From www.bloodjournal.org by guest on April 16, 2017. For personal use only.

Brief report

Genetic characterization of TET1, TET2, and TET3 alterations in myeloid malignancies

Omar Abdel-Wahab,[1,2] Ann Mullally,[3,4] Cyrus Hedvat,[5] Guillermo Garcia-Manero,[6] Jay Patel,[1] Martha Wadleigh,[3] Sebastien Malinge,[7] JinJuan Yao,[5] Outi Kilpivaara,[1] Rukhmi Bhat,[7] Kety Huberman,[1] Sabrena Thomas,[1] Igor Dolgalev,[1] Adriana Heguy,[1] Elisabeth Paietta,[8] Michelle M. Le Beau,[9] Miloslav Beran,[6] Martin S. Tallman,[7] Benjamin L. Ebert,[4,10,11] Hagop M. Kantarjian,[6] Richard M. Stone,[3] D. Gary Gilliland,[4,10-12] John D. Crispino,[7] and Ross L. Levine[1,2]

[1]Human Oncology and Pathogenesis Program, and [2]Leukemia Service, Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, NY; [3]Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA; [4]Division of Hematology, Department of Medicine, Brigham and Women's Hospital, Boston, MA; [5]Department of Pathology, Memorial Sloan-Kettering Cancer Center, New York, NY; [6]Department of Leukemia, M. D. Anderson Cancer Center, Houston, TX; [7]Division of Hematology/Oncology, Northwestern University Feinberg School of Medicine, Chicago, IL; [8]Montefiore Medical Center–North Division, New York Medical College, New York; [9]Section of Hematology/Oncology, University of Chicago, IL; [10]Harvard Stem Cell Institute, Boston, MA; [11]Broad Institute of Harvard and Massachusetts Institute of Technology, Boston; and [12]Howard Hughes Medical Institute, Harvard Medical School, Boston, MA

Disease alleles that activate signal transduction are common in myeloid malignancies; however, there are additional unidentified mutations that contribute to myeloid transformation. Based on the recent identification of *TET2* mutations, we evaluated the mutational status of *TET1*, *TET2*, and *TET3* in myeloproliferative neoplasms (MPNs), chronic myelomonocytic leukemia (CMML), and acute myeloid leukemia (AML). Sequencing of *TET2* in 408 paired tumor/normal samples distinguished between 68 somatic mutations and 6 novel single nucleotide polymorphisms and identified *TET2* mutations in MPN (27 of 354, 7.6%), CMML (29 of 69, 42%), AML (11 of 91, 12%), and M7 AML (1 of 28, 3.6%) samples. We did not identify somatic *TET1* or *TET3* mutations or *TET2* promoter hypermethylation in MPNs. *TET2* mutations did not cluster in genetically defined MPN, CMML, or AML subsets but were associated with decreased overall survival in AML (*P* = .029). These data indicate that *TET2* mutations are observed in different myeloid malignancies and may be important in AML prognosis. (Blood. 2009;114: 144-147)

Introduction

Our understanding of the molecular pathogenesis of myeloid malignancies, most notably acute myeloid leukemia (AML) and chronic myeloid leukemia (CML), has largely resulted from the identification and characterization of recurrent chromosomal translocations.[1] However, in many patients with myeloproliferative neoplasms (MPNs) and chronic myelomonocytic leukemia (CMML), recurrent clonal cytogenetic abnormalities are not observed. More recently, DNA resequencing studies of candidate genes,[2] gene families,[3,4] and the cancer genome[5] in MPN, CMML, and AML have identified somatic mutations in *FLT3*,[6] *JAK2*,[7-13] *MPL*,[14,15] and the *RAS* family of oncogenes.[16] These discoveries demonstrate activation of signal transduction pathways is a common pathogenic event in myeloid malignancies and have led to the development of molecularly targeted therapies. However, with the exception of CML, these therapies have yet to substantively improve outcomes for patients with myeloid malignancies.[17,18] This may reflect insufficient target inhibition, or, alternatively, this may indicate incomplete dependence on these activated pathways resulting from the presence of additional somatic mutations with prognostic, therapeutic, and biologic relevance.

The role of *TET* (Ten-Eleven Translocation) family gene members in hematopoietic transformation was thought to be restricted to the involvement of *TET1* as a translocation partner *MLL*-translocated AML, until the recent identification of inactivating mutations in *TET2* in MPN and MDS patients.[19] We therefore sought to evaluate a large set of MPN, CMML, and AML samples for somatic *TET2* alterations. We sequenced all coding exons of *TET2* in 408 paired tumor/normal samples and then assessed the frequency of somatic *TET2* mutations in 606 patients with MPN, CMML, and AML. We also investigated whether deletion or epigenetic inactivation of *TET2* are observed in MPN and evaluated MPN patients for somatic mutations in *TET1* and *TET3*.

