Precision Panel- 50 Genes

Test Description

The MolQ Precision Panel includes 50 genes, involving hotspot regions and 3159 unique variants, applicable to a wide range of tumor types for detection of SNV (single and multiple nucleotide variation), Insertion-Deletion, Copy Number Variation (CNV), and gene Fusions. Fusion and splice variants are detected in RNA.

Patient Demographic

Name: Ms. Megha Kalyan
Sex: Female
Date of Birth/Age: 41 years
Disease: Lung Adenocarcinoma

Clinician

Clinician Name: Dr Amit Verma
Medical Facility: Dr AV Institute of Personalized Cancer Therapy and Research
Pathologist: Not Provided

Specimen

Booking ID: 012305180274
Sample Type: FFPE
Tumor Content Percentage: 25%
Date of Collection: 18-05-2023
Date of Booking: 18-05-2023

CLINICAL SYNOPSIS

Megha Kalyan, is a known case of lung adenocarcinoma. She has been evaluated for pathogenic variations in the genes listed in Appendix 2.

RESULT SUMMARY

The assay has detected ***EGFR* exon 19 deletion** (**p.E746_A750del, VAF=32.00%**). In addition, ***PIK3CA*** (**p.R38H, VAF=20.89%**) alteration is also present. Multiple approved therapies are available in the specified cancer type to target *EGFR* exon 19 deletion. Preclinical data suggests that concurrent *PIK3CA* mutation is a poor prognostic factor in patients with advanced *EGFR*- or *KRAS*-mutant lung adenocarcinomas. However, there is no substantial evidence that the clinical benefit from EGFR TKI monotherapy is affected by a concurrent *PIK3CA* mutation in *EGFR* mutant lung cancers[179].

RESULTS

Variants in *EGFR* and *PIK3CA* genes were detected.

Gene/ Transcript (Locus)	Variant ID	Variant	Allele Frequency	Variant Effect	*Relevant Therapies (In this cancer type)	*Relevant Therapies (In other cancer type)	Tier[2]
EGFR NM_005228.5 (chr7:55242465)	COSM6225	c.2236_2250delGAA TTAAGAGAAGCA (p.Glu746_Ala750del)	32.00%	Nonframeshift Deletion	Afatinib[i,ii] bevacizumab*+ erlotinib[ii] dacomitinib[i,ii] erlotinib[i,ii]	None	Ia
PIK3CA NM_006218.4 (chr3:178916726)	COSM745	c.113G>A (p.Arg38His)	20.89%	Missense	None	None	IIc
IDH1	-	c.394C>T (p.Arg132Cys)	2.81%	-	None	ivosidenib[i]	IIc
BRAF	-	c.1406G>T (p.Gly469Val)	4.61%	-	None	bevacizumab + chemotherapy ipilimumab + nivolumab trametinib	IIc

ROS1	-	c.6076C>A (p.Leu2026Met)	4.58%		entrectinib[ii]	None	Ia
EGFR	-	c.2386G>T (p.Gly796Cys)	3.03%	-	None	None	IIc
FGFR3	-	Amplification	-	-	None	None	IIc
FGFR2	-	c.755C>T (p.Ser252Leu)	4.33%	-	None	None	IIc
PDGFRA	-	c.1977C>A (p.Asn659Lys)	4.12%	-	None	None	IIc
ERBB3	-	c.310G>A (p.Val104Met)	2.69%	-	None	None	IIc

* Public data sources included in relevant therapies: FDA[i], NCCN, EMA[ii], ESMO

RELEVANT LUNG CANCER FINDINGS

Gene	Findings	Gene	Findings	Gene	Findings
ALK	None detected	*KRAS*	None detected	*NTRK3*	None detected
BRAF	c.1406G>T; p.Gly469Val	*MET*	None detected	RET	None detected
EGFR	i. EGFR exon 19 deletion ii. c.2386G>T; p.Gly796Cys	*NTRK1*	None detected	ROS1	c.6076C>A; p.Leu2026Met
ERBB2	None detected	*NTRK2*	None detected		

CLINICAL CORRELATION AND VARIANT INTERPRETATION

BRAF **p.Gly469Val**

Gene description: The *BRAF* gene encodes the B-Raf proto-oncogene serine/threonine kinase, a member of the RAF family of serine/ threonine protein kinases which also includes ARAF and RAF1 (CRAF). BRAF is among the most commonly mutated kinases in cancer. Activation of the MAPK pathway occurs through *BRAF* mutations and leads to an increase in cell division, dedifferentiation, and survival[1,2]. *BRAF* mutations are categorized into three distinct functional classes namely, class 1, 2, and 3, and are defined by the dependency on the RAS pathway. Class 1 and 2 *BRAF* mutants are RAS-independent in that they signal as active monomers (Class 1) or dimers (Class 2) and become uncoupled from RAS GTPase signaling, resulting in constitutive activation of *BRAF*[3]. Class 3 mutants are RAS dependent as the kinase domain function is impaired or dead[3-5].

