ORIGINAL ARTICLE

Mutation in *TET2* in Myeloid Cancers

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ABSTRACT

BACKGROUND

The myelodysplastic syndromes and myeloproliferative disorders are associated with deregulated production of myeloid cells. The mechanisms underlying these disorders are not well defined.

METHODS

We conducted a combination of molecular, cytogenetic, comparative-genomic-hybridization, and single-nucleotide–polymorphism analyses to identify a candidate tumor-suppressor gene common to patients with myelodysplastic syndromes, myeloproliferative disorders, and acute myeloid leukemia (AML). The coding sequence of this gene, *TET2*, was determined in 320 patients. We analyzed the consequences of deletions or mutations in *TET2* with the use of in vitro clonal assays and transplantation of human tumor cells into mice.

RESULTS

We initially identified deletions or mutations in *TET2* in three patients with myelodysplastic syndromes, in three of five patients with myeloproliferative disorders, in two patients with primary AML, and in one patient with secondary AML. We selected the six patients with myelodysplastic syndromes or AML because they carried acquired rearrangements on chromosome 4q24; we selected the five patients with myeloproliferative disorders because they carried a dominant clone in hematopoietic progenitor cells that was positive for the V617F mutation in the Janus kinase 2 (*JAK2*) gene. *TET2* defects were observed in 15 of 81 patients with myelodysplastic syndromes (19%), in 24 of 198 patients with myeloproliferative disorders (12%) (with or without the *JAK2* V617F mutation), in 5 of 21 patients with secondary AML (24%), and in 2 of 9 patients with chronic myelomonocytic leukemia (22%). *TET2* defects were present in hematopoietic stem cells and preceded the *JAK2* V617F mutation in the five samples from patients with myeloproliferative disorders that we analyzed.

CONCLUSIONS

Somatic mutations in *TET2* occur in about 15% of patients with various myeloid cancers.

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N Engl J Med 2009;360:2289-301.
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The myelodysplastic syndromes and myeloproliferative disorders are clonal disorders that arise in hematopoietic stem cells,[1] whereas acute myeloid leukemia (AML) can arise from stem cells or more mature progenitor cells.[2] The main feature of the heterogeneous subtypes of the myelodysplastic syndromes is ineffective hematopoiesis that affects one or several lineages.[3] In about a third of patients, the disorder progresses to AML. Some of the molecular abnormalities that are associated with specific subtypes of myelodysplastic syndromes[4] or the transformation of myelodysplastic syndromes to AML[5] have been identified, but the basis of these related syndromes remains poorly understood.[6]

The myeloproliferative disorders, especially polycythemia vera and essential thrombocythemia, are frequently associated with a somatic mutation, V617F, in the Janus kinase 2 (*JAK2*) gene, which results in a constitutively active Janus protein kinase.[7-9] In mouse models, the presence of *JAK2* V617F alone can cause myeloproliferative disorders but does not give hematopoietic stem cells a proliferative advantage.[10-12] Recent evidence indicates that *JAK2* V617F does not account for all the pathophysiological effects of the myeloproliferative disorders.[13-16] For example, *JAK2* V617F was absent in some patients in whom a *JAK2* V617F–positive myeloproliferative disorder progressed to AML.[17]

We hypothesized that an early genetic event in a hematopoietic stem cell could be common to the myelodysplastic syndromes, myeloproliferative disorders, and AML. By using various genetic approaches, we identified a tumor-suppressor gene, *TET2*, that is deleted or mutated in patients with various myeloid cancers.

Figure 1 (facing page). Structural DNA Rearrangements and Mutations in *TET2* on Chromosome 4q24 in Subgroups of Patients.

In Panel A, a minimally deleted region on chromosome 4q24 is seen in subgroups of patients with myelodysplastic syndromes and acute myeloid leukemia (AML). This region was defined with the use of RP11-1061M121, RP11-356L5, and RP11-16G16 bacterial artificial chromosomes (represented by horizontal lines with double arrows) in fluorescence in situ hybridization. The region encompasses a unique gene, *TET2*. In Panel B, acquired abnormalities on chromosome 4q24 are shown for three of five patients with myeloproliferative disorders who carried the *JAK2* V617F mutation. The abnormalities were detected with the use of high-resolution single-nucleotide-polymorphism (SNP) and comparative-genomic-hybridization (CGH) arrays. The blue bars indicate the regions affected by loss of heterozygosity (LOH). Patient MPD05 had a smaller LOH because of an acquired 325-kb deletion. *TET2* was the only open reading frame observed in this region. In Panel C, nucleotide sequences of DNA from bone marrow (BM) samples from patients with AML and myelodysplastic syndromes are compared with wild-type and nontumor samples from the same patients (when available). The presence of wild-type nucleotide sequences in the samples was attributed to residual normal hematopoietic cells. Matched samples consisted of bone marrow cells obtained after transplantation, during a period of clinical health (Patient nAML1); B type lymphoid cells transformed by Epstein–Barr virus (Patient nAML2); and peripheral-blood mononuclear cells stimulated by a phorbol ester (Patient MDS03). In Panel D, wild-type *TET2* sequences are compared with *TET2* sequences in tumor cells and matched mononuclear cells from Patients MPD01 and MPD04. The dashed vertical lines frame the nine-nucleotide deletion shown for Patient MPD04. The arrow indicates the presence of a small, residual peak of wild-type allele in mononuclear cells from Patient MPD01. In Panels C and D, asterisks indicate nucleotide changes. In Panel E, alignment of the conserved region spanning amino acids 1842 through 1921 of human TET2 protein is shown. Invariant amino acids (identical in all species) are marked by a star, with conserved amino acids indicated by a double dot under the alignment. An arrow points from the amino acids targeted by mutational events in myeloid cancers to the resulting amino acid. In Panel F, the TET protein family is shown with the location of acquired missense mutations. The arrowhead indicates the fusion point of mixed-lineage leukemia (MLL) protein on TET1. The conserved regions that are shared by all TET family proteins appear as shaded boxes. DM denotes *Drosophila melanogaster*, Fugu *Takifugu rubripes*, Hs *Homo sapiens*, and Mm *Mus musculus*.

METHODS

PATIENTS

Table 1 in the Supplementary Appendix (available with the full text of this article at NEJM.org) lists the clinical features of the patients, whose conditions were diagnosed with the use of standard international criteria.[18-20] Samples of bone marrow or blood were obtained from 320 patients (84 with myelodysplastic syndromes, 203 with myeloproliferative disorders, 2 with primary AML, 22 with secondary AML, and 9 with chronic myelomonocytic leukemia) after the patients provided written informed consent and the local research ethics committees (at Hôtel-Dieu and Cochin hospitals) gave approval.