Methods

Patients

DNA was isolated from peripheral blood and/or bone marrow from 606 MPN, CMML, and AML samples. Matched normal DNA was available for 408 samples, including 354 sporadic MPN samples, 26 CMML samples, and 28 affected members of MPN pedigrees. Blood/bone marrow DNA but not matched normal DNA was available for 198 samples, including 3 sporadic MPN samples, 20 affected members of MPN kindreds, 96 AML samples, 45 CMML samples, and 34 M7 AML samples (16 from the Eastern Cooperative Oncology Group). Approval was obtained from the institutional review boards at the Dana-Farber Cancer Institute and at

Submitted March 10, 2009; accepted April 27, 2009. Prepublished online as *Blood* First Edition paper, May 6, 2009; DOI 10.1182/blood-2009-03-210039.

The online version of this article contains a data supplement.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 USC section 1734.

© 2009 by The American Society of Hematology

From www.bloodjournal.org by guest on April 16, 2017. For personal use only.

Memorial Sloan-Kettering Cancer Center for these studies, and informed consent was provided according to the Declaration of Helsinki.

Sequence analysis of TET1, TET2, and TET3

DNA resequencing of all coding exons of *TET1-3* was performed (primers/conditions are listed in supplemental Table 1, available on the *Blood* website; see the Supplemental Materials link at the top of the online article). Nonsynonymous alterations not present in single nucleotide polymorphism (database [db]SNP) were annotated as somatic mutations or SNPs based on sequence analysis of matched germ line DNA. Nonsynonymous alterations not in dbSNP nor determined to be somatic in paired samples or in recently reported data[19] were censored. All somatic mutations were validated by resequencing nonamplified DNA.

Copy number analysis of TET1, TET2, and TET3

A total of 207 MPN tumor samples were analyzed using Affymetrix 250K StyI Arrays.[20] The *JAK2V617F*-mutant AML cell lines HEL and SET2 were analyzed using Affymetrix 6.0 SNP Arrays.

Methylation-specific polymerase chain reaction

Methylation of 2 CpG islands in the promoter region of *TET2* was assessed in 37 MPN patients and 4 *JAK2V617F*-positive leukemia cell lines (SET2, MBO2, HEL, UKE1). Methylation-specific polymerase chain reaction was performed as previously described (primers are listed in supplemental Table 1).[21]

Statistics

Statistical analyses were performed using MedCalc (MedCalc).

Results and discussion

Sequence analysis of all coding exons of *TET2* in 408 paired tumor/normal samples identified 8 frameshift, 12 nonsense, and 37 nonsynonymous alterations not present in dbSNP. Analysis of germ line DNA distinguished between 31 somatic missense mutations and 6 unannotated SNPs (Table 1; supplemental Figure 1); all unannotated SNPs were observed in matched normal tissue in at least 2 samples. All frameshift and nonsense mutations were not present in matched normal tissue. The strategy of paired sequencing of normal and tumor tissue is critical for accurate annotation of candidate mutations as 2 novel SNPs, which were recently reported as *TET2* mutations (Q1084P and Y867H)[22] were present in the germ line in multiple patient samples consistent with their being unannotated SNPs. After defining the spectrum of somatic *TET2* mutations in paired tumor/normal samples, we determined the frequency of *TET2* mutations in MPN (7.6%, including 9.8% polycythemia vera, 4.4% essential thrombocythemia, and 7.7% primary myelofibrosis), CMML (42.1%), AML (12.1%), and acute megakaryoblastic leukemia (3.6%). We identified biallelic/homozygous TET2 mutations in 1 of 354 MPN patients and in 7 of 69 CMML patients ($P < .001$, Fisher exact test). Sequencing of *TET2* in 48 affected members from 28 MPN kindreds identified somatic *TET2* mutations in 7 affected persons. We also identified 4 germ line nonsynonymous variants in affected members of MPD kindreds present in dbSNP that could represent rare familial MPN alleles. However, 3 of these 4 SNPs were observed in only some affected members of kindred but not others, and the fourth variant (M1701I) is observed in many sporadic MPN, CMML, and AML cases. Somatic *TET2* mutations were not noted in the 4 *JAK2V617F*-positive leukemic cell lines.