Alterations and prevalence: Recurrent somatic mutations in *BRAF* are observed in 40-60% of melanoma and thyroid cancer, approximately 10% of colorectal cancer, and about 2% of non-small cell lung cancer (NSCLC)[6-10]. Mutations at V600 belong to class 1 and include V600E, the most recurrent somatic *BRAF* mutation across diverse cancer types[4,11]. Class 2 mutations include K601E/N/T, L597Q/V, G469A/V/R, G464V/E, and *BRAF* fusions[4]. Class 3 mutations include D287H, V459L, G466V/E/A, S467L, G469E, and N581S/I[4]. *BRAF* V600E is universally present in hairy cell leukemia, mature B-cell cancer, and prevalent in histiocytic neoplasms[12-14]. Other recurrent *BRAF* somatic mutations cluster in the glycine-rich phosphate-binding loop at codons 464-469 in exon 11 as well as additional codons flanking V600 in the activation loop[11]. In primary cancers, *BRAF* amplification is observed in 8% of ovarian cancer and about 1% of breast cancer[7,10]. *BRAF* fusions are mutually exclusive to *BRAF* V600 mutations and have been described in melanoma, thyroid cancer, pilocytic astrocytoma, NSCLC and several other cancer types[15-19]. Part of the oncogenic mechanism of *BRAF* gene fusions is the removal of the N-terminal auto-inhibitory domain leading to constitutive kinase activation[5,15,17].

Potential relevance: Vemurafenib[20] (2011) was the first targeted therapy approved for the treatment of patients with unresectable or metastatic melanoma with a *BRAF* V600E mutation. *BRAF* class 1 mutations, including V600E, are sensitive to vemurafenib, whereas class 2 and 3 mutations are insensitive[4]. BRAF kinase inhibitors including dabrafenib[21] (2013) and encorafenib[22] (2018) are also approved for the treatment of patients with unresectable or metastatic melanoma with *BRAF* V600E/K mutations. Encorafenib[22] is approved in combination with cetuximab[23] (2020) for the treatment of *BRAF* V600E mutated colorectal cancer. Due to the tight coupling of RAF and MEK signaling, several MEK inhibitors have been approved for patients harboring *BRAF* alterations[4]. Trametinib[24] (2013) and binimetinib[25] (2018) were approved for the treatment of metastatic melanoma with *BRAF* V600E/K mutations. Combination therapies of BRAF plus MEK inhibitors have been approved in melanoma and NSCLC. The combinations of dabrafenib/trametinib (2015) and vemurafenib/cobimetinib[26] (2015) were approved for the treatment of patients with unresectable or metastatic melanoma with a *BRAF* V600E/K mutation. Subsequently, the combination of dabrafenib and trametinib was approved for metastatic NSCLC (2017) with a BRAF V600E mutation. The PD-L1 antibody, atezolizumab[27], has also been approved in combination with cobimetinib and vemurafenib for *BRAF* V600 mutation-positive unresectable or metastatic melanoma. In 2018, binimetinib[28] was also granted breakthrough designation in combination with cetuximab and encorafenib for *BRAF* V600E mutant metastatic colorectal cancer. The pan-RAF kinase inhibitor, tovorafenib (DAY-101), was granted breakthrough therapy designation (2020) by the FDA for pediatric patients with advanced low-grade glioma harboring activating *RAF* alterations[29]. The ERK inhibitor ulixertinib[30] was also granted a fast-track designation in 2020 for the treatment of patients with non-colorectal solid tumors harboring *BRAF* mutations G469A/V, L485W, or L597Q. The FDA granted fast-track designation (2022) to the pan-RAF inhibitor, KIN-2787[31], for the treatment of *BRAF* class II or III alteration-positive malignant or unresectable melanoma. *BRAF* fusion is a suggested mechanism of resistance to BRAF targeted therapy in melanoma[32]. Additional mechanisms of resistance to BRAF targeted therapy include *BRAF* amplification and alternative splice transcripts as well as activation of PI3K signaling and activating mutations in *KRAS*, *NRAS*, and *MAP2K1/2* (*MEK1/2*)[33-39]. Clinical responses to sorafenib and trametinib in limited case studies of patients with *BRAF* fusions have been reported[19].

