Precise™ Tumor

Molecular Profile Test

Myriad Genetics
320 Wakara Way
Salt Lake City, UT 84108

Myriad genetics

PATIENT	PHYSICIAN	SPECIMEN	CASE
PATIENT Breast Example **SEX** Female **DISEASE** Human epidermal growth factor 2 negative carcinoma of breast **MRN#** MRN-22222 **DATE OF BIRTH** 06/14/1948	**ORDERING PHYSICIAN** Example Doctor	**SPECIMEN TYPE** Formalin-fixed paraffin-embedded tissue specimen **DATE COLLECTED** 01/17/2023 **DATE RECEIVED** 01/17/2023	**ACCESSION#** Breast-Precise **DATE REPORTED** **REVIEW STATUS** Final **DATE ORDERED** 01/06/2023

Interpretive Comments

Refer to the Clinically Relevant Results section of the report for a discussion of potential treatment approaches. Based on histopathologic review, the tumor cell percentage in the sequenced sample is 70%.

IA	IB	IIC	IIB	TMB	MSI	PD-L1	Trials
2	0	2	3	Low 5.5 muts/Mb	Stable 2.4% Unstable Sites	Negative TPS: <1% unit	12

Clinical Implications

TIER	VARIANT DETECTED (GENE/SYNTAX)	CLINICAL IMPACT		SELECT CLINICAL TRIALS
IA	**PIK3CA** p.G1007R	**May benefit from:**	Alpelisib	7
		In Tumor Type:	HR-positive, HER2-negative, PIK3CA-mutated breast cancer	
IA	**PIK3CA** p.N345T	**May benefit from:**	Alpelisib	7
		In Tumor Type:	HR-positive, HER2-negative, PIK3CA-mutated breast cancer	
IIC	**TP53** p.P142Afs*5			1
IIC	**FGFR1** Copy number gain in FGFR1 (9 copies)	**May benefit from:**	Lenvatinib, Pazopanib, or Sorafenib	5
		In Tumor Type:	Renal cell carcinoma (RCC)	

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Please contact Myriad Medical Services at 1-800-469-7423 X 3850 to discuss any questions regarding this result.

PATIENT	DOB	DISEASE	ACCESSION	MRN	REPORT DATE	REPORT STATUS
Breast Example	06/14/1948	Human epidermal growth factor 2 negative carcinoma of breast	Breast-Precise	MRN-22222		Final

TIER	VARIANT DETECTED (GENE/SYNTAX)	CLINICAL IMPACT		SELECT CLINICAL TRIALS
		May benefit from:	Ponatinib	
		In Tumor Type:	Acute lymphocytic leukemia (ALL) or Chronic myelocyticleukemia (CML)	
		May benefit from:	Erdafitinib	
		In Tumor Type:	Urothelial carcinoma with a susceptible FGFR2 or FGFR3alteration	
		May benefit from:	Nintedanib	
		In Tumor Type:	Idiopathic pulmonary fibrosis	
		May benefit from:	Infigratinib, Pemigatinib, or Futibatinib	
		In Tumor Type:	Cholangiocarcinoma with a FGFR2 fusion or otherrearrangement	

Pertinent Negatives

(Disease-relevant genes not containing detected mutations)

AR	*BRAF*	*BRCA1*	*BRCA2*	*EGFR*	*ERBB2*	*KIT*
KRAS	*MET*	*MLH1*	*MSH2*	*MSH6*	*NRAS*	*NTRK1*
NTRK2	*NTRK3*	*PDGFRA*	*PGR*	*PMS2*	*PTEN*	*RET*

Clinically Relevant Results

PIK3CA	**p.G1007R**	c.3019G>C	Tier IA	NM_006218.2	VAF: 63%	Depth: 891

PIK3CA p.G1007R is a missense alteration located in the PI3K/PI4K domain (aa 765 - 1051; UniProt.org) of the PIK3CA protein and has been identified as a recurrent hotspot (statistically significant) in a population-scale cohort of tumor samples of various cancer types(cancerhotspots.org). PIK3CA p.G1007R increased cell proliferation and migration and increased AKT phosphorylation compared to wildtype PIK3CA in culture (PMID: 34779417) and increased transformation ability in several different cell lines in culture (PMID: 29533785, PMID: 34779417). Therefore, this alteration is predicted to be activating and oncogenic.

