Dorothea Brooke

Diagnosis
breast cancer

Accession No.
TL-19-C8AC86

xT

Date of Birth
02/05/1981

Sex
Female

Physician
JAS Provider

Institution
JAS Test Institution
jas567565675

TEMPUS | xT 596 Genes

Normal specimen:
Blood
Collected 2/5/2019
Received 2/11/2019

Tumor specimen:
mastectomy, right
jaspath2018-99887, c1
Collected 6/13/2018
Received 2/11/2019
Tumor Percentage: 76%

GENOMIC VARIANTS

Somatic - Potentially Actionable — Variant Allele Fraction

TP53	p.Y236C	Missense variant - LOF	58.9%
PIK3CA	p.E542K	Missense variant (exon 10) - GOF	52.0%
CDKN2A	Copy number loss		
EGFR	Copy number gain		
CYTH3 - BRAF	Chromosomal rearrangement		
EWSR1 - CREB3L2	Chromosomal rearrangement		

Somatic - Biologically Relevant

FBXW7	p.K444fs	Frameshift - LOF	3.5%
BRCA1	Copy number loss		
PTEN	Copy number loss		
TMPRSS2-ERG	Chromosomal rearrangement		

Germline - Pathogenic / Likely Pathogenic — Clinical Significance

MUTYH	p.G396D	Chr1:45797228	• Pathogenic MYH-associated polyposis

IMMUNOTHERAPY MARKERS

Tumor Mutational Burden

16.9 m/MB 32nd percentile

Microsatellite Instability Status

Stable | Equivocal | High

TREATMENT IMPLICATIONS

WEE1 Inhibitor (AZD1775) ✓ FDA approved, current diagnosis	TP53 p.Y236C Loss-of-function Clinical research, solid tumors: PMID 27601554
WEE1 Inhibitor (AZD1775) + Carboplatin *Investigational drug*	TP53 p.Y236C Loss-of-function Clinical research, ovarian cancer: PMID 27998224

PI3K Inhibitor (PA799) *Investigational drug*	PIK3CA p.E542K Gain-of-function Clinical research, ovarian cancer: PMID 25231405
Pan-AKT Inhibitor (AZD5363) *Investigational drug*	PIK3CA p.E542K Gain-of-function Clinical research, gynecologic cancers: PMID 29066505
PI3K Inhibitor (Alpelisib) *Investigational drug*	PIK3CA p.E542K Gain-of-function Clinical research, ovarian cancer: PMID 29401002 Clinical research, endometrial cancer: PMID 29401002
Palbociclib (CDK4/6 Inhibitor) ✓ FDA approved, other indications	CDKN2A Copy number loss Loss-of-function Preclinical, renal cell carcinoma: PMID 23898052 Preclinical, melanoma: PMID 24495407
Anti-EGFR MAbs (Cetuximab, Panitumumab) ✓ FDA approved, current diagnosis	EGFR Copy number gain Clinical research, colorectal cancer: PMID 24653627
Gefitinib (EGFR Inhibitor) ✓ FDA approved, other indications	EGFR Copy number gain Clinical research, esophageal cancer: PMID 24950987 Clinical research, non-small cell lung cancer: PMID 20826716
Lapatinib (Pan-HER TKI) ✓ FDA approved, other indications	EGFR Copy number gain Clinical research, chordoma: PMID 23559153
Trametinib (MEK Inhibitor) ✓ FDA approved, other indications	CYTH3 - BRAF Chromosomal rearrangement Case study, melanoma: PMID 26072686
Sorafenib (TKI) ✓ FDA approved, other indications	CYTH3 - BRAF Chromosomal rearrangement Case study, melanoma: PMID 23890088
Associated	EWSR1 - CREB3L2 Chromosomal rearrangement Clinical research, sclerosing epithelioid fibrosarcoma: PMID 24441665