EGFR p.Glu746_Ala750del

Gene description: The *EGFR* gene encodes the epidermal growth factor receptor (EGFR) tyrosine kinase, a member of the ERBB/human epidermal growth factor receptor (HER) family. In addition to EGFR/ERBB1/HER1, other members of the ERBB/HER family include ERBB2/HER2, ERBB3/HER3, and ERBB4/HER4[40]. EGFR ligand induced dimerization results in kinase activation and leads to stimulation of oncogenic signaling pathways including the PI3K/AKT/MTOR and RAS/RAF/MEK/ERK pathways. Activation of these pathways promote cell proliferation, differentiation, and survival[41,42].

Alterations and prevalence: Recurrent somatic mutations in the tyrosine kinase domain (TKD) of EGFR are observed in approximately 10-20% of lung adenocarcinoma, and at higher frequencies in never-smoker, female, and Asian populations[7,9,10,43]. The most common mutations occur near the ATP-binding pocket of the TKD and include short in-frame deletions in exon 19 (EGFR exon 19 deletion) and the L858R amino acid substitution in exon 21[44]. These mutations constitutively activate EGFR resulting in downstream signaling, and represent 80% of the *EGFR* mutations observed in lung cancer. A second group of less prevalent activating mutations include E709K, G719X, S768I, L861Q, and short in-frame insertion mutations in exon 20[45-48]. *EGFR* activating mutations in lung cancer tend to be mutually exclusive to *KRAS* activating mutations[49]. In contrast, a different set of recurrent activating *EGFR* mutations in the extracellular domain include R108K, A289V and G598V and are primarily observed in glioblastoma[44,50]. Amplification of *EGFR* is observed in several cancer types including 30% of glioblastoma,

12% of esophageal cancer, 10% of head and neck cancer, 5% of bladder cancer, and 5% of lung squamous cell carcinoma[7,9,10,50,51]. Deletion of exons 2-7, encoding the extracellular domain of EGFR (EGFRvIII), results in overexpression of a ligand-independent constitutively active protein and is observed in approximately 30% of glioblastoma[52-54].

Potential relevance: Approved first-generation EGFR tyrosine kinase inhibitors (TKIs) include erlotinib[55] (2004) and gefitinib[56] (2015), which block the activation of downstream signaling by reversible interaction with the ATP-binding site. Although initially approved for advanced lung cancer, the discovery that drug sensitivity was associated with exon 19 and exon 21 activating mutations allowed first-generation TKIs to become subsequently approved for front-line therapy in lung cancer tumors containing exon 19 or exon 21 activating mutations. Second-generation TKIs afatinib[57] (2013) and dacomitinib[58] (2018) bind EGFR and other ERBB/HER gene family members irreversibly and were subsequently approved. First- and second-generation TKIs afatinib, dacomitinib, erlotinib, and gefitinib are recommended for the treatment NSCLC harboring *EGFR* exon 19 insertions, exon 19 deletions, point mutations L861Q, L858R, S768I, and codon 719 mutations, whereas most *EGFR* exon 20 insertions, except p.A763_Y764insFQEA, confer resistance to the same therapies[59-62]. However, in 2021, the irreversible tyrosine kinase inhibitor, mobocertinib[63] was FDA approved for the treatment of NSCLC with *EGFR* exon 20 insertion mutations. Additionally, in 2022, the FDA granted breakthrough therapy designation to the irreversible EGFR inhibitors, CLN-081 (TPC-064)[64] and sunvozertinib[65], for locally advanced or metastatic non-small cell lung cancer harboring EGFR exon 20 insertion mutations. In lung cancer containing *EGFR* exon 19 or 21 activating mutations, treatment with TKIs is eventually associated with the emergence of drug resistance[66]. The primary resistance mutation that emerges following treatment with first-generation TKI is T790M, accounting for 50-60% of resistant cases[44]. Third generation TKIs were developed to maintain sensitivity in the presence of T790M. Osimertinib[67] (2015) is an irreversible inhibitor indicated for metastatic *EGFR* T790M positive lung cancer and for the first-line treatment of metastatic NSCLC containing *EGFR* exon 19 deletions or exon 21 L858R mutations. Like first-generation TKIs, treatment with osimertinib is associated with acquired resistance. In this case, resistance is associated with the C797S mutation and occurs in 22-44% of cases[66]. The T790M and C797S mutations may be each selected following sequential treatment with a first-generation TKI followed by a third-generation TKI or vice versa[68]. T790M and C797S can occur in either *cis* or *trans* allelic orientation[68]. If C797S is observed following progression after treatment with a third-generation TKI in the first-line setting, sensitivity may be retained to first-generation TKIs[68]. If C797S co-occurs in *trans* with T790M following sequential treatment with first- and third-generation TKIs, patients may exhibit sensitivity to combination first- and third-generation TKIs, but resistance to third-generation TKIs alone[68,69]. However, C797S occurring in *cis* conformation with T790M, confers resistance to first- and third-generation TKIs[68]. Fourth-generation TKIs are in development to overcome acquired C797S and T790M resistance mutations after osimertinib treatment. EGFR targeting antibodies including cetuximab (2004), panitumumab (2006), and necitumumab (2016) are under investigation in combination with EGFR-targeting TKIs for efficacy against *EGFR* mutations. The bispecific antibody, amivantamab[70], targeting EGFR and MET was approved (2021) NSCLC tumors harboring *EGFR* exon 20 insertion mutations. The Oncoprex immunogene therapy quaratusugene ozeplasmid[71] in combination with osimertinib received a fast-track designation from the FDA (2020) for NSCLC tumors harboring *EGFR* mutations that progressed on osimertinib alone. BDTX-189[72] was granted a fast-track designation (2020) for the treatment of solid tumors harboring an *EGFR* exon 20 insertion mutation.

