T 833.537.1819 **F** 833.874.0918 **E** support@northstaronc.com **northstaronc.com**

Patient		Sample		Physician	
Name	**Jane Jones**	Specimen Type	**Blood**	Ordering Physician	**John Smith**
Date of Birth (Age)	**11/27/1940 (82 yrs)**	Collection Date	**06/01/2023**	Medical Facility	**BillionToOne Inc**
Assigned Sex at Birth	**Female**	Receipt Date	**06/05/2023**	Address	**1035 O'Brien Drive Suite 112 Menlo Park, California 94025**
Diagnosis	**Rectal adenocarcinoma**	Accession ID	**V010900AA001-1**	Phone	**(833) 537-1819**
Medical Record #	**ID400231**	Report Date	**07/05/2023**	Fax	**(833) 874-0918**
Internal Patient ID	**10000090**	Test Number	**1**		

Northstar Select Results

9 **informative genomic alterations identified**

Summary of Informative Genomic Alterations

Detected Genomic Findings[§]	Associated FDA-Approved Therapies: Approved in indication	Approved in other indication	Associated with resistance	Clinical Trials[§§]	% cfDNA / Copy number
MSI-High	dostarlimab, ipilimumab/nivolumab, nivolumab, pembrolizumab			10	Detected
KRAS G12V		binimetinib, cobimetinib, selumetinib, trametinib	cetuximab, panitumumab	1	51%
ATM N619fs*3		olaparib		4	21%
CHEK2 K373E		olaparib		2	2.81%
BRIP1 K606*		olaparib		1	2.38%
CHEK2 c.319+1G>T		olaparib		2	0.42%
BRCA2 T3033fs*29		bevacizumab/olaparib, niraparib, olaparib, rucaparib, talazoparib		2	0.09%
FBXW7 R393*				0	24%
APC Y1075*				0	0.73%

[§] For additional variant information, please see the Variant Details section of the report. Variants of Unknown Significance (VUS) are reported after the Variant Details section of the report.

[§§] Clinical trials are matched within 500 miles of the ordering physician, based on detected genomic alterations and provided patient demographics. For additional options, please visit **www.clinicaltrials.gov**.

Microsatellite Instability Status

Microsatellite Instability Status (MSI) is reported here based upon mutation detection at curated genome-wide MSI sites and subsequent bioinformatic algorithm calculation.

Stable **High**

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Clinical Trial Availability

Clinical trial matches are displayed based upon somatic variant detection and patient demographic and diagnostic information provided on the test requisition form. Trials are matched within 500 miles of the ordering provider's location. This list is neither comprehensive nor a guarantee of eligibility, as many other requirements must be met prior to enrollment.

For further details on eligibility, please visit **www.clinicaltrials.gov** and enter the NCT#.

Phase 3 NCT02997228	Colorectal Cancer Metastatic dMMR/MSI-H Immuno-Therapy (COMMIT) Study: A Randomized Phase III Study of mFOLFOX6/Bevacizumab/Atezolizumab Combination Versus Single Agent Atezolizumab in the First-Line Treatment of Patients With Deficient DNA Mismatch Repair (dMMR)/Microsatellite Instability-High (MSI-H) Metastatic Colorectal Cancer **MSI-High**	**Berkeley, California (94704)** Alta Bates Summit Medical Center-Herrick Campus
Phase 3 NCT04008030	A Phase 3 Randomized Clinical Trial of Nivolumab Alone, Nivolumab in Combination With Ipilimumab, or Investigator's Choice Chemotherapy in Participants With Microsatellite Instability High (MSI-H) or Mismatch Repair Deficient (dMMR) Metastatic Colorectal Cancer **MSI-High**	**Los Angeles, California (90033)** Usc/Norris Comprehensive Cancer Center
Phase 2 NCT03767348	An Open-Label, Multicenter, Phase 1/2 Study of RP1 as a Single Agent and in Combination With PD1 Blockade in Patients With Solid Tumors **MSI-High**	**San Francisco, California (94115)** University of California - San Francisco
Phase 2 NCT05608044	A Randomized, Open-Label, Phase 2 Study of Botensilimab (AGEN1181) as Monotherapy and in Combination With Balstilimab (AGEN2034) or Investigator's Choice Standard of Care (Regorafenib or Trifluridine and Tipiracil) for the Treatment of Refractory Metastatic Colorectal Cancer **MSI-High**	**Duarte, California (91010)** City of Hope National Medical Center
Phase 2 NCT05221320	A Phase 2 Basket Trial of Ulixertinib (BVD-523) in Combination With Hydroxychloroquine in Patients With Advanced GI Malignancies Harboring Mitogen-activated Protein Kinase (MAPK) Pathway Mutations (BVD-523-HCQ) **MSI-High**	**San Francisco, California (94143)** University of California San Francisco
Phase 2 NCT02628067	A Clinical Trial of Pembrolizumab (MK-3475) Evaluating Predictive Biomarkers in Subjects With Advanced Solid Tumors (KEYNOTE 158) **MSI-High**	**Los Angeles, California (90048)** Call for Information (Investigational Site 0017)
Phase 2 NCT02693535	Targeted Agent and Profiling Utilization Registry (TAPUR) Study **MSI-High** **ATM N619fs*3** **CHEK2 K373E** **CHEK2 c.319+1G>T** **BRCA2 T3033fs*29**	**Palo Alto, California (94301)** Sutter Palo Alto Medical Foundation: Palo Alto
Phase 2 NCT05593328	A Phase 2, Randomized, Open-label Study of Onvansertib in Combination With FOLFIRI and Bevacizumab Versus FOLFIRI and Bevacizumab for Second Line Treatment of Metastatic Colorectal Cancer in Patients With a KRAS or NRAS Mutation **KRAS G12V**	**Bakersfield, California (93309)** Comprehensive Blood and Cancer Center - Bakersfield
Phase 2 NCT04564027	A Modular Phase 2a Multicentre Open-Label Study to Investigate DNA-damage Response Agents (or Combinations) in Patients With Advanced Cancer Whose Tumours Contain Molecular Alterations (PLANETTE) **ATM N619fs*3**	**San Francisco, California (94115)** Research Site

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Phase 2 NCT04123366	A Phase 2 Study of Olaparib in Combination With Pembrolizumab in Participants With Previously Treated, Homologous Recombination Repair Mutation (HRRm) and/or Homologous Recombination Deficiency (HRD)-Positive Advanced Cancer **ATM N619fs*3** **CHEK2 K373E** **BRIP1 K606*** **CHEK2 c.319+1G>T** **BRCA2 T3033fs*29**	**San Francisco, California (94158)** University of California San Francisco (Site 0015)
Phase 2 NCT03682289	Phase II Trial of Ceralasertib (AZD6738) Alone and in Combination With Olaparib or Durvalumab in Patients With Selected Solid Tumor Malignancies **ATM N619fs*3**	**San Francisco, California (94143)** University of California, San Francisco
Phase 1/Phase 2 NCT04521413	A First-In-Human, Phase 1/2 Study Of CFI-402411, a Hematopoietic Progenitor Kinase-1 (HPK1) Inhibitor, as a Single Agent and in Combination With Pembrolizumab in Subjects With Advanced Solid Malignancies **MSI-High**	**Los Angeles, California (90025)** The Angeles Clinic
Phase 1/Phase 2 NCT04041310	A Phase I/II, Multicenter, Open-Label Study of Nous-209 Genetic Vaccine for the Treatment of Microsatellite Unstable Solid Tumors **MSI-High**	**Duarte, California (91010)** City of Hope Comprehensive Cancer Center
Phase 1/Phase 2 NCT04381650	A Phase 1b/2 Study of TAK-981 Plus Pembrolizumab to Evaluate the Safety, Tolerability, and Antitumor Activity of the Combination in Patients With Select Advanced or Metastatic Solid Tumors **MSI-High**	**Stanford, California (94305)** Stanford Cancer Institute (SCI)

Variant Details

Microsatellite Instability-High (MSI-H)

Tumors exhibiting microsatellite instability (MSI) have a higher mutational burden than microsatellite stable (MSS) tumors and express higher levels of immune checkpoint receptors [1, 2, 3, 4, 5]. Thus, checkpoint inhibitors, several of which have received agency approval for certain indications, may be clinically relevant for tumors exhibiting MSI [6, 7, 8, 9, 10, 11]. In fact, pembrolizumab has been FDA-approved as a second or later line of therapy for the treatment of pediatric and adult solid tumors with high microsatellite instability (MSI-H) or that are deficient in mismatch repair (dMMR), as a front-line therapy for colorectal carcinoma patients with MSI-H or dMMR, and endometrial carcinoma patients with MSI-H or dMMR, who are not eligible for curative surgery or radiation, following progression on systemic therapy [12, 10]. In addition, nivolumab and the combination of nivolumab and ipilimumab have been FDA-approved for the treatment of MSI-H or dMMR colorectal carcinoma [13, 14]. In contrast, the combination of lenvatinib and pembrolizumab has been FDA-approved for the treatment of advanced endometrial cancer patients with unresectable tumors lacking markers for MSI-H and dMMR following disease progression on systemic therapy [15].

