Liquid Precision Panel- 50 Genes

Test Description

The MolQ Liquid 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. Renu Kapoor
Sex: Female
Date of Birth/Age: 69 years
Disease: Non-small Cell Lung Carcinoma

Clinician

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

Specimen

Booking ID: 012311210098
Sample Type: Blood
Tumor Content Percentage: NA
Date of Collection: 21-11-2023
Date of Booking: 21-11-2023

CLINICAL SYNOPSIS

Renu Kapoor, is a known case of non-small cell lung carcinoma. She has been evaluated for pathogenic variations in the genes listed in Appendix 2.

RESULT SUMMARY

Variants detected as per NCCN Guidelines: Clinically relevant ***EGFR*** exon 19 deletion (p.Glu746_Ala750del, VAF= 7.40%), (p.Leu747_Thr751delinsP, VAF=1.73%) and (p.Leu747_Thr751delinsQ, VAF= 0.58%) are detected in the given specimen.

Other variants detected:

PTEN mutation (p.Ala126Ser, VAF= 0.16%) is present.

In addition, ***EGFR*** mutation (p.Leu858Arg, VAF= 2.4%) and ***PIK3CA*** mutation (p.His1047Arg, VAF= 5%) are also present in the filtered data.

Note: The sequencing was performed on 7.52 ng of cfTNA in the given specimen. The average coverage of sequencing was 22319 in this sample.

RESULTS

Clinically relevant alteration was detected.

Gene	Variant ID	Variant	Allele Frequency	Variant Effect	ClinVar#	Exon	*Relevant Therapies (In this cancer type)	*Relevant Therapies (In other cancer type)	Tier[1]
EGFR NM_005228.5 (chr7:55242465)	COSM6225	c.2236_2250del GAATTAAGAGA AGCA (p.Glu746_Ala7 50del)	7.40%	Non frameshift Deletion	-	19	Afatinib[i,ii] bevacizumab* + erlotinib[ii] dacomitinib[i,ii] erlotinib[i,ii] erlotinib + ramucirumab[i,ii] gefitinib[i,ii] osimertinib 1, 2 atezolizumab + bevacizumab + chemotherapy gefitinib + chemotherapy	None	Ia

EGFR NM_005228.5 (chr7:55242469)	COSM12383	c.2239_2251delTTAAGAGAAGCAAinsC (p.Leu747_Thr751delinsP)	1.73%	Non frameshift Block Substitution	-	19	Afatinib[i,ii] bevacizumab* + erlotinib[ii] dacomitinib[i,ii] erlotinib[i,ii] erlotinib + ramucirumab[i,ii] gefitinib[i,ii] osimertinib 1, 2 atezolizumab + bevacizumab + chemotherapy gefitinib + chemotherapy	None	Ia
EGFR NM_005228.5 (chr7:55242469)	COSM12420	c.2239_2252delTTAAGAGAAGCAACinsCA (p.Leu747_Thr751delinsQ)	0.58%	Non frameshift Block Substitution	-	19	Afatinib[i,ii] bevacizumab* + erlotinib[ii] dacomitinib[i,ii] erlotinib[i,ii] erlotinib + ramucirumab[i,ii] gefitinib[i,ii] osimertinib 1, 2 atezolizumab + bevacizumab + chemotherapy gefitinib + chemotherapy	None	Ia
PTEN NM_000314.8 (chr10:89692892)	COSM28889	c.376G>T (p.Ala126Ser)	0.16%	Missense	Uncertain significance	5	None	None	IIc

* Public data sources included in relevant therapies: FDA[i], NCCN, EMA[ii], ESMO. #Based on Clinvar version 20200329.

[1]Li et al. Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer: A Joint Consensus Recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists. J Mol Diagn. 2017 Jan;19(1):4-23.

* **Includes biosimilars/generics**

RELEVANT NON-SMALL CELL LUNG CARCINOMA FINDINGS

Gene	Findings	Gene	Findings
ALK	None detected	*NTRK1*	None detected
BRAF	None detected	*NTRK2*	None detected
EGFR	Exon 19 deletion	*NTRK3*	None detected
ERBB2	None detected	*RET*	None detected
KRAS	None detected	*ROS1*	None detected
MET	None detected		

CLINICAL CORRELATION AND VARIANT INTERPRETATION

PTEN **p.Ala126Ser** **Coverage Frequency 2460**

Gene description: The *PTEN* gene encodes the phosphatase and tensin homolog, a tumor suppressor protein with lipid and protein phosphatase activities[1]. PTEN antagonizes PI3K/AKT signaling by catalyzing the dephosphorylation of phosphatidylinositol (3,4,5)- trisphosphate (PIP3) to PIP2 at the cell membrane, which inhibits the activation of AKT[2,3]. In addition, PTEN has been proposed to influence RAD51 loading at double strand breaks during homologous recombination repair (HRR) and regulate the G_2/M checkpoint by influencing CHEK1 localization through AKT inhibition, thereby regulating HRR efficiency[4]. Germline mutations in *PTEN* are linked to hamartoma tumor syndromes, including Cowden disease, which are defined by uncontrolled cell growth and benign or malignant tumor formation[5]. *PTEN* germline mutations are also associated with inherited cancer risk in several cancer types[6].