ERBB3 p.Val104Met

Gene description*:* The *ERBB3* gene encodes the erb-b2 receptor tyrosine kinase 3, a member of the human epidermal growth factor receptor (HER) family. Along with ERBB3/HER3, EGFR/ERBB1/HER1, ERBB2/HER2, and ERBB4/HER4 make up the HER protein family[40]. ERBB3/ HER3 binds to extracellular factors, such as neuregulins, but has an impaired kinase domain[73]. Upon ligand binding, ERBB3 forms hetero-dimers with other ERBB/HER family members, including ERBB2/HER2 resulting in activation of tyrosine kinase activity primarily through its dimerization partner.

Alterations and prevalence: *ERBB3* gene amplification leading to an increase in expression occurs at low frequency (1-5%) in several cancer types including bladder, esophagus, lung adenocarcinoma, ovarian, pancreas, sarcoma, stomach, and uterine cancers[7,9,10,74-77]. *ERBB3* is also the target of relatively frequent (5-10%) and recurrent somatic mutations in diverse cancer types including bladder, cervical, colorectal, and stomach cancers[7,8,10,74,76]. Recurrent *ERBB3* mutations such as V104L/M, occur primarily in the extracellular domain.

Potential relevance: Currently, no therapies are approved for *ERBB3* aberrations. Overexpression and activation of ERBB3/HER3 is one mechanism of acquired resistance to therapies targeting EGFR and ERBB2/HER2[78,79]. Preclinical and

translational research studies have characterized the oncogenic potential of recurrent *ERBB3* mutations and their sensitivity to anti-ERBB antibodies and small molecule inhibitors[80-83]. A phase I study exhibited progression-free survival (PFS) of 2.5 months and overall survival (OS) of 9 months in 25 patients with *ERBB3* mutations treated by anti-ERBB antibodies or molecular-targeted agents[84].

FGFR2 p.Ser252Leu

Gene description: The *FGFR2* gene encodes fibroblast growth receptor 2, a member of the fibroblast growth-factor receptor (FGFR) family that also includes FGFR1, 3 and 4. These proteins are single-transmembrane receptors composed of three extracellular immunoglobulin (Ig)-type domains and an intracellular kinase domain. Upon FGF-mediated stimulation, FGFRs activate several oncogenic signaling pathways, including the RAS/RAF/MEK/ERK, PI3K/AKT/MTOR, PLC/PKC and JAK/STAT pathways influencing cell proliferation, migration, and survival[85-87].

Alterations and prevalence: Aberrations most common to the FGFR family are amplifications, followed by mutations and fusions. The majority of these aberrations result in gain of function[88]. Missense mutations are the most prevalent alterations in *FGFR2* and are observed in up to 15% of uterine carcinomas[7,10,89]. These mutations are predominantly activating, most often involve substitutions at S252 and P253, and confer sensitivity to pan-FGFR2 inhibitors[89,90]. *FGFR2* amplification occurs in up to 4% of gastric carcinoma, and is associated with poor prognosis as well as tumor invasion and metastasis[10,91-93]. *FGFR2* fusions have also been reported in up to 14% of cholangiocarcinoma and confer sensitivity to select FGFR inhibitors[10,94,95].

