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Avenio® Pan-Cancer CGP Panel Matched to FoundationONE® CDx
Variant Report

ORDER DETAILS

PATIENT NAME	TUMOR CELLULARITY
MEDICAL RECORD#	DATE COLLECTED
DATE OF BIRTH	DATE RECIEVED
GENDER	ORDERING PHYSICIAN
DIAGNOSIS	ORGANIZATION
SPECIMEN TYPE	REPORT DATE
SPECIMEN ID	REPORT STATUS

PROFILE SUMMARY†

GENOMIC ALTERATIONS	Rearrangements	Other Alterations
Small Variants **EGFR** p.E746_A750del **KRAS** p.G12C ***Gene amplifications*** **MYC** – Conclusive	***Fusions*** **BCR-ABL1**	***Microsatellite Instability*** 3.3% unstable sites Microsatellite Stable ***Tumor Mutation Burden*** TMB-High; 25 mutations per Mb ***Loss of Heterozygosity (LOH)*** N/A

† See methods for details

GENOMIC ALTERATION SUMMARY

Variants and Biomarkers

VARIANT	TIER	VAF%	APPROVED/RECOMMENDED THERAPY‡
EGFR p.E746_A750del Inframe Indel	I-A	**12**	**In current diagnosis** afatinib[a], bevacizumab + erlotinib, dacomitinib, erlotinib[a], erlotinib + ramucirumab, gefitinib[a], Osimertinib **In other indications** None
KRAS p.G12C Missense	I-A	**12**	**In current diagnosis** sotorasib, afatinib[r], erlotinib[r], gefitinib[r] **In other indications** None
TMB High	I-B	**N/A**	None
MYC Amplification – Conclusive	II-C	**N/A**	None
BCR-ABL1 Fusion	II-C	**N/A**	**In current diagnosis** None **In other indications** asciminib, bosutinib, dasatinib, imatinib, nilotinib, ponatinib

‡ Clinical impact of detected genomic alterations is in response and/or no response to certain FDA-approved drugs. The agents listed in this report may however have little or no evidence in the patient's tumor type, nor are they identified and listed in order of level of evidence for this patient's tumor type or potential or predicted efficacy for this patient.

[a] = therapy with altered response, [r] = therapy resistant to

GENOMIC ALTERATION COMBINATION SUMMARY

Variants and Biomarkers in Combination

COMBINATION	TIER	APPROVED/RECOMMENDED THERAPY§
EGFR p.E746_A750del Inframe Indel	I-B	**In current diagnosis** afatinib[r], erlotinib[r], gefitinib[r]
KRAS p.G12C Missense		**In other indications** None

EGFR exon 19 deletions are in-frame deletions or indels that result in increased EGFR kinase activity and induce oncogenic transformation of cells (PMID: 16912195, PMID: 17495523). KRAS codon 12 mutation indicates any amino acid change at codon 12 of the KRAS protein. KRAS codon 12 variants are hotspot mutations that often interfere with RAS GTPase activity, resulting in downstream pathway activation (PMID: 28666118, PMID: 22589270). For certain patients with non-small cell lung cancer (NSCLC), KRAS mutations are associated with reduced responsiveness to EGFR tyrosine kinase inhibitor (TKI) therapy (NCCN)

§ Clinical impact of detected genomic alterations is in response and/or no response to certain FDA-approved drugs. The agents listed in this report may however have little or no evidence in the patient's tumor type, nor are they identified and listed in order of level of evidence for this patient's tumor type or potential or predicted efficacy for this patient.