MSI has been associated with proximal colon location, mucinous histology, lower tumor grade, age at diagnosis of less than 50 years old, and the presence of BRAF mutation in colorectal carcinoma studies [16, 17, 18, 19, 20]. In addition, increased frequency of PD-L1 expression has been reported in colorectal carcinoma cases with MSI-H as compared with MSS/MSI-L cases [21, 22, 23, 24, 25]. Several studies have reported MSI-high status to be associated with better prognosis in colorectal carcinoma patients compared with patients lacking MSI-high [26, 27, 28, 29]. Adjuvant 5-FU may not benefit colorectal cancer patients with stage II/III MSI-H tumors when given as monotherapy [30, 31, 32].

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KRAS

KRAS encodes the signaling protein K-Ras, a member of the Ras family; activating KRAS alterations may result in activation of downstream signaling pathways, including the Raf/MEK/ERK pathway [35, 36]. Clinical studies have suggested limited efficacy of MEK inhibitors in KRAS mutant tumors; however, combinations of MEK inhibitors with other targeted therapies may still be relevant [37, 38, 39, 40, 41, 42, 43, 44]. Other clinical approaches are also under investigation in the context of KRAS-mutant tumors, including FAK and Shp-2 inhibitors [45, 46, 47, 48, 49, 50]. In addition, inhibitors specifically targeting KRAS G12C and cell-based therapies targeting KRAS G12V and G12D are being investigated clinically and preclinically [51, 52, 53, 54]. Sotorasib and adagrasib have been FDA-approved in patients with locally advanced or metastatic non-small cell lung carcinoma harboring a KRAS G12C mutation, as determined by an FDA-approved test, following treatment with at least one prior systemic therapy [55, 56]. In addition, the combination of adagrasib with cetuximab has been reported to provide clinical benefit in colorectal carcinoma patients with KRAS G12C mutation and has been granted “breakthrough” designation by the FDA for accelerated review [57].

KRAS G12V

Gene	**KRAS**
Nucleotide	**NM_004985.5: c.35G>T**
Amino Acid	**p.G12V**
Exon	**2**
Biomarker Type	**activating**

The KRAS G12V mutation lies within the first "G box" domain of the K-Ras protein, one of several conserved regions responsible for GTP binding and hydrolysis; disruption of this region creates a protein that is defective for GTP hydrolysis and is therefore constitutively active [58, 35, 59, 60]. KRAS G12V has been shown to induce hyperplastic lesions and metastatic tumor formation in mice, and is one of the more common KRAS mutations cited in colorectal cancer, where it has been associated with metastatic and aggressive tumors [61, 62, 63, 64, 65, 66].

ATM

ATM encodes the serine/threonine protein kinase Ataxia telangiectasia mutated (Atm), which is a member of the PI3K/PI4K family [67]. Based on preclinical and clinical evidence, ATM-deficient tumors may be sensitive to poly(ADP-ribose) polymerase (PARP) inhibitors, Atr inhibitors, and DNA-PKcs inhibitors, which are under investigation in clinical trials [68, 69, 70, 71, 72, 73, 74]. The PARP inhibitors olaparib, rucaparib, and talazoparib have been approved for certain indications in the context of BRCA1 or BRCA2 mutation, or alteration in one or more homologous recombination repair genes, including ATM mutation [75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85].

ATM N619fs*3

Gene	**ATM**
Nucleotide	**NM_000051.4: c.1856dupA**
Amino Acid	**p.N619fs*3**
Exon	**12**
Biomarker Type	**inactivating**

The ATM frameshift alteration reported here is expected to effectively truncate the 3056-amino acid Atm protein prior to the FAT, kinase, and FATC domains (UniProt). The FAT and FATC domains are involved in activation, and the FATC and kinase domains are critical to the function of Atm in DNA damage response [86, 87, 88, 89, 90, 91]. In addition, this alteration is likely to elicit nonsense-mediated decay [92, 93, 94, 95]. Therefore, this alteration is expected to be inactivating.

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CHEK2

CHEK2 encodes the tumor suppressor, checkpoint kinase 2 (Chk2), a serine/threonine kinase that plays an important role in cell cycle arrest in response to DNA damage [96, 97, 98, 99]. Depletion of CHEK2 has been reported to increase sensitivity to PARP inhibitors in preclinical models and PARP inhibitors are in clinical trials in cancers with DNA repair deficiencies, including CHEK2 alterations [100]. The PARP inhibitors olaparib, rucaparib, and talazoparib have been approved for certain indications in the context of BRCA1 or BRCA2 mutation, or alteration in one or more homologous recombination repair genes, including CHEK2 mutation [75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85].

CHEK2 K373E

Gene	**CHEK2**
Nucleotide	**NM_007194.4: c.1116_1117delinsTG**
Amino Acid	**p.K373E**
Exon	**11**
Biomarker Type	**inactivating**

CHEK2 K373E is a missense alteration that occurs in the kinase domain of the Chk2 protein, specifically in the activation loop (UniProt). K373E has been reported to impair Chk2 autophosphorylation and kinase activity and to result in increased cell survival after ionizing radiation [101]. Therefore, this alteration is predicted to be inactivating.

CHEK2 c.319+1G>T

Gene	**CHEK2**
Nucleotide	**NM_007194.4: c.319+1G>T**
Amino Acid	**p.?**
Exon	**2**
Biomarker Type	**unknown - predicted inactivating**

CHEK2 splice site 319+1G>T occurs in a conserved splice donor site at the junction of transcribed exons 2 and 3, corresponding to amino acid 107 of 543 in the Chk2 protein (IGV). Splice site mutations may lead to exon skipping or protein truncation. Exon skipping or protein truncation near this location would result in the loss of the majority of the Chk2 protein, including the entire kinase domain (UniProt). Therefore, although this alteration has not been functionally characterized, it may lead to a loss of protein function.

BRIP1

BRIP1, also known as BACH1, encodes the Fanconi anemia group J protein (FancJ). Inactivating BRIP1 mutations may result in impaired DNA damage repair; germline BRIP1 mutations have been associated with Fanconi anemia complementation group J (FA-J) and some forms of familial cancer [102, 103, 104, 105, 106, 107]. Inactivation of the FA/BRCA pathway, including FancJ deficiency, has been reported to sensitize cells to mitomycin C and cisplatin [108, 109, 110, 111, 112]. In addition, inactivation of FancJ may sensitize cells to inhibition of poly(ADP-ribose) polymerase (PARP) [113, 114]. PARP inhibitors are currently under investigation in both preclinical and clinical studies. The PARP inhibitors olaparib, rucaparib, and talazoparib have been approved for certain indications in the context of BRCA1 or BRCA2 mutation, or alteration in one or more homologous recombination repair genes, including BRIP1 mutation [75, 76, 77, 78, 79, 80, 81, 82, 83, 84].

BRIP1 K606*

Gene	**BRIP1**
Nucleotide	**NM_032043.3: c.1816A>T**
Amino Acid	**p.K606***
Exon	**13**
Biomarker Type	**inactivating**

The nonsense alteration reported here is expected to truncate the 1249 amino acid FancJ protein, resulting in the loss of the region involved in the interaction with Brca1, as well as several C-terminal phosphoserine residues (UniProt). The C-terminus of FancJ interacts with Brca1, and this interaction is important for double-strand break repair functions in cells [115]. In addition, a germline BRIP1 mutation resulting in a frameshift at K998 has been reported in a breast cancer patient with a family history of multiple cancers; this alteration was reported to lead to reduced protein stability and decreased Brca1 binding in vitro [116]. This alteration is also likely to elicit nonsense-mediated decay [92, 93, 94, 95]. Therefore, this alteration is predicted to be inactivating.