Alterations and prevalence: *PTEN* is frequently altered in cancer by inactivating loss-of-function mutations and by gene deletion. *PTEN* mutations are frequently observed in 50%-60% of uterine cancer[7,8]. Nearly half of somatic mutations in *PTEN* are stop-gain or frameshift mutations that result in truncation of the protein reading frame. Recurrent missense or stop-gain mutations at codons R130, R173, and R233 result in loss of phosphatase activity and inhibition of wild-type *PTEN*[3,9-12]. *PTEN* gene deletion is observed in 15% of prostate cancer, 9% of squamous lung cancer, 9% of glioblastoma, and 1-5% of melanoma, sarcoma, and ovarian cancer[7,8].

Potential relevance: Currently, no therapies are approved for *PTEN* aberrations. However, due to the role of PTEN in HRR, poly(ADPribose) polymerase inhibitors (PARPi) are being explored as a potential therapeutic strategy in PTEN deficient tumors[13,14]. In 2022, the FDA granted fast track designation to the small molecule inhibitor, pidnarulex[15], for *BRCA1/2*, *PALB2*, or other homologous recombination deficiency (HRD) mutations in breast and ovarian cancers.

EGFR **exon 19 deletion (p.Glu746_Ala750del, p.Leu747_Thr751delinsP, p.Leu747_Thr751delinsQ) Coverage Frequency 365, 346, 346**

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[16]. 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[17,18].

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[7,8,19,20]. 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[21]. 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[22-25]. *EGFR* activating mutations in lung cancer tend to be mutually exclusive to *KRAS* activating mutations[26]. 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[21,27]. 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[7,8,20,27,28]. 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[29-31].

Potential relevance: Approved first-generation EGFR tyrosine kinase inhibitors (TKIs) include erlotinib[32] (2004) and gefitinib[33] (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[34] (2013) and dacomitinib[35] (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[36-39]. However, in 2021, the irreversible tyrosine kinase inhibitor, mobocertinib[40] was FDA approved for the treatment of NSCLC with *EGFR* exon 20 insertion mutations. Additionally, in 2022, the FDA granted breakthrough therapy designation Biomarker Descriptions to the irreversible EGFR inhibitors, CLN-081 (TPC-064)[41] and sunvozertinib[42], 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[43]. The primary resistance mutation that emerges following treatment with first-generation TKI is T790M, accounting for 50-60% of resistant cases[21]. Third generation TKIs were developed to maintain sensitivity in the presence of T790M. Osimertinib[44] (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[43]. 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[45]. T790M and C797S can occur in either cis or trans allelic orientation[45]. 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[45]. 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[45,46]. However, C797S occurring in cis conformation with T790M, confers resistance to first- and third-generation TKIs[45]. 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[47], targeting EGFR and MET was approved (2021) for NSCLC tumors harboring *EGFR* exon 20 insertion mutations. CPO301[48] received a fast track designation (2023) from the FDA for EGFR mutations in patients with metastatic NSCLC who are relapsed/refractory or ineligible for EGFR targeting therapy such as 3rd-generation EGFR inhibitors including osimertinib. The Oncoprex immunogene therapy quaratusugene ozeplasmid[49] 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[50] was granted a fast track designation (2020) for the treatment of solid tumors harboring an *EGFR* exon 20 insertion mutation.

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.

Jatinder Kaur, PhD
Head, Molecular Biology & Genomics

Dr. Gulshan Yadav, MD
Head, Pathology

REFERENCES

1. Milella et al. PTEN: Multiple Functions in Human Malignant Tumors. Front Oncol. 2015 Feb 16;5:24. doi: 10.3389/fonc.2015.00024. eCollection 2015. PMID: 25763354
2. Song et al. The functions and regulation of the PTEN tumour suppressor. Nat. Rev. Mol. Cell Biol. 2012 Apr 4;13(5):283-96. PMID: 22473468
3. Chalhoub et al. PTEN and the PI3-kinase pathway in cancer. Annu Rev Pathol. 2009;4:127-50. PMID: 18767981
4. Mansour et al. Loss of PTEN-assisted G2/M checkpoint impedes homologous recombination repair and enhances radio-curability and PARP inhibitor