[a] = therapy with altered response, [r] = therapy resistant to

CLINICALLY RELEVANT RESULTS[ϕ]

Tier 1: Variants of Strong Clinical Significance

VARIANT	INTERPRETATION
EGFR p.E746_A750del Inframe Indel I-A	EGFR-E746_A750del is an activating mutation. The presence of an EGFR abnormality (mutation, amplification, or overexpression) can result in an overabundance or overactivity of Egfr protein, which can lead to excessive proliferation [PMID: 18337605]. EGFR activating mutations or amplification may predict sensitivity to Egfr-targeted therapies, including inhibitors of multiple ErbB family members, and several have received agency approval in some tumor types [PMID: 19692680, 19692684, 16014883]. EGFR mutations in non-small cell lung carcinoma (NSCLC) have been found to be more common in women, never-smokers, those of East Asian ethnicity, and in patients with adenocarcinoma histology [PMID: 15741570, 15118073, 23558737]. Studies have reported non-squamous NSCLC patients with metastatic disease and tumors harboring an EGFR exon 19 deletion or L858R mutation to be sensitive to 4simertinib, erlotinib, afatinib, gefitinib, dacomitinib, and the combination of erlotinib plus ramucirumab [PMID: 25589191, 22285168, 28958502]. Several studies have reported that resistance to Egfr TKIs in non-small cell lung cancer (NSCLC) is mediated by the transformation of NSCLC cell types to those of SCLC with neuroendocrine features; these transformed SCLC cases have been shown to be responsive to standard SCLC therapy regimens involving platinum and etoposide–based chemotherapy [PMID: 24101933, 21430269, 25908039]. EGFR mutations have been reported in 14-57% of NSCLC cases and are found more commonly in East Asian patients as compared with other ethnicities [PMID: 29543321, 29981927, 31208370]. In addition, EGFR mutations have been reported in 2-9% of small cell lung carcinoma (SCLC) samples [PMID: 29720878, 22103903, 21178714].
KRAS p.G12C Missense I-A	KRAS, a proto-oncogene, is a G-protein with intrinsic GTPase activity and activating mutations in this gene result in unregulated signaling through the MAP/ERK pathway (NCCN, NSCLC v2.2018). A missense alteration in KRAS, G12C, is identified. Mutations in KRAS at codon 12 (within the GTP binding region) result in reduced GTPase activity, which in turn leads to constitutive activation of KRAS and its downstream PI3K-AKT and MAPK signaling pathways (PMID: 26902995; 25705018; 26673580). Approximately 25% of patients with lung adenocarcinomas in a North American population have KRAS mutations (NCCN, NSCLC v2.2018). In NSCLC, the presence of a KRAS mutation is prognostic of poor survival when compared to patients with tumors without KRAS mutation. Mutations in KRAS have been associated with reduced responsiveness to EGFR TKI therapy; it does not appear to affect chemotherapeutic efficacy (NCCN, NSCLC v2.2018). In a study of lung adenocarcinomas, KRAS mutations were independent predictors of pleural invasion (PMID: 24694341). Another study reviewing data from 481 NSCLC patients who underwent thoracic surgery reported that patients with KRAS G12C developed significantly more bone metastases (PMID: 27336603). 83 patients from a phase II trial (docetaxel + placebo or MEK1/2 inhibitor selumetinib) with KRAS mutant tumors were retrospectively assessed for differences in OS, PFS, ORR and change in tumor size at week 6 according to type of KRAS mutation. G12C, G12D and G12V were the most common mutations (46%, 22%, 11%, respectively). Patients with G12C or G12V mutations had a trend towards longer OS, PFS and ORR compared to other KRAS mutations. Changes in tumor size were similar between groups with the best responses in tumors with G12V mutations. Few differences were observed between groups when treated with docetaxel + placebo (NCT00890825; PMID: 26125448)

[ϕ] The variant interpretation and classification system is based on the joint consensus recommendations of the AMP, ASCO and the CAP (J Mol Diagn 2017, 19:4-23). Clinical data aggregation and evidence mining is based on Navify Mutation Profiler's Clinical Decision Support Application , which is clinically validated in the lab. See methods for more details

TMB High I-B	TMB high indicates a high tumor mutational burden. TMB is generally defined as the number of somatic mutations per megabase of genome examined. High TMB may result from increased exposure to mutagens as well as mutations in genes such as those involved in DNA damage repair. TMB high tumors are more likely to express neoantigens that can be targeted by activated immune cells (PMID: 30792906). The cut-offs for TMB high differ across tumor types and studies. TMB high tumors have been associated with prognosis and therapeutic response in some tumor types (PMID: 30395155, PMID: 30505715). Pembrolizumab (FDA, TGA, TFDA) is approved for certain patients with solid tumors harboring high tumor mutational burden (TMB). Nivolumab in combination with ipilimumab (ESMO) is recommended for certain patients with non-small cell lung cancer (NSCLC) harboring high TMB.