Potential relevance: Several pan-FGFR inhibitors have been approved for *FGFR2* aberrations in cancer. Futibatinib[96], is approved (2022) for *FGFR2* rearrangement or fusion-positive locally advanced or metastatic intrahepatic cholangiocarcinoma. Infigratinib, was granted accelerated approval (2021) for previously treated, unresectable locally advanced or metastatic cholangiocarcinoma positive for *FGFR2* fusion or other rearrangement[97]. Erdafitinib[98], received FDA approval (2019) for the treatment of locally advanced or metastatic urothelial cancer that is positive for *FGFR2* fusions including, *FGFR2-BICC1* and *FGFR2-CASP7*, *FGFR3* fusions, or *FGFR3* mutation. Pemigatinib[99], received FDA approval (2020), for previously treated, advanced or unresectable cholangiocarcinoma harboring *FGFR2* fusions or other *FGFR2* rearrangements. The FDA has granted fast-track designation (2023) to the pan-FGFR inhibitor, KIN-3248, for unresectable, locally advanced or metastatic cholangiocarcinoma with *FGFR2* fusions or other alterations after receiving at least one prior systemic therapy[100]. The FDA also granted fast-track designation (2018) to Debio 1347[101] for solid tumors harboring *FGFR1*, *FGFR2*, or *FGFR3* aberrations. Additional FGFR inhibitors are under clinical evaluation for *FGFR2* aberrations. In a phase II study of patients with *FGFR2* fusion-positive intrahepatic cholangiocarcinoma, the pan-kinase inhibitor derazantinib, demonstrated an overall response rate (ORR) of 20.7% with progression-free survival (PFS) of 5.7 months[102]. Likewise, results of a phase II trial testing the pan- FGFR inhibitor, infigratinib (BGJ398) demonstrated an ORR of 14.8% (18.8% FGFR2 fusions only), disease control rate (DCR) of 75.4% (83.3% FGFR2 fusions only), and a median PFS of 5.8 months[103].

FGFR3 Amplification

Gene description: The *FGFR3* gene encodes fibroblast growth receptor 3, a member of the fibroblast growth-factor receptor (FGFR) family that also includes FGFR1, 2 and 4. These proteins are single-transmembrane receptors composed of three extracellular immunoglobulin (Ig)-type domains and an intracellular kinase domain. . Upon FGF-mediated stimulation, FGFRs activate several oncogenic signaling pathways, including the RAS/RAF/MEK/ERK, PI3K/AKT/MTOR, PLC/PKC and JAK/STAT pathways influencing cell proliferation, migration, and survival[85-87].

Alterations and prevalence: Aberrations most common to the FGFR family are amplifications, followed by mutations and fusions. The majority of these aberrations result in gain of function[88]. *FGFR3* amplification is observed in up to 19% of uterine carcinoma, with somatic mutations occurring in 10-20% of bladder cancer[7,10,74]. Missense mutations that occur in the extracellular immunoglobulin-like and transmembrane domains of FGFR3, including S249C, R248C, and Y375C, cause ligand-independent dimerization and constitutive activation of FGFR3[104-106].

Potential relevance: The pan-FGFR inhibitor, erdafitinib[98], received FDA approval (2019) for the treatment of locally advanced or metastatic urothelial cancer that is positive for *FGFR2* fusions, *FGFR3* fusions including *FGFR3-TACC3* and *FGFR3-BAIAP2L1*, and *FGFR3* gene mutations including R248C, S249C, G370C, and Y373C. The FGFR3 monoclonal antibody, vofatamab[107] was

granted fast-track designation (2019) by the FDA, for the treatment of advanced or metastatic bladder urothelial cell carcinoma that harbors *FGFR3* mutations or fusions. The FDA also granted fast-track designation (2018) to Debio 1347[101] for solid tumors harboring *FGFR1*, *FGFR2*, or *FGFR3* aberrations. Unregulated activation of FGFR3 has been associated with resistance to tamoxifen in ER-positive breast cancer[108].

IDH1 **p.Arg132Cys**

Gene description: The *IDH1* and *IDH2* genes encode homologous isocitrate dehydrogenase enzymes that catalyze the conversion of isocitrate to α-ketoglutarate (α-KG)[109]. The *IDH1* gene encodes the NADP+ dependent cytoplasmic isocitrate dehydrogenase enzyme; *IDH2* encodes the mitochondrial isoform.

Alterations and prevalence: Recurrent somatic mutations in *IDH1* and *IDH2* are mutually exclusive and observed in several malignancies including glioma, chondrosarcoma, intrahepatic cholangiocarcinoma, acute myeloid leukemia (AML), and myelodysplastic syndrome (MDS)[110]. Recurrent *IDH1* variants include predominately R132H/C plus other substitutions at lower frequencies. These gain of function variants confer neomorphic enzyme activity[111]. Although wild-type enzymatic activity is ablated, recurrent *IDH1* variants catalyze the conversion of α-KG to D-2-hydroxyglutarate, an oncometabolite with diverse effects on cellular metabolism, epigenetic regulation, redox states, and DNA repair[109,112]. Recurrent *IDH1* mutations are present in 5-10% of patients with AML and 5% of patients with MDS[113-115.] Recurrent *IDH1* mutations are present in nearly 80% of lower grade diffuse gliomas[7,10].

