REPORTING

Original Report Date:	FEB-24-2018
Addendum Date:	OCT-29-2018
Receipt Date:	FEB-17-2018
Collection Date:	FEB-15-2018
Specimen:	Blood
Status:	ADDENDUM

PHYSICIAN

SQAPortalPhysician O'SQAPortalPhysician
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Additional Recipient: N/A

Complete Tumor Response Map on page 2

Summary of Somatic Alterations & Associated Treatment Options

KEY Approved in indication | Approved in other indication | Lack of response

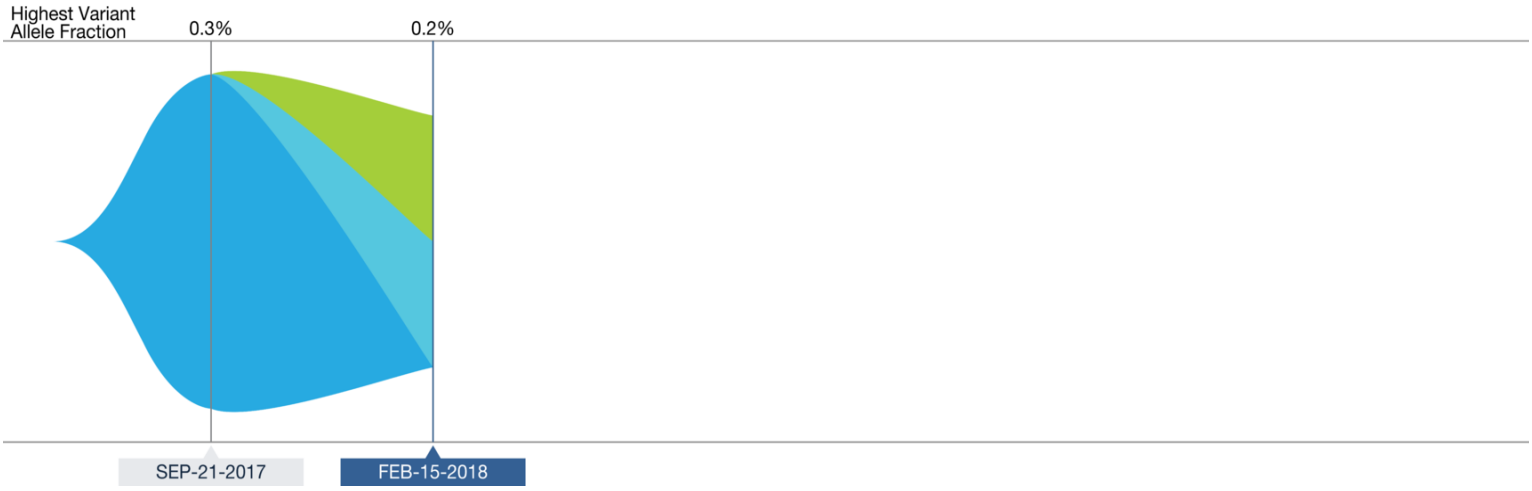
Alteration	% cfDNA or Amplification	Associated FDA-approved therapies	Clinical trial availability (see page 3)
TP53 R273C	0.2%	None	Yes

Variants of Uncertain Significance

NF1 S1834L (0.2%)
The functional consequences and clinical significance of alterations are unknown. Relevance of therapies targeting these alterations is uncertain.

Guardant360 Tumor Response Map

The Guardant360 Tumor Response Map illustrates the variant allele fraction (% cfDNA) of observed somatic variants at each sample submission time point. Amplifications are not plotted, and only the first and last five test dates are plotted. Please see the Physician Portal (**portal.guardanthealth.com**) for the Tumor Response Map with all test dates.



Alteration	% cfDNA or Amp	Alteration Trend	
TP53 R273C	0.2%	ND → 0.2%	
NF1 S1834L	0.2%	ND → 0.2%	Variant of Uncertain Significance §
IDH1 R132C	ND	0.3% → ND	

The table above annotates the variant allele fraction (% cfDNA) detected in this sample, listed in descending order.