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BRCA2

BRCA2 inactivation may impair the DNA damage repair process and result in a loss of cell cycle checkpoint control leading to tumorigenesis [117, 118]. Inactivating BRCA2 alterations have been reported to predict sensitivity to platinum-based chemotherapy and PARP inhibitors, including olaparib, rucaparib, niraparib, and talazoparib, which are FDA-approved in specific indications [84, 119, 76, 77, 120, 82]. In addition, talazoparib in combination with enzalutamide has been FDA-approved for the treatment of metastatic castration-resistant prostate cancer patients with tumors harboring germline or somatic alteration in one or more homologous recombination repair genes, including BRCA2 [85].

BRCA2 T3033fs*29

Gene	**BRCA2**
Nucleotide	**NM_000059.4: c.9097delA**
Amino Acid	**p.T3033fs*29**
Exon	**23**
Biomarker Type	**inactivating**

The alteration reported here is expected to effectively truncate the 3418-amino acid Brca2 protein prior to the C-terminal Rad51 binding domain and three nuclear localization signals crucial to Brca2 protein function; truncating mutations including T3195* and Y3308* have been reported to result in Brca2 inactivation [121, 122, 123, 124, 125]. In addition, this alteration is likely to elicit nonsense-mediated decay [92, 93, 94, 95]. Therefore, this alteration is expected to be inactivating.

FBXW7

FBXW7 encodes the F-box/WD repeat-containing protein 7, Fbxw7, which is a subunit of the SCF ubiquitin ligase complex responsible for recruitment of substrates for targeted degradation by the proteasome [126]. Loss of Fbxw7 function may result in the stabilization of some oncoproteins, including mTOR, Notch1, Myc, Jun, and Cyclin E1, all of which are substrates for Fbxw7-targeted degradation by the proteasome [127, 128, 129]. Preclinical studies have reported that inactivation of Fbxw7 results in stabilization of mTOR; however, FBXW7 alterations have been shown not to be predictive of response to everolimus or temsirolimus in clinical studies [127, 130, 131]. Other agents that target mTOR, including dual mTORC1/mTORC2 inhibitors and dual PI3K/mTOR inhibitors, are currently in preclinical and clinical development [132, 133, 134].

FBXW7 R393*

Gene	**FBXW7**
Nucleotide	**NM_033632.3: c.1177C>T**
Amino Acid	**p.R393***
Exon	**8**
Biomarker Type	**unknown - predicted inactivating**

The FBXW7 nonsense alteration reported here is expected to truncate the 707-amino acid Fbxw7 protein, resulting in a protein that retains the conserved F-box domain but lacks all or a portion of the WD40 repeat region (UniProt). The WD40 repeat region is responsible for substrate recognition; therefore, this mutation is predicted to result in a failure to target substrates for degradation, thus enhancing the stability of known oncogene substrates including Cyclin E, Notch1, Myc, Jun, and mTOR [128, 127]. Therefore, this mutation is expected to be inactivating.

APC

Inactivation of Apc results in the deregulation of Wnt signaling through beta-catenin [135]. There are currently no approved drugs targeted to APC defects in cancer. However, several potential approaches, including downstream Wnt pathway, Cox-2 inhibitors, and tankyrase 1/2 inhibitors are under investigation [136, 137, 138, 139, 140].

APC Y1075*

Gene	**APC**
Nucleotide	**NM_000038.6: c.3225T>A**
Amino Acid	**p.Y1075***
Exon	**16**
Biomarker Type	**inactivating**

The nonsense alteration reported here is expected to truncate the 2843-amino acid Apc protein within the beta-catenin binding domain, and is therefore likely to result in a disruption of the ability of the Apc protein to bind to beta-catenin, which in turn may upregulate Wnt signaling [141, 142, 143].

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Variants of Unknown Significance (VUS)

The clinical relevance of these variants is currently unknown. Therefore, the functional impact of targeting these variants cannot be determined at this time.

RAD51C c.506T>C (p.V169A), 52%
CDK12 c.3230A>C (p.E1077A), 23%
CHEK2 c.1604G>A (p.R535H), 14%
APC c.1394C>A (p.A465E), 0.92%
PALB2 c.1940A>G (p.H647R), 0.3%
FANCA c.2057C>T (p.A686V), 0.17%
CDK12 c.4382dupG (p.T1463fs*?), 0.08%
BRCA2 c.5023T>C (p.C1675R), 24%
RHOA c.101A>G (p.Y34C), 22%
ERBB2 c.1059delG (p.R354fs*77), 10%
APC c.6471A>C (p.K2157N), 0.87%
FGFR3 c.2387delC (p.P796fs*?), 0.21%
AR c.1951A>T (p.S651C), 0.12%
BRAF c.1177+1857G>C (p.?), 0.07%
TP53 c.697C>T (p.H233Y), 24%
AR c.173A>T (p.Q58L), 15%
MAP2K2 c.158G>A (p.R53Q), 3.85%
EGFR c.2538A>C (p.K846N), 0.48%
APC c.4844A>G (p.Y1615C), 0.18%
APC c.7973G>T (p.W2658L), 0.11%

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Interpretation

Genomic alterations (SNVs/indels/fusions) were detected in the cell-free DNA (cfDNA) isolated from the patient's blood specimen. The variant frequency of the mutations, reported as %cfDNA, is calculated and reported for detected SNVs and indels. These alterations may be informative to cancer treatment response and/or clinical trial eligibility. While somatic actionability is of primary concern when determining report inclusion, some variants may be included without matched actionable outcomes. These variants may be critical to carcinogenesis in the patient's tumor.

The cell-free DNA (cfDNA) isolated from the patient's blood specimen was identified as Microsatellite instability-High (MSI-H). Microsatellite instability is linked to deficient DNA mismatch repair (dMMR), caused by inactivating mutations in one or more mismatch repair (MMR) genes (MLH1, MSH2, MSH6, and PMS2) or by somatic MLH1 promoter hypermethylation. As a result of MMR deficiency, most MSI-high tumors are marked by elevated tumor mutational burdens, with an abundance of low allele frequency insertion/deletion mutations. The reported somatic alterations should be interpreted in this context of MSI-H.

The KRAS G12V mutation included in this report was observed at an allele fraction suspicious for potential germline origin. Northstar Select™ is validated for the detection of somatic variants, and was not developed to distinguish germline variants, which may predispose the patient to certain types of cancer, from detected somatic variants. Discussion with a qualified professional as well as additional investigation with a test validated for calling germline mutations is recommended.

Methods and Limitations

Northstar Select™ is a next generation sequencing (NGS)-based in vitro diagnostic test for detection of substitutions (SNVs), small insertion and deletion alterations (indels), selected genes' copy number alterations (CNAs, including amplifications and deletions) and selected gene-rearrangements in a total of 84 genes (Table 1), as well as microsatellite instability status. Cell-free DNA (cfDNA) is extracted from plasma, and the targeted regions, encompassing >250kb, are amplified and sequenced. The sensitivity of the test is 100% (95% CI: [99.84%,100.00%]) for SNVs and indels for variant allele fraction (VAF) ≥0.5% and fusions examined in the assay analytical validation, and 100% (95% CI: [76.84%,100.00%]) for CNAs. The limit of detection for the assay is 0.13-0.16% (VAF) for SNVs and indels. However, key actionable variants may be reported at lower VAFs where technically feasible. The overall base-wise specificity of the Northstar Select™ test is >99.99%, leading to high confidence in true positive reporting for variants with VAF >0.2%, despite the expansive genomic loci targeted and assayed. For CNAs, the LOD for gene amplifications is 2.125-2.16 copies and for loss is 1.78 copies. CNA LOD is subject to aneuploidy noise, with less aneuploid samples having a superior LOD. MSI score is calculated using a count of somatic indel mutations in targeted microsatellite sites; based upon meeting threshold, MSI-H status is reported when detected. The sensitivity of the MSI component is 100% (95% CI: [97.72%,100.00%]) for MSI-H at a tumor fraction of ≥0.5%, and the limit of detection of the assay for MSI-H is 0.07-0.4% tumor fraction. An MSI-Indeterminate result is an inconclusive result as it does not suggest the presence or absence of MSI-H in the patient. For certain cfDNA sample or variant characteristics, such as low cfDNA input or high level of cancer-associated chromosomal copy abnormality, the analytical sensitivity may be reduced.

Variants detected in the cfDNA are aligned to the hg19 reference genome. Informative genomic alterations are potentially actionable or biologically relevant variants based on evidence from medical and scientific literature. Variants of unknown significance (VUS) are genomic alterations that do not have sufficient evidence to determine biological/clinical significance.