Table 1. Novel TET2 somatic missense mutations and unannotated SNPs in 4q24

Alteration	Nucleotide change	Genomic coordinate	Amino acid change
Somatic mutation	434G→A	106374983	S145N
Somatic mutation	935A→G	106375484	N312S
Somatic mutation	1379C→T	106375928	S460F
Somatic mutation	1997A→G	106376546	D666G
Somatic mutation	2821C→T	106377370	P941S
Somatic mutation	3403G→A	106377953	C1135Y
Somatic mutation	3575T→G	106383519	C1194W
Somatic mutation	3609A→T	106384192	S1204C
Somatic mutation	3639C→T	106384222	R1214W
Somatic mutation	3724A→T	106384307	D1242V*
Somatic mutation	3733A→C	106384316	Y1245S
Somatic mutation	3780C→T	106384363	R1261C
Somatic mutation	3781G→A	106384364	R1261H*
Somatic mutation	3862G→T	106400285	G1289V
Somatic mutation	4074C→T	106410247	R1358C*
Somatic mutation	4080G→C	106410253	G1360R
Somatic mutation	4248G→T	106413237	V1417F
Somatic mutation	5151G→T	106416269	V1718L*
Somatic mutation	5268C→G	106416386	H1757D
Somatic mutation	5283A→T	106416401	Q1828L
Somatic mutation	5430T→C	106416548	C1811R
Somatic mutation	5617T→C	106416735	I1873T*
Somatic mutation	5641A→G	106416759	H1881R*
Somatic mutation	5698T→C	106416816	V1900A
Somatic mutation	5754C→T	106416873	A1919V
Somatic mutation	5776G→A	106416894	R1926H
Somatic mutation	5780G→A	106416898	R1927K
Somatic mutation	5820C→T	106416938	P1941S
Somatic mutation	5896G→A	106417014	R1966H
Somatic mutation	5920C→T	106417038	R1974M
Somatic mutation	5998G→A	106417116	R2000K
SNP	100C→T	106374649	L34F
SNP	520C→A	106375069	P174H
SNP	2599T→C	106377148	Y867H
SNP	3418A→T	106377767	E1073V
SNP	3451A→C	106377800	Q1084P
SNP	5166C→T	106416284	P1723S

Novel unannotated SNPs in 4q24. SNPs were defined as missense mutations that were seen in more than one tumor and paired buccal sample.

*Somatic missense mutations that occurred in more than 2 samples.

We did not identify methylation at either of 2 CpG islands of the *TET2* promoter in 37 MPN samples or in 4 *JAK2V617F*-positive leukemic cell lines (supplemental Figure 2). Copy number analysis of 207 MPN patients identified 3 patients with heterozygous deletions of one copy of the region containing *TET2* (4q24), suggesting that TET2 mutations are more common than large deletions in MPN patients. Sequencing data from these 3 patients revealed that one patient had a homozygous somatic missense mutation, consistent with heterozygous mutation followed by deletion of the remaining copy of *TET2* (supplemental Figure 3). The HEL cell line had a heterozygous deletion of the *TET2* locus. One MPN patient had a large deletion on chromosome 10, which included the *TET1* locus (10q21.3). Furthermore, although we identified several novel SNPs in *TET1* and *TET3* (supplemental Table 3), we did not identify somatic *TET1* or *TET3* mutations in 96 MPN patients. No MPN samples or cell lines had loss of the TET3 locus (2p13.1) or amplifications of *TET1*, *TET2*, and *TET3*.

The frequency of *TET2* mutations did not differ between *JAK2V617F*-positive (16.4%) and *JAK2V617F*-negative (2.5%) MPN ($P = .08$, Fisher exact test). Likewise, *TET2* mutations were equally frequent in MPN patients with and without the recently

From www.bloodjournal.org by guest on April 16, 2017. For personal use only.