PIK3CA mutations have been reported in 34% of Breast Cancer samples in cBioPortal for Cancer Genomics (cBioPortal.org; June2022). A pooled analysis of 10319 breast cancer patients from 19 studies has reported that PIK3CA mutation was associated with ER positivity, lower tumor grade, and smaller tumor size (PMID: 29470143).

PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha, activates PI3K/AKT/mTOR signaling to promote cell proliferation (PMID: 23411347, PMID: 31905960). PIK3CA activating mutations have been identified in a number of tumor types such as breast cancer (PMID: 32234362, PMID: 32404150), colon cancer (PMID: 32099598), endometrial cancer, glioblastoma, skin cancer, ovarian cancer (PMID: 20535651, PMID: 31892193), and mammary angiosarcoma (PMID: 32123305), and PIK3CA amplification has been observed in esophageal adenocarcinoma (PMID: 31865178).

Alpelisib (FDA approved PI3K inhibitor for the treatment of postmenopausal women with HR-positive HER2-negative, PIK3CAmutated,advanced or metastatic breast cancer following progression on or after an endocrine-based regimen) in combination with fulvestrant is recommended as preferred regimen (second-line therapy) for PIK3CA-mutated HR-positive/HER2-negative post menopausal women having recurrent or stage IV disease (category 1) (NCCN 'Breast cancer' v.8.2022; PMID: 31091374). Clinical trials investigating PIK3CA inhibitors, PI3K inhibitors, and Pan-AKT inhibitors, alone or in combination, are recruiting patients with PIK3CAmutations (NCT02465060, NCT02761694, NCT03337724, NCT03056755, NCT03006172, PMID: 31277699).

PIK3CA mutations and activation of the PI3K pathway may play a role in resistance to hormonal therapy in ER-positive breast cancers(PMID: 23087906). PI3K pathway activation (activating PIK3CA mutations) has been associated with resistance to Her2-targetedtherapies in some clinical studies, though in other studies no association was found (PMID: 22172323, PMID: 20501798, PMID: 21594665, PMID: 21676217, PMID: 22744290, AACR 2013, Abstract S4-06, PMID: 27428671).

Baseline plasma PIK3CA mutation has been associated with innate resistance to trastuzumab in a study of 24 Her2-positive gastric cancer patients (PMID: 30269082)

PIK3CA	**p.N345T**	c.1034A>C	Tier IA	NM_006218.2	VAF: 61.3%	Depth: 821

PIK3CA p.N345T is a missense alteration located in the C2 PI3K-type domain (aa 330 - 487; UniProt.org) of the PIK3CA protein and has been identified as a recurrent hotspot (statistically significant) in a population-scale cohort of tumor samples of various cancer types (cancerhotspots.org). PIK3CA p.N345T increased transformation ability in two different cell lines in culture (PMID: 29533785). In ClinVar, this alteration is reported as 'Pathogenic' (Variation ID: 376489). Therefore, this alteration is predicted to be activating andoncogenic.

PIK3CA mutations have been reported in 34% of Breast Cancer samples in cBioPortal for Cancer Genomics (cBioPortal.org; June2022). A pooled analysis of 10319 breast cancer patients from 19 studies has reported that PIK3CA mutation was associated with ER positivity, lower tumor grade, and smaller tumor size (PMID: 29470143).

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TP53	**p.P142Afs*5**	c.423_427del5	Tier IIC	NM_000546.5	VAF: 52.4%	Depth: 773

TP53 p.P142Afs*5 is expected to truncate the p53 protein within the DNA-binding domain (DBD) (PMID: 18410249, PMID: 32164171),resulting in the loss of a portion of the DBD, the entire tetramerization domain, and the C-terminal regulatory domain (PMID: 18410249). DBD mutations are thought to result in loss of function via the loss of transactivation of p53-dependent genes (PMID: 12826609). The tetramerization domain is thought to be critical to normal p53 function (PMID: 20978130). In addition, the C-terminal regulatory domain has been shown to be required for DNA binding and transcriptional activation by p53 (PMID: 22178617). Therefore, this alteration is predicted to be inactivating and oncogenic.