Reported treatments and trials should be comprehensively evaluated by the medical provider, as inclusion in the report is neither a guarantee of treatment/trial match, nor intended to be fully comprehensive. Treatments and trials are reported based upon the information provided at the time of test requisition, including diagnosis, sex, age, and location, and may not account for all elements of the patient's medical history. Certain treatments may have specifications surrounding other clinical factors, such as HR/HER2-status or germline mutation origin. The ordering provider should consult official FDA-approval information for full approval specifications. Inclusion in Northstar Select™ does not guarantee that reported treatments or trials will have an impact on clinical outcome.

This assay is validated for the detection of somatic variants, but was not developed to distinguish germline variants, which may predispose the patient to certain types of cancer, from detected somatic variants. For variants with a VAF reported between 40-60% cfDNA, separate germline testing may be recommended to identify and characterize such variants that may have hereditary implications.

Table 1: Genes on the Northstar Select™ panel

Northstar Select™ reports single nucleotide variants, insertion and deletion variants (indels), and splice site mutations for clinically relevant exons in 82 genes, copy number amplifications in 19 genes, and copy number loss in 5 genes, and fusion events in 9 genes, as detailed in the table below.

AKT1, AKT2, ALK$^{\wedge}$, APC, AR^{+}, ARAF, ARID1A, ATM^{-}, BRAF$^{\wedge+}$, BRCA1^{-}, BRCA2^{-}, BRIP1, CCND1, CCND2, CCNE1^{+}, CD274^{+}, CDH1, CDK12, CDK4^{+}, CDK6^{+}, CDKN2A^{-}, CDKN2B, CHEK2, CTNNB1, DDR2, EGFR^{+}, ERBB2^{+}, ESR1^{+}, EZH2, FANCA, FBXW7, FGFR1^{+}, FGFR2$^{\wedge+}$, FGFR3$^{\wedge}$, FGFR4, GATA3, GNA11, GNAQ, GNAS, HRAS, IDH1, IDH2, JAK2, JAK3, KIT^{+}, KRAS^{+}, MAP2K1, MAP2K2, MET^{+}, MLH1, MPL, MSH2, MSH6, MTOR, MYC^{+}, NF1, NOTCH1, NPM1, NRAS, NTRK1$^{\wedge}$, NTRK2$^{\wedge\wedge}$, NTRK3$^{\wedge\wedge}$, PALB2, PDGFRA^{+}, PIK3CA^{+}, PMS2, PTEN^{-}, PTPN11, RAD51C, RAD51D, RAF1^{+}, RB1, RET$^{\wedge+}$, RHOA, RIT1, ROS1$^{\wedge}$, SF3B1, SMAD4, SMO, STK11, TERT, TP53, TSC1, VHL

$^{\wedge}$ Northstar Select™ also reports fusion events for this gene
$^{\wedge\wedge}$ Northstar Select™ reports only reports only fusion events for these genes, no SNVs and indels will be reported; NTRK3 is limited to NTRK3-ETV6 fusions only
$^{+}$ Northstar Select™ also reports copy number amplifications of this gene
$^{-}$ Northstar Select™ also reports copy number deletions of this gene

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Diagnosis	**Rectal adenocarcinoma**	Internal Patient ID	**10000090**	Report Date	**07/05/2023**

References

1. Kim H, Jen J, Vogelstein B, Hamilton SR. Clinical and pathological characteristics of sporadic colorectal carcinomas with DNA replication errors in microsatellite sequences. *The American journal of pathology.* Am J Pathol 1994 Jul;145(1):148-56. PMID:8030745.
2. Alexander J, Watanabe T, Wu TT, Rashid A, Li S, Hamilton SR. Histopathological identification of colon cancer with microsatellite instability. *The American journal of pathology.* Am J Pathol 2001 Feb;158(2):527-35. PMID:11159189.
3. Howitt BE, Shukla SA, Sholl LM, et al. Association of Polymerase e-Mutated and Microsatellite-Instable Endometrial Cancers With Neoantigen Load, Number of Tumor-Infiltrating Lymphocytes, and Expression of PD-1 and PD-L1. *JAMA Oncol.* 2015 Dec;1(9):1319-23. PMID:26181000.
4. Timmermann B, Kerick M, Roehr C, et al. Somatic mutation profiles of MSI and MSS colorectal cancer identified by whole exome next generation sequencing and bioinformatics analysis. *PLoS One.* 2010 Dec 22;5(12):e15661. PMID:21203531.
5. Llosa NJ, Cruise M, Tam A, et al. The vigorous immune microenvironment of microsatellite instable colon cancer is balanced by multiple counter-inhibitory checkpoints. *Cancer discovery.* Cancer Discov 2015 Jan;5(1):43-51. PMID:25358689.
6. Migden MR, Rischin D, Schmults CD, et al. PD-1 Blockade with Cemiplimab in Advanced Cutaneous Squamous-Cell Carcinoma. *The New England journal of medicine.* N Engl J Med 2018 Jul 26;379(4):341-351. PMID:29863979.
7. Topalian SL, Drake CG, Pardoll DM. Targeting the PD-1/B7-H1(PD-L1) pathway to activate anti-tumor immunity. *Curr Opin Immunol.* 2012 Apr;24(2):207-12. Epub 2012 Jan 9. PMID:22236695.
8. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. *Nat Rev Cancer.* 2012 Mar 22;12(4):252-264. PMID:22437870.
9. Taube JM, Klein A, Brahmer JR, et al. Association of PD-1, PD-1 ligands, and other features of the tumor immune microenvironment with response to anti-PD-1 therapy. *Clin Cancer Res.* 2014 Oct 01;20(19):5064-74. Epub 2014 Apr 8. PMID:24714771.
10. Le DT, Uram JN, Wang H, et al. PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. *N Engl J Med.* 2015 Jun 25;372(26):2509-20. Epub 2015 May 30. PMID:26028255.
11. Wang RF, Wang HY. Immune targets and neoantigens for cancer immunotherapy and precision medicine. *Cell research.* Cell Res 2017 Jan;27(1):11-37. PMID:28025978.
12. Lee V, Le DT. Efficacy of PD-1 blockade in tumors with MMR deficiency. *Immunotherapy.* Immunotherapy 2016;8(1):1-3. PMID:26643016.
13. Overman MJ, Lonardi S, Wong KYM, et al. Durable Clinical Benefit With Nivolumab Plus Ipilimumab in DNA Mismatch Repair-Deficient/Microsatellite Instability-High Metastatic Colorectal Cancer. *J Clin Oncol.* 2018 Mar 10;36(8):773-779. Epub 2018 Jan 20. PMID:29355075.