CLINICAL TRIALS

Study of AG-270 in Subjects With Advanced Solid Tumors or Lymphoma With MTAP Loss (NCT03435250)	**Phase I** Nashville, TN - 729 mi ✓ **CDKN2A deletion** ✓ **MTAP deletion**
Study of COTI-2 as Monotherapy or Combination Therapy for the Treatment of Malignancies (NCT02433626)	**Phase I** Houston, TX - 937 mi ✓ **TP53 mutation**
OLAParib COmbinations (NCT02576444)	**Phase II** Cleveland, OH - 993 mi ✓ **TP53 mutation** ✓ **CDKN2A deletion**

Somatic	Mutation effect	Variant allele fraction
ARID1A	c.4221delA p.A1408fs Frameshift - LOF NM_006015	6.7%
PTCH1	c.457-1G>T Splice region variant - LOF NM_001083602	3.3%

Germline	Mutation effect	Condition
CHEK2	c.14C>T p.S5L Missense variant chr22:29130696 NM_007194	Breast cancer susceptibility, colon cancer susceptibility
MYH11	c.3755A>T p.H1252L Missense variant chr16:15820808 NM_002474	Marfan syndrome, Loeys-Dietz syndromes, and familial thoracic aortic aneurysms and dissections

LOW COVERAGE REGIONS

GFRA2	LOC285766	NOTCH1	PDPK1

SOMATIC VARIANT DETAILS - POTENTIALLY ACTIONABLE

TP53 c.707A>G p.Y236C NM_000546 Missense variant - LOF VAF: 58.9%

TP53 encodes a tumor suppressor that is commonly disabled across cancer types. It normally functions to activate cellular DNA repair mechanisms, plays a role in cell cycle progression in response to DNA damage, and can initiate apoptosis. Loss of function mutations, copy number loss, and epigenetic modifications resulting in underexpression of TP53 are associated with cancer progression.

PIK3CA c.1624G>A p.E542K NM_006218 Missense variant (exon 10) - GOF VAF: 52.0%

PIK3CA encodes the catalytic subunit, p110 alpha protein, of the phosphatidylinositol 3-kinase (PI3K) enzyme. The p110 subunit is responsible for the enzyme's phosphorylation activity, and is involved in the PI3K-AKT-mTOR and the Ras-Raf-MEK-ERK pathways that mediate cellular growth and survival. Activating mutations, copy number gains, and overexpression of PIK3CA are associated with cancer progression.

CDKN2A Copy number loss

CDKN2A encodes two proteins, p16(INK4a) and p14(ARF), that function in regulating cell growth. The p16(INK4a) protein regulates the cell cycle through the inhibition of CDK4 and CDK6, preventing them from stimulating cell proliferation. The p14(ARF) protein binds to MDM2 to keep p53 intact and stimulate the p53-dependent cell cycle arrest and apoptosis. Loss of function mutations, copy number loss, and underexpression of CDKN2A are associated with cancer progression.

EGFR Copy number gain

EGFR (ERBB1) encodes one of the four proteins in a family of transmembrane receptor tyrosine kinases that also includes ERBB2 (HER2/NEU), ERBB3 (HER3), and ERBB4 (HER4). Activation of EGFR through ligand binding results in downstream activation of multiple signaling pathways, including the Ras-Raf-MEK-ERK and the PI3K-AKT-mTOR pathways. Gain of function mutations, copy number gains, epigenetic variation, and fusions resulting in the constitutive activation or overexpression of EGFR are associated with cancer progression. Following treatment with anti-EGFR therapy, the tumor can acquire resistance to such therapy through mechanisms including MET amplification and cis EGFR p.T790M mutations.

CYTH3 - BRAF Chromosomal rearrangement

BRAF is a serine-threonine kinase in the RAF family of kinases, which includes ARAF, BRAF, and CRAF (RAF1). BRAF is a central component of the RAS/MAPK pathway involved in cell differentiation, proliferation, migration, and apoptosis. Fusion of CYTH3 with BRAF results in loss of key N-terminal BRAF autoregulatory domains. This is thought to result in constitutive activation of BRAF and tumorigenesis. In addition to fusions, activating mutations and recently identified kinase deficient and kinase dead mutations, as well as copy number gains of BRAF can lead to cancer progression.

