (Turner, N.C., 2023). Including all patients—those with wild-type tumors and those with PIK3CA, AKT1, or PTEN-mutant tumors (n=708)—the capivasertib plus fulvestrant group (n=355) demonstrated a median progression-free survival of 7.2 months (95% CI=5.5, 7.4), compared to 3.6 months (95% CI=2.8, 3.7) in the placebo plus fulvestrant group (n=353) (HR=0.60 [95% CI=0.51, 0.71]; P<0.001) (Turner, N.C., 2023).

## Description of Genomic Findings

### Gene Altered: *CDH1*
### Alteration: p.D367Pfs*25

CDH1 (cadherin 1, type 1) encodes E-Cadherin and functions as a tumor suppressor (Wang, L. et al., 2018). It is involved in epithelial cell-cell adhesion, cell signaling, differentiation, and polarity (van Roy, F., 2008; Abascal, M.F. et al., 2016). Germline mutations in CDH1 are linked to hereditary diffuse gastric cancer (Wang, L. et al., 2018), while somatic mutations occur in gastric, breast, colon, and NSCLC cancers (Jain, R. et al., 2016; Liu, X., 2014).

CDH1, also known as E-cadherin, is a calcium-dependent transmembrane glycoprotein primarily expressed in epithelial cells. It plays a role in cell-cell adhesion, signaling pathways, and epithelial-to-mesenchymal transition (EMT) (van Roy, F., 2008). The extracellular part of E-cadherin promotes homophilic cell adhesion by binding to cadherins on neighboring cells, while the intracellular domain connects to the actin cytoskeleton via interactions with catenins and helps activate signaling pathways involved in EMT (Shapiro, L. et al., 1995; Ozawa, M. et al., 1989).

The transcription factor SNAIL, a key regulator of the EMT during embryonic development, represses the expression of the E-cadherin gene in tumor cell lines (Cano, A. et al., 2000; Batlle, E. et al., 2000). The loss of E-cadherin function or expression facilitates cancer progression by altering cellular morphology, reducing cellular adhesion, and increasing cellular motility (Handschuh, G. et al., 1999; Vleminckx, K. et al., 1991; Perl, A.K. et al., 1998).

In addition to point mutations and loss of heterozygosity (LOH), epigenetic silencing through hypermethylation of the CDH1 promoter has been linked to the loss of E-cadherin gene expression during cancer progression (Yoshiura, K. et al., 1995). Individuals with a germline CDH1 mutation have a higher risk of developing diffuse gastric cancer and lobular breast cancer (Pharoah, P.D. et al., 2001; Samadder, N.J. et al., 2019; Blair, V.R. et al., 2020). E-cadherin loss has also been observed in various sporadic cancer types, including gastric, colorectal, and esophageal cancers (Machado, J.C. et al., 2001; Elzagheid, A. et al., 2012; Ling, Z. et al., 2011).

***p.D367Pfs*25***

The D367Pfs*25 variant is a frameshift mutation resulting from an insertion or deletion of nucleotides that alters the reading frame of the gene. The frameshift occurs at amino acid position 367, where aspartic acid (D) is expected. It leads to a change in the amino acid sequence from this point and introduces a premature stop codon 25 amino acids downstream in the altered reading frame. This premature termination is likely to result in a truncated E-cadherin protein with a loss of normal function due to the absence of critical *C*-terminal residues.

CDH1 truncating mutations can produce several forms of *C*-terminally truncated CDH1 proteins. These mutations have been found in breast cancer and as germline mutations in familial gastric cancer (Berx, G. et al., 1996; Norton, J.A. et al., 2007). CDH1 loss in a transplant mouse model demonstrated increased genomic instability and increased disease burden compared to wildtype.

## Description of Genomic Findings

### Gene Altered: *MYC*
### Alteration: Amplification

The MYC protooncogene encodes a DNA-binding factor that can activate and repress transcription. Through this process, MYC regulates the expression of many target genes that govern essential cellular functions, including cell growth, cell cycle progression, apoptosis, cellular transformation (Baluapuri, A. et al., 2020; Wahlström, T., 2015), and immune system responses (Casey, S.C. et al., 2018). MYC also plays a vital role in DNA replication. Deregulated MYC expression, caused by various genetic alterations, results in persistent MYC activity in several cancers and promotes oncogenesis (Dominguez-Sola, D. et al., 2007). Amplification, overexpression, and rearrangement of MYC are often observed in solid tumors and hematological malignancies (Kalkat, M. et al., 2017; Shih, D.J. et al., 2020), such as lung cancer (Chanvorachote, P. et al., 2020) and diffuse large B-cell lymphoma (Xia, Y., 2020).

MYC is altered in 4.53% of all cancers, with the highest prevalence of alterations found in breast invasive ductal carcinoma, lung adenocarcinoma, colon adenocarcinoma, prostate adenocarcinoma, and invasive breast carcinoma. The most common modifications in MYC are Amplification (3.97%), Mutation (0.92%), Fusion (0.09%), A59V (0.04%), and S161L (0.03%) (André, F. et al. 2017).

MYC is a transcription factor in the MYC protein family (c-MYC, n-MYC, and l-MYC) that heterodimerizes with the protein MAX to regulate the transcription of thousands of genes. MYC promotes tumor development by stimulating cell proliferation, preventing exit from the cell cycle, promoting blood vessel formation, and increasing genomic instability (Dang, C.V., 2012; Meyer, N., 2008; Nesbit, C.E. et al., 1999; Vita, M., 2006). The MYC gene has several distinct structural domains, including five highly conserved "Myc-boxes" found across multiple species within the MYC family (Meyer, N., 2008). Mutations in the MYC gene are less common than amplification and translocation. Known mutations usually occur near residue T58, a phosphorylation site on MYC that is crucial for ubiquitination and subsequent degradation of the MYC protein (Bahram, F. et al., 2000; Dang, C.V., 2012). Typically, phosphorylation of T58 controls MYC degradation, but mutation at this site extends the protein's half-life (Cai, Q. et al., 2015).

It has been reported that two common mutant MYC alleles derived from human Burkitt lymphoma disconnect proliferation from apoptosis and, as a result, are more effective than wildtype MYC at promoting B-cell lymphomagenesis in mice. Mutant MYC proteins can still stimulate proliferation and activate p53 but are unable to promote apoptosis because they fail to induce the BH3-only protein BIM and do not effectively inhibit BCL2. Disrupting apoptosis through the enforced expression of BCL2 or the loss of either BIM or p53 function allows wildtype MYC to produce lymphomas as efficiently as mutant MYC (Hemann, M.T. et al., 2005).

