ONCOLOGY genpath an OPKO Health Company

OnkoSight™ NGS AML Panel Sequencing Report

Final Report

SAMPLE, PHYSICIAN
ONCOLOGY HOSPITAL
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Patient Name: SAMPLE, PATIENT
DOB: 01/18/1967 **Age:** 54 Y **Sex:** M
Address:

Specimen ID: XXXXXXXXX
Date of Report: 05/10/2021 05:46 PM EDT
Date Collected: 05/05/2021
Date Received: 05/06/2021
Specimen Source: Peripheral Blood

RESULT SUMMARY: ABNORMAL

DETECTED GENOMIC ALTERATIONS:

Tier I: Variants of Strong Clinical Significance

NPM1 p.Trp288Cysfs*12

Tier II: Variants of Potential Clinical Significance

NRAS p.Gln61Arg

KRAS p.Gly13Asp

TUMOR TYPE: Suspected Acute Myeloid Leukemia

CLINICAL INFORMATION:
Reported a clinical history of AML.

PERTINENT NEGATIVE RESULTS:
The following genes are NEGATIVE for clinically relevant mutations. Mutational hotspots and surrounding exonic regions were interrogated for DNA level point mutations and indels (fusions not assayed).
ASXL1, BCOR, DNMT3A, EZH2, FLT3, IDH1, IDH2, KIT, PHF6, PTPN11, RUNX1, TET2, TP53, WT1.

TECHNICAL SUMMARY

Gene	Alteration	AMP Tier	Chr	Pos	Ref	Alt	Coverage	Allele Freq. or Fold Change	cDNA Change	Exon
NPM1	p.Trp288Cysfs*12	I	5	170837543	C	CTCTG	5623	31%	c.860_863dup	11
KRAS	p.Gly13Asp	II	12	25398281	C	T	11390	19%	c.38G>A	2
NRAS	p.Gln61Arg	II	1	115256529	T	C	432	8%	c.182A>G	3

PROGNOSTIC ASSOCIATIONS

Gene	Alteration	Prognostic Association	Disease Association
NPM1	p.Trp288Cysfs*12	Associated with Decreased Risk of Relapse and Increased Overall Survival in the Absence of FLT3-ITD Mutations in Cytogenetically Normal Acute Myeloid Leukemia	Acute Myeloid Leukemia

LEGEND: Likely Response for defined therapy; Unlikely Response for defined therapy; Unknown therapeutic response; Associated with increased survival; Associated with decreased survival; Investigational agent available

James Weisberger M.D.
Laboratory Director

GenPath is a business unit of BioReference Laboratories, Inc.

Electronically signed by Wojciech Swat, PhD, FACMG

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Patient Name: SAMPLE, PATIENT DOB: 01/18/1967 **Specimen ID: XXXXXXXXX**

INTERPRETATION SUMMARY

A hotspot mutation in KRAS (p.Gly13Asp) was detected in this patient's sample.

KRAS mutations have been reported in about 2% of acute myeloid leukemia (AML) cases (24737308; 22417203; 19075190). The overall clinical and therapeutic significance of KRAS mutations in AML remain under investigation, and KRAS mutational status has not been included in risk stratification algorithms for AML (22898602; NCCN Guidelines, Acute Myeloid Leukemia, Version 3.2021). RAS mutations are otherwise recurrent in MDS and MDS/MPN overlap syndromes, among others (WHO Haematopoietic and Lymphoid Tissues, 2017), and the presence of this abnormality in the setting of an AML would raise the possibility of progression from underlying lower grade disease.

A hotspot mutation in NPM1 (p.Trp288Cysfs*12) was detected in this patient's sample.

NPM1 mutations are associated with a more favorable prognosis with decreased risk of relapse and increased overall survival in the absence of FLT3 internal tandem duplication (ITD) in cytogenetically normal acute myeloid leukemia (AML) (27895058; 17957027; NCCN Guidelines, Acute Myeloid Leukemia, Version 3.2021). In addition, a recent study showed that patients with NPM1-mutated AML benefited from high dose daunorubicin treatment (26755712).

A hotspot mutation in NRAS (p.Gln61Arg) was detected in this patient's sample.