§ See definitions section for more detail

Available Clinical Trials (within the same state as the ordering physician)

There may be additional trials not listed here. Visit: **portal.guardanthealth.com** or email **clientservices@guardanthealth.com** with A90029 in the subject line of the email, for additional trials.

Alteration	Trial ID / Contact	Title	Phase	Site(s)
TP53 R273C	Visit portal.guardanthealth.com for trials not within the same state as the physician's office			

More clinical trial options available at **portal.guardanthealth.com**

Definitions

Somatic Alterations Not Detected (ND): Somatic alterations may be present that are below the limit of detection of this test. Certain sample or variant characteristics may result in reduced analytic sensitivity. The absence of detectable somatic alterations in circulating cell-free DNA does not preclude the presence of somatic alterations in the tumor.

Variant of Uncertain Significance: The functional consequences and clinical significance of alterations are unknown. Relevance of therapies targeting these alterations is uncertain.

Comments

None

Interpretation

Somatic alterations were detected in the circulating cell-free DNA isolated from this patient's blood specimen. These genomic alterations are cancer-associated somatic variants, some of which have been associated with either increased or reduced clinical response to specific treatments. The percentage of altered cell-free DNA circulating (% cfDNA) in blood is related to the unique tumor biology of each patient. Factors that may affect the % cfDNA of detected somatic alterations include tumor growth, turn over, size, heterogeneity, vascularization, disease progression, and treatment.

Method and Limitations

Guardant360 sequences 73 cancer-associated genes to identify somatic alterations. Cell-free DNA (cfDNA) is extracted from plasma, enriched for targeted regions, and sequenced using the Illumina platform and hg19 as the reference genome. All exons are sequenced in some genes; only clinically significant exons are sequenced in other genes. The types of genomic alterations detected by Guardant360 include single nucleotide variants, gene amplifications, fusions, short insertions/deletions, and splice site-disrupting events (see Table 1). Microsatellite Instability (MSI) is assessed for all cancer types by evaluating somatic changes in the length of repetitive sequences on the Guardant360 panel. A “Not Detected” result does not preclude MSI-High status in tissue. MSI status is currently not reported for specimens originating from New York State or for earlier panel versions. This version of the Guardant360 test is not validated for the detection of other types of genomic alterations, such as complex rearrangements or gene deletions. Certain sample or variant characteristics, such as low cfDNA concentration, may result in reduced analytic sensitivity. Guardant360 cannot discern the source of circulating cfDNA, and for some variants in the range of ~40 to 60% cfDNA, the test cannot easily distinguish germline variants from somatic alterations. Guardant360 is not validated for the detection of germline or de novo variants that are associated with hereditary cancer risk. Tissue genotyping should be considered when plasma genotyping is negative, if clinically appropriate.

Table 1: Genes on the Guardant360 Panel

Guardant360 reports single nucleotide variants and splice site mutations in all clinically relevant exons in 73 genes and reports other variant types in select genes as indicated below.