***Amplification***

Amplification of MYC is linked to increased expression of the MYC gene (Dang, C.V., 2012; Blancato, J. et al., 2004), which leads to the activation of MYC target genes. These target genes control various cellular processes involved in cancer development, including promoting cell proliferation, preventing exit from the cell cycle, stimulating blood vessel formation, and increasing genomic instability (Dang, C.V., 2012; Nesbit, C.E. et al., 1999; Vita, M., 2006; Meyer, N., 2008).

Genomic amplification of MYC occurs in many types of tumors. A study analyzing genomic amplification across different cancers found focal amplifications of MYC in 14% of all cancers, along with increased large-scale arm-level copy number changes involving the MYC gene locus (Beroukhim, R. et al., 2010).

## Description of Genomic Findings

### Gene Altered: *RAD21*
### Alteration: p.Q378*

RAD21 (RAD21 cohesin complex component) is a double-strand-break repair protein and a member of the cohesin complex, which is responsible for maintaining sister chromatid segregation during the S-phase of the mitotic cycle (Panigrahi, A.K. et al., 2012). Amplification and overexpression of RAD21 have been observed in several cancer types, including breast, prostate, and colorectal (Xu, H. et al., 2011; Deb, S. et al., 2014), and mutations have been identified in acute myeloid leukemia (Tsai, C. et al., 2017).

RAD21 is altered in 2.48% of all cancers, with breast invasive ductal carcinoma, lung adenocarcinoma, invasive breast carcinoma, prostate adenocarcinoma, and colon adenocarcinoma showing the highest prevalence of alterations. The most common alterations in RAD21 include RAD21 Amplification (1.68%), RAD21 Mutation (0.86%), RAD21 Loss (0.04%), RAD21 Fusion (0.16%), and RAD21 R65* (0.03%) (André, F. et al., 2017).

RAD21 is a subunit of the cohesin complex that aligns and stabilizes sister chromatids during metaphase (Losada, A., 2014). The cohesin ring consists of two large structural proteins, SMC1a and SMC3, and it opens and closes through the binding of alpha-kleisin subunits to the RAD21 adapter protein (Losada, A., 2014). The cohesin complex also helps maintain chromatin looping structures, or 3D arrangements of DNA that enable regulatory control of gene expression (Rao, S.S. et al., 2017). RAD21 has been linked to the repair of double-stranded DNA breaks (Xu, H. et al., 2010).

Germline mutations in RAD21 have been found in patients with cohesinopathies, causing a range of developmental defects (Deardorff, M.A. et al., 2012). Somatic RAD21 mutations have been identified in patients with acute myeloid leukemia and myelodysplastic syndromes (Thol, F. et al., 2014; Kon, A. et al., 2013; Thota, S. et al., 2014). Most RAD21 mutations are missense and are predicted to cause loss of function; however, it is still unclear if RAD21 mutations lead to aneuploidy as expected (Thota, S. et al., 2014).

***p.Q378****

The Q378* variant has not been characterized in the scientific literature; therefore, its effect on RAD21 protein function and its role in the disease phenotype cannot be definitively determined. However, it is a truncating mutation in a tumor suppressor gene and is therefore likely oncogenic.

Truncating mutations of RAD21 have been identified in patients with myeloid malignancies and are associated with significantly reduced RAD21 expression (Thota, S. et al., 2014). Assessment of the clonal evolution of RAD21 truncating mutations demonstrated that they most commonly occur as a late event in leukemia development. Since RAD21 is a subunit in the cohesion complex, loss of RAD21 is linked to a reduction in the expression of other cohesion proteins, including SMC1A, and leads to defective chromosomal separation and cell bridging (Li, Z. et al., 2017).

## Description of Genomic Findings

### Gene Altered: *TP53*
### Alteration: p.S183*

The TP53 tumor suppressor gene is located on the short arm of chromosome 17 (17p13). It contains 11 exons spanning 20 kilobases and encodes a 53-kDa nuclear phosphoprotein. The TP53 protein includes functional domains, such as the *N*-terminus transcriptional activation domain, the sequence-specific DNA-binding domain, the oligomerization domain, and the *C*-terminus negative regulatory domain (Strano, S. et al., 2007). The TP53 gene is involved in various biological processes, including DNA repair, apoptosis, cell cycle arrest, autophagy, metabolism, and cellular aging (Jin, S. et al., 2001; Levine, A.J., 1997; Riley, T. et al., 2008). Mutations in both alleles of the TP53 gene are found in up to 50% of all human cancers (Olivier, M. et al., 2010; Robles, A.I. et al., 2010; Soussi, T. et al., 2006).

On average, a mutated form of TP53 is present in 50% of cancers (Soussi, T. et al., 2005). Loss-of-function (LOF) and gain-of-function (GOF) mutations are the most common types of TP53 alterations (Miller, M. et al., 2016). The most extensively studied polymorphism is rs1042522 (c.215G>C), a G-to-C transversion in exon 4 at codon 72, leading to an amino acid change from arginine to proline (TP53 Arg72Pro). The rs1042522 polymorphism is located in a proline-rich region of the protein, which has long been recognized as crucial for growth suppression and apoptosis (Soussi, T. et al., 2005). This polymorphism induces a structural change in the protein, resulting in variants with different electrophoretic mobility (Pietsch, E.C. et al., 2006).

The biochemical and biological features of the two p53 isoforms differ because of a polymorphism at codon 72. The TP53 Arg72 variant seems to trigger apoptosis more effectively than the Pro72 form (Pim, D. et al., 2004). When paired with specific tumor-derived mutations, the Arg72 variation may boost tumor suppressive activity due to its higher ability to inactivate p73. The polymorphism influences gain-of-function mutations, as mutated TP53 Arg72 binds to p73 more easily than mutated TP53 Pro72 (Marin, M.C. et al., 2000). The Arg72 variant is a more potent inhibitor of chemotherapy-induced apoptosis than the Pro72 form (Bergamaschi, D. et al., 2003).

Patients with breast, lung, or head and neck cancer who are homozygous for the Arg72 allele tend to have more prolonged survival and better responses to chemotherapy and radiotherapy (Nelson, H.H. et al., 2005; Tommiska, J. et al., 2005; Xu, Y. et al., 2005).

