Overexpression of sPRDM16 coupled with loss of p53 induces myeloid leukemias in mice

Danielle C. Shing,[1] Maurizio Trubia,[2] Francesco Marchesi,[3] Enrico Radaelli,[3] Elena Belloni,[2] Cinzia Tapinassi,[2] Eugenio Scanziani,[3] Cristina Mecucci,[4] Barbara Crescenzi,[4] Idoya Lahortiga,[5] Maria D. Odero,[5] Giuseppe Zardo,[6] Alicja Gruszka,[1] Saverio Minucci,[1] Pier Paolo Di Fiore,[2] and Pier Giuseppe Pelicci[1,7]

[1]Department of Experimental Oncology, European Institute of Oncology, Milan, Italy. [2]Italian Foundation for Cancer Research (FIRC), Institute of Molecular Oncology (IFOM), Milan, Italy. [3]Department of Veterinary Pathology, Hygiene and Public Health, University of Milan, Milan, Italy. [4]Department of Clinical and Experimental Medicine, University of Perugia, Perugia, Italy. [5]Department of Genetics and Division of Oncology, University of Navarra, Pamplona, Spain. [6]San Raffaele Biomedical Park Foundation, Rome, Italy. [7]Department of Medicine, Surgery and Dentistry, University of Milan, Milan, Italy.

Transgenic expression of the abnormal products of acute myeloid leukemia–associated (AML-associated) primary chromosomal translocations in hematopoietic stem/progenitor cells initiates leukemogenesis in mice, yet additional mutations are needed for leukemia development. We report here aberrant expression of PR domain containing 16 (*PRDM16*) in AML cells with either translocations of 1p36 or normal karyotype. These carried, respectively, relatively high prevalence of mutations in the *TP53* tumor suppressor gene and in the nucleophosmin (*NPM*) gene, which regulates p53. Two protein isoforms are expressed from *PRDM16*, which differ in the presence or absence of the PR domain. Overexpression of the short isoform, sPRDM16, in mouse bone marrow induced AML with full penetrance, but only in the absence of p53. The mouse leukemias were characterized by multilineage cellular abnormalities and megakaryocyte dysplasia, a common feature of human AMLs with 1p36 translocations or *NPM* mutations. Overexpression of sPRDM16 increased the pool of HSCs in vivo, and in vitro blocked myeloid differentiation and prolonged progenitor life span. Loss of p53 augmented the effects of sPRDM16 on stem cell number and induced immortalization of progenitors. Thus, overexpression of sPRDM16 induces abnormal growth of stem cells and progenitors and cooperates with disruption of the p53 pathway in the induction of myeloid leukemia.

Introduction

Acute myeloid leukemia (AML) is initiated and maintained by a population of leukemic stem cells (SCs) that have an innate or acquired ability to self renew and is characterized by the accumulation of abnormally differentiated, immature myeloid cells in the bone marrow and peripheral blood (1, 2). Genetically, AML is a heterogeneous disease: 40% of cases carry primary chromosome translocations or inversions that encode fusion proteins; another 40% have normal karyotypes, the majority of which carry mutations of *NPM*; and the remaining carry rare and heterogeneous translocations (3, 4). Translocations and inversions are frequently present as a single chromosomal aberration, and transgenic expression of their abnormal products induces leukemia in mice, indicating a pathogenetic role in the disease (5). However, the clonal nature, low frequency, and long latency of transgenic mouse leukemias suggest that the fusion proteins alone are not sufficient for leukemia development. Instead, a "2-hit" model for myeloid leukemogenesis is favored (5, 6). Although the nature of the cooperating mutations remains largely unknown, candidate genes include *FLT3*, *TP53*, or *NRAS*, which are frequently mutated in AMLs regardless of the presence of other genetic abnormalities (7–9).

PR domain containing 16 (*PRDM16*; also known as *MEL1*) is involved in the rare AML-associated translocations t(1;3)(p36;q21) and t(1;21)(p36;q15). In both cases, expression of *PRDM16* is altered, either as a consequence of its juxtaposition to the enhancer element of *RPN1* at 3q21 (10, 11) or to its fusion with *AML1* (*AML1/PRDM16*) at 21q15 (12–14). AMLs carrying t(1;3)(p36;q21) translocations present with a characteristic disease phenotype of trilineage dysplasia, dysmegakaryocytopoiesis, normal to elevated platelet counts, poor response to chemotherapy, and poor prognosis (15). Relatively high levels of expression of *PRDM16* have also been observed in AMLs with intermediate risk karyotype (14, 16). However, the mechanism by which altered expression of *PRDM16* contributes to myeloid leukemogenesis is unknown.

