Specimen ID:
Control ID:

Acct#: **Phone:**
INTEGRATED ONCOLOGY
201 SUMMIT VIEW DR, STE 100
BRENTWOOD TN 37027

Patient Details
DOB:
Age (yyy/mm/dd):
Gender:
Patient ID:

Specimen Details
Date collected:
Date received:
Date entered:
Date reported: 4/20/2018 8:18 AM

Physician Details
Ordering: Referring:
ID:
NPI:

Specimen Type: Bone Marrow
Clinical Indication: Thrombocytopenia, Leukopenia

SUMMARY

At least one variant of **strong** clinical significance and one variant of **potential** clinical significance were detected.

Gene	Variant Detected	Amino Acid Change	Variant Frequency (%)	Diagnostic Clinical Significance	Prognostic Clinical Significance	Therapeutic Clinical Significance
SRSF2	c.284C>T	p.Pro95Leu	43	Yes	Yes	Yes
TET2	c.3967G>T	p.Glu1323X	48	Yes	Yes	Yes
TET2	c.4933_4955dup23	p.Gln1652HisfsX51	18	Yes	Yes	Yes
TET2	c.3818G>T	p.Cys1273Phe	8	Yes	Yes	Yes

See additional details below

Therapeutic Implications

Gene	Amino Acid Change	FDA Approved Therapies	FDA Approved Therapies for Other Indications	Possible Drug Resistance	Clinical Trials
SRSF2	p.Pro95Leu	None	None	None	None
TET2	p.Glu1323X	None	None	None	None
TET2	p.Gln1652HisfsX51	None	None	None	None
TET2	p.Cys1273Phe	None	None	None	None

INTERPRETATION

SRSF2 c.284C>T (p.P95L) is located in exon 1 of transcript NM_003016.4 for the gene SRSF2 on chromosome 17. Other variants in SRSF2 have been reported to lead to disease by causing a gain-of-function in the encoded protein and alteration of P95 is found in the vast majority of all cases of SRSF2 mutations in MDS. Evidence from an animal model indicates that alteration of codon Pro95 in SRSF2 contribute to a phenotype similar to myelodysplastic syndromes. Functional evidence shows variants in this codon alter SRSF2's normal sequence-specific RNA binding activity, thereby altering recognition of specific exonic splicing enhancer motifs to drive recurrent mis-splicing of key hematopoetic regulators.

The SRSF2 (Serine/Arginine-Rich Splicing Factor 2; OMIM 600813) gene encodes a protein component of spliceosome complex. Mutation of SRSF2 is most commonly seen in CMML (47-51%), often with co-mutated TET2. SRSF2 mutations are also found in 18% of MDS and are associated with leukemic transformation and poor survival. In MPN, SRSF2 mutations are associated with advanced diseases and leukemia transformation as well.

TET2 c.3967G>T (p.E1323X) is located in exon 8 of transcript NM_001127208.2 for the gene TET2 on chromosome 4. Other variants in TET2 have been reported to lead to disease by causing a loss-of-function in the encoded protein. The current variant results in a premature termination codon, predicted to cause a truncation of the encoded protein or absence of the protein due to nonsense mediated decay. Evidence from an animal model indicates that variants in TET2 contribute to a phenotype similar to Chronic myelomonocytic leukemia (CMML)-like disease.

TET2 c.4933_4955dup23 (p.Q1652HfsX51) is located in exon 11 of transcript NM_001127208.2 for the gene TET2 on chromosome 4. Other variants in TET2 have been reported to lead to disease by causing a loss-of-function in the encoded protein. The current variant results in a premature termination codon, predicted to result in a loss of function in the Tet2 protein due to the loss of the substrate binding domain. Evidence from an animal model indicates thatTET2 deficiency contributes to a phenotype similar to Chronic myelomonocytic leukemia (CMML)-like disease.

TET2 c.3818G>T (p.C1273F) is located in exon 7 of transcript NM_001127208.2 for the gene TET2 on chromosome 4. Other variants in TET2 have been reported to lead to disease by causing a loss-of-function in the encoded protein. The current variant results in a non-conservative amino acid change in the encoded protein sequence. Evidence from an animal model indicates that loss of function variants in TET2 contribute to a phenotype similar to Chronic myelomonocytic leukemia (CMML)-like disease.