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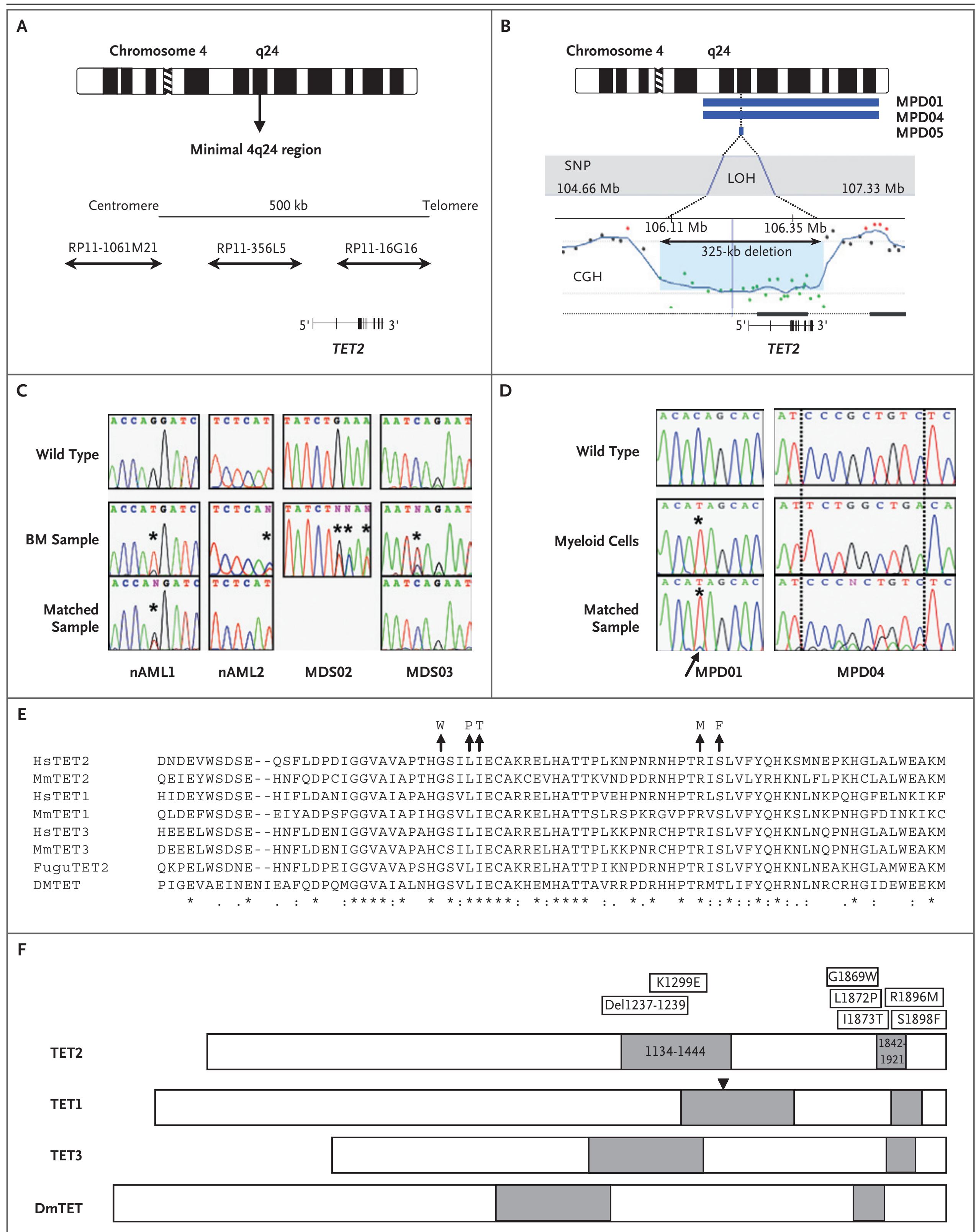

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Table 1. Clinical and Molecular Data for Patients with *TET2* Mutations.*

Patient Number	Disease	*JAK2* and *MPL* Status†	*TET2* Defect	Status of the *TET2* Locus‡	Acquired
Myelodysplastic syndromes					
nAML1	Primary AML (FAB M4)		Del/p.Arg1896Met	1 Copy	Yes/yes
nAML2	Primary AML (FAB M4)		Del/p.Ile1873Thr	1 Copy	Yes/yes
sAML1	Secondary AML		Del/wt	1 Copy	Yes/ND
MDS01	Refractory anemia		Del/del	Loss	Yes
MDS02	RAEB2		Del/p.Arg581fs	1 Copy	Yes/ND
MDS03	RAEB1		Del/p.Gln1624X	1 Copy	Yes
sAML2	Secondary AML		p.Ser1898Phe	ND	Yes
sAML4	Secondary AML after RARS		p.Pro869fs	ND	ND
sAML5	Secondary AML		p.Gln1834fs	ND	ND
sAML6	Secondary AML		p.[Cys1633fs]+[Gln891X]	ND	ND
sAML7	Secondary AML after RARS		p.Gln943X	ND	ND
MDS04	Refractory anemia		p.[Lys1299Glu]+[Arg544X]	ND	ND
MDS07	Refractory anemia		No amplification of 5′ of Ex11	ND	ND
MDS30	Refractory anemia		p.[Gln764fs]+[Arg1516X]	ND	ND
MDS09	RARS-T		p.His658fs	ND	ND
MDS35	RARS		p.[Ile1175Val]+[Tyr1255X]	ND	ND
MDS10	RCMD-RS		p.[Gln321X]+[Tyr1724X]	ND	ND
MDS28	RCMD-RS		p.Glu711fs	ND	ND
MDS18	RAEB1		p.[Glu692fs]+[Met1656fs]	ND	ND
MDS27	RAEB1		p.[Gln530fs]+[Lys1090fs]	ND	ND
MDS33	RAEB1		p.[Glu1137fs]+[Ile1873Thr]	ND	ND
MDS39	RAEB1		p.Leu1872Pro	ND	ND
MDS40	RAEB1		P.Tyr1560fs	ND	ND
MDS42	RAEB1		p.[Leu1872Pro]+[Ile1873Thr]	ND	ND
MDS34	RAEB2		g.4366-1G→T Mutation of splice acceptor site Ex5	1 Copy	ND
MDS41	RAEB2		p.[Gln942X]+[Thr1883fs]	ND	ND
CMML01	Chronic myelomonocytic leukemia		p.Gln685X	ND	ND
CMML02	Chronic myelomonocytic leukemia		p.[Glu846fs]+[Arg1067X]	ND	ND
Myeloproliferative disorders					
MPD01	Primary myelofibrosis	*JAK2* V617F	p.Gln557X	LOH (2 copies)	Yes
MPD04	Polycythemia vera	*JAK2* V617F	p.Pro1237_Ser1239del	LOH (2 copies)	Yes
MPD05	Polycythemia vera	*JAK2* V617F	Del/wt	LOH (1 copy)	Yes
MPD18	Polycythemia vera	*JAK2* V617F	p.Arg1216X	ND	ND
MPD20	Polycythemia vera	*JAK2* V617F	p.Pro1540fs	2 Copies	Yes
MPD35	Essential thrombocythemia	*JAK2* V617F	p.Ser354X	2 Copies	Yes
MPD43	Essential thrombocythemia and subsequent myelofibrosis	*JAK2* V617F	p.[Arg550fs]+[Asn857fs]	2 Copies	Yes
MPD45	Polycythemia vera	*JAK2* V617F	p.Pro763fs	ND	ND
MPD69	Polycythemia vera	*JAK2* V617F	p.[Cys1273fs]+[Pro1617fs]	ND	ND
MPD74	Primary myelofibrosis	WT	p.Thr1086fs	ND	ND