TP53 mutations have been reported in 60% of Breast cancer samples in cBioPortal for Cancer Genomics (cBioPortal.org; May 2022). A preclinical study has reported that the knockdown of TP53 and PTEN expression in human breast cells promotes the formation of cancer stem cells and a gene expression profile resembling that of basal-like triple-negative breast tumors (PMID: 24531711). Another preclinical study of isogenic patient-derived xenograft TNBC models reported increased growth in both primary and metastatic sites in p53-deficient tumors as compared with wildtype tumors (PMID: 26818199).

TP53 encodes the p53 tumor suppressor protein, a transcription factor that responds to cellular stresses, including DNA damage and oncogenic activation, by inducing downstream anti-tumor responses such as DNA repair and apoptosis (PMID: 11099028). The p53protein consists of an N-terminal transactivation domain, a central DNA-binding domain, an oligomerization domain, and a C-terminal regulatory domain (PMID: 22713868). Loss of tumor suppressor p53, which is encoded by the TP53 gene, is common in aggressive advanced cancers (PMID: 19935675). Carriers of a germline mutation in TP53 have Li-Fraumeni Syndrome, an inherited cancer syndrome resulting in multiple tumors in early adulthood, including breast cancer, brain tumors, and leukemias (PMID: 1978757, PMID:2259385, PMID: 1683921). Expression of p53 in normal cells is low; however, TP53 alterations, including those that result in loss of p53tumor suppressor function, may lead to stabilization and increased expression of p53, particularly in the nucleus, and several studies have shown that it may have oncogenic gain-of-function effects (PMID: 15625370, PMID: 11400116, PMID: 12826609, PMID: 21760960,PMID: 18802452).

Currently, there are no approved therapies targeting TP53 alterations, despite their high prevalence in cancer. Therapeutic approaches under investigation include gene therapy for TP53 and (dendritic cell-based) TP53 vaccines (PMID: 24583792, PMID: 21541192, PMID: 24982341). Inhibition of components of the DNA damage checkpoint, including Checkpoint Kinase 1 (Chk1) and Wee1,has been reported to enhance the activity of DNA-damaging agents in preclinical cancer models with deficiency of p53 function(PMID: 21087899, PMID: 20107315, PMID: 21799033). Clinical trials of the Wee1 inhibitor adavosertib (MK-1775) are currently underway for patients with solid tumors and hematologic malignancies. Olaparib in combination with AZD1775 (WEE1 inhibitor) is in phase II clinical trial to determine tumor overall response rate in patients with advanced solid tumors harboring TP53 mutations(NCT02576444, Active, not recruiting). Studies have reported Aurora kinase A to be activated in cells harboring TP53 mutation, and Aurora kinase A and B inhibitors have been reported to activate wild-type p53 in cellular assays; thus, tumors retaining a wild-typeTP53 allele may benefit from Aurora kinase inhibitors (PMID: 25398437, PMID: 25512615, PMID: 21761334, PMID: 25758253, PMID:22611192, PMID: 23955083). Some studies suggest that Hsp90 inhibitors may be effective in tumors with oncogenic TP53 alterations(PMID: 26009011, PMID: 17982489).

Mutations in TP53 may increase resistance to ionizing radiation therapy (PMID: 14576853, PMID: 25913131). TP53 mutations have been significantly associated with platinum resistance in studies of ovarian cancer cases (PMID: 25385265, PMID: 28148293).

A phase II trial study is available to determine how well modified vaccinia virus ankara vaccine expressing p53 (p53MVA) and pembrolizumab work in treating patients with ovarian, primary peritoneal, or fallopian tube cancer that has come back (NCT03113487).AMG 650 is currently being investigated in phase I clinical trial (dose exploration phase only) for patients with advanced or metastatic solid tumor harboring TP53 mutations (metastatic high grade serous ovarian cancer is further eligible for dose-expansion phase) (NCT04293094).

FGFR1	Copy number gain in FGFR1 (9 copies)	Tier IIC		VAF:	Depth:

FGFR1 amplification has been associated with increased FGFR1 mRNA and protein expression, and has been reported to lead to aberrant downstream signaling (PMID: 20094046, PMID: 20179196, PMID: 26088290, PMID: 26801869).