Tier 2: Variants of Potential Clinical Significance

MYC Amplification II-C	MYC-amplification is an activating alteration. MYC encodes the protein Myc (also known as c-Myc), a transcription factor that regulates many genes related to cell-cycle regulation and cell growth. It is an oncogene and may be activated in 20% or more of human cancers [PMID: 16904903]. MYC copy number increase or amplification has been correlated with increased Myc expression in several tumor types [51]. Putative high-level amplification of MYC has been reported in 5.4-8.7% of Non-small cell lung carcinoma (NSCLC) cases (cBioPortal for Cancer Genomics, Jan 2019). MYC is located on chromosome 8q24, and MYC deregulation (amplification, overexpression, translocation) has been identified in several different cancer types, including breast, prostate, colorectal, and lymphoma [PMID:10378696]. Preclinical studies have suggested several synthetic lethal strategies to indirectly target Myc, including synthetic lethal interactions between Myc overexpression and inhibition of Cdk1, Cdk2, or Aurora kinases. Cdk inhibitors and Aurora kinase inhibitors are under investigation in clinical trials [PMID: 22430491]. Increased Myc expression has been reported in SCLC cell lines with acquired resistance to everolimus, as compared with control cell lines; reduction of Myc expression restored everolimus sensitivity [PMID: 25884680].
BCR-ABL1 Fusion II-C	ABL1 alterations are found in cancer, including hematologic cancers, melanoma, cervical cancer, and colorectal cancer (COSMIC). The most commonly reported ABL1 alteration in cancer is fusion with the breakpoint cluster region (BCR) gene (referred to as the Philadelphia chromosome) and is the hallmark of chronic myeloid leukemia (CML), which results in constitutive ABL1 kinase activity (PMID: 23842646). Therapies targeting ABL1 are approved or recommended or are under investigation in ABL1-altered cancers (PMID: 34638304, PMID: 34922630, PMID: 34959482). Alterations in BCR including translocations, indels and missense mutations are found in hematopoietic and lymphoid cancers, as well as in intestinal and skin cancers (COSMIC) (PMID: 28572459). Patients with solid tumors harboring activating ABL1 alterations match inclusion criteria for clinical trials, such as trials with tyrosine kinase inhibitors. No therapies are approved or recommended for solid tumors based on ABL1 fusion status

CLINICAL TRIALS#

TITLE	TRIAL IDENTIFIER	PHASE	VARIANT
A Study to Evaluate the Efficacy and Safety of Multiple Targeted Therapies as Treatments for Participants With Non-Small Cell Lung Cancer (NSCLC)	NCT03178552	II	KRAS p.G12C
Phase 2 Trial of MRTX849 Monotherapy and in Combination With Pembrolizumab and a Phase 3 Trial of Adagrasib in Combination in Patients KRAS G12C Mutation KRYSTAL-7	NCT04613596	II	**KRAS** p.G12C
A Study of Amivantamab and Lazertinib in Combination With Platinum-Based Chemotherapy Compared With Platinum-Based Chemotherapy in Patients With Epidermal Growth Factor Receptor (EGFR)-Mutated Locally Advanced or Metastatic Non- Small Cell Lung Cancer After Osimertinib Failure (MARIPOSA-2)	NCT04988295	III	**EGFR** Exon 19 Deletion

VARIANTS OF UNCERTAIN CLINICAL SIGNIFICANCE

*A research use only list of variants of uncertain significance (single nucleotide variants and indels) can be provided upon request.