Potential relevance: The IDH1 inhibitors, ivosidenib[116] (2018) and olutasidenib[117] (2022), are FDA approved for the treatment of AML patients with IDH1 R132C/G/H/L/S variants[118]. Ivosidenib has also been granted breakthrough designation (2020) for *IDH1* mutated relapsed or refractory myelodysplastic syndrome (MDS)[119]. *IDH1* mutations are associated with inferior leukemia-free survival in primary myelofibrosis (PMF) and inferior overall survival in polycythemia vera (PV) but have been shown to confer improved prognosis in lower grade gliomas[120-122].

PDGFRA **p.Asn659Lys**

Gene description: The *PDGFRA* gene encodes the platelet derived growth factor receptor alpha, a member of the PDGF receptor type III receptor tyrosine kinase family, which includes PDGFRB, CSF1R, FLT1, FLT3, FLT4, KDR and KIT[123,124]. PDGFRA is a receptor for platelet derived growth factors, which are mitogens for cells of mesenchymal origin[125]. PDGFRA may function as a homodimer or heterodimer with PDGFRB depending on the ligand[126]. The *PDGFRA* gene is physically adjacent to *KIT* and *KDR* on chromosome 4q12. Ligand binding to PDGFRA results in kinase activation and stimulation of downstream pathways including the RAS/RAF/MEK/ERK and PI3K/AKT/MTOR pathways promoting cell proliferation and survival.

Alterations and prevalence: Recurrent somatic *PDGFRA* alterations are observed in both solid and hematological cancers and include activating mutations, gene amplification, and translocations generating *PDGFRA* gene fusions. Recurrent *PDGFRA* activating mutations, including D842V, V561D, N659K, and in-frame deletions in exon 18, are common in 30-40% of KIT negative gastrointestinal stromal tumors (GISTs) and approximately 7% overall[127-130]. *PDGFRA* recurrent mutations are also described in adult and pediatric glioblastoma and high-grade gliomas[50,130]. In these cases, *PDGFRA* amplification is common (about 10% of cases) and recurrent mutations frequently co-occur with gene amplification[7,10]. *PDGFRA* fusions are observed in gliomas and glioblastomas as well as eosinophilic leukemias, of which the *FIP1L1-PDGFRA* fusion defines approximately half of patients with hypereosinophilic syndrome[131-133].

Potential relevance: The FDA has granted fast-track designation to crenolanib[134] (2017) for GISTs harboring *PDGFRA* D842V mutation. Avapritinib[135] is a tyrosine kinase inhibitor (TKI) that is approved (2020) by the FDA for metastatic or unresectable GIST harboring PDGFRA exon 18 mutations including PDGFRA D842V mutation. Another TKI, imatinib[136], is approved (2001) for patients diagnosed with chronic eosinophilic leukemia harboring *FIP1L1-PDGFRA* fusions. Additionally, imatinib is recommended for the treatment of GISTs harboring PDGFRA exon 18 mutations with the exception of D842V[137]. The TKI, dasatinib, is recommended as a second-line therapy for the treatment of GISTs harboring a PDGFRA exon 18 mutation that is insensitive to imatinib, including the D842V mutation[137].

PIK3CA **p.Arg38His**

Gene description: The *PIK3CA* gene encodes the phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha of the class I phosphatidylinositol 3-kinase (PI3K) enzyme[138]. PI3K is a heterodimer that contains a p85 regulatory subunit, which couples one of four p110 catalytic subunits to activated tyrosine protein kinases[139,140]. The p110 catalytic subunits include p110α, β, δ, γ and are encoded by genes *PIK3CA*, *PIK3CB*, *PIK3CD*, and *PIK3CG*, respectively[139]. PI3K catalyzes the conversion of phosphatidylinositol (4,5)-bisphosphate (PI(4,5)P2) into phosphatidylinositol (3,4,5)-trisphosphate (PI(3,4,5)P3) while the phosphatase and tensin homolog (PTEN) catalyzes the reverse reaction[141,142]. The reversible phosphorylation of inositol lipids regulates diverse aspects of cell growth and metabolism[141-144]. Recurrent somatic alterations in *PIK3CA* are frequent in cancer and result in the activation of PI3K/AKT/MTOR pathway, which can influence several hallmarks of cancer including cell proliferation, apoptosis, cancer cell metabolism and invasion, and genetic instability[145-147].

Alterations and prevalence: Recurrent somatic activating mutations in *PIK3CA* are common in diverse cancers and are observed in 20-30% of breast, cervical, and uterine cancers and 10-20% of bladder, gastric, head and neck, and colorectal cancers[7,10]. Activating mutations in *PIK3CA* commonly occur in exons 10 and 21 (previously referred to as exons 9 and 20 due to exon 1 being untranslated)[148,149]. These mutations typically cluster in the exon 10 helical (codons E542/E545) and exon 21 kinase (codon H1047) domains, each having distinct mechanisms of activation[150-152]. *PIK3CA* resides in the 3q26 cytoband, a region frequently amplified (10-30%) in diverse cancers including squamous carcinomas of the lung, cervix, head and neck, and esophagus, and in serous ovarian and uterine cancers[7,10].