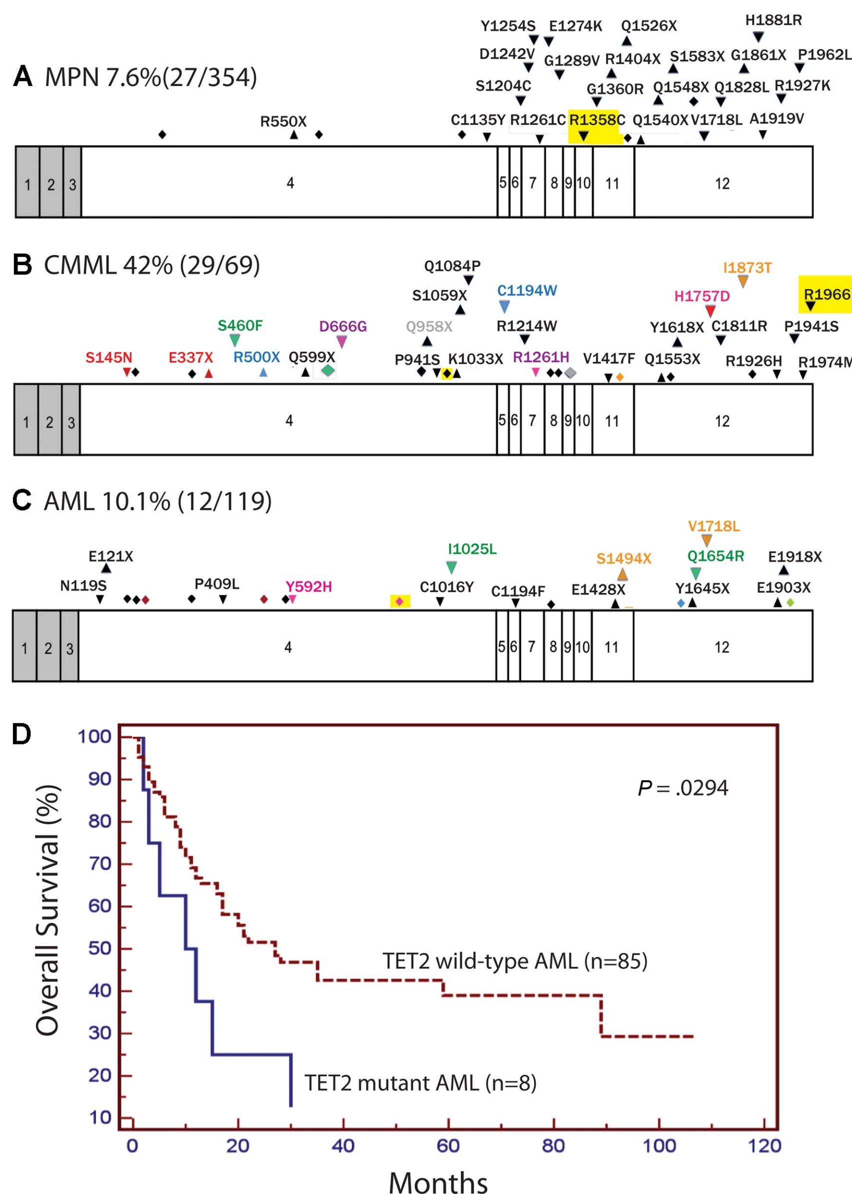

Figure 1. Exons of TET2 with locations of mutations and effect of mutations on overall survival in AML. Locations of mutations in MPN (A), CMML (B), and AML/acute megakaryoblastic leukemia samples (C) as well Kaplan-Meier estimates of overall survival in AML (D) are shown. Shaded regions represent non–protein-coding exons and introns are not shown. Exons are drawn to relative scale. Missense mutations (down arrowheads), nonsense mutations (up arrowheads), and frameshifts (diamonds) are shown at their approximate location along the exon. Mutations occurring within the same patient sample are represented in the same color. Mutations that were homozygous are highlighted in yellow.

described *JAK2V617F*-positive *MPN* predisposition haplotype ($P = .9$).[20] We did not note a correlation between *TET2* alterations and mutations in *FLT3*, *JAK2*, and *RAS* in CMML, nor did we observe a correlation between *TET2* mutations and specific cytogenetic subgroups *MLL*, *FLT3*, *CEBPA*, or *NPM1* mutations, or a history of antecedent MPN/MDS in AML ($P > .5$, Fisher exact test). However, we did note that *TET2* mutations are associated with decreased overall survival in AML compared with *TET2*-wild-type AML patients ($P = .03$, Figure 1D; supplemental Table 2).

In this report, we sequenced all coding exons of *TET2* to define the spectrum of somatic *TET2* mutations in myeloid malignancies. The broad range of myeloid disorders linked to mutations in *TET2* suggests that mutations in *TET2* have a pleiotropic role in myeloid transformation. Although our data suggest that *TET2* mutations may hold prognostic significance in AML, larger clinical correlative studies will be needed to more accurately assess the effect of *TET2* mutations on prognosis, diagnosis, and therapeutic relevance to myeloid malignancies. Whether *TET2* mutations dysregulate pathways already known to contribute to hematopoietic transformation, or represent a novel pathway, remains to be elucidated, and the role of *TET* family alterations in neoplasms other than myeloid malignancies is not yet known.