AKT1	*ALK* #	*APC* Ω	*AR* †	*ARAF*	*ARID1A* Ω	*ATM* Ω	*BRAF* †	*BRCA1* Ω
BRCA2 Ω	*CCND1* †	*CCND2* †	*CCNE1* †	*CDH1* Ω	*CDK4* †	*CDK6* †	*CDKN2A* Ω	*CTNNB1*
DDR2	*EGFR* †Ω	*ERBB2* †Ω	*ESR1*	*EZH2*	*FBXW7*	*FGFR1* †	*FGFR2* †#	*FGFR3* #
GATA3 Ω	*GNA11*	*GNAQ*	*GNAS*	*HNF1A*	*HRAS*	*IDH1*	*IDH2*	*JAK2*
JAK3	*KIT* †Ω	*KRAS* †	*MAP2K1*	*MAP2K2*	*MAPK1*	*MAPK3*	*MET* †Ω	*MLH1* Ω
MPL	*MTOR* Ω	*MYC* †	*NF1* Ω	*NFE2L2*	*NOTCH1*	*NPM1*	*NRAS*	*NTRK1* #
NTRK3	*PDGFRA* †Ω	*PIK3CA* †	*PTEN* Ω	*PTPN11*	*RAF1* †	*RB1* Ω	*RET* #	*RHEB*
RHOA	*RIT1*	*ROS1* #	*SMAD4* Ω	*SMO*	*STK11* Ω	*TERT* ‡	*TP53* Ω	*TSC1* Ω
VHL Ω								

Ω Guardant360 reports insertion and deletion variants (indels) in this gene.
Guardant360 reports fusion events involving this gene for all known gene partners.
‡ Guardant360 reports alterations in the promoter region of this gene.
† Guardant360 reports amplifications of this gene.

About the Test

The Guardant360 assay was developed and its performance characteristics were determined by Guardant Health, Inc. This test has not been cleared or approved by the U.S. Food and Drug Administration (FDA). The FDA has determined that such clearance or approval is not necessary. This test may be used for clinical purposes and should not be regarded as investigational or for research only. Guardant Health's clinical reference laboratory is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) as qualified to perform high complexity clinical laboratory testing. The laboratory report should be interpreted in the context of other clinical information and laboratory, pathology, and imaging studies by a qualified medical professional prior to initiating or changing a patient's treatment plan. The selection of any, all, or none of the drugs associated with potential clinical benefit (or potential lack of clinical benefit) is entirely at the discretion of the treating medical professional. Drug and trial information are based on the diagnosis written on the submitted test request form; this information is not based on any supplemental information provided by the requesting medical professional, including pathology reports or other molecular studies. Some drugs listed in this report may not be approved or cleared by the FDA for the indicated use. Guardant Health makes no endorsement, express or implied, of any product, physician, or procedure contained in this report. This report makes no promises or guarantees that a particular medication will affect (or not affect) the clinical outcome of any patient.

Testing performed at: Guardant Health

Laboratory Director: Arthur Baca, MD PhD | CLIA ID: 05D2070300 | CAP #: 8765297 | 505 Penobscot Drive, Redwood City, CA, 94063, United States

Additional information is available

Any therapeutic annotations are based on publicly available information. This information is described in the "Detailed Therapy Results" and "Relevance of Detected Alterations" sections.

Visit portal.guardanthealth.com or email clientservices@guardanthealth.com with A90029 in the subject line of the email for:

– Additional clinical trials
– Detailed Therapy Results
– Relevance of Detected Alterations
– References

If you would like to receive this additional information with every Guardant360 report, please call client services at 855.698.8887 to opt-in.

Additional information begins on the next page.

List of Available Clinical Trials

Alteration	Trial ID / Contact	Title	Phase	Site (number in parenthesis is count of trial sites)
TP53 R273C	NCT02503709	Onalespib and CDKI AT7519 in Treating Patients With Solid Tumors That Are Metastatic or Cannot Be Removed by Surgery	Phase 1	Columbus, OH; Bethesda, MD (2); Boston, MA (2)
	NCT02898207	Olaparib and Onalespib in Treating Patients With Solid Tumors That Are Metastatic or Cannot Be Removed by Surgery or Recurrent Ovarian, Fallopian Tube, Primary Peritoneal, or Triple-Negative Breast Cancer	Phase 1	Rochester, MN; Scottsdale, AZ; Jacksonville, FL; Boston, MA (4)