#The information presented here is not meant to be a complete list of available clinical trials pertinent to detected genomic alterations. The accuracy of information is as of currently available databases in the public domain, which is continually updated, and further mining is strongly recommended. The order of listed information does not in any way relate to the applicability of a trial to the patient's tumor, or strength of its relevance to the genomic alteration in this tumor. More information about a specific clinical trial can be obtained using the trial identifier on www.clinicaltrials.gov.

REVIEW AND APPROVAL

Reviewed, verified and electronically signed:

Dr. Meer-Taher Shabani-Rad, MD, FRCPC, DABP

Dr. Faisal M Khan, PhD, D(ABHI)

VARIANTS 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.

I	II	III	IV
Variants of strong clinical significance	**Variants of potential clinical significance**	**Variants of uncertain clinical significance**	**Likely benign or benign variants**
I A FDA approved therapy Included in professional guidelines **I B** Well-powered studies with expert consensus	**II C** FDA approved therapies for different tumor types. Multiple published studies with some consensus **IID** Preclinical trials or a few case reports without consensus	Variants in cancer genes with unknown clinical significance No convincing published evidence of cancer association	Likelihood of rare germline variants No existing published evidence of cancer association

METHODOLOGY

Experimental Methodology

This test uses targeted next-generation sequencing to analyze coding regions of the most inclusive annotated RefSeq transcript for each of the targeted genes. Target enrichment was performed using AVENIO® Tumor Tissue CGP Kit (matched to FoundationONE® CDx panel (Roche). Sequencing of enriched libraries was performed in multiplex on the Illumina NextSeq 500/550 of NovaSeq 6000 using the paired-end, 150 base-pair configurations.

Informatics Methodology: Secondary analysis was performed a using the AVENIO® Connect software, a secondary analysis and workflow manager supported by FoundationONE® analysis platform. Appropriate coverage (>250x) was confirmed in >95% of regions spanning known hotspots of clinical importance in the targeted genes. Variants passing the quality filters of minimum read depth 250x and variant frequency >5% are reported. ITDs, insertions and deletions >50bp may not be detected by the NGS assay. Analysis output files include VCF for SNV/Indels, JSON for copy number variations (CNVs), select rearrangements, tumor mutational burden (TMB), Microsatellite Instability (MSI) and loss of heterozygosity (LOH), and are used for downstream tertiary analysis using Navify Mutation Profiler.

In the absence of confirmed somatic status in current databases, this assay cannot distinguish somatic heterozygous from germline variants. A follow-up germline testing may therefore be indicated. Small variants of uncertain significance (VUS) are not listed in the report but are available upon request. Benign or likely benign variants are not reported. The CNV detection algorithm works in the gene amplification and homozygous gene deletion mode. Whole-gene duplications with a sufficiently high copy number score (above 10) are reported as borderline, to confidently report amplification scores of 20 and higher as conclusive.

Microsatellite instability (MSI) status is determined by the assessment of homopolymer repeat loci in or near the target gene regions of this test and is reported as MSI-High or MS-Stable. Rarely MSI-Ambiguous or MSI-Unknown may be reported, when relevant, for example if the sample of insufficient quality to confidently determine the MSI status.

Tumor mutational burden (TMB) is determined by counting all somatic mutations occurring at a frequency >5% in the sequenced genes and the total number is represented as mutations per megabase (mut/Mb) unit. TMB results are reported as TMB-High (≥ 20 Muts/Mb), TMB-Intermediate (6-19 Muts/Mb), TMB-Low ≤ 5 Muts/Mb). Tumor Mutation Burden may be reported as "Unknown" if the sample is not of sufficient quality to confidently determine Tumor Mutation Burden.

Loss of Heterozygosity (LOH) is determined only for Ovarian cancer patients. Positive homologous recombination deficiency (HRD) status is defined as tBRCA-positive and/or LOH high.

Interpretation of pathogenicity of detected variants is as of current reports and databases, which are continually updated.

Sensitivity of the test is 5% for somatic variants detection. Details on low coverage regions can be provided upon request.