Acknowledgments

The authors thank the patients who have contributed to our understanding of these disorders.

This work was supported by the National Institutes of Health (grant CA10863101, G.G.-M., H.M.K., and D.G.G.; HL082677, R.L.L.), the Howard Hughes Medical Institute, the Starr Cancer Consortium (New York, NY), the Myeloproliferative Disorders Foundation (Chicago, IL), the Doris Duke Charitable Foundation (New York, NY), and the ECOG Leukemia Tissue Bank (Boston, MA; CA021115 and CA114737). O.A.-W. is supported by a Dana Fellowship from the Memorial Sloan Kettering Clinical Scholars Program. O.K. is supported by a grant from the Academy of Finland (Helsinki, Finland). D.G.G. is an Investigator of the Howard Hughes Medical Institute and is a Doris Duke Charitable Foundation Distinguished Clinical Scientist. R.L.L. is an Early

From www.bloodjournal.org by guest on April 16, 2017. For personal use only.

Career Award recipient of the Howard Hughes Medical Institute, a Clinical Scientist Development Award recipient of the Doris Duke Charitable Foundation, and the Geoffrey Beene Junior Chair at Memorial Sloan Kettering Cancer Center. J.D.C. is a Scholar of the Leukemia & Lymphoma Society (White Plains, NY).

Authorship

Contribution: O.A.-W., A.M., J.P., D.G.G., J.D.C., and R.L.L. designed the study; C.H., G.G.-M., M.W., S.M., J.Y., R.B., E.P., M.M.L.B., M.B., M.S.T., H.M.K., and R.L.L. collected and processed samples and provided genetic and clinical annotation; O.A.-W., J.P., K.H., S.T., I.D., A.H., and R.L.L. performed sequence analysis, analyzed sequence traces, and validated mutations; O.A.-W., B.L.E., R.M.S., and R.L.L. acquired and analyzed SNP array data; O.A.-W., J.P., and O.K. performed methylation-specific polymerase chain reaction; O.A.-W. and R.L.L. wrote the manuscript with assistance from A.M., C.H., and J.D.C.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

Correspondence: Ross L. Levine, Human Oncology and Pathogenesis Program, Memorial Sloan Kettering Cancer Center, 1275 York Ave, Box 20, New York, NY 10065; e-mail: leviner@mskcc.org.