Potential relevance: The PI3K inhibitor, alpelisib[153], is FDA approved (2019) in combination with fulvestrant for the treatment of patients with *PIK3CA*-mutated, hormone receptor (HR)-positive, human epidermal growth factor receptor 2 (HER2)-negative, advanced or metastatic breast cancer. Additionally, a phase Ib study of alpelisib with letrozole in patients with metastatic estrogen receptor (ER)- positive breast cancer, the clinical benefit rate, defined as lack of disease progression ≥ 6 months, was 44% (7/16) in *PIK3CA*-mutated tumors and 20% (2/20) in *PIK3CA* wild-type tumors[154]. Specifically, exon 20 H1047R mutations were associated with more durable clinical responses in comparison to exon 9 E545K mutations[154]. However, alpelisib did not improve response when administered with letrozole in patients with ER^+ early breast cancer with *PIK3CA* mutations[155]. Case studies with MTOR inhibitors sirolimus and temsirolimus report isolated cases of clinical response in *PIK3CA* mutated refractory cancers[156,157].

ROS1 p.Leu2026Met

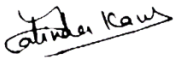
Gene description: The *ROS1* gene encodes the ROS proto-oncogene receptor tyrosine kinase 1 which exhibits structural similarity to anaplastic lymphoma kinase (ALK)[158,159]. Like *ALK*, *ROS1* is the target of recurrent chromosomal rearrangements that generate fusion proteins containing the intact ROS1 tyrosine kinase domain combined with numerous fusion partner genes[160]. ROS1 fusion kinases are constitutively activated and drive oncogenic transformation[161].

Alterations and prevalence: *ROS1* fusions occur in approximately 1-2% of patients with non-small cell lung cancer (NSCLC) and are also observed in cholangiocarcinoma, gastric cancer, ovarian cancer, and glioblastoma[158,162-166].

Potential relevance: The tyrosine kinase inhibitor, entrectinib[167], is approved (2019) for the treatment of *ROS1* fusion positive metastatic NSCLC. Crizotinib[168], originally approved for the treatment of ALK positive NSCLC (2011), is also approved (2016) for the treatment of ROS1 positive NSCLC[169]. Acquired resistance to crizotinib in ROS1 positive NSCLC is associated with kinase domain mutations S1986F/Y, G2032R, D2033N and L2155S[170-172]. The ROS1 tyrosine kinase inhibitor, repotrectinib[173], was granted fast-track and breakthrough designations (2020) for ROS1 positive NSCLC. The ROS-1 inhibitor, taletrectinib[174], was also granted breakthrough therapy designation (2022) for the treatment of adult patients with advanced or metastatic ROS1-positive non-small cell lung cancer (NSCLC) who are ROS1 tyrosine kinase inhibitor (TKI) treatment naïve or previously treated with crizotinib. Ceritinib is a second generation ALK inhibitor approved (2017) for ALK positive NSCLC that has also shown efficacy in ROS1 positive NSCLC. In a phase II study, ceritinib demonstrated systemic and intra-cranial activity with an objective response rate (ORR) of 62% in patients with advanced ROS1 positive NSCLC[175]. In addition to crizotinib and entrectinib, ceritinib is recommended for first-line treatment of ROS1-positive NSCLC[59]. Lorlatinib is a CNS-penetrant third-generation ALK and ROS1 inhibitor with preclinical activity against almost all known ALK and ROS1 resistance mutations[176,177]. Lorlatinib is currently FDA approved (2018) for ALK positive metastatic NSCLC. In a phase I study testing lorlatinib in advanced ROS1-positive NSCLC, objective response was observed in 6/12 (50%) of patients[178]. Lorlatinib is recommended for subsequent therapy in *ROS1* fusion-positive NSCLC in patients who have progressed after treatment with crizotinib, entrectinib, or ceritinib[59].

RECOMMENDATIONS

- Validation of the variant(s) by Sanger sequencing is recommended to rule out false positives.
- Genetic counselling is advised for interpretation on the consequences of the variant(s).
- If results obtained do not match the clinical findings, additional testing should be considered as per referring clinician's recommendations.