Putative high-level amplification of FGFR1 has been reported in 12% of breast cancer samples in cBioPortal for Cancer Genomics(cBioPortal.org; June 2022). FGFR1 alterations, including mutations, amplifications, and translocations have been observed in a wide range of cancer types. Furthermore, FGFR1 amplification has been identified as a driver mutation in some cancer types, including breast and lung carcinoma (PMID: 19601767, PMID: 19182515, PMID: 22273505, PMID: 21160078, PMID: 20179196, PMID: 19340397, PMID:10498824). Studies have reported that earlier-stage tumors exhibit greater FGFR1 expression than later-stage tumors (PMID: 9374233,PMID: 16807070).

FGFR1 is a receptor tyrosine kinase that is a member of the fibroblast growth factor receptor (FGFR) family. FGFR1 is involved in embryonic development, cell proliferation, differentiation, migration, skeletogenesis, mitogenesis, and angiogenesis (PMID: 22508544).Binding of FGF ligands to FGFR1 results in the rapid dimerization and activation of downstream signaling pathways including the Ras,PI3K/AKT, and MAPK pathways (PMID: 16597617, PMID: 20094046). Cell-type-specific FGFR1 regulation is dependent on tissue distribution and ligand availability (PMID: 16597617). Germline mutations in FGFR1 are associated with congenic disorders that present with physical malformations, mental retardation, and neurologic deficits (PMID: 23812909). Amplifying or activating mutations in FGFR1occur in varying frequency in multiple cancers including those of the lung, breast, prostate, head and neck, and esophagus (PMID:12147242). In metastatic renal cell carcinoma, FGF signaling mediates acquired treatment resistance from VEGF-directed therapies(PMID: 24387233). Currently, a number of small-molecule inhibitors of the FGFR proteins are in use, with the major difference among them being their specificity to FGFR versus other receptor tyrosine kinases (RTKs) (PMID: 24265351).

Tumors with FGFR1 amplification or activating mutations may be sensitive to Fgfr family inhibitors, and clinical trials of these agents are currently underway in solid tumors (PMID: 20094046, PMID: 21641723). Several multi-kinase inhibitors that target Fgfrs, including pazopanib, ponatinib, regorafenib, and lenvatinib, have been FDA approved for certain indications and continue to be studied in clinical trials (PMID: 20100962, PMID: 26482279, PMID: 25671254, PMID: 22595799, PMID: 24180494, PMID: 23177514, PMID: 23177515).Additional agents that target Fgfrs are also being studied in clinical trials (PMID: 27870574, PMID: 29182496, PMID: 29177434, ASCO2015, Abstract 2508, PMID: 26324363, PMID: 24411639, PMID: 28303906). FGFR1 amplification and signaling through Fgfr1 have been implicated in resistance to endocrine therapy and Cdk4/6 inhibitors in breast cancer preclinical models (PMID: 30914635, PMID: 20179196, PMID: 24425047, PMID: 28751448). A preclinical study of ER-positive and FGFR1-amplified breast cancer cells and patient derived xenografts reported that combined treatment with fulvestrant and the Fgfr inhibitor lucitanib resulted in greater growth inhibition as compared with each drug alone (PMID: 28751448).

Other Biomarkers

BIOMARKER	RESULT	CLINICAL IMPACT
TMB	Low 5.5 muts/Mb	
MSI	Stable 2.4% Unstable Sites	

BIOMARKER	RESULT	CLINICAL IMPACT
PD-L1	**Negative** TPS: <1% unit	Immunohistochemical staining for PD-L1 was performed using anti-PD-L1 clone SP263.

Clinical Trials

Clinical Trials associated with this patient's genomic profile and tumor type are displayed below.

TITLE	TRIAL IDENTIFIER	PHASE	VARIANT
TAPUR: Testing the Use of Food and Drug Administration (FDA) Approved Drugs That Target a Specific Abnormality in a Tumor Gene in People With Advanced Stage Cancer	NCT02693535	II	**FGFR1** Copy number gain in FGFR1 (9 copies)
Targeted Therapy Directed by Genetic Testing in Treating Patients With Advanced Refractory Solid Tumors, Lymphomas, or Multiple Myeloma (The MATCH Screening Trial)	NCT02465060	II	**PIK3CA** p.G1007R c.3019G>C **PIK3CA** p.N345T c.1034A>C **FGFR1** Copy number gain in FGFR1 (9 copies)
A Study of TAS-120 in Patients With Metastatic Breast Cancer	NCT04024436	II	**FGFR1** Copy number gain in FGFR1 (9 copies)
Infigratinib for the Treatment of Advanced or Metastatic Solid Tumors in Patients With FGFR Gene Mutations	NCT04233567	II	**FGFR1** Copy number gain in FGFR1 (9 copies)
Testing the Addition of an Anti-cancer Drug, Copanlisib, to the Usual Immunotherapy (Nivolumab With or Without Ipilimumab) in Patients With Advanced Solid Cancers That Have Changes in the Following Genes: PIK3CA and PTEN	NCT04317105	I/II	**PIK3CA** p.G1007R c.3019G>C **PIK3CA** p.N345T c.1034A>C