EWSR1 - CREB3L2 Chromosomal rearrangement

EWSR1 encodes a multifunctional protein that is involved in a variety of cellular processes, including gene expression, cell signaling, and RNA processing and transport. Fusions between EWSR1 and different 3' partners are regularly identified in soft tissue sarcomas and can aid in differential diagnosis. The fusion protein includes an N-terminal transcriptional activation domain and a C-terminal RNA-binding domain and is associated with cancer progression.

SOMATIC VARIANT DETAILS - BIOLOGICALLY RELEVANT

FBXW7 c.1332_1335delAGTG p.K444fs NM_033632 Frameshift - LOF VAF: 3.5%

FBXW7 encodes a protein that coordinates the ubiquitin-dependent proteolysis of multiple critical cellular regulators, controlling essential processes of the cell cycle, cellular differentiation, and apoptosis. Loss of function mutations, copy number loss, epigenetic variation, and underexpression of FBXW7 are associated with cancer progression.

BRCA1 Copy number loss

BRCA1 encodes a nuclear phosphoprotein which helps maintain DNA stability through homologous recombination based DNA double stranded break repair and involvement in DNA damage checkpoint control. Loss of function mutations and copy number loss in BRCA1 are associated with cancer progression.

PTEN Copy number loss

PTEN encodes a phosphatase protein that acts as a tumor suppressor by negatively regulating the PI3K-AKT-mTOR pathway via dephosphorylation of phosphatidylinositol (3,4,5)-trisphosphate (PIP3). This pathway is a key regulator of cell proliferation and survival. Loss of function mutations and copy number loss of PTEN are associated with cancer progression.

TMPRSS2-ERG Chromosomal rearrangement

ERG is an oncogene and transcription factor in the erythroblast transformation-specific (ETS) family. It normally functions in embryonic development, cell differentiation, apoptosis, and angiogenesis. ERG forms an oncogenic fusion gene with the 5’-UTR of TMPRSS2. The 5'-UTR of TMPRSS2 contains androgen responsive regulatory elements that drive ERG overexpression. In addition to this fusion, amplification and overexpression of ERG are associated with cancer progression.

GERMLINE VARIANT DETAILS

MUTYH c.1187G>A p.G396D NM_001128425 Chr1:45797228 Splice region variant Clinical Significance: Pathogenic

2017

Diagnosis
05/05/2017

Assay Description

The Tempus xT assay is a custom oncology testing panel consisting of 596 genes with single nucleotide variants, indels and translocations measured by hybrid capture next-generation sequencing (NGS). For the complete gene list, see the Tempus website. The limit of detection of the assay is 5% variant allele fraction (VAF) with sensitivity of 99.1% for single nucleotide variants, 10% VAF with sensitivity of 98.1% for indels and 99.9% sensitivity for translocations. (Certain driver or resistance genes may be reported to lower VAFs when technically possible.)

Potentially Actionable alterations are protein-altering variants with an associated therapy based on evidence from the medical literature. **Biologically Relevant** alterations are protein-altering variants that may have functional significance or have been observed in the medical literature but are not associated with a specific therapy in the Tempus knowledge database. **Variants of Unknown Significance (VUSs)** are protein-altering variants exhibiting an unclear effect on function and/or without sufficient evidence to determine their pathogenicity. **Benign variants** are not reported. Variants are identified through aligning the patient's DNA sequence to the human genome reference sequence version hg19 (GRCh37). The clinical summary (first page of the report) shows actionable and biologically relevant somatic variants, and certain pathogenic or likely pathogenic inherited variants that are reported as incidental findings (if a matched normal sample was provided and the patient has consented to receive germline findings).

Tumor mutational burden (TMB) measures the quantity of somatic mutations, of any pathogenicity, including benign, carried in a tumor as the number of single nucleotide protein-altering mutations per million base pairs. Studies have shown that tumors with higher TMB have an increased likelihood of response to immunotherapy [1, 2].