14. Overman MJ, McDermott R, Leach JL, et al. Nivolumab in patients with metastatic DNA mismatch repair-deficient or microsatellite instability-high colorectal cancer (CheckMate 142): an open-label, multicentre, phase 2 study. *Lancet Oncol.* 2017 Sep;18(9):1182-1191. Epub 2017 Jul 19. PMID:28734759.
15. Makker V, Rasco D, Vogelzang NJ, et al. Lenvatinib plus pembrolizumab in patients with advanced endometrial cancer: an interim analysis of a multicentre, open-label, single-arm, phase 2 trial. *Lancet Oncol.* 2019 May;20(5):711-718. Epub 2019 Mar 25. PMID:30922731.
16. Song Y, Wang L, Ran W, et al. Effect of Tumor Location on Clinicopathological and Molecular Markers in Colorectal Cancer in Eastern China Patients: An Analysis of 2,356 Cases. *Front Genet.* 2020;11:96. Epub 2020 Feb 25. PMID:32161617.
17. Tran CG, Goffredo P, Mott SL, et al. The impact of KRAS mutation, microsatellite instability, and tumor laterality on the prognosis of nonmetastatic colon cancer. *Surgery.* Surgery 2022 Mar;171(3):657-665. PMID:34865865.
18. Thibodeau SN, Bren G, Schaid D. Microsatellite instability in cancer of the proximal colon. *Science (New York, N.Y.).* Science 1993 May 7;260(5109):816-9. PMID:8484122.
19. Chang CC, Lin PC, Lin CC, et al. Molecular and Clinicopathological Differences by Age at the Diagnosis of Colorectal Cancer. *Int J Mol Sci.* 2017 Jul 05;18(7). Epub 2017 Jul 5. PMID:28678173.
20. Fujiyoshi K, Yamaguchi T, Kakuta M, et al. Predictive model for high-frequency microsatellite instability in colorectal cancer patients over 50 years of age. *Cancer Med.* 2017 Jun;6(6):1255-1263. Epub 2017 May 23. PMID:28544821.
21. Korehisa S, Oki E, Iimori M, et al. Clinical significance of programmed cell death-ligand 1 expression and the immune microenvironment at the invasive front of colorectal cancers with high microsatellite instability. *International journal of cancer.* Int J Cancer 2018 Feb 15;142(4):822-832. PMID:29044503.
22. Valentini AM, Di Pinto F, Cariola F, et al. PD-L1 expression in colorectal cancer defines three subsets of tumor immune microenvironments. *Oncotarget.* Oncotarget 2018 Feb 2;9(9):8584-8596. PMID:29492219.
23. Salem ME, Puccini A, Grothey A, et al. Landscape of Tumor Mutation Load, Mismatch Repair Deficiency, and PD-L1 Expression in a Large Patient Cohort of Gastrointestinal Cancers. *Molecular cancer research : MCR.* Mol Cancer Res 2018 May;16(5):805-812. PMID:29523759.
24. Lee KS, Kwak Y, Ahn S, et al. Prognostic implication of CD274 (PD-L1) protein expression in tumor-infiltrating immune cells for microsatellite unstable and stable colorectal cancer. *Cancer Immunol Immunother.* 2017 Jul;66(7):927-939. Epub 2017 Apr 12. PMID:28405764.
25. Millen R, Hendry S, Narasimhan V, et al. CD8(+) tumor-infiltrating lymphocytes within the primary tumor of patients with synchronous de novo metastatic colorectal carcinoma do not track with survival. *Clinical & translational immunology.* Clin Transl Immunology 2020;9(7):e1155. PMID:32953115.
26. Vilar E, Gruber SB. Microsatellite instability in colorectal cancer-the stable evidence. *Nat Rev Clin Oncol.* 2010 Mar;7(3):153-62. Epub 2010 Feb 9. PMID:20142816.
27. Gryfe R, Kim H, Hsieh ET, et al. Tumor microsatellite instability and clinical outcome in young patients with colorectal cancer. *The New England journal of medicine.* N Engl J Med 2000 Jan 13;342(2):69-77. PMID:10631274.
28. Watanabe T, Wu TT, Catalano PJ, et al. Molecular predictors of survival after adjuvant chemotherapy for colon cancer. *N Engl J Med.* 2001 Apr 19;344(16):1196-206. PMID:11309634.
29. Popat S, Hubner R, Houlston RS. Systematic review of microsatellite instability and colorectal cancer prognosis. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology.* J Clin Oncol 2005 Jan 20;23(3):609-18. PMID:15659508.
30. Ribic CM, Sargent DJ, Moore MJ, et al. Tumor microsatellite-instability status as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer. *The New England journal of medicine.* N Engl J Med 2003 Jul 17;349(3):247-57. PMID:12867608.
31. Sargent DJ, Marsoni S, Monges G, et al. Defective mismatch repair as a predictive marker for lack of efficacy of fluorouracil-based adjuvant therapy in colon cancer. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology.* J Clin Oncol 2010 Jul 10;28(20):3219-26. PMID:20498393.
32. Tan WJ, Hamzah JL, Acharyya S, et al. Evaluation of Long-Term Outcomes of Microsatellite Instability Status in an Asian Cohort of Sporadic Colorectal Cancers. *Journal of gastrointestinal cancer.* J Gastrointest Cancer 2018 Sep;49(3):311-318. PMID:28550452.
33. Boland CR, Thibodeau SN, Hamilton SR, et al. A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. *Cancer Res.* 1998 Nov 15;58(22):5248-57. PMID:9823339.
34. Umar A, Boland CR, Terdiman JP, et al. Revised Bethesda Guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. *J Natl Cancer Inst.* 2004 Feb 18;96(4):261-8. PMID:14970275.
35. Pylayeva-Gupta Y, Grabocka E, Bar-Sagi D. RAS oncogenes: weaving a tumorigenic web. *Nat Rev Cancer.* 2011 Oct 13;11(11):761-74. PMID:21993244.
36. Nakano H, Yamamoto F, Neville C, Evans D, Mizuno T, Perucho M. Isolation of transforming sequences of two human lung carcinomas: structural and functional analysis of the activated c-K-ras oncogenes. *Proc Natl Acad Sci U S A.* 1984 Jan;81(1):71-5. PMID:6320174.