References

1. Fröhling S, Scholl C, Gilliland DG, Levine RL. Genetics of myeloid malignancies: pathogenetic and clinical implications. *J Clin Oncol.* 2005;23:6285-6295.
2. Ley TJ, Minx PJ, Walter MJ, et al. A pilot study of high-throughput, sequence-based mutational profiling of primary human acute myeloid leukemia cell genomes. *Proc Natl Acad Sci U S A.* 2003; 100:14275-14280.
3. Loriaux MM, Levine RL, Tyner JW, et al. High-throughput sequence analysis of the tyrosine kinome in acute myeloid leukemia. *Blood.* 2008; 111:4788-4796.
4. Tomasson MH, Xiang Z, Walgren R, et al. Somatic mutations and germline sequence variants in the expressed tyrosine kinase genes of patients with de novo acute myeloid leukemia. *Blood.* 2008;111:4797-4808.
5. Ley TJ, Mardis ER, Ding L, et al. DNA sequencing of a cytogenetically normal acute myeloid leukaemia genome. *Nature.* 2008;456:66-72.
6. Kiyoi H, Naoe T, Yokota S, et al. Internal tandem duplication of FLT3 associated with leukocytosis in acute promyelocytic leukemia: Leukemia Study Group of the Ministry of Health and Welfare (Kohseisho). *Leukemia.* 1997;11:1447-1452.
7. James C, Ugo V, Le Couedic JP, et al. A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera. *Nature.* 2005; 434:1144-1148.
8. Baxter EJ, Scott LM, Campbell PJ, et al. Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. *Lancet.* 2005;365: 1054-1061.
9. Kralovics R, Passamonti F, Buser AS, et al. A gain-of-function mutation of JAK2 in myeloproliferative disorders. *N Engl J Med.* 2005;352: 1779-1790.
10. Zhao R, Xing S, Li Z, et al. Identification of an acquired JAK2 mutation in polycythemia vera. *J Biol Chem.* 2005;280:22788-22792.
11. Levine RL, Loriaux M, Huntly BJ, et al. The JAK2V617F activating mutation occurs in chronic myelomonocytic leukemia and acute myeloid leukemia, but not in acute lymphoblastic leukemia or chronic lymphocytic leukemia. *Blood.* 2005;106: 3377-3379.
12. Levine RL, Wadleigh M, Cools J, et al. Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis. *Cancer Cell.* 2005;7:387-397.
13. Scott LM, Scott MA, Campbell PJ, Green AR. Progenitors homozygous for the V617F mutation occur in most patients with polycythemia vera, but not essential thrombocythemia. *Blood.* 2006; 108:2435-2437.
14. Pardanani AD, Levine RL, Lasho T, et al. MPL515 mutations in myeloproliferative and other myeloid disorders: a study of 1182 patients. *Blood.* 2006; 108:3472-3476.
15. Pikman Y, Lee BH, Mercher T, et al. MPLW515L is a novel somatic activating mutation in myelofibrosis with myeloid metaplasia. *PLoS Med.* 2006; 3:e270.
16. Murray MJ, Cunningham JM, Parada LF, Dautry F, Lebowitz P, Weinberg RA. The HL-60 transforming sequence: a ras oncogene coexisting with altered myc genes in hematopoietic tumors. *Cell.* 1983;33:749-757.
17. Verstovek S, Kantarjian H, Pardanani A, et al. A phase I/II study of ICNB018424, an oral, selective JAK inhibitor, in patients with primary myelofibrosis and postPV/ET myelofibrosis. *Am Soc Clin Oncol Annual Meeting.* 2008:abstract 7004.
18. Stone RM, DeAngelo DJ, Klimek V, et al. Patients with acute myeloid leukemia and an activating mutation in FLT3 respond to a small-molecule FLT3 tyrosine kinase inhibitor, PKC412. *Blood.* 2005;105:54-60.
19. Delhommeau F, Dupont S, James C, et al. TET2 is a novel tumor suppressor gene inactivated in myeloproliferative neoplasms: identification of a pre-JAK2 V617F event [abstract]. *Blood.* 2008; 112:1-3.
20. Kilpivaara O, Mukherjee S, Schram AM, et al. Genome-wide analysis of myeloproliferative neoplasms (MPN) identifies a germline JAK2 SNP that is associated with predisposition to the development of JAK2V617F-positive MPN and modifier loci that influence MPN phenotype. *Nat Genet.* 2009;41:455-459.
21. Herman JG, Graff JR, Myohanen S, Nelkin BD, Baylin SB. Methylation-specific PCR: a novel PCR assay for methylation status of CpG islands. *Proc Natl Acad Sci U S A.* 1996;93:9821-9826.
22. Jankowska AM, Szpurka H, Tiu RV, et al. Loss of heterozygosity 4q24 and TET2 mutations associated with myelodysplastic/myeloproliferative neoplasms. *Blood.* 2009 Apr 16. [Epub ahead of print]

From www.bloodjournal.org by guest on April 16, 2017. For personal use only.

blood

2009 114: 144-147
doi:10.1182/blood-2009-03-210039 originally published online May 6, 2009

Genetic characterization of TET1, TET2, and TET3 alterations in myeloid malignancies

Omar Abdel-Wahab, Ann Mullally, Cyrus Hedvat, Guillermo Garcia-Manero, Jay Patel, Martha Wadleigh, Sebastien Malinge, JinJuan Yao, Outi Kilpivaara, Rukhmi Bhat, Kety Huberman, Sabrena Thomas, Igor Dolgalev, Adriana Heguy, Elisabeth Paietta, Michelle M. Le Beau, Miloslav Beran, Martin S. Tallman, Benjamin L. Ebert, Hagop M. Kantarjian, Richard M. Stone, D. Gary Gilliland, John D. Crispino and Ross L. Levine

---

Updated information and services can be found at:
http://www.bloodjournal.org/content/114/1/144.full.html

Articles on similar topics can be found in the following Blood collections
Brief Reports (1931 articles)
Myeloid Neoplasia (1656 articles)

Information about reproducing this article in parts or in its entirety may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#repub_requests

Information about ordering reprints may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#reprints

Information about subscriptions and ASH membership may be found online at:
http://www.bloodjournal.org/site/subscriptions/index.xhtml

Blood (print ISSN 0006-4971, online ISSN 1528-0020), is published weekly by the American Society of Hematology, 2021 L St, NW, Suite 900, Washington DC 20036.
Copyright 2011 by The American Society of Hematology; all rights reserved.