Microsatellite instability (MSI) refers to hypermutability caused by genetic or acquired defects in the DNA mismatch repair pathway. MSI status is divided into **MSI-high (MSI-H)** tumors, which have changes in microsatellite repeat lengths due to defective DNA mismatch repair activity. **Microsatellite stable (MSS)** tumors do not have detectable defects in DNA mismatch repair. **Microsatellite equivocal (MSE)** tumors have an intermediate phenotype which cannot be clearly classified as MSI-H or MSS based on the statistical cutoff used to define those categories. If MSI status will affect clinical management, immunohistochemical staining for DNA mismatch repair proteins, or another method of ascertaining MSI status, is recommended.

1. Nivolumab plus Ipilimumab in Lung Cancer with a High Tumor Mutational Burden.
https://www.ncbi.nlm.nih.gov/pubmed/29658845

2. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer.
https://www.ncbi.nlm.nih.gov/pubmed/25765070

Tempus Disclaimer

The analysis of nucleic acids by next-generation sequencing (NGS) can be affected by multiple factors including formalin-fixation degrading DNA and RNA quality, and low tumor purity limiting sensitivity. Additionally, the chance of detecting genetic alterations may be reduced in regions of the genome which are structurally difficult to sequence, in homologous genes, or due to sequencing errors.

Genetic alterations are defined as clinically significant based on peer-reviewed published literature and other authoritative sources such as NCCN guidelines. These references are not comprehensive, therefore clinically unknown findings may occur.

These test results and Information contained within the report are current as of the report date. Tempus will not update reports or send notification regarding reclassification of genomic alterations. This test was developed and its performance characteristics determined by Tempus. It has not been cleared or approved by the FDA. The laboratory is CLIA certified to perform high-complexity testing. Any decisions related to patient care and treatment choices should be based on the independent judgment of the treating physician and should take into account all information related to the patient, including without limitation, the patient and family history, direct physical examination and other tests. Tempus is not liable for medical judgment with regards to diagnosis, prognosis or treatment in connection with the test results.

Tempus Disclaimer (continued)

If the patient has consented to germline reporting, then consistent with the recommendations of the ACMG [1], Tempus reports certain germline secondary/incidental findings. These incidental findings include germline sequencing results associated with serious conditions that may or may not be related to the patient's current cancer diagnosis but are considered medically actionable. The clinical significance of reported variants is based on germline classification criteria created by the ACMG [2].

Since these are incidental findings and not a stand alone germline test, the rate of false negatives has not been assessed and certain mutations, such as exon level rearrangements may be missed. Additionally, detection of genetic variation in genes with high homology to other regions of the genome may be decreased or not reliably detected by NGS (including but not limited to these genes: NF1, PMS2, SBDS, and SUZ12) and large insertions and deletions may also not be detected by NGS. Because of these limitations, these germline tests results cannot be used to definitively rule out cancer or other genetic predisposition syndromes, and the results set forth herein should not be used as a substitute for tests validated to determine genetic risk.

Results of genetic testing, including the incidental germline findings described above, may have implications for both the patient and family members. Tempus does not provide genetic counseling; however, genetic counseling is strongly suggested, particularly in the event that deleterious mutations are reported. The ordering physician or the patient is responsible for contacting a genetic counselor to discuss test results.

1. Kalia SS, Adelman K, Bale SJ, Chung WK, Eng C, Evans JP, Herman GE, Hufnagel SB, Klein TE, Korf BR, McKelvey KD, Ormond KE, Richards CS, Vlangos CN, Watson M, Martin CL, Miller DT., Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2016 update (ACMG SF v2.0): a policy statement of the American College of Medical Genetics and Genomics. Genet Med. 2016 Nov 17. Doi:

2. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, Voelkerding K, Rehm HL; Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. ACMG Laboratory Quality Assurance Committee. Genet Med. 2015 May;17(5):405-24. doi: 10.1038/gim.2015.30.