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APPENDIX 1: TEST METHODOLOGY

Method

DNA and RNA were extracted from samples using the Qiagen FFPE DNA kit and Promega ReliaPrep FFPE Total RNA Miniprep system. Isolated DNA/RNA was directly loaded on Genexus Next Generation Sequencer and subjected to automated library preparation and template preparation followed by sequencing at average depth of ~4000X.
It utilizes unique molecular tags to enable high sensitivity detection of variants. Analysis is done using Ion Torrent Reporter Software, the data is visualized on Integrative Genomics Viewer (IGV) and analyzed. The final report is generated using Oncomine curated knowledgebase reporter and includes clinical trials information continuously being updated for the best of the patient management as per clinical guidelines.

DISCLAIMER

- This report was generated using the materials and methods as recommended which required the use of quality 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 incorporated inferences 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 of such diseases). Such information and correlations are subject to change over time in response to future scientific and medical findings. MolQ Laboratory makes no representation or warranty of any kind, expressed or implied, regarding the accuracy of the information provided by or contained in such manuscripts, references, and other sources is later determined to be inaccurate, the accuracy and quality of the Report may be adversely impacted. MolQ Laboratory is not obligated to notify you of any of the impact that future scientific or medical findings may have on the report.
- 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 or developing and implementing a plan of care for the patient. The report should never be considered or relied upon alone in making any diagnosis or prognosis. 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 the report. This report is based on a Next Generation Assay which does not distinguish between a somatic and a germline variant. If germline variant is in question, further testing is recommended. The report provided by MolQ Laboratory is on a "as is" basis. MolQ Laboratory makes no representation or warranty of any kind, expressed or implied, regarding the report. In no event will MolQ Laboratory 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.
- Medical knowledge and annotation are constantly updated and reflects the current knowledge at the time.
- Due to inherent technology limitations of the assay, not all bases of the exome can be covered by this test. Accordingly, variants in regions of insufficient coverage may not be identified and/or interpreted. Therefore, it is possible that certain variants are present in one or more of the genes analyzed, but have not been detected. The variants not detected by the assay that was performed may/ may not impact the phenotype.
- It is also possible that a pathogenic variant is present in a gene that was not selected for analysis and/or interpretation in cases where insufficient phenotypic information is available.
- The report shall be generated within turnaround time (TAT), however, such TAT may vary depending upon the complexity of test(s) requested. MolQ Laboratory under no circumstances will be liable for any delay beyond afore mentioned TAT.
- It is hereby clarified that the report(s) generated from the test(s) do not provide any diagnosis or opinion or recommends any cure in any manner. MolQ Laboratory hereby recommends the patient and/or the guardians of the patients, as the case may be, to take assistance of the clinician or a certified physician or doctor, to interpret the report(s) thus generated. MolQ Laboratory hereby disclaims all liability arising in connection with the report(s).
- In a very few cases genetic test may not show the correct results, e.g. because of the quality of the material provided to MolQ Laboratory. In case where any test provided by MolQ Laboratory fails for unforeseeable or unknown reasons that cannot be influenced by MolQ Laboratory in advance, MolQ Laboratory shall not be responsible for the incomplete, potentially misleading or even wrong result of any testing if such could not be recognized by MolQ Laboratory in advance.

- This is a laboratory developed test and the development and the performance characteristics of this test was determined by reference laboratory as required by the CLIA 1988 regulations. The report, and the tests used to generate the Report have not been cleared or approved by the US Food and Drug Administration (FDA). The FDA has determined that such clearance or approval is not necessary. The test results have scientifically shown to be clinically useful.

APPENDIX 2: GENE LIST WITH COVERAGE

DNA Hotspots					
AKT1	*AKT2*	*AKT3*	*ALK*	*AR*	*ARAF*
BRAF	*CDK4*	*CDKN2A*	*CHEK2*	*CTNNB1*	*EGFR*
ERBB2	*ERBB3*	*ERBB4*	*ESR1*	*FGFR1*	*FGFR2*
FGFR3	*FGFR4*	*FLT3*	*GNA11*	*GNAQ*	*GNAS*
HRAS	*IDH1*	*IDH2*	*KIT*	*KRAS*	*MAPK1*
MAPK2	*MET*	*MTOR*	*NRAS*	*NTRK1*	*NTRK2*
NTRK3	*PDGFRA*	*PIK3CA*	*PTEN*	*RAF1*	*RET*
ROS1	*SMO*	*TP53*			
CNVs					
ALK	*AR*	*CD274*	*CDKN2A*	*EGFR*	*ERBB2*
ERBB3	*FGFR1*	*FGFR2*	*FGFR3*	*KRAS*	*MET*
PIK3CA	*PTEN*				
Inter-genetic Fusions					
ALK	BRAF	*ESR1*	*FGFR1*	*FGFR2*	*FGFR3*
MET	*NRG1*	*NTRK1*	*NTRK2*	*NTRK3*	*NUTM1*
RET	*ROS1*	*RSPO2*	*RSPO3*		
Intra-genetic Fusions					
AR	*EGFR*	*MET*			