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Table 1. (Continued.)

Patient Number	Disease	*JAK2* and *MPL* Status†	*TET2* Defect	Status of the *TET2* Locus‡	Acquired
MPD81	Essential thrombocythemia	*JAK2* V617F	p.Ile252fs	ND	ND
MPD86	Polycythemia vera	*JAK2* V617F	p.[Val1180fs]+[Arg1404X]	ND	ND
MPD89	Polycythemia vera	*JAK2* V617F	p.Lys1422fs	ND	ND
MPD92	Primary myelofibrosis	*JAK2* V617F	p.Arg1302Gly	ND	ND
MPD96	Essential thrombocythemia	*JAK2* V617F	p.Trp1847X	ND	Yes
MPD99	Essential thrombocythemia	*JAK2* V617F	p.Leu676fs	ND	Yes
MPD120	Polycythemia vera	*JAK2* V617F	p.Ser460fs	ND	ND
MPD130	Essential thrombocythemia	*JAK2* V617F	p.Gly614fs	ND	ND
MPD132	Polycythemia vera	*JAK2* V617F	p.Cys1135fs	ND	ND
MPD133	Essential thrombocythemia	*JAK2* V617F	p.Gly1869Trp	ND	ND
MPD142	Polycythemia vera	*JAK2* V617F	p.Lys1117fs	ND	ND
MPD149	Essential thrombocythemia	*JAK2* V617F	p.Gly1256fs	ND	ND
MPD158	Polycythemia vera	*JAK2* V617F	p.Phe883fs	ND	Yes
MPD163	Essential thrombocythemia	MPL W515L	p.Gln1542X	ND	ND
MPD164	Primary myelofibrosis	*JAK2* V617F	p.Ser936fs	ND	ND
MPD183	Polycythemia vera	*JAK2* V617F	p.[Ile274fs]+[Gln635X]	ND	ND
MPD200	Essential thrombocythemia	WT	p.[Ala211fs]+[Asn1487fs]	ND	ND

* Patient MDS03 was studied at the RAEB1 and RAEB2 phases. Two successive samples from patient MDS34 were analyzed. AML denotes acute myeloid leukemia, del deletion, FAB French–American–British classification, fs frame shift, LOH loss of heterozygosity, ND not determined, RAEB refractory anemia with excess blasts, RAEB1 blasts 5 to 9%, RAEB2 blasts 10 to 19%, RARS refractory anemia with ringed sideroblasts, RARS-T RARS with thrombocytosis, RCMD-RS refractory cytopenia with multilineage dysplasia and ringed sideroblasts, and WT wild type (i.e., negative for *JAK2* V617F and *MPL* W515L mutations).

† Of the 27 patients with myelodysplastic syndromes and *TET2* mutations, the 22 who were tested for *JAK2* V617F and *MPL* W515L mutations had negative results.

‡ The copy number was determined either by fluorescence in situ hybridization or by the sequence analysis of *TET2* defects and single-nucleotide polymorphisms in individual colony experiments.

GENETIC ANALYSES

A detailed description of the materials and methods used in these experiments is provided in the Supplementary Appendix. Briefly, bone marrow mononuclear cells, lymphocytes, or granulocytes were isolated and stored in liquid nitrogen. Cellular experiments were performed as described previously.[10,11] Purified CD34+ cells (1×10^5 to 10×10^5 cells) were injected intravenously into mice that had undergone sublethal irradiation.[14]

Polymerase-chain-reaction (PCR) assays and direct sequencing reactions were performed with primers that are listed in Table 2 of the Supplementary Appendix. *JAK2* mutational status was determined as described previously.[10]

In comparative-genomic-hybridization and single-nucleotide–polymorphism (SNP) analyses, paired DNA samples from malignant and nonmalignant cells were analyzed with the use of 244K oligonucleotide arrays (Agilent Technologies) and Affymetrix 250K SNP (Affymetrix) arrays.

No commercial entity was involved in the conduct of the study, the analysis or storage of the data, or the preparation of the manuscript. The authors vouch for the completeness and accuracy of the data and the analysis.

RESULTS

DELETED REGION ON CHROMOSOME 4Q24

We previously reported on six patients with myelodysplastic syndromes or AML who had acquired rearrangements on chromosome 4q24.[21] In two of these patients, the chromosomal abnormality was found in both myeloid and lymphoid cells, indicating the involvement of a lymphomyeloid progenitor. In this study, we discovered a deleted region on chromosome 4q24 containing a single

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gene, *TET2*, in cells from the three patients with AML, by means of fluorescence in situ hybridization (Fig. 1A). Bone marrow cells from the three patients with myelodysplastic syndromes (Patients MDS01, MDS02, and MDS03) had a similar deletion. Taken together, five patients had a heterozygous loss, and one had a deletion of both copies.