Evidence for the clinical significance of NRAS mutations in AML is still emerging, and NRAS mutational status has not been included in risk stratification algorithms (22417203; NCCN Guidelines, Acute Myeloid Leukemia, Version 3.2021). RAS mutations are known to be recurrent in MDS or MDS/MPN conditions (WHO Haematopoietic and Lymphoid Tissues, 2017). The presence of this abnormality in the setting of overt involvement by AML would raise the possibility of progression from underlying lower grade disease.

Clinical and pathologic correlation is required to interpret these findings.

DETAILED GENETIC INTERPRETATION

Alteration	Interpretation
KRAS **p.Gly13Asp** **c.38G>A** **19% allele frequency** **Exon 2**	p.Gly13Asp represents a hotspot missense mutation in exon 2 of KRAS converting the wild type amino acid Glycine, into amino acid Aspartic Acid at residue 13. This mutation leads to impaired GTPase activity and constitutive activation of RAS signaling. Structurally, KRAS p.Gly13Asp, like other KRAS codon 12 and 13 mutations, acts through steric hindrance of the van der Waals bonding between KRAS and GTP-ase activating proteins (GAPs) leading to an alteration in the proper orientation of the catalytic glutamine (Q61). This results in markedly decreased GTP hydrolysis and the persistence of the GTP-bound state of KRAS. As a result, there is incessant activation of a multitude of KRAS-dependent downstream effector pathways (9219684; 21993244). The KRAS gene (Kirsten rat sarcoma viral oncogene homolog) is located on chromosome 12p12.1. The gene is a member of the Ras oncogene family and encodes a GTPase. RAS proteins are involved in regulation of cell proliferation and cell survival pathways. Somatic missense KRAS mutations are most commonly found in exons 2 and 3 (codons 12, 13 and 61), promote constitutive activation of the protein in the GTP-bound state, and result in increased signaling (17384584; 19349489; 18372904; 23622131; 22898602).
NPM1 **p.Trp288Cysfs*12** **c.860_863dup** **31% allele frequency** **Exon 11**	p.Trp288Cysfs*12 represents a frameshift mutation in exon 11 of NPM1. This variation results in a shift of the reading frame and hence a pre-mature stop to the protein coding sequence. This hotspot mutation, also classified as a Type A NPM1 mutation (15659725), targets a tryptophan at hotspot codon position 288 in the C-terminal region of the protein, one of the residues required for nucleolar localization of NPM1, leading to cytoplasmic dislocation of NPM (12450141; 17008539). This variant is most often reported in de novo acute myeloid leukemia (AML). The nucleophosmin (nucleolar phosphoprotein B23, Numatrin), or NPM1 gene, is located on chromosome 5q35.1. The gene encodes a chaperone protein involved in a variety of cellular processes including ribosome biogenesis, cellular proliferation and regulation of p53 pathway.

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Alteration	Interpretation
	Somatic mutations in NPM1 are often frameshift mutations that target nucleolar localization signal (NLS) at the C-terminus of the protein, and result in loss of function and abnormal cytoplasmic localization (15659725; 19569254). NPM1 is used in risk stratification in acute myeloid leukemia (AML) (23146058).
NRAS **p.Gln61Arg** **c.182A>G** **8% allele frequency** **Exon 3**	p.Gln61Arg represents a hotspot missense mutation in exon 3 of NRAS converting the wild type amino acid, Glutamine, into amino acid Arginine at residue 61. This mutation leads to impaired GTPase activity and constitutive activation of RAS signaling. Structurally, NRAS p.Gln61Arg, like other NRAS codon 61 mutations, interferes with the coordination of a water molecule that is required for the hydrolysis of GTP (21993244; 10574788; 20194776) . As a result, GTP-bound NRAS persists and there is constitutive activation of a number of RAS-dependent pathways (21993244). The NRAS gene (neuroblastoma RAS viral (V-Ras) oncogene homolog) is located on chromosome 1p13.2. The gene is a member of the Ras oncogene family and encodes a GTPase. RAS proteins are involved in regulation of cell proliferation and cell survival pathways. Somatic missense NRAS mutations are most commonly found in exons 2 and 3 (codons 12, 13 and 61), promote constitutive activation of the protein in the GTP-bound state, and result in increased signaling (17384584; 16291983; 22898602).

