Canexia Health™

Find It® Report

Patient Name: **Date of Birth:** **Patient ID:** CG05006

Report ID: 5477 **Surgical ID:** 20-10689 A8 **Panel:** Full

PATIENT INFORMATION

Patient Name:
Date of Birth: **Sex:** Unknown
Care Card #: Province of issue: Diagnosis:
Colorectal cancer
Reason for Referral: Other
Previous Molecular Tests: MLH1 absent
Test Requested: Find It®
Date of Receipt: 2021-02-01
Date of Report: 2021-06-09 08:13:21 PDT

HEALTHCARE PROVIDER INFORMATION

Referring Physician:
Institution:
Address:
Phone: **Fax:**
Pathologist:
Institution:
Address:
Phone: **Fax:**
cc: **Fax:**

SPECIMEN INFORMATION

Specimen Collection Date:
Specimen Source:
Specimen Type: FFPE
Primary Site of Tumour: Colon **Histologic Type:** Adenocarcinoma **Surgical ID #:** 20-10689 A8

Tumour Surface Area on Slide:
Tumour Distribution Pattern:
Tumour Cellularity (%):
Tumour Necrosis (%):
Sample: DNA-27662-CG001Qv51Next049-16

SUMMARY OF TEST RESULTS

Key Mutations Present*							
Gene	**cDNA Change**	**Amino Acid Change**	**Exon**	**VAF%**	**Therapeutic Implication**	**Level of Evidence**	**Trials Available**
EGFR	c.1787C>T NM_005228.3	p.(P596L)	15	50.1	May be sensitive to EGFR TK inhibitors	Tier: II.D ●Literature	0
PIK3CA	c.1633G>A NM_006218.3	p.(E545K)	10	7.0	May be responsive to PI3K/AKT/mTOR inhibitors	Tier: II.D ●Literature	1

TABLE 1: Mutations present including SNV's and Indel Mutations

Key Mutations Absent*

TABLE 2: Key Mutations Absent

* All hotspot mutations detected in this sample are shown in Table 1, above. All hotspot mutations tested are listed in the "Hotspot Panel" Table (below, after Methodology). Mutations listed in the "Hotspot Panel" but not presented in Table1 were tested, but were not detected in this sample.

Gene Amplification
No evidence of CNV for genes CCNE1, EGFR, ERBB2, FGFR1, FGFR2, KIT, KRAS, MET and PIK3CA.

TABLE 3: Amplification

Microsatellite Status		
Status	**Score**	**Therapeutic Implication**
MSI-H	0.8711	May be sensitive to immune modulation-checkpoint inhibitor treatment.

TABLE 4: Microsatellite Section

RECOMMENDATIONS

For Microsatellite Instability (MSI) positive results, confirmatory testing using a validated orthogonal method such as IHC or mismatch repair gene sequencing is recommended along with consideration of referral to a cancer genetics clinic if a germline mutation is suspected. The presence of a BRAF mutation makes it less likely that MSI is due to a germline mutation/Lynch syndrome.

INTERPRETATION

Mutations - SNV - Indel
PRESENCE of a PIK3CA mutation

An activating PIK3CA mutation, as described in the table above, was detected in the DNA of this sample.

PIK3CA, the catalytic subunit of PI3-kinase, is frequently mutated in a diverse range of cancers including breast, endometrial and cervical cancers. Specifcally, the PIK3CA E545K mutation is known to be oncogenic. PIK3CA E545K is present in 2.61% of AACR GENIE cases, with breast invasive ductal carcinoma, colon adenocarcinoma, lung adenocarcinoma, bladder urothelial carcinoma, and breast invasive lobular carcinoma having the greatest prevalence (AACR Project GENIE, 2017).

PIK3CA mutations occur in10-20% of colorectal cancer (Hamada T, 2017),

While alpelisib in combination with fulvestrant is FDA-approved for the treatment of patients with PIK3CA-mutant ER+/HER2- breast cancer, its clinical utility in patients with PIK3CA E545K mutant colorectal cancer is unknown (Chakravarty D, 2017),

PRESENCE of an EGFR mutation

An activating EGFR, as described in the table above, was detected in the DNA of this sample.