Diagnostic Significance: Variants in TET2 are highly associated with CMML. They support a diagnosis of CMML in the correct clinicopathologic setting, especially if there is a concurrent **SRSF2** mutation. TET2 mutations are also frequently seen in AML, MPN and MDS, as well as age-related clonal hematopoiesis. Prognostic Significance: Evidence for the prognostic significance of this or similar variants in this gene for MDS is conflicting, with different sources describing a favorable or no prognostic effect. Evidence for the prognostic significance of this or similar variants in this gene for CMML is conflicting, with different sources describing an unfavorable, no, or favorable prognostic effect. Multiple sources, including several clinical studies, suggest that this or similar variants in this gene are an unfavorable prognostic indicator for AML, especially in patients intermediate-risk cytogenetics, especially when it is combined with other adverse molecular markers and in cytogenetically normal patients with mutated CEBPA and/or mutated NPM1 without FLT3 internal tandem duplication [FLT3-ITD]. Therapeutic Significance: Multiple small clinical studies suggest that this or similar variants in this gene indicate responsiveness of MDS to azacitidine and decitabine, especially in patients without clonal ASXL1 mutations. At least one small clinical study suggests that this or similar variants in this gene indicate responsiveness of AML to azacitidine, especially in higher risk patients with low blast count. Based on these data, the variant is considered to be of possible clinical significance.

The TET2 (Ten eleven translocation 2; OMIM 612839) gene encodes the methylcytosine dioxygenase 2 enzyme that regulates DNA methylation. Loss of TET is an early event in hematologic malignancies and is frequently associated with clonal hematopoiesis in elderly population. Inactivation mutations of TET2 are found in a wide spectrum of hematologic malignancies, including about 50% of CMML, up to 25% of MDS and AML, >10% of MPN, >25% of BPDCN, as well as T cell lymphomas and diffuse large B cell lymphoma. TET2 mutations may be associated with response to therapy with hypomethylating agents in the absence of ASXL1 and TP53 mutations.

METHODOLOGY AND LIMITATIONS

IntelliGEN® Myeloid utilizes amplicon-based next generation sequencing to identify alterations in 50 genes that have diagnostic, prognostic, and therapeutic significance in myeloid neoplasms. The sensitivity of this assay is 5-10% variant allele fraction for single nucleotide variants (SNV) and insertion/deletions (InDels). This assay can detect whole-gene copy number alterations (CNAs) of 25% or greater. Mutations outside the targeted regions and gene rearrangements will not be detected. Clinical significance of

Patient: Specimen ID:
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METHODOLOGY AND LIMITATIONS (cont)

variants is classified following a joint consensus recommendation from the AMP, ASCO and CAP. Results should be interpreted in conjunction with clinical and other laboratory findings for the most accurate interpretation.

This test was developed and its performance characteristics determined by LabCorp. It has not been cleared or approved by the Food and Drug Administration.

The following amplicons did not meet the quality requirements of the assay in this specimen. Variants in these regions could not be detected with optimal sensitivity.

Gene	Exon	Chromosome	Start Position	Stop Position
STAG2	9	X	123181329	123181329
STAG2	22	X	123200023	123200044
KDM6A	26	X	44950005	44950053

LIST OF ALL GENES IN PANEL				
ABL1	CUX1	IKZF1	NPM1	SF3B1
ASXL1	DNMT3A	JAK2	NRAS	SMC1A
BCOR	ETV6	JAK3	PDGFRA	SMC3
BCORL1	EZH2	KDM6A	PHF6	**SRSF2**
BRAF	FBXW7	KIT	PML	STAG2
CALR	FLT3	KMT2A	PTEN	**TET2**
CBL	GATA1	KRAS	PTPN11	TP53
CDKN2A	GATA2	MPL	RAD21	U2AF1
CEBPA	IDH1	NF1	RUNX1	WT1
CSF3R	IDH2	NOTCH1	SETBP1	ZRSR2