We discovered involvement of the same region on chromosome 4q24 using a different method in patients with myeloproliferative disorders. Two subgroups of patients with myeloproliferative disorders and different profiles of *JAK2* V617F clonal expansion have been described previously.[10] The first subgroup had a low proportion of CD34+ cells bearing the *JAK2* V617F mutation and a high proportion of granulocytes with the mutation, whereas the second subgroup had a high proportion of CD34+ cells bearing *JAK2* V617F. We analyzed samples from five patients from the second subgroup (Patients MPD01 through MPD05) using comparative genomic hybridization and SNP arrays to compare DNA from cells that were presumed to be affected (granulocytes) with DNA from cells that were presumed to be normal (mononuclear cells or lymphocytes) (Fig. 1B). In this subgroup, one patient with primary myelofibrosis (Patient MPD01) and one with polycythemia vera (Patient MPD04) had loss of heterozygosity (LOH) without copy-number modification in chromosome 4, ranging from q22 to the end of the long arm of the chromosome, which was not found in presumably normal cells.[22] A third patient (Patient MPD05) had an acquired 325-kb deletion in the region on chromosome 4q24 containing *TET2* as a single candidate gene. This region was normal in Patients MPD02 and MPD03.

Figure 2 (facing page). *TET2* Mutations in Fractionated CD34+ Cells from Patients with Myelodysplastic Syndromes.

In samples from patients with myelodysplastic syndromes, mutated TET2 sequence is observed in immature CD34+ cells and is associated with in vivo expansion of the mutated clone. In Panel A, sequencing chromatograms of sorted CD34+ cells from Patient MDS03 are shown in samples obtained during phases of refractory anemia with excess blasts of 5 to 9% (RAEB1) and with excess blasts of 10 to 19% (RAEB2). Nucleotide sequences that were observed in an unsorted bone marrow sample from the patients and in a wild-type control sample are shown for comparison purposes. Asterisks indicate the mutated nucleotide. In Panel B, polymerase-chain-reaction (PCR) assay followed by restriction-fragment–length polymorphism (RFLP) analysis shows DNA that was isolated from sorted CD34+CD38− and CD34+CD38+ cells from Patient MDS03 at the RAEB1 phase. Amplified fragments were digested with the use of the Taq endonuclease (Tas1) and underwent size fractionation with the use of agarose-gel electrophoresis. The proportion of TET2 that was mutated was evaluated by measuring the intensity of the mutated or wild-type signal relative to that of the signal generated by both alleles (wild type plus mutated), as compared with a control sample. MW denotes molecular weight (see Fig. 5 in the Supplementary Appendix for details). In Panel C, the results of PCR–RFLP analysis of *TET2* that was directly performed from sorted CD34+CD38− and CD34+CD38+ cells from Patient MDS09 with the use of the *Bacillus stearothermophilus* endonuclease (BseL1) are shown. In Panel D, PCR-RFLP genotyping analysis with the use of BseL1 endonuclease shows sorted CD34+CD38− and CD34+CD38+ clones from Patient MDS09; the clones were grown at one cell per well. The proportion of mutated clones differs significantly between the two cell populations ($P=0.01$ by Fisher's exact test). The histograms represent the fraction of clones with wild-type or mutated *TET2*. The absence of wild-type fragment in CD34+CD38+ clones is indicated by asterisks.

ACQUIRED MUTATIONS IN *TET2*

The *TET2* gene comprises 11 exons spread over 150 kb and is widely expressed.[23,24] In humans, the predicted TET2 protein belongs to a three-member family containing two highly conserved regions (Fig. 1, and Fig. 1 and 2 in the Supplementary Appendix). *TET2* has not previously been implicated in human cancers, but *TET1* is fused to the mixed-lineage leukemia (*MLL*) gene in the chromosomal translocation t(10;11)(p12;q23), which occurs rarely in patients with acute leukemia.[23,24] The function of the TET proteins is unknown, but the presence of the conserved carboxy-terminal domain in the MLL–TET1 fusion protein suggests its importance in cellular transformation.

The loss of both copies of *TET2* in Patient MDS01 and the loss of one copy in eight other patients suggested that *TET2* is a tumor-suppressor gene. We therefore searched for nucleotide sequence variation in the coding region of the remaining copy of *TET2* in the eight patients with either a heterozygous deletion or a copy-neutral loss of heterozygosity. A comparison of these eight sequences with human sequences in the databases identified nucleotide changes in the remaining *TET2* genes in six patients. One change caused a frame shift, two generated stop codons, one was an in-frame deletion, and two resulted in amino acid substitutions of extremely conserved residues