TITLE	TRIAL IDENTIFIER	PHASE	VARIANT
Study of eFT226 in Subjects With Selected Advanced Solid Tumor Malignancies	NCT04092673	I/II	**FGFR1** Copy number gain in FGFR1 (9 copies)
GDC-0084 With Radiation Therapy for People With PIK3CA-Mutated Solid Tumor Brain Metastases or Leptomeningeal Metastases	NCT04192981	I	**PIK3CA** p.N345T c.1034A>C **PIK3CA** p.G1007R c.3019G>C
To Evaluate the Safety, Tolerability, and Pharmacokinetics of Inavolisib Single Agent in Participants With Solid Tumors and in Combination With Endocrine and Targeted Therapies in Participants With Breast Cancer	NCT03006172	I	**PIK3CA** p.G1007R c.3019G>C **PIK3CA** p.N345T c.1034A>C
Study of AMG 650 in Adult Participants With Advanced Solid Tumors	NCT04293094	I	**TP53** p.P142Afs*5 c.423_427del5
A Study of LY3484356 in Participants With Advanced or Metastatic Breast Cancer or Endometrial Cancer	NCT04188548	I	**PIK3CA** p.G1007R c.3019G>C **PIK3CA** p.N345T c.1034A>C
Study of the CDK4/6 Inhibitor Palbociclib (PD-0332991) in Combination With the PI3K/mTOR Inhibitor Gedatolisib (PF-05212384) for Patients With Advanced Squamous Cell Lung, Pancreatic, Head & Neck and Other Solid Tumors	NCT03065062	I	**PIK3CA** p.G1007R c.3019G>C **PIK3CA** p.N345T c.1034A>C
Study of CYH33 in Combination With Olaparib an Oral PARP Inhibitor in Patients With Advanced Solid Tumors.	NCT04586335	I	**PIK3CA** p.G1007R c.3019G>C **PIK3CA** p.N345T c.1034A>C

Classification and Levels of Evidence

The variant classification system used in this report is based on joint consensus recommendations of the Association for Molecular Pathology, American Society of Clinical Oncology, and the College of American Pathologists (J Mol Diagn 2017, 19:4-23). Tiers IA, IB, IIC, IID, III and IV describe variant categories of descending clinical significance in the patient. Variants in Tier IV are not reported in accordance with the consensus recommendations.

IA

Variant of strong clinical significance, Level A evidence (FDA approved therapy or practice guideline in patient's tumor type)

IB

Variant of strong clinical significance, Level B Evidence (consensus in the field based on well-powered studies in patient's tumor type)

IIC

Variant of potential clinical significance, Level C evidence (FDA approved therapy or practice guideline in other tumor type(s), evidence from multiple small published studies, or based on availability of investigational therapies)

IID

Variant of potential clinical significance, Level D evidence (case reports or preclinical studies)

III Variant of uncertain clinical significance

IV Benign or likely benign variant

Tier IID Variants

ESR1	*FOXP1*	*TBX3*
p.E247*	p.?	p.P134Hfs*4
NM_001122742.1	NM_032682.5	NM_016569.3
c.739G>T	c.975-1G>A	c.399_402delTCCT

Tier III - Variants of Uncertain Significance

FGF19	*GPR124*	*GTF2E2, NRG1*	*LRP1B*	*LRP1B*	*MLLT3*
p.G181S NM_005117.2 c.541G>A	p.G391S NM_032777.9 c.1171G>A	*GTF2E2-NRG1* fusion transcript	p.I1546M NM_018557.2 c.4638C>G	p.W944R NM_018557.2 c.2830T>C	p.S312G NM_004529.2 c.934A>G