Adjuvant combination chemotherapy for early breast cancer includes anthracyclines (Khasraw, M. et al., 2012). Epirubicin and doxorubicin are two commonly used anthracyclines in breast cancer treatment. A doxorubicin epimer plays a crucial role in managing both early and metastatic breast cancer. Epirubicin's effectiveness is comparable to that of doxorubicin; however, it has a distinct toxicity profile, particularly in terms of cardiotoxicity. Epirubicin acts by intercalating into DNA, inhibiting topoisomerase II, producing oxygen and drug-free radicals, and disrupting DNA, RNA, and protein synthesis, thereby exhibiting cytocidal activity (Glück, S., 2005). In 1999, the Food and Drug Administration (FDA) approved Epirubicin in the United States for the treatment of breast cancer (Khasraw, M. et al., 2012).

Recent research has shown that the p53 codon 72 polymorphism influences the apoptotic capacity of wildtype p53 and may consequently impact chemotherapy response (Xu, Y. et al., 2005). Several recent in vivo and in vitro studies have emphasized a functional difference between the Pro72 and Arg72 variants of wildtype p53, with the Arg72 form demonstrating a stronger ability to induce apoptosis than the Pro72 variant. These functional differences among polymorphic variants may affect tumor response to systemic chemotherapy by altering apoptotic potential (Dumont, P. et al., 2003; Sullivan, A. et al., 2004).

Xu, Y. et al. conducted a study to determine if the p53 codon 72 polymorphism affected the pathological response to neoadjuvant treatment in primary breast cancer. One hundred and ten patients with operable breast cancer received anthracycline-based neoadjuvant chemotherapy, and their p53 codon 72 polymorphism status was identified by PCR-RFLP. The authors performed a clinical trial using the CTF regimen (5-fluorouracil, pirarubicin, and cyclophosphamide) for patients with operable breast cancer; if a patient declined participation, they received an anthracycline-based regimen such as FEC (5-fluorouracil, epirubicin, and cyclophosphamide) or CAF (5-fluorouracil, epirubicin, doxorubicin, and cyclophosphamide). The polymorphic variants did not differ significantly in terms of initial clinical stage, tumor size, estrogen receptor or progesterone receptor status, menopausal status, or erbB2 expression. However, only 13% (3 of 23) of patients with the Pro/Pro variant showed a favorable pathologic response, defined as complete response or minimal residual disease, according to the investigators. In contrast, individuals with the Pro/Arg or Arg/Arg variations showed a satisfactory pathologic response in 40% (22 of 55) or 37.5% (12 of 32) of cases (P = 0.019). Additionally, patients with the Pro/Pro variant were more likely to have positive axillary lymph node

status (P = 0.007) compared to those with the Pro/Arg or Arg/Arg variants (Xu, Y. et al., 2005).

Furthermore, the p53 codon 72 polymorphism was identified as a significant predictor of pathological response in multivariate analysis (odds ratio, 6.7; 95% confidence interval, 1.4-31.2; P = 0.016). The research indicated that breast cancer patients with the Pro/Pro variant might be less responsive to anthracycline-based treatment than those with the Pro/Arg or Arg/Arg variants. This p53 codon 72 polymorphism analysis could serve as a simple predictive marker for selecting suitable breast cancer patients for anthracycline-based neoadjuvant chemotherapy in clinical practice (Xu, Y. et al., 2005).

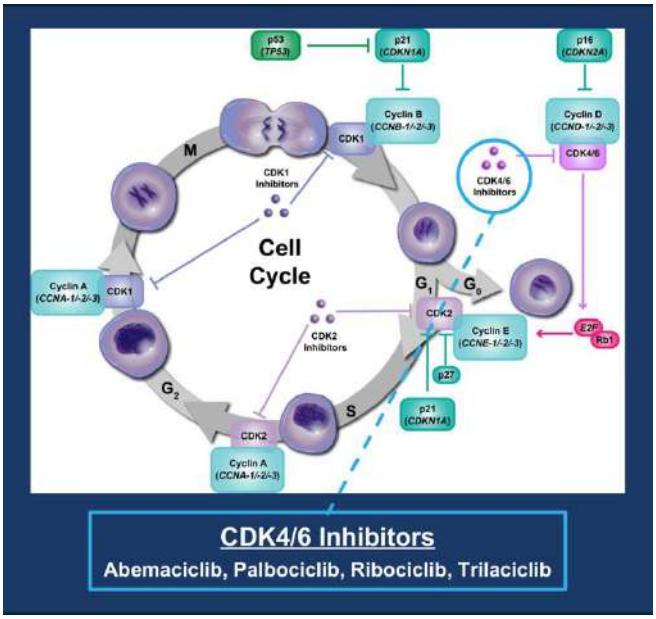
Ovarian cancer patients with TP53 missense mutations showed greater resistance to platinum-based chemotherapy (66% vs. 34% in wildtype, P = 0.008) and experienced a shorter time to disease progression (n = 54, P = 0.037). A study of ovarian carcinoma patients (n = 178) reported decreased overall survival (HR: 1.59, 95% CI: 1.09-2.33, P = 0.014) for patients with TP53 mutations (Reles, A. et al., 2001).

***p.S183****

The S183*variant has not been characterized in the scientific literature; therefore, its effect on TTP53 protein function and its role in the disease phenotype cannot be definitively determined. However, it is a truncating mutation in a tumor suppressor gene and is therefore likely oncogenic. Based on the effects of truncating mutations downstream of S183 (Kharaziha, P. et al., 2019; Tong, D.R. et al., 2021), it is predicted to cause a loss of TP53 protein function.

Truncating mutations of TP53 occur throughout the gene, leading to the production of several C-terminally truncated protein forms. These alterations are predicted to be inactivating and are associated with poor prognosis (Bullock, A.N., 2001; DiGiammarino, E.L. et al., 2002; Kawaguchi, T. et al., 2005; Plas, M.L. et al., 2011; Holstege, H. et al., 2009).

Experimental studies have shown that truncating mutations promote cancer cell proliferation, survival, and metastasis, as evidenced by the increased cell motility and tumor formation observed upon ectopic expression of these mutations in melanoma cells in vivo. This was partly due to the aberrant localization of truncated proteins to the mitochondria, regulating genes involved in cell survival, including CypD (Shirole, N.H. et al., 2016).