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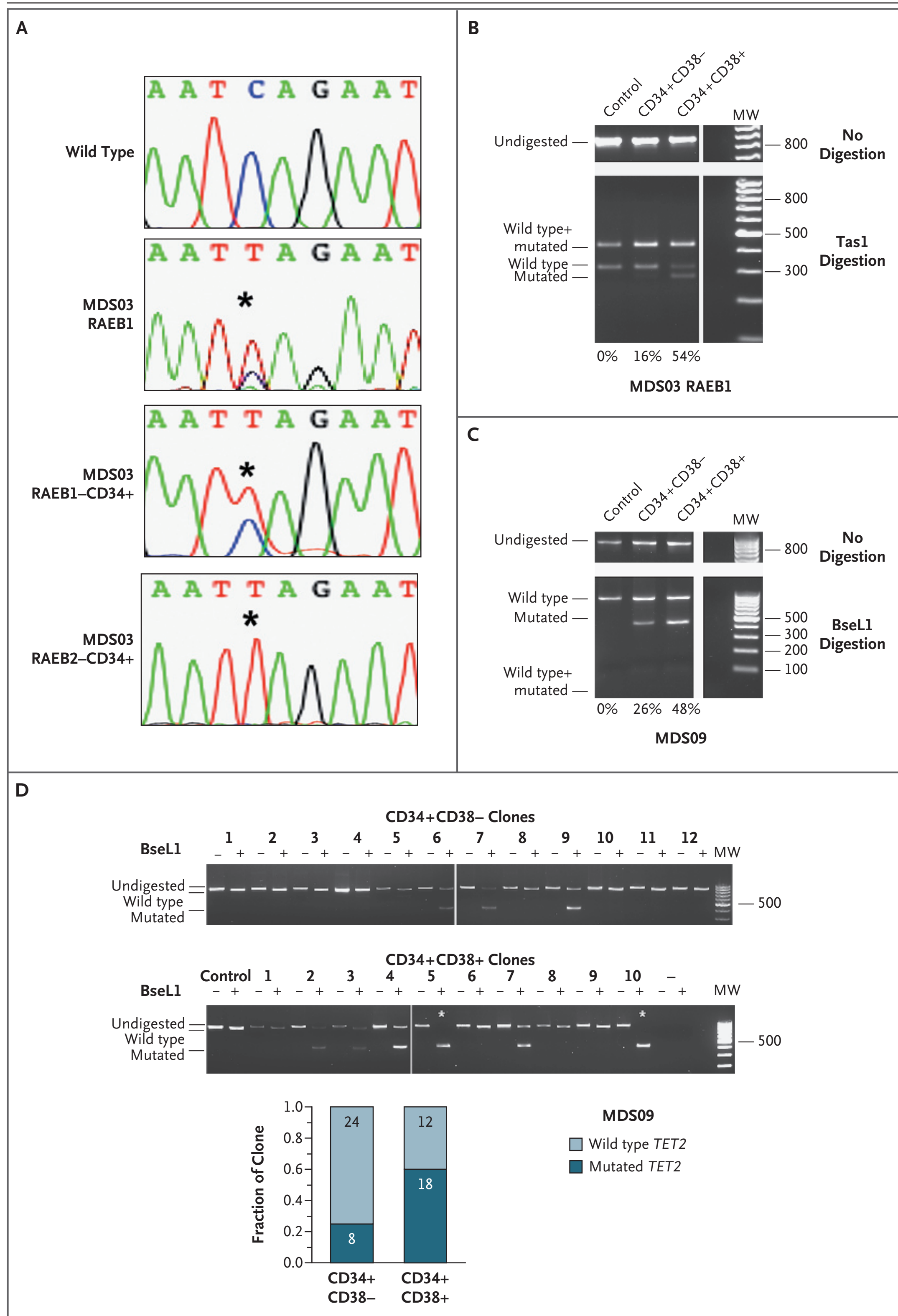

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(Table 1 and Fig. 1C, 1D, and 1E, and Fig. 2 in the Supplementary Appendix). No variations were found in the coding sequence of *TET2* in Patients sAML1 and MPD05. *TET2* coding sequences were wild type in Patients MPD02 and MPD03, who retained both copies of *TET2*.

To investigate whether these variations were somatically acquired in myeloid cells, we studied DNA obtained from lymphocytes from four patients. In analyses of the coding sequences of *TET2* in these DNA samples, we found no mutations in samples from Patients MDS03, MPD04, and nAML2 (Fig. 1C and 1D) and no deletion in a sample from Patient MPD05 (data not shown). In addition, wild-type sequence was detected in mononuclear cells from Patient MPD01 (Fig. 1D). In Patient nAML1, the wild-type sequence predominated, as compared with the mutated sequence, in a sample obtained when the patient was in remission. Together, these data indicate that the *TET2* defects were somatically acquired.

To determine the prevalence of *TET2* mutations in malignant myeloid disorders, we analyzed the *TET2* coding sequence in 309 available samples from 81 patients with various subtypes of myelodysplastic syndromes, 21 patients with secondary AML, 9 patients with chronic myelomonocytic leukemia, 181 patients with *JAK2* V617F–positive myeloproliferative disorders, and 17 patients with *JAK2* V617F–negative myeloproliferative disorders. Sequence variants of *TET2*, resulting in conserved amino acid substitution, stop codons, or frame shifts, were detected in 45 of 309 patients (15%) (Table 1 and Fig. 1E and 1F, and Fig. 3 in the Supplementary Appendix). In Patient MDS07, amplification of the 5′ region of exon 11 on PCR failed, suggesting an uncharacterized structural genomic rearrangement in this region. *TET2* defects were found in 22 of 111 patients with various types of myelodysplastic syndromes, in 21 of 181 patients with myeloproliferative disorders associated with *JAK2* V617F, in 1 of 6 patients with disease associated with a W515L/K mutation in the myeloproliferative leukemia virus oncogene (*MPL*), and in 2 of 11 patients who did not carry either an *MPL* W515L/K or *JAK2* V617F mutation.

Overall, we found *TET2* defects in diverse myeloid disorders, with a prevalence of 15% (46 of 309 patients). Because most of such mutations are predicted to truncate the protein, they could result in partial or total loss of function of the TET2 protein. In 25 of 55 patients with *TET2* defects, two different mutations were detected, which probably targeted both copies of *TET2* (Table 1). This inference was confirmed in Patient MDS42 by subcloning and analysis of individual DNA molecules (Fig. 4 in the Supplementary Appendix). A single defect was observed in 30 of 55 samples, suggesting that haploinsufficiency of *TET2* has a role in these cancers.

Figure 3 (facing page). *TET2* and *JAK2* Defects in Clones Containing Lymphoid and Myeloid Cells.

The *TET2* mutation is a primary event in myeloproliferative disorders and is associated with in vivo expansion of the mutated clone. Sequence analyses of *TET2* and *JAK2* mutations in patients with myeloproliferative disorders were performed in CD34+CD38− cells grown in culture derived from clonal B cells, myeloid cells, and natural killer cells (BMNK) and in CD34+CD38+ cells in methylcellulose culture for differentiation of burst-forming unit–erythroid (BFU-E) or colony-forming unit–granulocyte–macrophage (CFU-GM) activity. In Panel A, the sequencing chromatograms show representative results from three clones per patient. Asterisks indicate the positions of nucleotide changes. For Patient MPD05, sequence analysis was performed with the use of a single-nucleotide polymorphism to detect the loss of heterozygosity (LOH) resulting from the deletion in *TET2*. In Panel B, the histograms show the fractions of immature progenitors (BMNK) and mature progenitors (BFU-E and CFU-GM) carrying defects in both *TET2* and *JAK2*, clones with mutated *TET2*, and nonmutated clones. Panel C shows the fractions of immature progenitors (BMNK) and mature myeloid progenitors (BFU-E and CFU-GM) carrying *TET2* defects in *JAK2* wild-type progenitor cells from Patients MPD05 and MPD20. In Panels B and C, the numbers of analyzed clones are shown on the bars.