EGFR is altered in 6.83% of all cancers with lung adenocarcinoma, conventional glioblastoma multiforme, glioblastoma, breast invasive ductal carcinoma, and colon adenocarcinoma having the greatest prevalence of alterations. EGFR is altered in 3.22% of colorectal carcinoma patients. EGFR P596L is present in 0.02% of AACR GENIE cases, with glioblastoma, anaplastic astrocytoma, conventional glioblastoma multiforme, and skin squamous cell carcinoma having the greatest prevalence (AACR Project GENIE, 2017).

EGFR P596L lies within the extracellular domain of the EGFR protein and results in increased transformation ability in two different cell lines in one study (Ng PK, 2018). This mutation is predicted to lead to a gain of EGFR protein function.

While the EGFR tyrosine kinase inhibitors (TKIs) erlotinib, afatinib and gefitinib are FDA-approved for the treatment of patients with non-small cell lung cancer, their clinical utility in patients with colorectal cancer harboring the EGFR P596L mutation is unknown (Chakravarty D, 2017),

Copy Number Alterations
No evidence of CNV for genes CCNE1, EGFR, ERBB2, FGFR1, FGFR2, KIT, KRAS, MET and PIK3CA.

Microsatellite Status
This sample has been tested as MSI-High (strong evidence). In tissue samples, this finding is indicative of sensitivity to immune modulation-checkpoint inhibitor treatment. MSI testing by any method is not fully concordant with mismatch repair deficiency testing. Further testing may be required (see below). For Microsatellite Instability (MSI) positive results, confirmatory testing using a validated orthogonal method such as IHC or mismatch repair gene sequencing is recommended along with consideration of referral to a cancer genetics clinic if a germline mutation is suspected. The presence of a BRAF p.V600E mutation makes it less likely that MSI is due to a germline mutation/Lynch syndrome and more likely due to somatic MLH1 promoter methylation.

OTHER FINDINGS

Additional targeted treatment options, if available, are listed in the clinical trial section. It is the responsibility of the oncology team to select the most suitable clinical trial for the patient. Additional tumour testing may be needed to determine patient's eligibility for a particular trial.

The therapeutic implication of these results should be considered in conjunction with other clinical information and/or tests. A list of benign changes, if identified in the tumour DNA of this patient, is available upon request. The ability to detect a particular variant in a given specimen will depend upon the allele proportion of the variant in the extracted DNA combined with the lower limit of detection of the assay.

CLINICAL TRIALS

Study	Genes	Phase	Countries	Title
NCT03006172	PIK3CA	Phase 1	Canada	A Phase I, Open-Label, Dose-Escalation Study Evaluating the Safety, Tolerability, and Pharmacokinetics of GDC-0077 as a Single Agent in Patients With Locally Advanced or Metastatic PIK3CA-Mutant Solid Tumors and in Combination With Endocrine and Targeted Therapies in Patients With Locally Advanced or Metastatic PIK3CA-Mutant Hormone-Receptor Positive Breast Cancer

TABLE 5: The clinical trials included in the report are sourced from Canadian trials listed on clinicaltrials.gov. We select trials based on tumour histotype and mutation status, with a specific focus on trials of targeted therapy. The inclusion of a trial in our report does not necessarily mean that the patient would be eligible. Patients' eligibility for a trial, and the benefit that they may derive from it, will depend on additional factors that must be assessed by the oncologist. Conversely, the list of potentially relevant trials in our report may not be complete. We may have overlooked relevant trials on these websites, or there may be relevant trials listed elsewhere. Please let us know if you identify a trial of targeted therapy that could have been included in a patient's report.