# Description of Genomic Findings

## Gene Altered: *CCND1*
## Alteration: Amplification

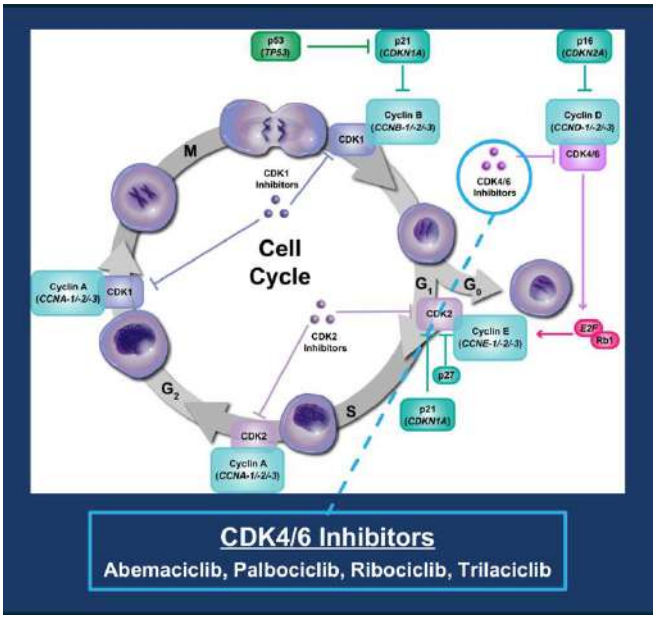
CCND1 (cyclin D1) plays an essential role in controlling the cell cycle, especially in the transition from G1 to S phase, by interacting with CDK4 and CDK6 (John, R.R. et al., 2017; Qie, S., 2016). Amplification and/or overexpression of CCND1 have been linked to many tumor types, including breast, lung, liver, colorectal cancer, and mantle cell lymphoma (John, R.R. et al., 2017), where overexpression may drive tumor formation (Shan, J. et al., 2009), and CCND1 fusions with IGH are common in hematological cancers (Li, J.Y. et al., 1999; Rahman, Z.A. et al., 2020; Yamamoto, K. et al., 2020; Nishijima, A. et al., 2019).

CCND1 is altered in 4.10% of all cancers, with breast invasive ductal carcinoma, invasive breast carcinoma, bladder urothelial carcinoma, breast invasive lobular carcinoma, and endometrial endometrioid adenocarcinoma showing the highest prevalence of alterations. Fusions, rearrangements, missense mutations, nonsense mutations, silent mutations, and in-frame deletions and insertions are observed in cancers such as endometrial, intestinal, and stomach cancers. The most common alterations in CCND1 are CCND1 Amplification (3.83%), CCND1 Mutation (0.62%), CCND1 Loss (0.04%), CCND1 P287S (0.05%), and CCND1 Fusion (0.05%) (André, F. et al., 2017).

CCND1 is a protein that links external growth signals to cell cycle entry by activating cyclin-dependent kinases 4 (CDK4) and 6 (CDK6) (Meyerson, M., 1994). When it forms a complex with cyclin D1, CDK4 and CDK6 phosphorylate and inactivate retinoblastoma (RB), leading to the activation of a gene expression program regulated by the E2F family of transcription factors. This process is crucial for the transition from G1 to S phase in the cell cycle (Diehl, J.A., 2002; Resnitzky, D. et al., 1994). Cyclin D1 functions include controlling cell growth, proliferation, transcription, DNA repair, and migration (Musgrove, E.A. et al., 2011). As an oncogene, cyclin D1 is often overexpressed or amplified in many cancers, such as breast, lung, melanoma, and oral squamous cell carcinomas (Beroukhim, R. et al., 2010; Santarius, T. et al., 2010). Although cyclin D1 is not essential for cell cycle entry (Kozar, K. et al., 2004), its amplification or overexpression in human tumors is oncogenic because it enables cancer cells to grow independently of external growth signals (Choi, Y.J., 2014; Santarius, T. et al., 2010).

***Amplification***

CCND1 amplification leads to overexpression of the protein. This mutation is one of the most common copy-number alterations in human tumors (Beroukhim, R. et al., 2010). Overexpression of CCND1 in vitro and in a mouse model has shown that it is activating, as indicated by increased pathway activity and cell proliferation (Meyerson, M., 1994; Mueller, A. et al., 1997).




*Resistance: Capivasertib*

In a preclinical study, patient-derived xenograft (PDX) models of HER2-negative breast cancer with CCND1 amplification showed resistance to capivasertib treatment (Gris-Oliver, A. et al., 2020).

*Capivasertib + Paclitaxel*

In a clinical study, capivasertib combined with paclitaxel led to a partial response in an ERBB2 (HER2)-negative breast cancer patient and slowed tumor growth in a patient-derived xenograft (PDX) model with CCND1 amplification (Gris-Oliver, A. et al., 2020).