EARLY PROGENITOR CELLS WITH *TET2* MUTATIONS

We sought *TET2* defects in CD34+ cells, which include hematopoietic stem cells and hematopoietic progenitors, from four patients with myelodysplastic syndromes (Patients MDS03, MDS09, MDS28, and MDS35). In all four patients, a mutated *TET2* sequence was detected (Fig. 2, and Fig. 5 and 6 in the Supplementary Appendix). In one patient (Patient MDS03), CD34+ cells were analyzed in the first phase of refractory anemia with excess blasts (RAEB1, 5 to 9% blasts) and in the second phase (RAEB2, 10 to 19% blasts). The wild-type sequence was detected in the RAEB1 phase, but only a mutated sequence was found in the RAEB2 phase, suggesting the presence of expanded clones of

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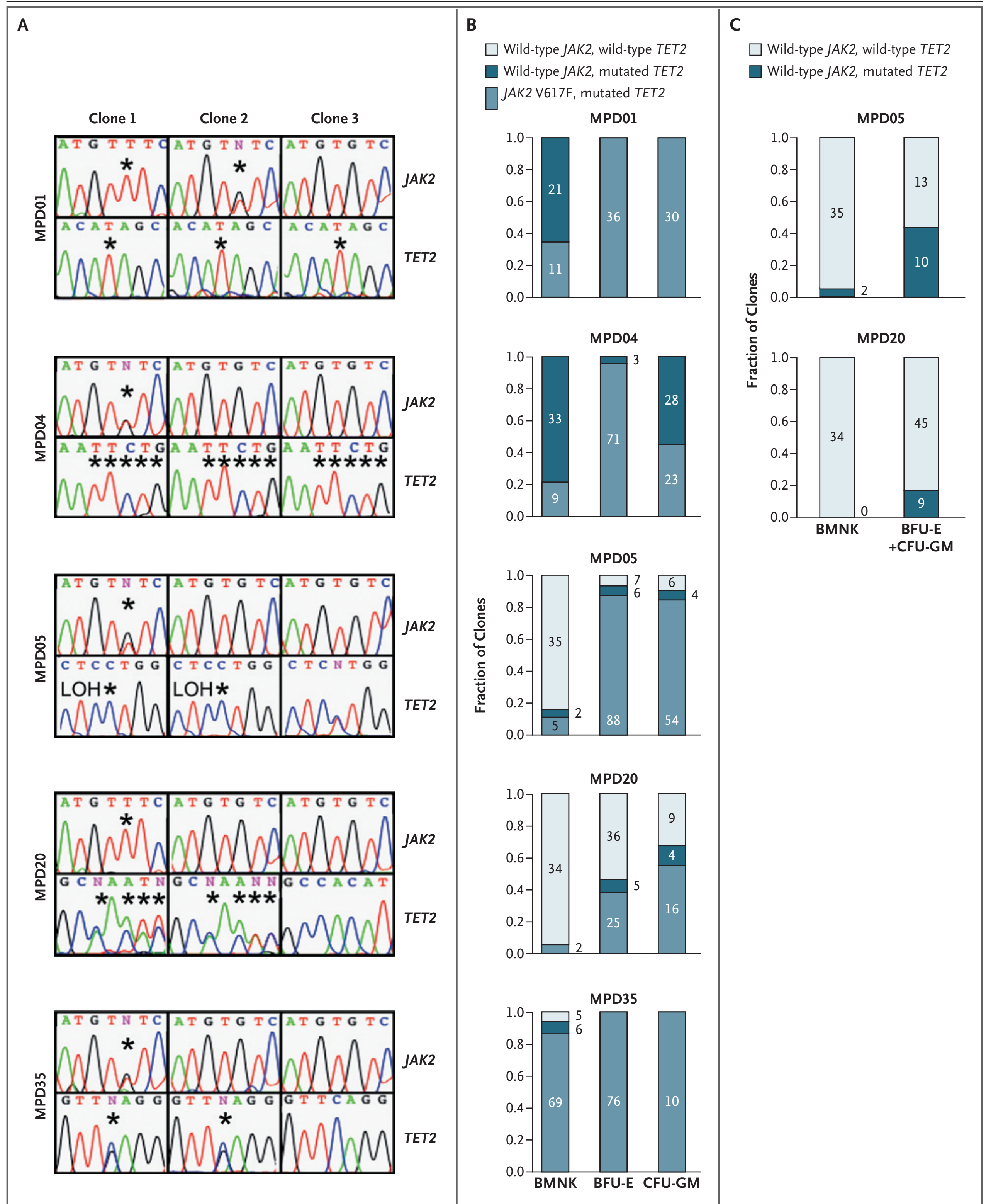

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progenitors bearing the mutation during disease progression (Fig. 2A).

We fractionated the CD34+ cells from these four patients into CD34+CD38– populations (corresponding to stem cells and multipotent progenitors) and into CD34+CD38+ populations (corresponding to more mature progenitors). In Patient MDS03, mutations in *TET2* were found in 16% of cells in the CD34+CD38– population and in 54% of cells in the CD34+CD38+ population (Fig. 2B). The corresponding proportions for Patient MDS09 were 26% and 48%, respectively (Fig. 2C). Further analysis was performed by seeding single hematopoietic progenitors from Patient MDS09 on a stromal-cell layer or in a methylcellulose medium. A *TET2* mutation was identified in 8 of 32 clones (25%) derived from CD34+CD38– cells and in 18 of 30 clones (60%) derived from CD34+CD38+ cells (Fig. 2D). Wild-type *TET2* was not always detected in clones bearing a mutated *TET2*, suggesting its loss in a minority of the cells.

In Patients MDS28 and MDS35, the difference in the occurrence of *TET2* mutations in CD34+CD38– and CD34+CD38+ cells was evaluated from the respective peak intensities and confirmed by subcloning and sequencing of bacterial clones (Fig. 6 in the Supplementary Appendix). In Patient MDS28, the mutated *TET2* sequence was barely detectable in CD34+CD38– cells, whereas it represented one third of the *TET2* sequences from the CD34+CD38+ cells. The data for these four patients with myelodysplastic syndromes indicate that *TET2* mutations were present in a small number of immature CD34+CD38– cells and increased in the population of mature progenitors.