CLINICAL TRIALS

Context	NCTID	Title	Conditions	Location	Sponsor
NPM1	NCT03013998	Study of Biomarker-Based Treatment of Acute Myeloid Leukemia	Previously Untreated Acute Myeloid Leukemia	Multiple locations in United States	Beat AML, LLC

METHODS

Nucleic acid from the submitted specimen was subjected to a PCR-based, Amplicon target enrichment using the Illumina TruSeq Amplicon Assay (San Diego, CA). Coding and non-coding regions of the selected genes were enriched and subsequently sequenced on an Illumina MiSeq instrument (San Diego, CA) with paired end, 186 base pair reads. Following mapping of the read data to the human genome (reference build GRCh37/hg19), single nucleotide variants, insertions and deletions with an allele frequency greater than 5% were detected utilizing a customized bioinformatic analytical pipeline. FLT3 insertions greater than 15 base pairs are detected to a 0.5% allelic burden. Reported variants include known disease associated mutations and unclear variants with little or no literature support. Benign population polymorphisms are not included in the report. The mutation hotspots of the following genes were interrogated by this test: ASXL1, BCOR, DNMT3A, EZH2, FLT3, IDH1, IDH2, KIT, KRAS, NPM1, NRAS, PHF6, PTPN11, RUNX1, TET2, TP53, WT1.

Variant Tier categorization is rendered in accordance with the AMP/ASCO/CAP consensus recommendations (see 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. The Journal of molecular diagnostics : JMD. 2017 Jan;19(1):4-23. doi: 10.1016/j.jmoldx.2016.10.002. PubMed Central PMCID: PMC5707196. PubMed PMID: 927993330).

Onkosight™ was developed and its performance characteristics were determined by GenPath, a division of BioReference Laboratories. This test has not been cleared or approved by the US Food and Drug Administration (FDA). The FDA has determined that such a clearance or approval is not necessary. Pursuant to the requirements of CLIA'88, this laboratory has established and verified the test's accuracy and precision. However, a false positive or false negative result incurred during any phase of the testing cannot be completely excluded. Large insertion/deletion (eg. FLT3-ITD aberrations) may not be detected by this assay due to the limit of sequencing read length and bioinformatics processing. This assay does not detect translocation/gene fusion. This assay does not determine variant causality, or whether a variant is inherited or somatically acquired. These results may be used for clinical or research purposes and therefore should be carefully considered within the context of other clinical and laboratory data. In the absence of an appropriate clinical context, the clinical utility of OnkoSight ™ testing is not clearly defined. The information contained in this report reflects the current interpretation of the findings as of the date of the report, based on the available scientific information. This information, which comes from numerous sources, is subject to change over time in response to future scientific and medical findings and correlations. BioReference Laboratories, Inc. makes no representation or warranty of any kind regarding the accuracy of information provided or contained in these manuscripts, references or other sources of information. If any of the information provided by or contained in the referenced material is later deemed to be inaccurate, this may impact the accuracy of this report and interpretation of the findings.