## Description of Biomarker Findings

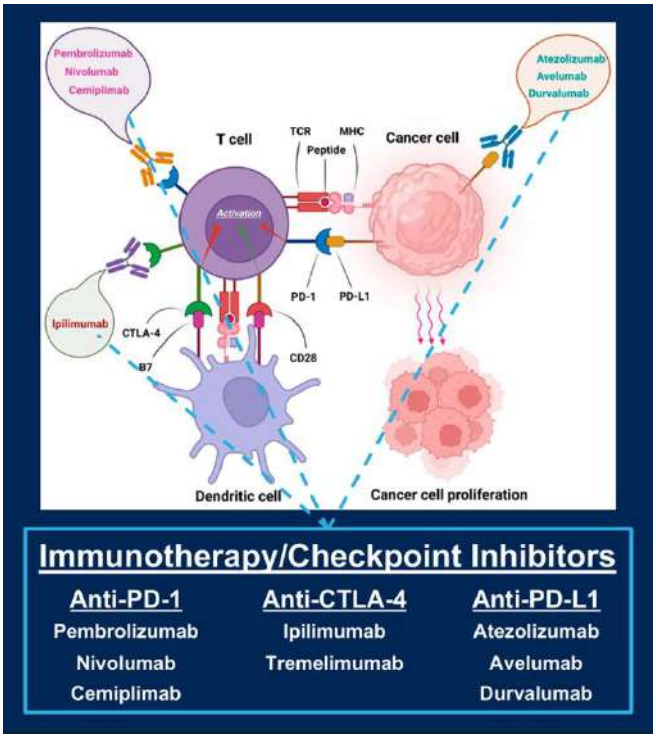
### Biomarker Result: TMB – High

Based on clinical evidence in solid tumors, increased TMB may be associated with greater sensitivity to immunotherapeutic agents, including anti-PD-L1 and anti-PD-1 therapies (Cristescu, R. et al., 2018; Goodman, A.M. et al., 2017; Goodman, A.M. et al., 2019; Samstein, R.M. et al., 2019). Multiple clinical trials of PD-1- or PD-L1-targeting immune checkpoint inhibitors in NSCLC have reported that patients with tumors harboring TMB ≥10 Muts/Mb derive greater clinical benefit from these therapies than those with TMB <10 Muts/Mb; similarly, higher efficacy of anti-PD-1 or anti-PD-L1 immunotherapy for the treatment of patients with NSCLC, compared with the use of chemotherapy, has been observed more significantly in cases of TMB ≥10 Muts/Mb (Carbone, D.P. et al., 2017; Chae, Y.K. et al., 2019; Colli, L.M. et al., 2016; Forde, P.M. et al., 2018; Goodman, A.M. et al., 2019; Hellmann, M.D. et al., 2018; Miao, D. et al., 2018; Ready, N. et al., 2019; Rizvi, H. et al., 2018; Rizvi, N.A. et al., 2015; Samstein, R.M. et al., 2019; Wang, V.E. et al., 2017).

The overall survival (OS) of patients with non-small cell lung cancer (NSCLC) treated with pembrolizumab plus chemotherapy is improved relative to chemotherapy alone or those treated with nivolumab plus ipilimumab, as well as chemotherapy (Hellmann, M.D. et al., 2019), across all tumor mutational burden (TMB) levels. A large-scale genomic analysis found that unspecified lung non-small cell lung carcinoma (NSCLC), lung adenocarcinoma, and lung squamous cell carcinoma (SCC) samples harbored median TMBs between 6.3 and 9 Muts/Mb, and 12% to 17% of cases had an elevated TMB of greater than 20 Muts/Mb (Chalmers, Z.R. et al., 2017). Lower TMB is observed more commonly in NSCLCs harboring known driver mutations (EGFR, ALK, ROS1, or MET), except for BRAF or KRAS mutations, which are commonly observed in cases with elevated TMB.

Although some studies have reported a lack of association between smoking and mutational burden in NSCLC (Shim, H.S. et al., 2015; Xiao, D. et al., 2016), several other large studies did find a strong association with increased TMB (Ding, L. et al., 2008; Govindan, R. et al., 2012; Imielinski, M. et al., 2012; Kim, Y. et al., 2014). TMB >10 Muts/Mb was found to be more frequent in NSCLC metastases compared with primary tumors for both adenocarcinoma (38% vs. 25%) and squamous cell carcinoma (SCC) (41% vs. 35%) subtypes (Stein, M.K. et al., 2019).

An extensive study of Chinese patients with lung adenocarcinoma reported a shorter median overall survival (OS) for tumors with a higher number of mutations in a limited gene set compared with those with a lower mutation number (48.4 vs. 61.0 months) (Xiao, D. et al., 2016). Another study of patients with NSCLC correlated elevated TMB with a poorer prognosis and found that significantly lower TMB, in combination with PD-L1-negative status, was associated with more prolonged median survival in patients with lung adenocarcinoma (Chen, Y. et al., 2019). However, no significant prognostic association has been reported between TMB and/or PD-L1 status and survival in patients with lung squamous cell carcinoma (SCC) (Yu, H. et al., 2019).



## Clinical Trial Findings

| Biomarker | Clinical Trial Title | Clinical Trial ID | Phase |
|---|---|---|---|
| *PIK3CA/ PTEN* | PIK3CA/PTEN-altered Advanced Breast Cancer Treated With MEN1611 Monotherapy or in Combination With Eribulin (SABINA) | NCT05810870 | II |
| *PIK3CA/ CDH1* | Alpelisib/iNOS Inhibitor/Nab-paclitaxel in Patients With HER2 Negative Metaplastic Breast Cancer (MpBC) | NCT05660083 | II |
| *PIK3CA* | First in Human Phase 1 Study of AG01 Anti-Progranulin/GP88 Antibody in Advanced Solid Tumor Malignancies | NCT05627960 | I |
| *PIK3CA/ PTEN* | Serial Circulating Tumor DNA (ctDNA) Monitoring During Adjuvant Capecitabine in Early Triple-negative Breast Cancer | NCT04768426 | II |
| *CHD1* | Abemaciclib in Treating Patients With Surgically Resectable, Chemotherapy Resistant, Triple Negative Breast Cancer | NCT03979508 | II |
| *TP53* | NordicTrip, a Translational Study of Preoperative Chemotherapy in TNBC | NCT04335669 | III |
| *TMB–H* | A Beta-only IL-2 ImmunoTherapY Study (ABILITY-1) | NCT05086692 | I/II |
| *TMB–H* | A Phase I/II Study of Regorafenib Plus Avelumab in Solid Tumors (REGOMUNE) | NCT03475953 | I/II |