METHODOLOGY

This test includes targeted sequence analysis of hotspot mutations/coding exons of the requested genes and transcripts (listed below). DNA is extracted and targets of interest amplified using a highly multiplexed in-house designed PCR assay. The targeted regions are sequenced using Illumina technology with 151bp paired-end reads. Sequence reads that pass defined quality threshold metrics are aligned to the reference sequence (Genome Build hg19) and variants are identified and annotated using a validated, custom-built bioinformatics pipeline. Standard acceptance criteria for reporting of analytical runs are a minimum read depth of ≥5000x (NextSeq), a base quality score of ≥30, and a mapping quality score of ≥30. For single nucleotide changes, the acceptance criteria include a variant allele fraction (VAF) of ≥1% and a probability score of ≥0.70. The VAF is defined as the proportion of alleles with a mutation to the total number of alleles present in a sample and is expressed as a percentage. The probability score is the likelihood that a detected mutation is a true positive. For indels, the acceptance criteria include a VAF of ≥1% and a penalty score ≤0.45. CNVs with z-score>1.5 will be reported with 2.4<CN<3.5 indicating low evidence amplifications and CN≥3.5 indicating high evidence. MSI status is reported based on a probability score (i.e. probability of the sample being MSI-H) for samples that meet the minimum coverage requirement for microsatellite amplicons. Samples that do not meet the coverage criterion are not reportable for MSI. The reportable MSI statuses are: "MSI-H"(score>0.6); "Possible evidence of MSI" (0.4<score≤0.6); and "MS-Stable" (score≤0.4).
Hotspot variants are categorized into clinical significance tiers as per Li et al, J Mol Diagn 2017, 19(1):4-23. Variants of strong or potential clinical significance (tier I or II) will be reported. VUS and likely benign variants (tier III and IV) will not be reported. Please contact the laboratory if tier III or IV variants are required.

Result	Gene	Hotspot	Transcript	Result	Gene	Hotspot	Transcript
Neg	AKT1	E17	NM_001014432.1	Neg	IDH2	R140, R172	NM_002168.3
Neg	ALK	T1151, L1152, C1156, F1174, L1196, L1198, G1202, D1203, S1206, G1269, R1275, Y1278	NM_004304.4	Neg	KIT	S476, K550-V555, Y553, W557, V559, V560, L576, K642, V654, T670, D816, D820, N822, Y823, A829, Exons 9, 11, 13	NM_000222.2
Neg	AR	L702, V716, S741, W742, Q784, H875, F877, T878, M896	NM_000044.3	Neg	KRAS	K5, A11, G12, G13, L19, Q22, A59, G60, Q61, K117, A146	NM_004985.4
Neg	BRAF	Q201, G464, G466, F468, G469, Y472, D594, F595, G596, L597, V600, K601, K601-M620, G606	NM_004333.4	Neg	MAP2K1	F53, Q56, K57, K59, V60, D67, I103, I111, C121, N122, P124, P387	NM_002755.3
Neg	CTNNB1	D32, S33, G34, I35, H36, S37, T41, S45	NM_001904.3	Neg	MAP2K2	F57, Q60, K61, L119, H123, G132	NM_030662.3
Neg	DDR2	L239, I638, S768	NM_001014796.1	Neg	MET	T1010, V1110, H1112, V1206, L1213, D1246, Y1248, Y1253, Exons 13, 14, 18	NM_001127500.2
Neg	DICER1	D1705, D1709, G1809, D1810, E1813	NM_177438.2	Neg	NRAS	G12, G13, A59, G60, Q61, K117, A146	NM_002524.4
Pos	EGFR	R108, A289, S492, P596, G598, E709, L718, G719, L747, A750, K754, S768, T790, L792, G796, C797, L798, L833, L838, L858, L861, Exons 18-21	NM_005228.3	Neg	NTRK1	F589, G595, G667	NM_002529.3
Neg	ERBB2	G309, S310, K753, L755, I767, D769, V773, G776, V777, Exons 20	NM_004448.3	Neg	NTRK3	G623, G696	NM_001012338.2
Neg	ESR1	K303, E380, S463, V534, P535, L536, Y537, D538	NM_001122742.1	Neg	PDGFRA	R560-E571, V561, P577, N659, L839-Y849, D842, H845, D846	NM_006206.4
Neg	FGFR1	N546, K656	NM_023110.2	**Pos**	PIK3CA	R88, C90, R93, P104, G106, N107, R108, K111, R115, N345, I354-P377, R357, G364, E365, C420, E453, P539, E542, E545, Q546, D549, E970, E978, M1043, N1044, A1046, H1047, G1049	NM_006218.3
Neg	FGFR2	S252, P253, W290, A315, S372, Y375, C382, N549, K659, E731, E777	NM_000141.4	Neg	POLE	P286, M295, S297, F367, D368, V411, L424, M444, A456, S459	NM_006231.3
Neg	FGFR3	R248, S249, G370, S371, Y373, G380, A391, K650	NM_000142.4	Neg	PTCH1	W844, G1093	NM_000264.3
Neg	FOXL2	C134	NM_023067.3	Neg	PTEN	A126, G129, R130, R173, R233, K254-K267	NM_000314.6
Neg	GNA11	Q209	NM_002067.4	Neg	RET	G533, K603, C609, C611, C618, C620, C630, D631, C634, G691, E768, L790, Y791, V804, Y806, A883, R886, S891, S904, M918, A919, Exons 10, 13, 15	NM_020975.4
Neg	GNAQ	Q209	NM_002072.4	Neg	ROS1	S1986, L2026, G2032	NM_002944.2
Neg	GNAS	R201	NM_000516.5	Neg	STK11	Q37, P281	NM_000455.4
Neg	HRAS	G12, G13, Q61	NM_005343.3	Neg	TP53	P72fs, L111fs, C135, P151, R158, A159, Y163, R175, L194, I195, S215, Y220, Y234, C238, S241, R249, D259, R273, P278, R280, Exons 2-11	NM_000546.5
Neg	IDH1	R132	NM_005896.3				