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REFERENCES

1. Genome Aggregation Database (gnomAD), Cambridge, MA (URL: https://gnomad.broadinstitute.org) [May, 2021]
2. Catalogue of Somatic Mutations in Cancer (COSMIC), (URL: http://cancer.sanger.ac.uk/cosmic) [May, 2021]
3. Li MM, Datto M, Duncavage EJ, Kulkarni S, Lindeman NI, Roy S, Tsimberidou AM, Vnencak-Jones CL, Wolff DJ, Younes A, Nikiforova MN. 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. The Journal of molecular diagnostics : JMD. 2017 Jan;19(1):4-23. doi: 10.1016/j.jmoldx.2016.10.002. PubMed PMID: 27993330. PubMed Central PMCID: PMC5707196.
4. Ward AF, Braun BS, Shannon KM. Targeting oncogenic Ras signaling in hematologic malignancies. Blood. 2012 Oct 25;120(17):3397-406. Epub 2012 Aug 16. doi: 10.1182/blood-2012-05-378596. PubMed PMID: 22898602. PubMed Central PMCID: PMC3482854.
5. Haigis KM, Kendall KR, Wang Y, Cheung A, Haigis MC, Glickman JN, Niwa-Kawakita M, Sweet-Cordero A, Sebolt-Leopold J, Shannon KM, Settleman J, Giovannini M, Jacks T. Differential effects of oncogenic K-Ras and N-Ras on proliferation, differentiation and tumor progression in the colon. Nature genetics. 2008 May;40(5):600-8. Epub 2008 Mar 30. doi: 10.1038/ng.115. PubMed PMID: 18372904. PubMed Central PMCID: PMC2410301.
6. Pylayeva-Gupta Y, Grabocka E, Bar-Sagi D. RAS oncogenes: weaving a tumorigenic web. Nature reviews. Cancer. 2011 Oct 13;11(11):761-74. Epub 2011 Oct 13. doi: 10.1038/nrc3106. PubMed PMID: 21993244. PubMed Central PMCID: PMC3632399.
7. Curtin JA, Fridlyand J, Kageshita T, Patel HN, Busam KJ, Kutzner H, Cho KH, Aiba S, Bröcker EB, LeBoit PE, Pinkel D, Bastian BC. Distinct sets of genetic alterations in melanoma. The New England journal of medicine. 2005 Nov 17;353(20):2135-47. doi: 10.1056/NEJMoa050092. PubMed PMID: 16291983.
8. Falini B, Nicoletti I, Martelli MF, Mecucci C. Acute myeloid leukemia carrying cytoplasmic/mutated nucleophosmin (NPMc+ AML): biologic and clinical features. Blood. 2007 Feb 01;109(3):874-85. Epub 2006 Sep 28. doi: 10.1182/blood-2006-07-012252. PubMed PMID: 17008539.
9. Schubbert S, Shannon K, Bollag G. Hyperactive Ras in developmental disorders and cancer. Nature reviews. Cancer. 2007 Apr;7(4):295-308. doi: 10.1038/nrc2109. PubMed PMID: 17384584.
10. Falini B, Mecucci C, Tiacci E, Alcalay M, Rosati R, Pasqualucci L, La Starza R, Diverio D, Colombo E, Santucci A, Bigerna B, Pacini R, Pucciarini A, Liso A, Vignetti M, Fazi P, Meani N, Pettirossi V, Saglio G, Mandelli F, Lo-Coco F, Pelicci PG, Martelli MF, Cytoplasmic nucleophosmin in acute myelogenous leukemia with a normal karyotype. The New England journal of medicine. 2005 Jan 20;352(3):254-66. doi: 10.1056/NEJMoa041974. PubMed PMID: 15659725.
11. Scheffzek K, Ahmadian MR, Kabsch W, Wiesmüller L, Lautwein A, Schmitz F, Wittinghofer A. The Ras-RasGAP complex: structural basis for GTPase activation and its loss in oncogenic Ras mutants. Science (New York, N.Y.). 1997 Jul 18;277(5324):333-8. doi: 10.1126/science.277.5324.333. PubMed PMID: 9219684.
12. Walker A, Marcucci G. Molecular prognostic factors in cytogenetically normal acute myeloid leukemia. Expert review of hematology. 2012 Oct;5(5):547-58. doi: 10.1586/ehm.12.45. PubMed PMID: 23146058. PubMed Central PMCID: PMC3582378.
13. Riely GJ, Marks J, Pao W. KRAS mutations in non-small cell lung cancer. Proceedings of the American Thoracic Society. 2009 Apr 15;6(2):201-5. doi: 10.1513/pats.200809-107LC. PubMed PMID: 19349489.
14. di Magliano MP, Logsdon CD. Roles for KRAS in pancreatic tumor development and progression. Gastroenterology. 2013 Jun;144(6):1220-9. doi: 10.1053/j.gastro.2013.01.071. PubMed PMID: 23622131. PubMed Central PMCID: PMC3902845.
15. Buhrman G, Holzapfel G, Fetics S, Mattos C. Allosteric modulation of Ras positions Q61 for a direct role in catalysis. Proceedings of the National Academy of Sciences of the United States of America. 2010 Mar 16;107(11):4931-6. Epub 2010 Mar 01. doi: 10.1073/pnas.0912226107. PubMed PMID: 20194776. PubMed Central PMCID: PMC2841912.

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16. Scheidig AJ, Burmester C, Goody RS. The pre-hydrolysis state of p21(ras) in complex with GTP: new insights into the role of water molecules in the GTP hydrolysis reaction of ras-like proteins. Structure (London, England : 1993). 1999 Nov 15;7(11):1311-24. doi: 10.1016/s0969-2126(00)80021-0. PubMed PMID: 10574788.