TIMING OF *TET2* AND *JAK2* V617F MUTATIONS

We analyzed hematopoietic progenitors from five patients who had myeloproliferative disorders with mutations in *TET2* and *JAK2*. Immature CD34+CD38– cells were seeded at one cell per well in culture conditions that supported lymphomyeloid differentiation, whereas CD34+CD38+ cells were grown in erythroid–granulocytic methylcellulose assays. In all cells that were tested, both *TET2* and *JAK2* defects were present in clones containing lymphoid and myeloid cells together (Fig. 3A and 3B). Overall, after testing of 232 clones from immature progenitors, the *JAK2* V617F mutation was not observed in the absence of the *TET2* mutation. These results demonstrate that in patients with myeloproliferative disorders (as in patients with myelodysplastic syndromes), the *TET2* mutation is present in immature progenitors. In addition, the findings indicate that in the five patients with myeloproliferative disorders, *TET2* defects preceded *JAK2* mutations during the evolution of the disease. Of course, this finding does not formally rule out an alternative sequence of mutations in other patients with myeloproliferative disorders.

In Patients MPD01, MPD04, and MPD35, almost all the colonies derived from immature and mature progenitors carried a *TET2* mutation, indicating the dominance of the *TET2*-mutated clone at early stages of hematopoiesis (Fig. 3B). In Patients MPD05 and MPD20, most immature progenitor cells were wild type, whereas more mature progenitor cells had a mutated *TET2*. Within *JAK2* wild-type progenitors from these two patients, the proportion of clones with *TET2* defects in the immature population was 2 of 37 cells in Patient MPD05 and none of 34 cells in Patient MPD20; in the mature population, the proportion was 10 of 23 cells in Patient MPD05 and 9 of 54 cells in Patient MPD20 (Fig. 3C). The proportions of mutated clones differed significantly between the two populations ($P<0.001$ for Patient MPD05 and $P=0.01$ for Patient MPD20, by Fisher's exact test), indicating that the increase in cells with *TET2* mutations does not require the presence of the *JAK2* V617F mutation.

STUDIES OF *TET2* MUTATION IN MICE

To add to the evidence that the *TET2* mutation occurs initially in hematopoietic stem cells, we grafted CD34+ cells from patients with polycythemia vera or myelofibrosis in nonobese diabetic mice with severe combined immunodeficiency (NOD–SCID) and depletion of natural killer cells, independently of the proportion of *JAK2* V617F mutations.[14] Purified CD34+ cells isolated from patients with myeloproliferative disorders and the *JAK2* V617F mutation either with the *TET2* mutation (Patients MPD01 and MPD04) or without the *TET2* mutation (Patients MPD09, MPD11, and MPD27) were injected into NOD–SCID mice (Fig. 7 in the Supplementary Appendix).

The percentage of human cells from the three samples without a *TET2* mutation decreased with time, whereas the percentage of human cells from the two *TET2*-mutated samples increased with time (Fig. 4A). Furthermore, human hematopoietic reconstitution from *TET2*-mutated samples was skewed toward myeloid progenitors, at the expense of lymphoid progenitors, as judged from

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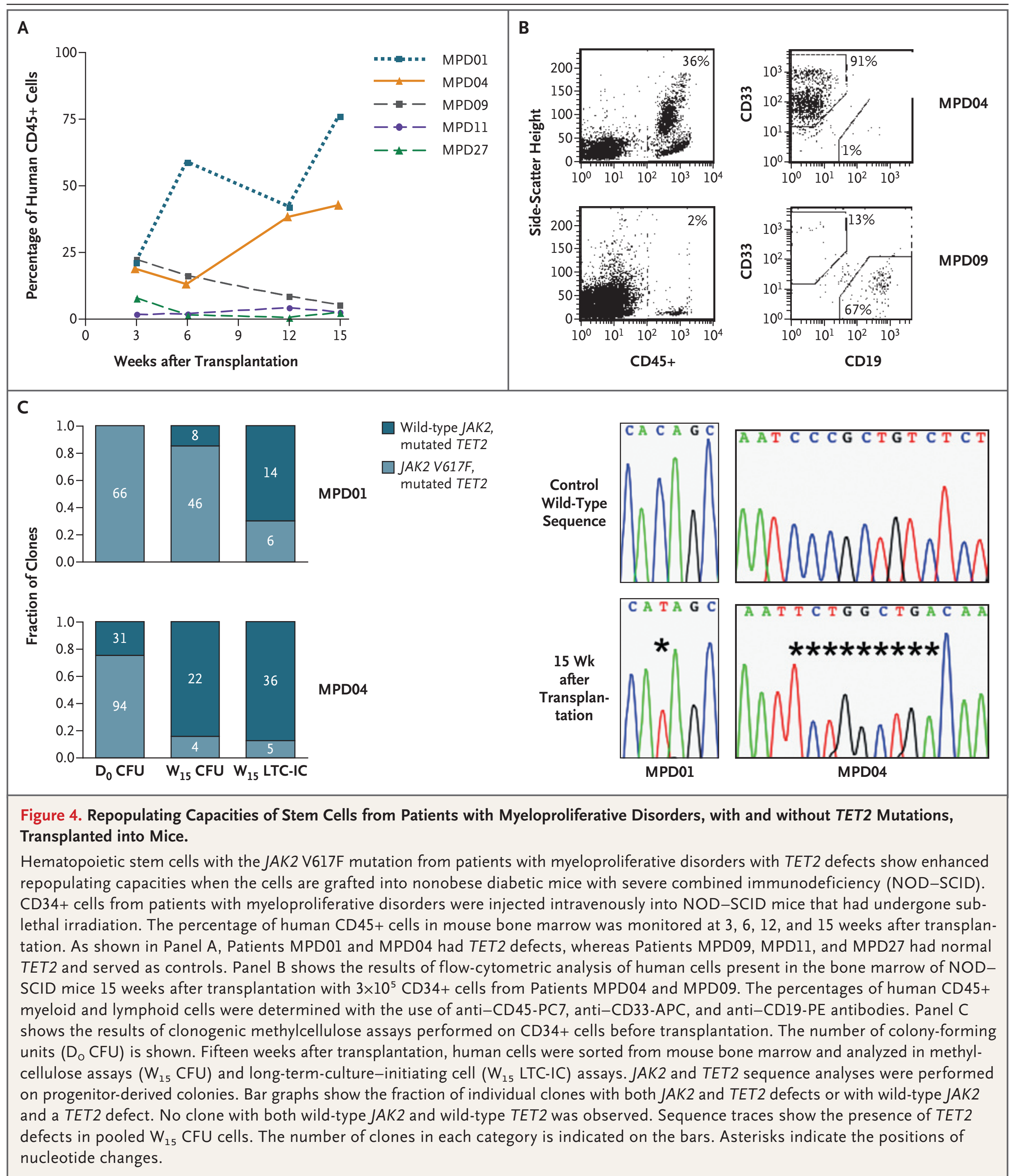

Figure 4. Repopulating Capacities of Stem Cells from Patients with Myeloproliferative Disorders, with and without *TET2* Mutations, Transplanted into Mice.