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37. Jänne PA, van den Heuvel MM, Barlesi F, et al. Selumetinib Plus Docetaxel Compared With Docetaxel Alone and Progression-Free Survival in Patients With KRAS-Mutant Advanced Non-Small Cell Lung Cancer: The SELECT-1 Randomized Clinical Trial. *JAMA.* 2017 May 09;317(18):1844-1853. PMID:28492898.
38. Adjei AA, Cohen RB, Franklin W, et al. Phase I pharmacokinetic and pharmacodynamic study of the oral, small-molecule mitogen-activated protein kinase kinase 1/2 inhibitor AZD6244 (ARRY-142886) in patients with advanced cancers. *J Clin Oncol.* 2008 May 01;26(13):2139-46. Epub 2008 Apr 7. PMID:18390968.
39. Manchado E, Weissmueller S, Morris JP, et al. A combinatorial strategy for treating KRAS-mutant lung cancer. *Nature.* 2016 Jun 30;534(7609):647-51. Epub 2016 Jun 22. PMID:27338794.
40. Infante JR, Somer BG, Park JO, et al. A randomised, double-blind, placebo-controlled trial of trametinib, an oral MEK inhibitor, in combination with gemcitabine for patients with untreated metastatic adenocarcinoma of the pancreas. *Eur J Cancer.* 2014 Aug;50(12):2072-2081. Epub 2014 Jun 7. PMID:24915778.
41. Lito P, Saborowski A, Yue J, et al. Disruption of CRAF-mediated MEK activation is required for effective MEK inhibition in KRAS mutant tumors. *Cancer Cell.* 2014 May 12;25(5):697-710. Epub 2014 Apr 17. PMID:24746704.
42. Hochster HS, Uboha N, Messersmith W, et al. Phase II study of selumetinib (AZD6244, ARRY-142886) plus irinotecan as second-line therapy in patients with K-RAS mutated colorectal cancer. *Cancer Chemother Pharmacol.* 2015 Jan;75(1):17-23. Epub 2014 Oct 17. PMID:25322874.
43. Blumenschein GR, Smit EF, Planchard D, et al. A randomized phase II study of the MEK1/MEK2 inhibitor trametinib (GSK1120212) compared with docetaxel in KRAS-mutant advanced non-small-cell lung cancer (NSCLC). *Ann Oncol.* 2015 May;26(5):894-901. Epub 2015 Feb 26. PMID:25722381.
44. Zhu Z, Aref AR, Cohoon TJ, et al. Inhibition of KRAS-driven tumorigenicity by interruption of an autocrine cytokine circuit. *Cancer Discov.* 2014 Apr;4(4):452-65. Epub 2014 Jan 20. PMID:24444711.
45. Gerber DE, Camidge DR, Morgensztern D, et al. Phase 2 study of the focal adhesion kinase inhibitor defactinib (VS-6063) in previously treated advanced KRAS mutant non-small cell lung cancer. *Lung Cancer.* 2020 Jan;139:60-67. Epub 2019 Nov 4. PMID:31739184.
46. Mainardi S, Mulero-Sánchez A, Prahallad A, et al. SHP2 is required for growth of KRAS-mutant non-small-cell lung cancer in vivo. *Nat Med.* 2018 Jul;24(7):961-967. Epub 2018 May 28. PMID:29808006.
47. Ruess DA, Heynen GJ, Ciecielski KJ, et al. Mutant KRAS-driven cancers depend on PTPN11/SHP2 phosphatase. *Nat Med.* 2018 Jul;24(7):954-960. Epub 2018 May 28. PMID:29808009.
48. Konstantinidou G, Ramadori G, Torti F, et al. RHOA-FAK is a required signaling axis for the maintenance of KRAS-driven lung adenocarcinomas. *Cancer Discov.* 2013 Apr;3(4):444-57. Epub 2013 Jan 28. PMID:23358651.
49. Tang KJ, Constanzo JD, Venkateswaran N, et al. Focal Adhesion Kinase Regulates the DNA Damage Response and Its Inhibition Radiosensitizes Mutant KRAS Lung Cancer. *Clinical cancer research : an official journal of the American Association for Cancer Research.* Clin Cancer Res 2016 Dec 1;22(23):5851-5863. PMID:27220963.
50. Chen YN, LaMarche MJ, Chan HM, et al. Allosteric inhibition of SHP2 phosphatase inhibits cancers driven by receptor tyrosine kinases. *Nature.* Nature 2016 Jul 7;535(7610):148-52. PMID:27362227.
51. Lindsay CR, Blackhall FH. Direct Ras G12C inhibitors: crossing the rubicon. *Br J Cancer.* 2019 Jul;121(3):197-198. Epub 2019 Jun 26. PMID:31239544.
52. Janes MR, Zhang J, Li LS, et al. Targeting KRAS Mutant Cancers with a Covalent G12C-Specific Inhibitor. *Cell.* 2018 Jan 25;172(3):578-589.e17. PMID:29373830.
53. Ostrem JM, Peters U, Sos ML, Wells JA, Shokat KM. K-Ras(G12C) inhibitors allosterically control GTP affinity and effector interactions. *Nature.* 2013 Nov 28;503(7477):548-51. Epub 2013 Nov 20. PMID:24256730.
54. Patricelli MP, Janes MR, Li LS, et al. Selective Inhibition of Oncogenic KRAS Output with Small Molecules Targeting the Inactive State. *Cancer Discov.* 2016 Mar;6(3):316-29. Epub 2016 Jan 6. PMID:26739882.
55. Sotorasib Edges Closer to Approval. *Cancer discovery.* Cancer Discov 2021 May;11(5):OF2. PMID:33547148.
56. Jänne PA, Riely GJ, Gadgeel SM, et al. Adagrasib in Non-Small-Cell Lung Cancer Harboring a KRAS(G12C) Mutation. *The New England journal of medicine.* N Engl J Med 2022 Jul 14;387(2):120-131. PMID:35658005.
57. Yaeger R, Weiss J, Pelster MS, et al. Adagrasib with or without Cetuximab in Colorectal Cancer with Mutated KRAS G12C. *The New England journal of medicine.* N Engl J Med 2023 Jan 5;388(1):44-54. PMID:36546659.
58. McCoy MS, Bargmann CI, Weinberg RA. Human colon carcinoma Ki-ras2 oncogene and its corresponding proto-oncogene. *Mol Cell Biol.* 1984 Aug;4(8):1577-82. PMID:6092920.
59. Niv H, Gutman O, Kloog Y, Henis YI. Activated K-Ras and H-Ras display different interactions with saturable nonraft sites at the surface of live cells. *The Journal of cell biology.* J Cell Biol 2002 May 27;157(5):865-72. PMID:12021258.
60. Colicelli J. Human RAS superfamily proteins and related GTPases. *Sci STKE.* 2004 Sep 07;2004(250):RE13. Epub 2004 Sep 7. PMID:15367757.
61. Andreyev HJ, Norman AR, Cunningham D, et al. Kirsten ras mutations in patients with colorectal cancer: the 'RASCAL II' study. *Br J Cancer.* 2001 Sep 01;85(5):692-6. PMID:11531254.
62. Guerrero S, Casanova I, Farré L, Mazo A, Capellà G, Mangues R. K-ras codon 12 mutation induces higher level of resistance to apoptosis and predisposition to anchorage-independent growth than codon 13 mutation or proto-oncogene overexpression. *Cancer Res.* 2000 Dec 01;60(23):6750-6. PMID:11118062.
63. Brembeck FH, Schreiber FS, Deramaudt TB, et al. The mutant K-ras oncogene causes pancreatic periductal lymphocytic infiltration and gastric mucous neck cell hyperplasia in transgenic mice. *Cancer Res.* 2003 May 01;63(9):2005-9. PMID:12727809.
64. Guerra C, Mijimolle N, Dhawahir A, et al. Tumor induction by an endogenous K-ras oncogene is highly dependent on cellular context. *Cancer cell.* Cancer Cell 2003 Aug;4(2):111-20. PMID:12957286.
65. Alamo P, Gallardo A, Di Nicolantonio F, et al. Higher metastatic efficiency of KRas G12V than KRas G13D in a colorectal cancer model. *FASEB J.* 2015 Feb;29(2):464-76. Epub 2014 Oct 30. PMID:25359494.
66. Radulovich N, Qian JY, Tsao MS. Human pancreatic duct epithelial cell model for KRAS transformation. *Methods Enzymol.* 2008;439:1-13. PMID:18374152.
67. Shiloh Y. ATM and related protein kinases: safeguarding genome integrity. *Nat Rev Cancer.* 2003 Mar;3(3):155-68. PMID:12612651.
68. Peng G, Lin SY. Exploiting the homologous recombination DNA repair network for targeted cancer therapy. *World J Clin Oncol.* 2011 Feb 10;2(2):73-9. PMID:21603316.
69. Weston VJ, Oldreive CE, Skowronska A, et al. The PARP inhibitor olaparib induces significant killing of ATM-deficient lymphoid tumor cells in vitro and in vivo. *Blood.* 2010 Nov 25;116(22):4578-87. Epub 2010 Aug 25. PMID:20739657.
70. Riabinska A, Daheim M, Herter-Sprie GS, et al. Therapeutic targeting of a robust non-oncogene addiction to PRKDC in ATM-defective tumors. *Sci Transl Med.* 2013 Jun 12;5(189):189ra78. PMID:23761041.
71. Menezes DL, Holt J, Tang Y, et al. A synthetic lethal screen reveals enhanced sensitivity to ATR inhibitor treatment in mantle cell lymphoma with ATM loss-of-function. *Mol Cancer Res.* 2015 Jan;13(1):120-9. Epub 2014 Sep 17. PMID:25232030.
72. Vendetti FP, Lau A, Schamus S, Conrads TP, O'Connor MJ, Bakkenist CJ. The orally active and bioavailable ATR kinase inhibitor AZD6738 potentiates the anti-tumor effects of cisplatin to resolve ATM-deficient non-small cell lung cancer in vivo. *Oncotarget.* 2015 Dec 29;6(42):44289-44305. PMID:26517239.
73. Schmitt A, Knittel G, Welcker D, et al. ATM Deficiency Is Associated with Sensitivity to PARP1- and ATR Inhibitors in Lung Adenocarcinoma. *Cancer Res.* 2017 Jun 01;77(11):3040-3056. Epub 2017 Mar 31. PMID:28363999.
74. Perkhofer L, Schmitt A, Romero Carrasco MC, et al. ATM Deficiency Generating Genomic Instability Sensitizes Pancreatic Ductal Adenocarcinoma Cells to Therapy-Induced DNA Damage. *Cancer research.* Cancer Res 2017 Oct 15;77(20):5576-5590. PMID:28790064.