Low Coverage Regions

GENE	CODING BASES WITH NO/LOW COVERAGE	EXONS WITH LOW COVERAGE
PDPK1	59.71%	Exon1, Exon2, Exon3, Exon4, Exon5, Exon6, Exon8, Exon9, Exon10
FOXL2	51.28%	Exon1
BBC3	47.88%	Exon3, Exon2
GATA4	45.82%	Exon2
FGF4	45.38%	Exon2, Exon1
GATA6	39.07%	Exon2, Exon3
NKX2-1	36.12%	Exon2
IRS2	34.81%	Exon1
WT1	33.58%	Exon3, Exon1
SOX17	32.48%	Exon1, Exon2
MYOD1	31.08%	Exon1, Exon3
SOCS1	28.59%	Exon2
CEBPA	28%	Exon1

GENE	CODING BASES WITH NO/LOW COVERAGE	EXONS WITH LOW COVERAGE
FGF3	25.11%	Exon1
FOXO1	23.99%	Exon1
NKX3-1	23%	Exon1
GID4	22.55%	Exon1
ZBTB7A	21.82%	Exon3, Exon2
PIK3R2	20.2%	Exon2, Exon5, Exon6, Exon16
SDHD	19.74%	Exon4
CENPA	18.47%	Exon1
PGR	17.69%	Exon1
PNRC1	17.04%	Exon1
PHOX2B	16.28%	Exon3
FGF2	15.76%	Exon1
JUN	15.54%	Exon1
SH2B3	15.11%	Exon2
CDKN2B	14.76%	Exon1
FOXA1	14.28%	Exon2
STAT5B	13.81%	Exon8, Exon7, Exon6
STAT5A	13.57%	Exon7, Exon8, Exon9
ARID1A	12.83%	Exon1
DNAJB1	12.59%	Exon3
FGF8	12.59%	Exon3, Exon2, Exon1
MYCN	12.52%	Exon2
GNAS	11.8%	Exon1
TMEM127	11.8%	Exon2

GENE	CODING BASES WITH NO/LOW COVERAGE	EXONS WITH LOW COVERAGE
LATS2	11.65%	Exon8, Exon4
SMARCD1	11.1%	Exon1
PMAIP1	10.98%	Exon1
YAP1	10.58%	Exon1
SRC	10.57%	Exon4
ARID1B	10.37%	Exon1
FANCE	10.36%	Exon1
MYCL1	10.31%	Exon2, Exon1
SOX10	10.19%	Exon4, Exon2

Test Information

REPORTED GENES: A total of 523 genes were subjected to targeted next generation sequencing analysis. Details available upon request. **CGW VERSION:** CGW_v6.21 **DATABASE DETAILS:** The versions, releases, builds, dates of the following databases were used to generate this report: Genomic Build: GRCh37.p13 | Genomic Annotation Sources: NCBI RefSeq v105 | dbNSFP: 4.3c | gnomAD: r2.1 | ExAC: v1.0 | ClinVar: 20221001 | COSMIC: v96 | NHLBI ESP: v.0.0.30 | dbSNP: 149

METHODOLOGY
Precise Tumor is powered by Intermountain Precision Genomics, a service of Intermountain Healthcare, using the TheraMap assay. The TheraMap assay is performed at 600 South Medical Center Drive, St. George, UT 84790. Immunohistochemical staining for PD-L1, if ordered, is performed at Intermountain Central Laboratory, located at 5252 South Intermountain Drive, PO Box 57970, Murray, UT 84157.

TheraMap Characteristics and Performance: Performance Characteristics at ≥20% Tumor Nuclei are as follows: for Single Nucleotide Variants (SNV) the sensitivity (PPA) at ≥5% mutant allele frequency is 99.4% (95% CI: 98.7-99.7%), for Insertions/Deletions (indels smaller than 40bp) the sensitivity (PPA) at ≥5% mutant allele frequency is 97.2% (95% CI: 93.5-99.1%), for Copy Number Variant-Gains (CNV-Gains) the sensitivity (PPA) at ≥2.5 Fold Change of coverage is >99.9% (95% CI: 95.9-100%), for Fusions/Rearrangements (from RNA) the sensitivity (PPA) at ≥10 copies of mutant RNA transcript per ng input is >99.9% (95% CI: 95.56-100%), for MET exon 14 skipping variants from RNA the sensitivity (PPA) at ≥17 copies of mutant RNA transcript per ng input is >99.9% (CI: 64.7-99.0%), for microsatellite instability the sensitivity (PPA) at >10% unstable loci is >99.9% (CI: 72.25-100%). For all variants the positive predictive value (PPV) of the assay is 99.8%, the specificity (NPA) is >99.9%, and Negative Predictive Value (NPV) is >99.9%, the reproducibility is 99.0%, and the repeatability is 99.2%.