treatment response in prostate cancer. Sci Rep. 2018 Mar 2;8(1):3947. PMID: 29500400
5. Leslie et al. Inherited PTEN mutations and the prediction of phenotype. Semin. Cell Dev. Biol. 2016 Apr;52:30-8. PMID: 26827793
6. Tan et al. Lifetime cancer risks in individuals with germline PTEN mutations. Clin. Cancer Res. 2012 Jan 15;18(2):400-7. PMID: 22252256
7. Cerami et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov. 2012 May;2(5):401-4. PMID: 22588877
8. Weinstein et al. The Cancer Genome Atlas Pan-Cancer analysis project. Nat. Genet. 2013 Oct;45(10):1113-20. PMID: 24071849
9. Dillon et al. Therapeutic targeting of cancers with loss of PTEN function. Curr Drug Targets. 2014 Jan;15(1):65-79. PMID: 24387334
10. Papa et al. Cancer-associated PTEN mutants act in a dominant-negative manner to suppress PTEN protein function. Cell. 2014 Apr 24;157(3):595-610. PMID: 24766807
11. Kato et al. Functional evaluation of p53 and PTEN gene mutations in gliomas. Clin. Cancer Res. 2000 Oct;6(10):3937-43. PMID: 11051241
12. Han et al. Functional evaluation of PTEN missense mutations using in vitro phosphoinositide phosphatase assay. Cancer Res. 2000 Jun 15;60(12):3147-51. PMID: 10866302
13. Mendes-Pereira et al. Synthetic lethal targeting of PTEN mutant cells with PARP inhibitors. EMBO Mol Med. 2009 Sep;1(6-7):315-22. PMID: 20049735
14. Bian et al. PTEN deficiency sensitizes endometrioid endometrial cancer to compound PARP-PI3K inhibition but not PARP inhibition as monotherapy. Oncogene. 2018 Jan 18;37(3):341-351. PMID: 28945226
15. https://www.senhwabio.com//en/news/20220125
16. King et al. Amplification of a novel v-erbB-related gene in a human mammary carcinoma. Science. 1985 Sep 6;229(4717):974-6. PMID: 2992089
17. Zhixiang. ErbB Receptors and Cancer. Methods Mol. Biol. 2017;1652:3-35. PMID: 28791631
18. Gutierrez et al. HER2: biology, detection, and clinical implications. Arch. Pathol. Lab. Med. 2011 Jan;135(1):55-62. PMID: 21204711
19. Pines et al. Oncogenic mutant forms of EGFR: lessons in signal transduction and targets for cancer therapy. FEBS Lett. 2010 Jun 18;584(12):2699-706. PMID: 20388509
20. Cancer Genome Atlas Research Network. Comprehensive molecular profiling of lung adenocarcinoma. Nature. 2014 Jul 31;511(7511):543-50. doi: 10.1038/nature13385. Epub 2014 Jul 9. PMID: 25079552
21. da et al. EGFR mutations and lung cancer. Annu Rev Pathol. 2011;6:49-69. doi: 10.1146/annurev-pathol-011110-130206. PMID: 20887192
22. Arcila et al. EGFR exon 20 insertion mutations in lung adenocarcinomas: prevalence, molecular heterogeneity, and clinicopathologic characteristics. Mol. Cancer Ther. 2013 Feb;12(2):220-9. PMID: 23371856
23. Kobayashi et al. EGFR Exon 18 Mutations in Lung Cancer: Molecular Predictors of Augmented Sensitivity to Afatinib or Neratinib as Compared with First- or Third-Generation TKIs. Clin Cancer Res. 2015 Dec 1;21(23):5305-13. doi:10.1158/1078-0432.CCR-15-1046. Epub 2015 Jul 23. PMID: 26206867
24. Yasuda et al. Structural, biochemical, and clinical characterization of epidermal growth factor receptor (EGFR) exon 20 insertion mutations in lung cancer. Sci Transl Med. 2013 Dec 18;5(216):216ra177. PMID: 24353160
25. Chiu et al. Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitor Treatment Response in Advanced Lung Adenocarcinomas with G719X/L861Q/S768I Mutations. J Thorac Oncol. 2015 May;10(5):793-9. PMID: 25668120
26. Karachaliou et al. KRAS mutations in lung cancer. Clin Lung Cancer. 2013 May;14(3):205-14. PMID: 23122493
27. Brennan et al. The somatic genomic landscape of glioblastoma. Cell. 2013 Oct 10;155(2):462-77. PMID: 24120142
28. Cancer Genome Atlas Network. Comprehensive genomic characterization of head and neck squamous cell carcinomas. Nature. 2015 Jan 29;517(7536):576-82. PMID: 25631445