Detailed Therapy Results

Alteration	Drug	Trade Name	Target	Current Status
TP53 R273C	AT13387		Small molecule inhibitor of Hsp90.	Phase 1 (Solid Tumor) Phase 2 (GIST (Gastrointestinal stromal tumor), Lymphoma, Non-small cell lung carcinoma (NSCLC), Prostate carcinoma, Lung cancer, Diffuse large B-cell lymphoma (DLBCL))
	Ganetespib		Small molecule inhibitor of Hsp90, also may inhibit Kit/Egfr/Bcr-Abl.	Phase 2 (Colorectal carcinoma (CRC)) Phase 3 (Non-small cell lung carcinoma (NSCLC), Acute myelocytic leukemia (AML), Lung cancer, Myelodysplastic Syndrome (MDS))
	Luminespib		Hsp90 inhibitor.	Phase 1 (Colorectal carcinoma (CRC)) Phase 2 (GIST (Gastrointestinal stromal tumor), Pancreatic carcinoma, Non-small cell lung carcinoma (NSCLC), Breast carcinoma)
	PU-H71		Hsp90 inhibitor.	Phase 1 (Colorectal carcinoma (CRC)) Phase 1 (Lymphoma, Solid Tumor, Non-Hodgkin lymphoma (NHL))
	SNX-5422		Hsp90 inhibitor.	Phase 2 (Solid Tumor) Phase 1 (Neuroendocrine carcinoma, Lymphoma, Non-small cell lung carcinoma (NSCLC), Hematologic malignancies, Chronic lymphocytic leukemia (CLL), Lung cancer)

Relevance of Detected Alterations

Alteration	Role in Disease	Effect on Drug Sensitivity	Effect on Drug Resistance
TP53 R273C	Loss of tumor suppressor p53, which is encoded by the TP53 gene, is common in aggressive advanced cancers (1). 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 (2-4). Expression of p53 in normal cells is low; however, TP53 alterations, including those that result in loss of p53 tumor 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 (5-9). TP53 mutations have been associated with distal tumor location, microsatellite stability (MSS), and high levels of chromosomal instability in studies of colorectal carcinoma (CRC) (10-14). Additionally, p53 expression in CRC has been positively correlated with distal tumor location, advanced TNM stage, and high Ki67 expression (15-20).	Some studies suggest that Hsp90 inhibitors may be effective in tumors with oncogenic TP53 alterations (21-23).	Mutations in TP53 may increase resistance to ionizing radiation therapy (24,25).
NF1 S1834L	Neurofibromin acts as a tumor suppressor by depressing Ras signaling, and NF1 mutations have been identified across the entire gene (26). Tumors with mutations in NF1 have therefore been predicted to have activation of Ras and downstream pathways, including the Ras-MAPK pathway and the mTOR pathway (27). Germline mutations of NF1, which are associated with neurofibromatosis type 1, have been found to be associated with increased frequency of several tumor types, including sarcoma, glioma, breast carcinoma, gastric cancer, neuroendocrine tumors, and hematological neoplasms (28-32). NF1 alterations have been reported to be associated with microsatellite instability resulting from mismatch repair (MMR) defects, which are often associated with hereditary nonpolyposis colon cancer (HNPCC); these data suggest that NF1 may be a mutational target in MMR deficient cells (33-35).	Loss of neurofibromin function may result in increased signaling through the Ras pathway and downstream MAPK and mTOR pathways (36). Tumors bearing NF1 mutations may therefore be sensitive to mTOR and MAPK inhibitors. The mTOR inhibitors everolimus and temsirolimus have been approved by the FDA for some indications and are currently in clinical trials for solid tumors (37,38). Several inhibitors designed to target both the mTORC1/Raptor and mTORC2/Rictor complexes are being tested in early phase clinical trials for advanced solid tumors (39). The MEK inhibitors trametinib and cobimetinib (in combination with vemurafenib) have been FDA-approved for BRAF V600E- and V600K-mutant melanoma and are under investigation in clinical trials (40,41). In the case of this uncharacterized variant, the relevance of any available therapeutic approaches is unknown.	

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