REFERENCES

1. Arber DA, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016; 127 (20): 2391-405.
2. Bejar R, et al. TET2 mutations predict response to hypomethylating agents in myelodysplastic syndrome patients. Blood. 2014; 124 (17): 2705-12.
3. Chou W, et al. TET2 mutation is an unfavorable prognostic factor in acute myeloid leukemia patients with intermediate-risk cytogenetics. Blood. 2011; 118 (14): 38033810.
4. COSMIC Database. Available at http://cancer.sanger.ac.uk/cosmic Accessed on Oct 10, 2017.
5. DA Arber, et al. The 2016 revision to the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia. Blood. prepub.
6. Damm F, et al. Mutations affecting mRNA splicing define distinct clinical phenotypes and correlate with patient outcome in myelodysplastic syndromes. Blood. 2012; 119 (14): 3211-8.
7. Genetics Home Reference. Available at http://ghr.nlm.nih.gov/gene/SRSF2 Accessed on Oct 10, 2017.
8. Genetics Home Reference. Available at http://ghr.nlm.nih.gov/gene/TET2 Accessed on Oct 10, 2017.
9. Guglielmelli P, et al. Impact of mutational status on outcomes in myelofibrosis patients treated with ruxolitinib in the COMFORT-II study. Blood. 2014; 123 (14): 2157-2160.
10. Hou HA, et al. Splicing factor mutations predict poor prognosis in patients with de novo acute myeloid leukemia. Oncotarget. 2016; 7 (8): 9084-101.
11. Itzykson R KO, et al. Impact of TET2 mutations on response rate to azacitidine in myelodysplastic syndromes and low blast count acute myeloid leukemias. Leukemia. 2011; 25 (7): 1147-52.
12. Jankowska AM, et al. Loss of heterozygosity 4q24 and TET2 mutations associated with myelodysplastic/myeloproliferative

neoplasms. Blood. 2009; 113 (25): 6403-6410.
13. Kohlmann A, et al. Next-Generation Sequencing Technology Reveals a Characteristic Pattern of Molecular Mutations in 72.8% of Chronic Myelomonocytic Leukemia by Detecting Frequent Alterations in TET2, CBL, RAS, and RUNX1. Journal of Clinical Oncology. 2010; 28 (24): 3858-3865.
14. Kosmider O GV, et al. TET2 mutation is an independent favorable prognostic factor in myelodysplastic syndromes (MDSs). Blood. 2009; 114 (15): 3285-91.
15. Kosmider O, et al. TET2 gene mutation is a frequent and adverse event in chronic myelomonocytic leukemia. Haematologica. 2009; 94 (12): 1676-81.
16. Li MM, 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; 19 (1): 4-23.
17. Liu W, et al. Prognostic significance of Tet methylcytosine dioxygenase 2 (TET2) gene mutations in adult patients with acute myeloid leukemia: a meta-analysis. Leukemia & lymphoma. 2014; 55 (12): 26912698.
18. Makishima H, et al. Mutations in the spliceosome machinery, a novel and ubiquitous pathway in leukemogenesis. Blood. 2012; 119 (14): 3203-10.
19. MetzelerKH, et al. TET2mutations improve the new European LeukemiaNet risk classification of acute myeloid leukemia: a Cancer and Leukemia Group B study. J Clin Oncol. 2011; 29 (10): 1373-81.
20. National Comprehensive Cancer Network. Myelodysplastic Syndromes (Version I.2018).https://www.nccn.org/professionals/physician_gls/pdf/mds.pdf. 2017.
21. National Comprehensive Cancer Network. Myeloproliferative Neoplasms (Version 2.2018). 2017.
22. Palomo L, et al. Targeted deep sequencing improves outcome stratification in chronic myelomonocytic leukemia with low risk cytogenetic features. Oncotarget. 2016; 7 (35): 57021-57035.
23. Papaemmanuil E, et al. Clinical and biological implications of driver mutations in myelodysplastic syndromes. Blood. 2013; 122 (22): 3616-27.
24. Papaemmanuil E, et al. Genomic Classification and Prognosis in Acute Myeloid Leukemia. N Engl J Med. 2016; 374 (23): 2209-21.
25. Smith AE, et al. Next-generation sequencing of the TET2 gene in 355 MDS and CMML patients reveals low-abundance mutant clones with early origins, but indicates no definite prognostic value. Blood. 2010; 116 (19): 3923-3932.
26. Thol F, et al. Frequency and prognostic impact of mutations in SRSF2, U2AF1, and ZRSR2 in patients with myelodysplastic syndromes. Blood. 2012; 119 (15): 3578-84.
27. Vannucchi AM, et al. Mutations and prognosis in primary myelofibrosis. Leukemia. 2013; 27 (9): 1861-1869.

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