## Gene List

### Entire Coding Sequence to detect SNVs, Indels, CNVs, and fusions

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ABCB1 | BCL9 | CDKN2B | DNMT3A | EZR | GLI1 | IKZF3 | LGR5 | MYC | PCBP1 | PTCH2 | RSPO3 | SRC | TRAF7 |
| ABL1 | BCLAF1 | CDKN2C | DNMT3B | FAF1 | GLI2 | IL10 | LIFR | MYCL | PDCD1 | PTEN | RTEL1 | SRSF2 | TRIP13 |
| ABL2 | BCOR | CEBPA | DNTT | FANCA | GNA11 | IL2 | LMNA | MYCN | PDCD11 | PTMA | RUNX1 | SS18 | TSC1 |
| ABRAXAS1 | BCORL1 | CENPA | DOT1L | FANCB | GNA12 | IL3 | LMO1 | MYD88 | PDCD1LG2 | PTP4A1 | RXRA | STAG1 | TSC2 |
| ACTA2 | BCR | CFTR | DPYD | FANCC | GNA13 | IL6R | LRP1B | MYH11 | PDGFB | PTPDC1 | RYBP | STAG2 | TSHR |
| ACTB | BIRC3 | CHD2 | DROSHA | FANCD2 | GNAQ | IL6ST | LRP5 | MYH9 | PDGFRA | PTPN1 | S1PR2 | STAT1 | TUSC3 |
| ACVR1 | BLM | CHD3 | DTX1 | FANCE | GNAS | IL7R | LRP6 | MYOD1 | PDGFRB | PTPN11 | SALL4 | STAT2 | TXNIP |
| ACVR1B | BMPR1A | CHD4 | DUSP2 | FANCF | GNB1 | ING1 | LTB | NAB2 | PDK1 | PTPN13 | SAMHD1 | STAT3 | TYK2 |
| ACVR2A | BRAF | CHD7 | DUSP22 | FANCG | GPC3 | INHA | LTK | NADK | PDPK1 | PTPN2 | SBDS | STAT4 | TYRO3 |
| AFP | BRCA1 | CHEK1 | DUSP4 | FANCI | GPS2 | INHBA | LYN | NBN | PDS5B | PTPN6 | SCAF4 | STAT5A | U2AF1 |
| AGO1 | BRCA2 | CHEK2 | DUSP9 | FANCL | GREM1 | INPP4A | LZTR1 | NCOA2 | PGBD5 | PTPRC | SCG5 | STAT5B | U2AF2 |
| AGO2 | BRD3 | CIC | E2F3 | FANCM | GRIN2A | INPP4B | MAD2L2 | NCOA3 | PGR | PTPRD | SDC4 | STAT6 | UBE2T |
| AJUBA | BRD4 | CIITA | EBF1 | FAS | GRIN2D | INPP5D | MAF | NCOR1 | PHF6 | PTPRO | SDHA | STK11 | UGT1A1 |
| AKT1 | BRD7 | CKS1B | ECT2L | FAT1 | GRM3 | INPPL1 | MAFB | NCOR2 | PHOX2B | PTPRS | SDHAF2 | STK19 | UNCX |
| AKT2 | BRIP1 | CLIP1 | EED | FBXO11 | GSK3B | INSR | MAGED1 | NCSTN | PICALM | PTPRT | SDHB | STK40 | USP6 |
| AKT3 | BRSK1 | CMTR2 | EEF1A1 | FBXO31 | GTF2I | IRAK1 | MAGI2 | NECTIN4 | PIGA | QKI | SDHC | SUFU | USP8 |
| ALB | BTG1 | CNBD1 | EEF2 | FBXW7 | GTSE1 | IRF1 | MALT1 | NEGR1 | PIK3C2B | RAB35 | SDHD | SUZ12 | VAV1 |
| ALK | BTG2 | COL1A1 | EGFL7 | FCGR2A | H1-2 | IRF2 | MAML2 | NF1 | PIK3C2G | RABEP1 | SERP2 | SYK | VEGFA |
| ALOX12B | BTK | COL5A1 | EGFR | FCGR3A | H3-3A | IRF4 | MAP2K1 | NF2 | PIK3C3 | RAC1 | SERPINA1 | TACSTD2 | VHL |
| AMER1 | BTLA | CPS1 | EGLN1 | FGF1 | H3-4 | IRF6 | MAP2K2 | NFATC2 | PIK3CA | RAC2 | SERPINB3 | TAF1 | VTCN1 |
| ANKRD11 | BUB1B | CRBN | EGR1 | FGF10 | H3-5 | IRF8 | MAP2K4 | NFE2 | PIK3CB | RAD21 | SERPINB4 | TAF15 | WAS |
| ANKRD26 | C3ORF70 | CREB3L3 | EGR3 | FGF12 | H3C1 | IRS1 | MAP3K1 | NFE2L2 | PIK3CD | RAD50 | SESN2 | TAL1 | WDR90 |
| APC | C8ORF34 | CREBBP | EIF1AX | FGF14 | H3C2 | IRS2 | MAP3K13 | NFKBIA | PIK3CG | RAD51 | SESN3 | TAP1 | WEE1 |
| APH1A | CA9 | CRKL | EIF3E | FGF19 | HAVCR2 | IRS4 | MAP3K14 | NFKBIE | PIK3R1 | RAD51B | SETBP1 | TAP2 | WIF1 |
| APLNR | CAD | CRLF2 | EIF4A2 | FGF2 | HDAC1 | ITK | MAP3K4 | NHP2 | PIK3R2 | RAD51C | SETD1B | TBL1XR1 | WNK2 |
| APOB | CALR | CRTC1 | EIF4E | FGF23 | HDAC2 | ITPKB | MAP3K6 | NKX2-1 | PIK3R3 | RAD51D | SETD2 | TBX3 | WRN |
| AR | CAMTA1 | CSDE1 | ELANE | FGF3 | HDAC4 | JAK1 | MAP3K7 | NKX3-1 | PIM1 | RAD52 | SETDB1 | TCF12 | WT1 |
| ARAF | CARD11 | CSF1R | ELF3 | FGF4 | HDAC7 | JAK2 | MAPK1 | NOD1 | PKN1 | RAD54L | SETDB2 | TCF3 | WWTR1 |
| ARFRP1 | CARM1 | CSF3R | ELOC | FGF5 | HGF | JAK3 | MAPK3 | NOP10 | PLAG1 | RAF1 | SF1 | TCF7L2 | XIAP |
| ARHGAP26 | CASP8 | CSNK1A1 | ELP2 | FGF6 | HIF1A | JARID2 | MAST1 | NOTCH1 | PLCB4 | RANBP17 | SF3B1 | TCL1A | XPA |
| ARHGAP35 | CBFA2T3 | CTAG1B | EML4 | FGF8 | HLA-A | JAZF1 | MAST2 | NOTCH2 | PLCG1 | RANBP2 | SGK1 | TCL1B | XPC |
| ARHGEF12 | CBFB | CTC1 | EMSY | FGF9 | HLA-B | JUN | MAX | NOTCH3 | PLCG2 | RARA | SH2B3 | TEK | XPO1 |
| ARID1A | CBL | CTCF | ENG | FGFR1 | HLA-C | KANSL1 | MC1R | NOTCH4 | PLK2 | RASA1 | SH2D1A | TENT5C | XRCC1 |
| ARID1B | CBLB | CTLA4 | EP300 | FGFR2 | HLA-DMA | KAT6A | MCL1 | NPM1 | PLXNB2 | RASGEF1A | SHH | TERT | XRCC2 |
| ARID2 | CBLC | CTNNA1 | EPAS1 | FGFR3 | HLA-DMB | KAT6B | MDC1 | NR4A3 | PMAIP1 | RB1 | SHOC2 | TET1 | XRCC3 |