17. Nishimura Y, Ohkubo T, Furuichi Y, Umekawa H. Tryptophans 286 and 288 in the C-terminal region of protein B23.1 are important for its nucleolar localization. Bioscience, biotechnology, and biochemistry. 2002 Oct;66(10):2239-42. doi: 10.1271/bbb.66.2239. PubMed PMID: 12450141.

18. Rau R, Brown P. Nucleophosmin (NPM1) mutations in adult and childhood acute myeloid leukaemia: towards definition of a new leukaemia entity. Hematological oncology. 2009 Dec;27(4):171-81. doi: 10.1002/hon.904. PubMed PMID: 19569254. PubMed Central PMCID: PMC3069851.

19. Reuter CW, Krauter J, Onono FO, Bunke T, Damm F, Thol F, Wagner K, Göhring G, Schlegelberger B, Heuser M, Ganser A, Morgan MA. Lack of noncanonical RAS mutations in cytogenetically normal acute myeloid leukemia. Annals of hematology. 2014 Jun;93(6):977-82. Epub 2014 Apr 16. doi: 10.1007/s00277-014-2061-9. PubMed PMID: 24737308.

20. Patel JP, Gönen M, Figueroa ME, Fernandez H, Sun Z, Racevskis J, Van Vlierberghe P, Dolgalev I, Thomas S, Aminova O, Huberman K, Cheng J, Viale A, Socci ND, Heguy A, Cherry A, Vance G, Higgins RR, Ketterling RP, Gallagher RE, Litzow M, van den Brink MR, Lazarus HM, Rowe JM, Luger S, Ferrando A, Paietta E, Tallman MS, Melnick A, Abdel-Wahab O, Levine RL. Prognostic relevance of integrated genetic profiling in acute myeloid leukemia. The New England journal of medicine. 2012 Mar 22;366(12):1079-89. Epub 2012 Mar 14. doi: 10.1056/NEJMoa1112304. PubMed PMID: 22417203. PubMed Central PMCID: PMC3545649.

21. Tyner JW, Erickson H, Deininger MW, Willis SG, Eide CA, Levine RL, Heinrich MC, Gattermann N, Gilliland DG, Druker BJ, Loriaux MM. High-throughput sequencing screen reveals novel, transforming RAS mutations in myeloid leukemia patients. Blood. 2009 Feb 19;113(8):1749-55. Epub 2008 Dec 15. doi: 10.1182/blood-2008-04-152157. PubMed PMID: 19075190. PubMed Central PMCID: PMC2647674.

22. NCCN Guidelines, Myelodysplastic Syndromes, Version 3.2021

23. NCCN Guidelines, Acute Myeloid Leukemia, Version 3.2021

24. Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J (Eds): WHO Haematopoietic and Lymphoid Tissues (Revised 4th edition). IARC: Lyon 2017

25. Gale RE, Green C, Allen C, Mead AJ, Burnett AK, Hills RK, Linch DC, The impact of FLT3 internal tandem duplication mutant level, number, size, and interaction with NPM1 mutations in a large cohort of young adult patients with acute myeloid leukemia. Blood. 2008 Mar 01;111(5):2776-84. Epub 2007 Oct 23. doi: 10.1182/blood-2007-08-109090. PubMed PMID: 17957027.

26. Luskin MR, Lee JW, Fernandez HF, Abdel-Wahab O, Bennett JM, Ketterling RP, Lazarus HM, Levine RL, Litzow MR, Paietta EM, Patel JP, Racevskis J, Rowe JM, Tallman MS, Sun Z, Luger SM. Benefit of high-dose daunorubicin in AML induction extends across cytogenetic and molecular groups. Blood. 2016 Mar 24;127(12):1551-8. Epub 2016 Jan 11. doi: 10.1182/blood-2015-07-657403. PubMed PMID: 26755712. PubMed Central PMCID: PMC4807422.

27. Döhner H, Estey E, Grimwade D, Amadori S, Appelbaum FR, Büchner T, Dombret H, Ebert BL, Fenaux P, Larson RA, Levine RL, Lo-Coco F, Naoe T, Niederwieser D, Ossenkoppele GJ, Sanz M, Sierra J, Tallman MS, Tien HF, Wei AH, Löwenberg B, Bloomfield CD. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood. 2017 Jan 26;129(4):424-447. Epub 2016 Nov 28. doi: 10.1182/blood-2016-08-733196. PubMed PMID: 27895058. PubMed Central PMCID: PMC5291965.