Hematopoietic stem cells with the *JAK2* V617F mutation from patients with myeloproliferative disorders with *TET2* defects show enhanced repopulating capacities when the cells are grafted into nonobese diabetic mice with severe combined immunodeficiency (NOD–SCID). CD34+ cells from patients with myeloproliferative disorders were injected intravenously into NOD–SCID mice that had undergone sublethal irradiation. The percentage of human CD45+ cells in mouse bone marrow was monitored at 3, 6, 12, and 15 weeks after transplantation. As shown in Panel A, Patients MPD01 and MPD04 had *TET2* defects, whereas Patients MPD09, MPD11, and MPD27 had normal *TET2* and served as controls. Panel B shows the results of flow-cytometric analysis of human cells present in the bone marrow of NOD–SCID mice 15 weeks after transplantation with 3×10^5 CD34+ cells from Patients MPD04 and MPD09. The percentages of human CD45+ myeloid and lymphoid cells were determined with the use of anti–CD45-PC7, anti–CD33-APC, and anti–CD19-PE antibodies. Panel C shows the results of clonogenic methylcellulose assays performed on CD34+ cells before transplantation. The number of colony-forming units (D_0 CFU) is shown. Fifteen weeks after transplantation, human cells were sorted from mouse bone marrow and analyzed in methylcellulose assays (W_{15} CFU) and long-term-culture–initiating cell (W_{15} LTC-IC) assays. *JAK2* and *TET2* sequence analyses were performed on progenitor-derived colonies. Bar graphs show the fraction of individual clones with both *JAK2* and *TET2* defects or with wild-type *JAK2* and a *TET2* defect. No clone with both wild-type *JAK2* and wild-type *TET2* was observed. Sequence traces show the presence of *TET2* defects in pooled W_{15} CFU cells. The number of clones in each category is indicated on the bars. Asterisks indicate the positions of nucleotide changes.

CD33 and CD19 antigen expression, unlike the predominantly lymphoid reconstitution observed with wild-type hematopoietic stem cells[25] (Fig. 4B).

Mouse bone marrow samples that contained human cells 15 weeks after transplantation were tested in vitro for their content of mature progenitors and long-term-culture–initiating cells, a surrogate assay for hematopoietic stem cells, and were analyzed for the presence of *TET2* and *JAK2* mutations. *TET2* defects were found in all human

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long-term-culture–initiating cells and progenitors in the mice (Fig. 4C).

Because only hematopoietic stem cells can sustain long-term hematopoietic reconstitution in NOD–SCID mice, these results are consistent with the occurrence of a *TET2* mutation in a true hematopoietic stem cell. The proportion of progenitor cells carrying only the *TET2* mutation increased at the expense of cells carrying both *TET2* and *JAK2* V617F mutations from baseline to 15 weeks after transplantation, which suggests that cells with a mutated *TET2* can proliferate in vivo independently of the *JAK2* V617F mutation (Fig. 4C).

DISCUSSION

We report that deletions or mutations in *TET2* are early events in some patients with myelodysplastic syndromes, myeloproliferative disorders, or secondary AML. The frequency of *TET2* mutations in unselected patients was 19% (15 of 81 patients) with myelodysplastic syndromes, 12% (24 of 198 patients) with myeloproliferative disorders, 24% (5 of 21 patients) with secondary AML, and 22% (2 of 9 patients) with chronic myelomonocytic leukemia. The detection of acquired genetic defects targeting both *TET2* copies in 24 of 55 patients indicates that *TET2* is a bona fide tumor-suppressor gene in some myeloid cancers. In samples from patients with myeloproliferative disorders who had both *TET2* and *JAK2* mutations, *TET2* mutations occurred first in the course of the disease. *TET2* mutations were observed in various subtypes of myelodysplastic syndromes, in keeping with reported loss of heterozygosity and interstitial deletions on chromosome 4q24 in these subtypes.[26,27] Similar events affecting chromosome 4q24 have been reported in patients with myeloproliferative disorders[28] and those with relapsed AML.[29]

TET2 mutations were observed in primitive CD34+CD38– cells, and xenograft transplantation demonstrated the presence of mutant *TET2* in hematopoietic stem cells in samples from patients with myeloproliferative disorders. In both myelodysplastic syndromes and myeloproliferative disorders, the *TET2* mutation appeared to be associated with the amplification of the mutated clone at the early stages of hematopoietic differentiation.

TET2 defects were observed in both myelodysplastic syndromes and myeloproliferative disorders, which are two distinct myeloid diseases. The presence of clinical and biologic phenotypes characteristic of these diseases probably requires at least one additional contributing event, as exemplified by our finding that the *JAK2* V617F or *MPL* W515L mutations, which are responsible for some biologic features of myeloproliferative disorders, were associated with *TET2* mutations.

Our results support a role for wild-type *TET2* in the control of balance between survival, growth, and differentiation in normal hematopoiesis. Elucidation of the biochemical pathway underlying the function of *TET2* will require additional work and may help to define novel mechanisms of renewal and differentiation of hematopoietic stem cells.

Supported by grants from INSERM, La Ligue Nationale contre le Cancer, La Fondation de France, Institut National du Cancer, Canceropole Ile de France (Cellules Souches Cancéreuses and Apoptosis 2007), L'association Française contre les Myopathies, GIS-Institut des Maladies Rares, la Direction Régionale de la Recherche Clinique AP–HP, Ministère de la Recherche et de la Technologie (to Dr. Dupont), and the European Hematology Association (to Dr. Delhommeau).

No potential conflict of interest relevant to this article was reported.

We thank J. Prchal, S. Constantinescu, T. Mercher, F. Wendling, and E. Solary for their critical reading of the manuscript; D. Bouscary and I. Callebaut for their help and support; O. Beyne-Rauzy, C. Delacroix, C. Preudhomme, B. Quesnel, and E. Solary for the recruitment of patients with myelodysplastic syndromes; P.J. Weiller, L. Roy, J.-A. Ribeil, and E. Lippert for the recruitment of patients with myeloproliferative disorders; and I. Teyssandier, H. Bouamar, and C. Pierre-Eugene for the preparation of samples.

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