Test performance characteristics for this laboratory validated test has been determined by the College of Physicians and Surgeons of Alberta (CPSA) - accredited laboratory.

DISCLAIMER

This report assumes that the sample received is from the individual noted by the unique identifiers and has not been contaminated with that of another individual prior to receipt at the Laboratory. Rare diagnostic errors may result from sample contamination, genotyping errors, or sequence polymorphisms in PCR primer binding sites. HGVS classification is provisional.

A negative result does not rule out the presence of a mutation below the limits of detection of the assay.

The CNV detection algorithm is based on the statistical inference of CNVs from comparing samples to each other and assumes that, for each region, the CNV are only present in a small fraction of samples. It may fail to detect a CNV in batches with a large fraction of samples having CNVs at the same position. Depending on the quality of the DNA material, the reported amplification scores between 10 and 20 may include a considerable fraction of false positives. Samples with <25% tumor nuclei may have decreased sensitivity for the detection of CNVs

The interpretation of variants is compiled from currently available sources. The content is subject to change and will be adapted from time to time as such sources are updated. HTL is not obligated to notify of any impact that future scientific or medical research findings may have on the report. Clinical associations described in this report are based on individual SNVs, Indels, fusions, splice variants, transcript variants and amplifications, and, when relevant, their combinations. Report is based on an NGS assay which does not distinguish between somatic and germline variants. Report generated here is using the materials and methods which required the use of various reagents, instruments, software, databases, and other items. A defect or malfunction in any may compromise the quality or accuracy of the report. The report should be interpreted and considered within the clinical context, and never be considered or relied upon alone in making any diagnosis or prognosis. The interpretation must always consider 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 manifestations 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 this report (or that are otherwise unknown). A finding of biomarker alteration does not necessarily indicate pharmacologic effectiveness (or lack thereof) of any drug or treatment regimen; a finding of no biomarker alteration does not necessarily indicate lack of pharmacologic effectiveness (or effectiveness) of any drug or treatment regimen. In this report, neither any biomarker alteration, nor any drug associated with potential clinical benefit (or potential lack of clinical benefit), are ranked in order of potential or predicted efficacy. Clinical associations based on a “lack of a variant” is not provided. Some drugs identified in the description of variant significance may not be approved by regulatory bodies (including, but not limited to FDA, EMA or NICE) for a particular use or validated for that use. The user is therefore required to independently validate that such drug may be lawfully used in the territory of prescription. The decision on patient and treatment care must be based on the independent medical judgement of the treating physician, taking into consideration all applicable information concerning the patient’s condition such as the patient and family history, physical examinations, information from other diagnostic tests, and patient preferences, in accordance with the standard of care in a given geographical region and community.

This assay is not by itself diagnostic and is not approved by the US FDA or Health Canada and is marketed for research use only panel. The assay has however undergone rigorous validation for clinical use including frequent participation in proficiency testing with interlaboratory data sharing and assessment in HTL, a laboratory accredited by the College of Physicians and Surgeons of Alberta (CPSA). Variant interpretation and classification systems are based on Navify Mutation Profiler’s Clinical Decision Support Software and have undergone rigorous clinical validation with reference to other third-party interpretation solutions and our in-house variant interpretation workflow.

DNA genes with full exonic regions for the detection of substitutions, indels, and CNVs