Sequencing Laboratory Methods: DNA and RNA are isolated from formalin-fixed, paraffin-embedded (FFPE) tissues. Sequencing libraries are generated using the Illumina TruSight Oncology 500 panel library preparation reagents. A panel of biotinylated single stranded probes are used to enrich the DNA derived libraries for all exons of 523 cancer-related genes and 130 microsatellite instability loci. A separate panel of biotinylated single stranded probes are used to enrich the RNA derived libraries for all exons of 56 cancer-related genes. The enriched libraries are sequenced on Illumina NextSeq sequencing platform. Given the overlap between the RNA and DNA capture, a total of 523 genes are interrogated across the RNA and DNA components of the test. This test targets coding regions and may not detect intronic variants, including those that may affect splicing. The genes analyzed for each variant type in the assay are listed on the Intermountain Precision Genomics website:

https://intermountainhealthcare.org/services/genomics/providers/theramap

Bioinformatics and Software Methods: DNA and RNA sequence data files are analyzed using our in-house TheraMap Oncology TSO500 pipeline v3.4, which includes the TruSight Oncology 500 v2.2 Local App and produces alignments and variant calls using GRCh37 as a reference. The software is optimized for the detection of single nucleotide variants, insertions and deletions (indels), CNV-Gains, gene fusion events, microsatellite instability, and tumor mutational burden.

The variant files are uploaded to PierianDx Clinical Genomics Workbench (CGW) for variant annotation, filtering, selection, therapy matching and clinical trial matching. Variants are reported according to HGVS nomenclature (www.hgvs.org/mutnomen) and classified as per the AMP classification system into tiers IA, IB, IIC, IID, III and IV. These tiers are stratified by clinical utility ('actionability&rsquo for clinical decision-making as to diagnosis, prognosis, treatment options, and carrier status) and previously reported data in the medical literature. Variations found in gnomAD (https://gnomad.broadinstitute.org/) that have ≥1% minor allele frequency (except those that are also in Clinvar denoted as clinically relevant, used in a clinical diagnostic assay, or reported as a mutation in a publication) are classified as known polymorphisms.

To generate the low coverage table, we calculate the percentage of the gene that is covered below 100X for the regions covered by the assay. A gene is listed as low coverage if 10% of its exon region is below 100x. Transcripts used to calculate no/low coverage regions are predefined in the TSO500 Local App v2. Exons from some transcripts included in the RefSeq annotation release v105 found in genes reported in certain gene subsets of this test are not targeted by the assay. The untargeted exons are disclaimed and identified as follows: HIST2H3A NM_001005464 exon 1, HIST2H3C NM_021059 exon 1, MYB NM_001130173 exon 1, PAX8 NM_003466 exon 8, PDPK1 NM_002613 exons 3-6, 8-10, RANBP2 NM_006267 exons 8 & 13, REL NM_002908 exon 9, RICTOR NM_152756 exon 19, SUZ12 NM_015355 exon 3. Additionally, all small variant calls in the HLA-A, KMT2B, KMT2C, and KMT2D genes are filtered out due to potential mismapping as a result of sequence homology with other genomic regions. Finally, the following low coverage regions occur in more than 80% of cases and are excluded from the low coverage table: CEBPA NM_004364 exon 1, WT1 NM_001198551 exon 1, SH2B3 NM_005475 exon 2, PDPK1 NM_001261816 exons 1-6, 8-10, CENPA NM_001042426 exon 1, SDHD NM_001276506 exon 4, FGF2 NM_002006 exon 1, BBC3 NM_001127240 exons 2-3, FOXO1 NM_002015 exon 1, FOXL2 NM_023067 exon 1, GATA6 NM_005257 exon 2, GATA4 NM_002052 exon 2, IRS2 NM_003749 exon 1, GID4 NM_024052 exon 1, PHOX2B NM_003924 exon 3, NKX2-1 NM_003317 exon 2, PNRC1 NM_006813 exon 1, NKX3-1 NM_006167 exon 1.