29. Mitsudomi et al. Epidermal growth factor receptor in relation to tumor development: EGFR gene and cancer. FEBS J. 2010 Jan;277(2):301-8. PMID: 19922469
30. Gazdar. Activating and resistance mutations of EGFR in non-small-cell lung cancer: role in clinical response to EGFR tyrosine kinase inhibitors. Oncogene. 2009 Aug;28 Suppl 1:S24-31. PMID: 19680293
31. Gan et al. The EGFRvIII variant in glioblastoma multiforme. J Clin Neurosci. 2009 Jun;16(6):748-54. PMID: 19324552
32. https://www.accessdata.fda.gov/drugsatfda_docs/label/2016/021743s025lbl.pdf
33. https://www.accessdata.fda.gov/drugsatfda_docs/label/2021/206995s004lbl.pdf
34. https://www.accessdata.fda.gov/drugsatfda_docs/label/2022/201292s017lbl.pdf
35. https://www.accessdata.fda.gov/drugsatfda_docs/label/2020/211288s003lbl.pdf
36. NCCN Guidelines® - NCCN-Non-Small Cell Lung Cancer [Version 3.2023]
37. Naidoo et al. Epidermal growth factor receptor exon 20 insertions in advanced lung adenocarcinomas: Clinical outcomes and response to erlotinib. Cancer. 2015 Sep 15;121(18):3212-3220. PMID: 26096453
38. Vyse et al. Targeting EGFR exon 20 insertion mutations in non-small cell lung cancer. Signal Transduct Target Ther. 2019;4:5. PMID: 30854234
39. Yi et al. A comparison of epidermal growth factor receptor mutation testing methods in different tissue types in non-small cell lung cancer. Int J Mol Med. 2014 Aug;34(2):464-74. PMID: 24891042
40. https://www.accessdata.fda.gov/drugsatfda_docs/label/2023/215310s003lbl.pdf
41. https://investors.cullinanoncology.com/news-releases/news-release-details/fda-grants-breakthrough-therapy-designationcullinan-oncologys
42. https://www.prnewswire.com/news-releases/fda-grants-breakthrough-therapy-designation-for-dizal-pharmaceuticals-dzd9008-inpatients-with-locally-advanced-or-metastatic-non-small-cell-lung-cancer-harboring-egfr-exon20-insertion-301469692.html
43. Madic et al. EGFR C797S, EGFR T790M and EGFR sensitizing mutations in non-small cell lung cancer revealed by six-color crystal digital PCR. Oncotarget. 2018 Dec 21;9(100):37393-37406. PMID: 30647840
44. https://www.accessdata.fda.gov/drugsatfda_docs/label/2023/208065Orig1s028lbl.pdf
45. Niederst et al. The Allelic Context of the C797S Mutation Acquired upon Treatment with Third-Generation EGFR Inhibitors Impacts Sensitivity to Subsequent Treatment Strategies. Clin. Cancer Res. 2015 Sep 1;21(17):3924-33. PMID: 25964297
46. Wang et al. Lung Adenocarcinoma Harboring EGFR T790M and In Trans C797S Responds to Combination Therapy of First- and Third-Generation EGFR TKIs and Shifts Allelic Configuration at Resistance. J Thorac Oncol. 2017 Nov;12(11):1723-1727. PMID: 28662863
47. https://www.accessdata.fda.gov/drugsatfda_docs/label/2022/761210s002lbl.pdf
48. http://iis.aastocks.com/20230612/10770455-0.PDF

49. https://www.genprex.com/news/genprex-receives-u-s-fda-fast-track-designation-for-gene-therapy-that-targets-lung-cancer/
50. https://investors.blackdiamondtherapeutics.com/news-releases/news-release-details/black-diamond-therapeutics-granted-fasttrack-designation-fda

APPENDIX 1: TEST METHODOLOGY

Method

Circulating cell-free total nucleic acid (cfTNA) were isolated from samples using the MagMAX Cell-Free Total Nucleic Acid Isolation Kit. Quantity and quality is checked by Qubit assay and Tape station, respectively. After quality check the isolated and purified sample was directly loaded on Ion Torrent Genexus Next Generation Sequencer and subjected to automated library preparation and template preparation followed by in-depth sequencing.
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.

- A negative value in liquid biopsy does not mean true absence of mutation. It may not be detectable in the blood sample but may still be positive in tissue biopsy.
- 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*			