| ARID3A | CCDC6 | CTNNB1 | EPCAM | FGFR4 | HLA-DOA | KBTBD4 | MDM2 | NRAS | PML | RBM10 | SHQ1 | TET2 | YAP1 |
| ARID4B | CCNB3 | CTNND1 | EPHA2 | FGR | HLA-DOB | KCNJ5 | MDM4 | NRG1 | PMS1 | RBM15 | SIGLEC1 | TET3 | YEATS4 |
| ARID5B | CCND1 | CTR9 | EPHA3 | FH | HLA-DPB1 | KDM2B | MECOM | NSD1 | PMS2 | RECQL | SIN3A | TFE3 | YES1 |
| ASMTL | CCND2 | CUL1 | EPHA5 | FHIT | HLA-DQA1 | KDM4C | MED12 | NSD2 | PNRC1 | RECQL4 | SLC26A3 | TFEB | YWHAE |
| ASPSCR1 | CCND3 | CUL3 | EPHA7 | FLCN | HLA-DQA2 | KDM5A | MEF2B | NT5C2 | POLD1 | RECQL5 | SLC34A2 | TFG | YY1AP1 |
| ASXL1 | CCNE1 | CUL4A | EPHB1 | FLI1 | HLA-DQB1 | KDM5C | MEF2C | NT5E | POLD3 | REL | SLFN11 | TFRC | ZBTB2 |
| ASXL2 | CCNE2 | CUL4B | EPHB4 | FLT1 | HLA-DQB2 | KDM6A | MEN1 | NTHL1 | POLE | RELA | SLIT2 | TGFBR1 | ZBTB20 |
| ATF7IP | CCR4 | CUX1 | EPOR | FLT3 | HLA-DRA | KDR | MERTK | NTRK1 | POLH | REST | SLX4 | TGFBR2 | ZBTB7A |
| ATIC | CCR5 | CXCR4 | EPS8 | FLT4 | HLA-DRB1 | KEAP1 | MET | NTRK2 | POLQ | RET | SMAD2 | TGIF1 | ZBTB7B |
| ATM | CCT6B | CYLD | ERBB2 | FLYWCH1 | HLA-DRB5 | KEL | MGMT | NTRK3 | POLRMT | RFC1 | SMAD3 | THADA | ZC3H12A |
| ATP6AP1 | CD19 | CYP17A1 | ERBB3 | FOLH1 | HLA-E | KIAA1549 | MIB1 | NUDT15 | POT1 | RHEB | SMAD4 | THRAP3 | ZCCHC12 |
| ATP6V1B2 | CD22 | CYP19A1 | ERBB4 | FOLR1 | HLA-F | KIF1A | MITF | NUF2 | POU2F2 | RHOA | SMARCA1 | TIPARP | ZFHX3 |
| ATP7B | CD27 | CYP2D6 | ERCC1 | FOXA1 | HLA-G | KIF1B | MKI67 | NUMBL | POU2F3 | RHOB | SMARCA2 | TLL2 | ZFP36L1 |
| ATR | CD274 | CYSLTR2 | ERCC2 | FOXA2 | HMGA2 | KIF5B | MKNK1 | NUP133 | PPARG | RHPN2 | SMARCA4 | TLR4 | ZFP36L2 |
| ATRX | CD276 | DACH1 | ERCC3 | FOXL2 | HNF1A | KIT | MLH1 | NUP214 | PPM1D | RICTOR | SMARCB1 | TLX3 | ZMYM2 |
| ATXN3 | CD28 | DAXX | ERCC4 | FOXO1 | HNRNPK | KLF2 | MLH3 | NUP93 | PPP2R1A | RINT1 | SMARCD1 | TMEM127 | ZMYM3 |
| ATXN7 | CD33 | DAZAP1 | ERCC5 | FOXO3 | HOXA11 | KLF4 | MLLT1 | NUP98 | PPP2R2A | RIT1 | SMARCE1 | TMEM30A | ZNF133 |
| AURKA | CD36 | DCUN1D1 | ERCC6 | FOXP1 | HOXB13 | KLF5 | MLLT10 | NUTM1 | PPP4R2 | RNF111 | SMC1A | TMPRSS2 | ZNF217 |
| AURKB | CD40 | DDB2 | ERF | FOXQ1 | HRAS | KLHL6 | MLLT3 | OLIG2 | PPP6C | RNF139 | SMC3 | TMSB4X | ZNF24 |
| AXIN1 | CD58 | DDR1 | ERG | FRS2 | HSD3B1 | KLLN | MN1 | P2RY8 | PRCC | RNF43 | SMO | TNFAIP3 | ZNF384 |
| AXIN2 | CD69 | DDR2 | ERRFI1 | FUBP1 | HSP90AA1 | KMT2A | MPL | PAG1 | PRDM1 | ROBO1 | SMYD3 | TNFRSF11A | ZNF703 |
| AXL | CD70 | DDX3X | ESR1 | FUS | HSP90AB1 | KMT2B | MRE11 | PAK1 | PRDM14 | ROS1 | SNCAIP | TNFRSF14 | ZNRF3 |
| B2M | CD74 | DDX41 | ESR2 | FYN | ICOSLG | KMT2C | MS4A1 | PAK3 | PREX2 | RPA1 | SOCS1 | TNFRSF17 | ZRSR2 |
| BABAM1 | CD79A | DEK | ETNK1 | G6PD | ID3 | KMT2D | MSH2 | PAK5 | PRF1 | RPL10 | SOCS2 | TNFRSF25 | |
| BAP1 | CD79B | DHH | ETS1 | GAB1 | IDH1 | KNSTRN | MSH3 | PALB2 | PRKACA | RPL22 | SOCS3 | TNFRSF8 | |
| BARD1 | CDC73 | DHX15 | ETV1 | GAB2 | IDH2 | KRAS | MSH6 | PALLD | PRKAR1A | RPL5 | SOS1 | TNFRSF9 | |
| BAX | CDH1 | DHX9 | ETV4 | GABRA6 | IDO1 | KRT222 | MSI2 | PARP1 | PRKCA | RPS15 | SOX10 | TOP1 | |
| BBC3 | CDK12 | DIAPH2 | ETV5 | GADD45B | IFNAR1 | L2HGDH | MSMB | PARP2 | PRKCB | RPS6KA3 | SOX17 | TOP2A | |
| BCL10 | CDK2 | DICER1 | ETV6 | GALNT12 | IFNGR1 | LAG3 | MSR1 | PARP3 | PRKCI | RPS6KA4 | SOX2 | TP53 | |
| BCL11B | CDK4 | DIS3 | EWSR1 | GATA1 | IFNGR2 | LAMP1 | MST1 | PASK | PRKD1 | RPS6KB1 | SOX9 | TP53BP1 | |
| BCL2 | CDK6 | DIS3L2 | EXO1 | GATA2 | IGF1 | LATS1 | MST1R | PAX3 | PRKDC | RPS6KB2 | SP140 | TP63 | |
| BCL2L1 | CDK8 | DKC1 | EXOSC6 | GATA3 | IGF1R | LATS2 | MTAP | PAX5 | PRKN | RPTOR | SPEN | TPM1 | |
| BCL2L11 | CDKN1A | DKK4 | EXT1 | GATA4 | IGF2 | LCK | MTOR | PAX7 | PRSS1 | RRAGC | SPOP | TPMT | |
| BCL2L2 | CDKN1B | DNAJB1 | EXT2 | GATA6 | IKBKE | LDLR | MUSK | PAX8 | PRSS8 | RRAS | SPRED1 | TRAF2 | |
| BCL6 | CDKN1C | DNM2 | EZH1 | GEN1 | IKZF1 | LEF1 | MUTYH | PBRM1 | PSIP1 | RRAS2 | SPRTN | TRAF3 | |
| BCL7A | CDKN2A | DNMT1 | EZH2 | GID4 | IKZF2 | LEMD2 | MYB | PC | PTCH1 | RSPO2 | SPTA1 | TRAF5 | |