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75. Ledermann J, Harter P, Gourley C, et al. Olaparib maintenance therapy in platinum-sensitive relapsed ovarian cancer. *N Engl J Med.* 2012 Apr 12;366(15):1382-92. Epub 2012 Mar 27. PMID:22452356.
76. Kim G, Ison G, McKee AE, et al. FDA Approval Summary: Olaparib Monotherapy in Patients with Deleterious Germline BRCA-Mutated Advanced Ovarian Cancer Treated with Three or More Lines of Chemotherapy. *Clin Cancer Res.* 2015 Oct 01;21(19):4257-61. Epub 2015 Jul 17. PMID:26187614.
77. Swisher EM, Lin KK, Oza AM, et al. Rucaparib in relapsed, platinum-sensitive high-grade ovarian carcinoma (ARIEL2 Part 1): an international, multicentre, open-label, phase 2 trial. *Lancet Oncol.* 2017 Jan;18(1):75-87. Epub 2016 Nov 29. PMID:27908594.
78. Balasubramaniam S, Beaver JA, Horton S, et al. FDA Approval Summary: Rucaparib for the Treatment of Patients with Deleterious BRCA Mutation-Associated Advanced Ovarian Cancer. *Clin Cancer Res.* 2017 Dec 01;23(23):7165-7170. Epub 2017 Jul 27. PMID:28751443.
79. Coleman RL, Oza AM, Lorusso D, et al. Rucaparib maintenance treatment for recurrent ovarian carcinoma after response to platinum therapy (ARIEL3): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet.* 2017 Oct 28;390(10106):1949-1961. Epub 2017 Sep 12. PMID:28916367.
80. Robson M, Im SA, Senkus E, et al. Olaparib for Metastatic Breast Cancer in Patients with a Germline BRCA Mutation. *The New England journal of medicine.* N Engl J Med 2017 Aug 10;377(6):523-533. PMID:28578601.
81. Robson ME, Tung N, Conte P, et al. OlympiAD final overall survival and tolerability results: Olaparib versus chemotherapy treatment of physician's choice in patients with a germline BRCA mutation and HER2-negative metastatic breast cancer. *Ann Oncol.* 2019 Apr 01;30(4):558-566. PMID:30689707.
82. Golan T, Hammel P, Reni M, et al. Maintenance Olaparib for Germline BRCA-Mutated Metastatic Pancreatic Cancer. *N Engl J Med.* 2019 Jul 25;381(4):317-327. Epub 2019 Jun 2. PMID:31157963.
83. Abida W, Patnaik A, Campbell D, et al. Rucaparib in Men With Metastatic Castration-Resistant Prostate Cancer Harboring a BRCA1 or BRCA2 Gene Alteration. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology.* J Clin Oncol 2020 Nov 10;38(32):3763-3772. PMID:32795228.
84. Litton JK, Rugo HS, Ettl J, et al. Talazoparib in Patients with Advanced Breast Cancer and a Germline BRCA Mutation. *N Engl J Med.* 2018 Aug 23;379(8):753-763. Epub 2018 Aug 15. PMID:30110579.
85. Agarwal N, Azad AA, Carles J, et al. Talazoparib plus enzalutamide in men with first-line metastatic castration-resistant prostate cancer (TALAPRO-2): a randomised, placebo-controlled, phase 3 trial. *Lancet (London, England).* Lancet 2023 Jun 2;. PMID:37285865.
86. Sommer LA, Schaad M, Dames SA. NMR- and circular dichroism-monitored lipid binding studies suggest a general role for the FATC domain as membrane anchor of phosphatidylinositol 3-kinase-related kinases (PIKK). *The Journal of biological chemistry.* J Biol Chem 2013 Jul 5;288(27):20046-63. PMID:23671275.
87. Jiang X, Sun Y, Chen S, Roy K, Price BD. The FATC domains of PIKK proteins are functionally equivalent and participate in the Tip60-dependent activation of DNA-PKcs and ATM. *J Biol Chem.* 2006 Jun 09;281(23):15741-6. Epub 2006 Apr 9. PMID:16603769.
88. Gamper AM, Choi S, Matsumoto Y, Banerjee D, Tomkinson AE, Bakkenist CJ. ATM protein physically and functionally interacts with proliferating cell nuclear antigen to regulate DNA synthesis. *J Biol Chem.* 2012 Apr 06;287(15):12445-54. Epub 2012 Feb 23. PMID:22362778.
89. Khanna KK, Keating KE, Kozlov S, et al. ATM associates with and phosphorylates p53: mapping the region of interaction. *Nat Genet.* 1998 Dec;20(4):398-400. PMID:9843217.
90. Guo Z, Deshpande R, Paull TT. ATM activation in the presence of oxidative stress. *Cell Cycle.* 2010 Dec 15;9(24):4805-11. PMID:21150274.
91. Tsai WB, Chung YM, Takahashi Y, Xu Z, Hu MC. Functional interaction between FOXO3a and ATM regulates DNA damage response. *Nat Cell Biol.* 2008 Apr;10(4):460-7. Epub 2008 Mar 16. PMID:18344987.
92. Lindeboom RG, Supek F, Lehner B. The rules and impact of nonsense-mediated mRNA decay in human cancers. *Nature genetics.* Nat Genet 2016 Oct;48(10):1112-8. PMID:27618451.
93. Popp MW, Maquat LE. Nonsense-mediated mRNA Decay and Cancer. *Current opinion in genetics & development.* Curr Opin Genet Dev 2018 Feb;48:44-50. PMID:29121514.
94. Nagy E, Maquat LE. A rule for termination-codon position within intron-containing genes: when nonsense affects RNA abundance. *Trends in biochemical sciences.* Trends Biochem Sci 1998 Jun;23(6):198-9. PMID:9644970.
95. Richards S, Aziz N, Bale S, et al. 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. *Genet Med.* 2015 May;17(5):405-24. Epub 2015 Mar 5. PMID:25741868.
96. Tomlinson IP, Houlston RS, Montgomery GW, Sieber OM, Dunlop MG. Investigation of the effects of DNA repair gene polymorphisms on the risk of colorectal cancer. *Mutagenesis.* 2012 Mar;27(2):219-23. PMID:22294770.
97. Xu HP, Riggs M, Rodgers L, Wigler M. A gene from S. pombe with homology to E. coli RNAse III blocks conjugation and sporulation when overexpressed in wild type cells. *Nucleic acids research.* Nucleic Acids Res 1990 Sep 11;18(17):5304. PMID:2205842.
98. van der Groep P, van der Wall E, van Diest PJ. Pathology of hereditary breast cancer. *Cell Oncol (Dordr).* 2011 Apr;34(2):71-88. Epub 2011 Feb 19. PMID:21336636.
99. Schutte M, Seal S, Barfoot R, et al. Variants in CHEK2 other than 1100delC do not make a major contribution to breast cancer susceptibility. *Am J Hum Genet.* 2003 Apr;72(4):1023-8. Epub 2003 Feb 27. PMID:12610780.
100. McCabe N, Turner NC, Lord CJ, et al. Deficiency in the repair of DNA damage by homologous recombination and sensitivity to poly(ADP-ribose) polymerase inhibition. *Cancer Res.* 2006 Aug 15;66(16):8109-15. PMID:16912188.
101. Higashiguchi M, Nagatomo I, Kijima T, et al. Clarifying the biological significance of the CHK2 K373E somatic mutation discovered in The Cancer Genome Atlas database. *FEBS Lett.* 2016 Dec;590(23):4275-4286. Epub 2016 Oct 25. PMID:27716909.
102. Norquist BM, Harrell MI, Brady MF, et al. Inherited Mutations in Women With Ovarian Carcinoma. *JAMA Oncol.* 2016 Apr;2(4):482-90. PMID:26720728.
103. Ramus SJ, Song H, Dicks E, et al. Germline Mutations in the BRIP1, BARD1, PALB2, and NBN Genes in Women With Ovarian Cancer. *J Natl Cancer Inst.* 2015 Nov;107(11). Epub 2015 Aug 27. PMID:26315354.
104. Rafnar T, Gudbjartsson DF, Sulem P, et al. Mutations in BRIP1 confer high risk of ovarian cancer. *Nat Genet.* 2011 Oct 02;43(11):1104-7. Epub 2011 Oct 2. PMID:21964575.
105. Pritchard CC, Mateo J, Walsh MF, et al. Inherited DNA-Repair Gene Mutations in Men with Metastatic Prostate Cancer. *N Engl J Med.* 2016 Aug 04;375(5):443-53. Epub 2016 Jul 6. PMID:27433846.
106. Abida W, Armenia J, Gopalan A, et al. Prospective Genomic Profiling of Prostate Cancer Across Disease States Reveals Germline and Somatic Alterations That May Affect Clinical Decision Making. *JCO Precis Oncol.* 2017 Jul;2017. Epub 2017 May 31. PMID:28825054.
107. Mathew CG. Fanconi anaemia genes and susceptibility to cancer. *Oncogene.* 2006 Sep 25;25(43):5875-84. PMID:16998502.
108. Wang LC, Gautier J. The Fanconi anemia pathway and ICL repair: implications for cancer therapy. *Crit Rev Biochem Mol Biol.* 2010 Oct;45(5):424-39. PMID:20807115.
109. Kitao H, Yamamoto K, Matsushita N, Ohzeki M, Ishiai M, Takata M. Functional interplay between BRCA2/FancD1 and FancC in DNA repair. *J Biol Chem.* 2006 Jul 28;281(30):21312-21320. Epub 2006 May 10. PMID:16687415.
110. Pang Q, Christianson TA, Keeble W, et al. The Fanconi anemia complementation group C gene product: structural evidence of multifunctionality. *Blood.* 2001 Sep 01;98(5):1392-401. PMID:11520787.
111. Bridge WL, Vandenberg CJ, Franklin RJ, Hiom K. The BRIP1 helicase functions independently of BRCA1 in the Fanconi anemia pathway for DNA crosslink repair. *Nat Genet.* 2005 Sep;37(9):953-7. Epub 2005 Aug 21. PMID:16116421.
112. Litman R, Peng M, Jin Z, et al. BACH1 is critical for homologous recombination and appears to be the Fanconi anemia gene product FANCJ. *Cancer Cell.* 2005 Sep;8(3):255-65. PMID:16153896.