Tumor Mutational Burden (TMB) Method: Tumor mutational burden (TMB) estimates the number of somatic variants per megabase of sequenced DNA. Results are reported as low or high. A TMB value of 10 or greater is considered "High", while values below 10 are considered "Low". The cutoff of 10 mutations/megabase was established in NSCLC and may not be applicable in other tumor types at this time. Preliminary data supporting the pan-tumor FDA approval (PMID: 32919526A) has shown that a TMB score >10 mutations/megabase of DNA correlates with response to checkpoint inhibitor therapy. Intermountain Precision Genomics is a participant in the Friends of Cancer Research TMB Harmonization Project (PMID: 32217756).

Microsatellite Instability (MSI) Method: Microsatellite instability (MSI) is a hyper mutation phenotype caused by the loss of DNA mismatch repair activity. TheraMap evaluates 130 loci. The percentage of unstable microsatellites is determined by the number of unstable sites divided by the total number of usable sites. Samples with >10% unstable sites are considered "MSI-High". Samples with ≤ 10% unstable sites are considered "MSI-Stable". The assay does not distinguish between "MSI-Stable" and "MSI-Low" due to the limitations of tumor only MSI testing. MSI status may be reported as "Indeterminate" if the total number of usable sites is less than 60.

DISCLAIMER

Tumors, by nature, are genetically heterogeneous. Variants below the limit of detection, insertions/deletions >40 bp, and fusions that do not alter expressed messenger RNA may not be detected by this assay. A negative result does not exclude the presence of a variant beyond these detection limitations. The assay is not informative for mutations outside the 523 cancer-related genes or for those regions for which the assay achieves limited coverage.

This assay is not validated for large (> 40bp) indels, complex structural variants, or gene-level deletion events. Further, the assay is not validated to detect fusions that do not alter the expressed transcript (such as those that occur in the MYC gene). MSI analysis can identify MSI-High and MSI-Stable; however, MSI-Low may not be detected. The threshold for reporting amplifications in genes is 2.5 fold normalized increase in coverage.

This Report was generated using the materials and methods described above, which required the use of various reagents, protocols, instruments, software, databases, and other items, some of which were provided or made accessible by third parties. A defect or malfunction in any such reagents, protocols, instruments, software, databases, and/or other items may compromise the quality or accuracy of the Report.

The Report has been created based on, or incorporates references to, various scientific manuscripts, references, and other sources of information, including without limitation manuscripts, references, and other sources of information that were prepared by third parties that describe correlations between certain genetic mutations and particular diseases (and/or certain therapeutics that may be useful in ameliorating the effects

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The Report must always be interpreted and considered within the clinical context, and a physician should always consider the Report along with all other pertinent information and data that a physician would prudently consider prior to providing a diagnosis to a patient or developing and implementing a plan of care for a patient. The Report should never be considered or relied upon alone in making any diagnosis or prognosis. The manifestation of many diseases are caused by more than one gene variant, a single gene variant may be relevant to more than one disease, and certain relevant gene variants may not have been considered in the Report. In addition, many diseases are caused or influenced by modifier genes, epigenetic factors, environmental factors, and other variables that are not addressed by the Report (or that are otherwise unknown). This Report is based on a next generation sequencing assay which does not distinguish between somatic and germline variants. If a germline variant is in question, further testing may be recommended. As such, the relevance of the Report should be interpreted in the context of a patient's clinical manifestations. The Report provided by Intermountain Precision Genomics (IPG) is provided on an “AS IS” basis. IPG makes no representation or warranty of any kind, expressed or implied, regarding the Report. In no event shall IPG be liable for any actual damages, indirect damages, and/or special or consequential damages arising out of or in any way connected with the Report, your use of the Report, your reliance on the Report, or any defect or inaccurate information included within the Report.

This is a laboratory developed test, and its performance characteristics have been determined by Intermountain Precision Genomics. It has not been cleared or approved by the U.S. Food and Drug Administration. The U.S. Food and Drug Administration does not require this test to go through premarket 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 (1988) as qualified to perform high complexity testing.