TABLE 6: Hotspot Panel: CG001v5.1_Hotspot_Manifest_Panel5.1.3_20210428.tsv. Neg=Negative, Pos=Positive

QUALITY METRICS

The figure below displays the correlation between the expected variant allelic fraction (VAF) and the observed VAF for the quality control sample.

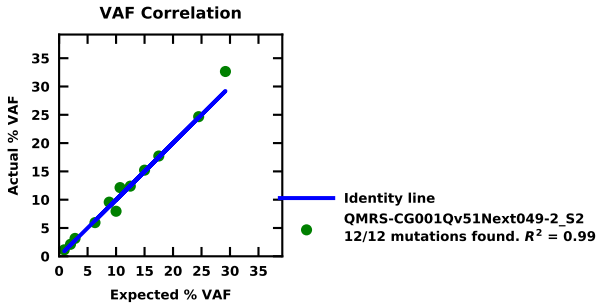

Figure 1: Correlation between the expected and observed variant allelic fraction (VAF) for the quality control sample for this run.

The table below summarizes the average amplicon coverage for this patient.

Gene	Hotspots	Average Run Depth	Average Sample Depth	Coverage Depth
KRAS	K5 A11 G12 G13 L19 Q22	7984.3	6389.81	5000 - 10000
FGFR3	R248 S249	8362.03	9280.14	5000 - 10000
KIT	MSI:BAT-25	7597.46	8768.7	5000 - 10000
PIK3CA	P539 E542 E545 Q546 D549	13263.12	9245.89	5000 - 10000
PIK3CA	R88 C90 R93	11033.82	8639.05	5000 - 10000
EGFR	S492	10160.37	8400.34	5000 - 10000
The other 168 amplicons				≥ 10000

TABLE 7: Amplicon Coverage

LIMITATIONS

Single nucleotide changes, insertions and deletions at the targeted hotspots and exons will be reported. Gene amplifications will be reported for select genes only (CCNE1, EGFR, ERBB2, FGFR1, FGFR2, KIT, KRAS, MET, PIK3CA). Sample MSI status will also be reported. Only single nucleotide variants with ≥1% allelic ratio and insertion and deletion ≥1% allelic ratio will be reported. Rare genetic variation can interfere with this assay. The ability to detect a particular variant in a given specimen will depend upon the allele proportion of the variant in the extracted DNA combined with the lower limit of detection of the assay. This assay does not differentiate between germline and somatic mutations and does not detect gene deletions.

This assay does not assess for mutations in BRCA or other genes associated with homologous recombination and DNA damage repair which could be associated with response to PARP inhibitors.

SIGNATURES

Dr. Brenda Murphy, Ph.D, FCCMG Molecular Geneticist
2021-06-09

Copies sent to:

REFERENCES

- "AACR Project GENIE: Powering Precision Medicine through an International Consortium." 2017 08 . Cancer Discov - PMID28572459
- Chakravarty D, Gao J, Phillips SM, et al. "OncoKB: A Precision Oncology Knowledge Base." 2017 Jul . JCO Precis Oncol - PMID28890946
- Hamada T, Nowak JA, Ogino S "PIK3CA mutation and colorectal cancer precision medicine." 2017 04 04. Oncotarget - PMID28423591
- Ng PK, Li J, Jeong KJ, et al. "Systematic Functional Annotation of Somatic Mutations in Cancer." 2018 03 12. Cancer Cell - PMID29533785