ABL1	BRCA2	CDKN2C	ERCC4	GATA4	KDM6A	MSH2	PARP3	RAD51B	SPEN
ACVR1B	BRD4	CEBPA	ERG	GATA6	KDR	MSH3	PAX5	RAD51C	SPOP
AKT1	BRIP1	CHEK1	ERRFI1	GID4	KEAP1	MSH6	PBRM1	RAD51D	SRC
AKT2	BTG1	CHEK2	ESR1	GNA11	KEL	MST1R	PDCD1	RAD52	STAG2
AKT3	BTG2	CIC	EZH2	GNA13	KIT	MTAP	PDCD1LG2	RAD54L	STAT3
ALK	BTK	CREBBP	FAM46C	GNAQ	KLHL6	MTOP	PDGFRA	RAF1	STK11
ALOX12B	C11orf30	CRKL	FANCA	GNAS	KMT2A	MUTYH	PDGFRB	RARA	SYK
AMER1	CALR	CSF1R	FANCC	GRM3	KMT2D	MYC	PDK1	RB1	TBX3
APC	CARD11	CSF3R	FANCG	GSK3B	KRAS	MYCL	PIK3C3B	RBM10	TEK
AR	CASP8	CTCF	FANCL	H3F3A	LTK	MYCN	PIK3C3G	REL	TET2
ARAF	CBFB	CTNNA1	FAS	HDAC1	LYN	MYD88	PIK3CA	RET	TIPARP
ARFRP1	CBL	CTNNB1	FBXW7	HGF	MAF	NBN	PIK3CB	RICTOR	TNFAIP3
ARID1A	CCND1	CUL3	FGF10	HNF1A	MAP2K1	NF1	PIK3R1	RNF43	TNFRSF1
ASXL1	CCND2	CUL4A	FGF12	HRAS	MAP2K2	NF2	PIM1	ROS1	4
ATM	CCND3	CXCR4	FGF14	HSD3B1	MAP2K4	NFE2L2	PMS2	RPTOR	TP53
ATR	CCNE1	CYP17A1	FGF19	ID3	MAP3K1	NFKBIA	POLD1	SDHA	TSC1
ATRX	CD22	DAXX	FGF23	IDH1	MAP3K13	NKX2-1	POLE	SDHB	TSC2
AURKA	CD274	DDR1	FGF3	IDH2	MAPK1	NOTCH1	PPARG	SDHC	TYRO3
AURKB	CD70	DDR2	FGF4	IGF1R	MCL1	NOTCH2	PPP2R1A	SDHD	U2AF1
AXIN1	CD79A	DIS3	FGF6	IKBKE	MDM2	NOTCH3	PPP2R2A	SETD2	VEGFA
AXL	CD79B	DNMT3A	FGFR1	IKZF1	MDM4	NPM1	PRDM1	SF3B1	VHL
BAP1	CDC73	DOT1L	FGFR2	INPP4B	MED12	NRAS	PRKAR1A	SGK1	WHSC1
BARD1	CDH1	EED	FGFR3	IRF2	MEF2B	NT5C2	PRKCI	SMAD2	WHSC1L1
BCL2	CDK12	EGFR	FGFR4	IRF4	MEN1	NTRK1	PTCH1	SMAD4	WT1
BCL2L1	CDK4	EP300	FH	IRS2	MERTK	NTRK2	PTEN	SMARCA4	XPO1
BCL2L2	CDK6	EPHA3	FLCN	JAK1	MET	NTRK3	PTPN11	SMARCB1	XRCC2
BCL6	CDK8	EPHB1	FLT3	JAK2	MITF	P2RY8	PTPRO	SMO	ZNF217
BCOR	CDKN1A	EPHB4	FOXL2	JAK3	MKNK1	PALB2	QKI	SNCAIP	ZNF703
BCORL1	CDKN1B	ERBB2	FUBP1	JUN	MLH1	PARK2	RAC1	SOCS1	
BRAF	CDKN2A	ERBB3	GABRA6	KDM5A	MPL	PARP1	RAD21	SOX2	
BRCA1	CDKN2B	ERBB4	GATA3	KDM5C	MRE11A	PARP2	RAD51	SOX9	

DNA genes with select intronic regions for the detection of gene rearrangements

ALK	BRCA1	ETV4	EZR	KIT	MYC	NUTM1	RET	SLC34A2
BCL2	BRCA2	ETV5	FGFR1	KMT2A (MLL)	NOTCH2	PDGFRA	ROS1	TERC
BCR	CD74	ETV6	FGFR2	MSH2	NTRK1	RAF1	RSPO2	TERT
BRAF	EGFR	EWSR1	FGFR3	MYB	NTRK2	RARA	SDC4	TMPRSS2

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