113. Pennington KP, Walsh T, Harrell MI, et al. Germline and somatic mutations in homologous recombination genes predict platinum response and survival in ovarian, fallopian tube, and peritoneal carcinomas. *Clin Cancer Res.* 2014 Feb 01;20(3):764-75. Epub 2013 Nov 15. PMID:24240112.
114. Hernández G, Ramírez MJ, Minguillón J, et al. Decapping protein EDC4 regulates DNA repair and phenocopies BRCA1. *Nat Commun.* 2018 Mar 06;9(1):967. Epub 2018 Mar 6. PMID:29511213.
115. Cantor SB, Bell DW, Ganesan S, et al. BACH1, a novel helicase-like protein, interacts directly with BRCA1 and contributes to its DNA repair function. *Cell.* 2001 Apr 06;105(1):149-60. PMID:11301010.
116. De Nicolo A, Tancredi M, Lombardi G, et al. A novel breast cancer-associated BRIP1 (FANCJ/BACH1) germ-line mutation impairs protein stability and function. *Clin Cancer Res.* 2008 Jul 15;14(14):4672-80. PMID:18628483.
117. Holloman WK. Unraveling the mechanism of BRCA2 in homologous recombination. *Nat Struct Mol Biol.* 2011 Jul 06;18(7):748-54. Epub 2011 Jul 6. PMID:21731065.
118. Kolinjivadi AM, Sannino V, de Antoni A, Técher H, Baldi G, Costanzo V. Moonlighting at replication forks - a new life for homologous recombination proteins BRCA1, BRCA2 and RAD51. *FEBS Lett.* 2017 Apr;591(8):1083-1100. Epub 2017 Jan 30. PMID:28079255.
119. Hollis RL, Churchman M, Gourley C. Distinct implications of different BRCA mutations: efficacy of cytotoxic chemotherapy, PARP inhibition and clinical outcome in ovarian cancer. *Onco Targets Ther.* 2017;10:2539-2551. Epub 2017 May 11. PMID:28546758.
120. Scott LJ. Niraparib: First Global Approval. *Drugs.* 2017 Jun;77(9):1029-1034. PMID:28474297.
121. Kuznetsov SG, Liu P, Sharan SK. Mouse embryonic stem cell-based functional assay to evaluate mutations in BRCA2. *Nat Med.* 2008 Aug;14(8):875-81. Epub 2008 Jul 6. PMID:18607349.
122. Davies OR, Pellegrini L. Interaction with the BRCA2 C terminus protects RAD51-DNA filaments from disassembly by BRC repeats. *Nat Struct Mol Biol.* 2007 Jun;14(6):475-83. PMID:17515903.
123. Spain BH, Larson CJ, Shihabuddin LS, Gage FH, Verma IM. Truncated BRCA2 is cytoplasmic: implications for cancer-linked mutations. *Proc Natl Acad Sci U S A.* 1999 Nov 23;96(24):13920-5. PMID:10570174.
124. Kim TM, Son MY, Dodds S, Hu L, Hasty P. Deletion of BRCA2 exon 27 causes defects in response to both stalled and collapsed replication forks. *Mutation research.* Mutat Res 2014 Aug-Sep;766-767:66-72. PMID:25847274.
125. Hucl T, Rago C, Gallmeier E, Brody JR, Gorospe M, Kern SE. A syngeneic variance library for functional annotation of human variation: application to BRCA2. *Cancer Res.* 2008 Jul 01;68(13):5023-30. PMID:18593900.
126. Welcker M, Clurman BE. FBW7 ubiquitin ligase: a tumour suppressor at the crossroads of cell division, growth and differentiation. *Nat Rev Cancer.* 2008 Feb;8(2):83-93. PMID:18094723.
127. Mao JH, Kim IJ, Wu D, et al. FBXW7 targets mTOR for degradation and cooperates with PTEN in tumor suppression. *Science.* 2008 Sep 12;321(5895):1499-502. PMID:18787170.
128. Akhoondi S, Sun D, von der Lehr N, et al. FBXW7/hCDC4 is a general tumor suppressor in human cancer. *Cancer Res.* 2007 Oct 01;67(19):9006-12. PMID:17909001.
129. Cao J, Ge MH, Ling ZQ. Fbxw7 Tumor Suppressor: A Vital Regulator Contributes to Human Tumorigenesis. *Medicine (Baltimore).* 2016 Feb;95(7):e2496. PMID:26886596.
130. Jardim DL, Wheler JJ, Hess K, et al. FBXW7 mutations in patients with advanced cancers: clinical and molecular characteristics and outcomes with mTOR inhibitors. *PLoS One.* 2014;9(2):e89388. Epub 2014 Feb 19. PMID:24586741.
131. Myers AP, Filiaci VL, Zhang Y, et al. Tumor mutational analysis of GOG248, a phase II study of temsirolimus or temsirolimus and alternating megestrol acetate and tamoxifen for advanced endometrial cancer (EC): An NRG Oncology/Gynecologic Oncology Group study. *Gynecol Oncol.* 2016 Apr;141(1):43-8. PMID:27016228.
132. Wallin JJ, Edgar KA, Guan J, et al. GDC-0980 is a novel class I PI3K/mTOR kinase inhibitor with robust activity in cancer models driven by the PI3K pathway. *Mol Cancer Ther.* 2011 Dec;10(12):2426-36. Epub 2011 Oct 13. PMID:21998291.
133. Mohan S, Vander Broek R, Shah S, et al. MEK Inhibitor PD-0325901 Overcomes Resistance to PI3K/mTOR Inhibitor PF-5212384 and Potentiates Antitumor Effects in Human Head and Neck Squamous Cell Carcinoma. *Clin Cancer Res.* 2015 Sep 01;21(17):3946-56. Epub 2015 May 14. PMID:25977343.
134. Mortensen DS, Fultz KE, Xu S, et al. CC-223, a Potent and Selective Inhibitor of mTOR Kinase: In Vitro and In Vivo Characterization. *Molecular cancer therapeutics.* Mol Cancer Ther 2015 Jun;14(6):1295-305. PMID:25855786.
135. Fu Y, Zheng S, An N, et al. β-catenin as a potential key target for tumor suppression. *Int J Cancer.* 2011 Oct 01;129(7):1541-51. Epub 2011 Jun 21. PMID:21455986.
136. Kim DY, Kwon YJ, Seo WY, et al. Tankyrase-selective inhibitor STP1002 shows preclinical antitumour efficacy without on-target toxicity in the gastrointestinal tract. *European journal of cancer (Oxford, England : 1990).* Eur J Cancer 2022 Sep;173:41-51. PMID:35849876.
137. Tuynman JB, Vermeulen L, Boon EM, et al. Cyclooxygenase-2 inhibition inhibits c-Met kinase activity and Wnt activity in colon cancer. *Cancer Res.* 2008 Feb 15;68(4):1213-20. PMID:18281498.
138. Lu W, Tinsley HN, Keeton A, Qu Z, Piazza GA, Li Y. Suppression of Wnt/beta-catenin signaling inhibits prostate cancer cell proliferation. *European journal of pharmacology.* Eur J Pharmacol 2009 Jan 5;602(1):8-14. PMID:19026633.
139. Yamada T, Masuda M. Emergence of TNIK inhibitors in cancer therapeutics. *Cancer science.* Cancer Sci 2017 May;108(5):818-823. PMID:28208209.
140. Katoh M, Katoh M. Molecular genetics and targeted therapy of WNT-related human diseases (Review). *Int J Mol Med.* 2017 Sep;40(3):587-606. Epub 2017 Jul 19. PMID:28731148.
141. Dikovskaya D, Li Z, Newton IP, et al. Microtubule assembly by the Apc protein is regulated by importin-beta--RanGTP. *J Cell Sci.* 2010 Mar 01;123(Pt 5):736-46. Epub 2010 Feb 9. PMID:20144988.
142. Eklof Spink K, Fridman SG, Weis WI. Molecular mechanisms of beta-catenin recognition by adenomatous polyposis coli revealed by the structure of an APC-beta-catenin complex. *EMBO J.* 2001 Nov 15;20(22):6203-12. PMID:11707392.
143. Liu J, Xing Y, Hinds TR, Zheng J, Xu W. The third 20 amino acid repeat is the tightest binding site of APC for beta-catenin. *J Mol Biol.* 2006 Jun 30;360(1):133-44. Epub 2006 May 15. PMID:16753179.

Patient Name	**Jane Jones**	Date of Birth (Age)	**11/27/1940 (82 yrs)**	Assigned Sex at Birth	**Female**
Diagnosis	**Rectal adenocarcinoma**	Internal Patient ID	**10000090**	Report Date	**07/05/2023**

This NGS-based assay was developed and its performance characteristics determined by BillionToOne, Inc. It has not been cleared or approved by the U.S. Food and Drug Administration. BillionToOne, Inc. is regulated under CLIA. This test is used for clinical purposes. It should not be regarded as investigational or for research. This test was performed using BillionToOne's patented technology (www.billiontoone.com/patents).

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