*Genes highlighted in light blue color have been sequenced with additional intronic probes spaced approximately 10kb apart for higher accuracy in detecting CNVs.*

| Specific Rearrangements with Intronic Breakpoints (Fusions) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *ABL1* | *BRAF* | *ERBB4* | *FGFR1* | *FUS* | *MYB* | *NTRK2* | *PAX7* | *PML* | *ROS1* |
| *ALK* | *CD74* | *ETV4* | *FGFR2* | *JAK2* | *MYC* | *NTRK3* | *PAX8* | *RAF1* | *RSPO2* |
| *BCL2* | *EGFR* | *ETV6* | *FGFR3* | *KMT2A* | *NOTCH2* | *NUTM1* | *PDGFRA* | *RARA* | *TERT* |
| *BCR* | *EML4* | *EWSR1* | *FOXO1* | *MSH2* | *NTRK1* | *PAX3* | *PDGFRB* | *RET* | *TMPRSS2* |

**Test Description:** This test offers a cutting-edge genomic assay for cancer, utilizing the sophisticated Next Generation Sequencing (NGS) method. Designed to swiftly and precisely identify essential oncologic biomarkers, it serves as a roadmap for therapeutic interventions such as Chemotherapy, Targeted Therapies, and Immunotherapies. Central to this assay is our robust 1091-gene panel, which merges about 600 traditionally analyzed genes with an added 500 newly recognized genes of clinical relevance and 637 pharmacogenomic-specific variants. The assay delves deep into both exonic and select intronic regions, prioritizing those associated with tumoral development and progression. Its capacity spans detecting Single Nucleotide Variants (SNVs), Small Insertions & Deletions (Indels), Copy Number Variations, and select Translocations, even when only limited clinical specimens are available. Concurrently, each sample undergoes rigorous assessment for metrics like Tumor Mutation Burden (TMB), Microsatellite Instability (MSI), Homologous Recombination Deficiency (HRD), and TCR clonality pivotal in determining suitability for immunotherapeutic interventions. Concluding the testing process, our seasoned molecular science team curates a comprehensive yet clinician-friendly report, underscoring salient biomarkers and potential therapeutic avenues, thus enriching the oncologist's decision-making arsenal.

**Note on CNVs:** An AI-driven probabilistic model is utilized to compute the tumor fraction, allowing for the simultaneous identification of genome segments and precise prediction of significant copy number changes. The AI model takes into account variations in clonality and copy numbers at each specific location, ensuring a thorough and comprehensive analysis. CNVs are estimated based on probes covering the entire exonic regions of our gene panel in addition to intronic probes spaced approximately 10kb apart for higher accuracy.

**Methodology and Limitations:** Macrodissection of relevant tumor areas is performed to enrich the patient specimen when the tumor content falls below the limit of detection of the assay. Specimens are de-paraffinized, and nucleic acid is isolated using common techniques and commercially available kits and reagents. Libraries are prepared using hybrid capture methodology targeting relevant regions, and next-generation sequencing is performed using Illumina Inc. technology. Data is aligned to the human genome build GRCh38.

**General Note:** Therapies and the clinical trials listed in this report may not be complete and exhaustive. Genomic alterations identified using this test may be associated with the activity of certain approved therapies; however, the agents listed in this report may have varied clinical evidence in the patient's tumor type. It is crucial to consider epigenetic factors, modified genes, and environmental influences, which may contribute to various forms and characteristics of cancer not covered in this analysis. Physicians should carefully interpret the report alongside other relevant information before devising a treatment plan for patients. The report should not be the sole basis for making diagnoses or treatment decisions. All treatment decisions remain the full and final responsibility of the treating physician, and physicians should refer to approved prescribing information for all therapies. Therapies contained in this report may have been approved by the U.S. FDA.

**Analytical Performance Metrics:** Analytic and Clinical Performance has been validated for the following variant types in solid tumor specimens >30% tumor and >1% VAF: SNV Accuracy >99%, Sensitivity >99%, Specificity >99% at 1-71% VAF; Indels (1 bp -15 bp): Accuracy >99%, Sensitivity >99%, Specificity >99% at 1.7-43% VAF; CNV and large Indels (1.3 mb - 61.6 mb): Accuracy >99%, Sensitivity >99%, Specificity >99% at 5-11 copy number (CN); Fusions and Structural Variants: Accuracy >99%, Sensitivity >99%, Specificity >99% (Minimum supporting reads = 18); Clinical Performance for all variant types was determined to be Accuracy >99%, Sensitivity >99%, Specificity >99% at 1-71% VAF. Please note that mutations with a VAF of less than 5% require extra caution during clinical decision making, as their determination may be prone to technical variability. This test was developed and its performance characteristics determined by Canary Oncoceutics, Inc. It has not been cleared or approved by the US Food and Drug Administration. The FDA does not require this test to go through premarket FDA review. This test is used for clinical purposes. It should not be regarded as investigational or for research. This laboratory is certified under the Clinical Laboratory Improvement Amendments (CLIA) as qualified to perform high complexity clinical laboratory testing.

* Public data sources included in relevant therapies: FDA, NCCN, EMA, ESMO
* Li et al. Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer: A Joint Consensus Recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists. J Mol Diagn. 2017 Jan;19(1):4-23.

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