The *PRDM16* locus encodes 2 proteins: PRDM16 (or MEL1) and the short isoform, sPRDM16 (or MEL1S) (17). They differ at their N terminus in the presence or absence of the PR domain, a 134–amino acid region with high homology to the SET domain, the structural hallmark of histone methyltransferases (17). In the PR domain family of proteins, the PR domain–negative isoform has the potential to be oncogenic (18). For the *MDS1/EVI1* gene at 3q26, the closest homolog of *PRDM16*, only the PR domain–negative isoform, EVI1, has leukemogenic properties (19, 20).

Nonstandard abbreviations used: AML, acute myeloid leukemia; EPO, erythropoietin; Hb, hemoglobin; lin$^-$, lineage negative; LTC-IC, long-term culture–initiating cell; MGG, May-Grünwald-Giemsa; MSCV, murine SC virus; NK, normal karyotype; PBL, peripheral blood lymphocyte; PE, phycoerythrin; PRDM16, PR domain containing 16; Q-PCR, quantitative RT-PCR; 5′-RACE, 5′–rapid amplification of cDNA ends; SC, stem cell; SCF, SC factor; sPRDM16, short isoform of *PRDM16*.

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Table 1
Diagnoses and karyotypes of the 5 leukemias

Case	Diagnosis	Karyotype
1	AML-M4	46, XX, t(1;3)(p36;q21)
2	AML-M1	46, XX [1]/46, XX, t(1;3)(p36;q21) [14]
3	NHL in leukemic phase	45, XY, t(1;6)(p36;q15), der(2), –3, –8, t(8;14)(q24;q11), t(9;?)(p24;?), +12, –14, +21
4	AML with trilineage dysplasia	45, XY, t(1;21)(p36;q22), –7
5	AML-M6	46, XX
	AML-M2	46, XX [44]/46, XX, t(1;6)(p36;q23) [56]

Indirect evidence suggests that only the short isoform of PRDM16, sPRDM16, has oncogenic potential. First, selective overexpression of *sPRDM16* is observed in AMLs with t(1;3)(p36;q21) translocations due to its juxtaposition to enhancer sequences at 3q21 (11, 17, 21). Second, *sPRDM16* is overexpressed in adult T cell leukemias due to hypomethylation of its promoter. Instead, the promoter of full-length *PRDM16* is silenced by DNA hypermethylation (22). Third, overexpression of sPRDM16, but not PRDM16, in myeloid L-G3 cells blocks granulocytic differentiation (17). Finally, aberrant expression of *sPRDM16*, but not *PRDM16*, by retroviral insertion promotes immortalization of murine bone marrow progenitors (23). However, a direct comparison of the in vivo leukemogenic potential of the 2 PRDM16 isoforms is still missing. In this study, we investigated the pattern of expression of *PRDM16* in AMLs with or without rearrangements of 1p36 and determined the leukemogenetic potential of each isoform in vitro and in vivo.

Results

Identification of leukemias with chromosomal rearrangements involving PRDM16. We identified 5 leukemias, of which 4 presented with AML, with 1p36 translocations involving *PRDM16* (Table 1 and Supplemental Table 1; supplemental material available online with this article; doi:10.1172/JCI32390DS1). In 4 cases the translocation was the only cytogenetic abnormality present. Breakpoint regions were refined by FISH mapping using genomic clones located upstream of, or spanning, *PRDM16* (Figure 1A and Supplemental Figure 1, A–D). We used 5′–rapid amplification of cDNA ends (5′-RACE) and RT-PCR to characterize *PRDM16* transcripts in the patients' blasts (Figure 1, B–E).

Cases 1 and 2, presenting with AML-M4 and AML-M1, carried a t(1;3)(p36;q21). The 1p36 breakpoint mapped upstream of *PRDM16* at 1p36, and the rearrangement resulted in its juxtaposition to the region downstream of *RPN1* at 3q21 (Figure 1, A and B). Expression of full-length *PRDM16* was confirmed by 5′-RACE (Figure 1B). Case 3, presenting with a B cell non-Hodgkin lymphoma in leukemic phase, carried a novel t(1;6)(p36;q15) that fused the 5′ untranslated region of *BACH2* (exons 1–5) at 6q15 to *PRDM16* exons 4–17. A *BACH2/PRDM16* fusion transcript resulted that led to expression of the PR domain–negative sPRDM16 isoform (Figure 1C). In case 4, presenting with AML with trilineage dysplasia, a t(1;21)(p36;q22) formed a fusion transcript composed of *AML1* exons 1–5 and *PRDM16* exons 2–17, including the PR domain (Figure 1D). Case 5 carried a novel t(1;6)(p36;q23) translocation that was present in the AML-M2 relapse of this patient but not in the primary AML-M6 leukemia (Table 1). The breakpoints were mapped downstream of *AHI1* at 6q23 and upstream of *PRDM16*, leading to expression of full-length *PRDM16* (Figure 1E). The breakpoint at 6q23 was located near the *MYB* proto-oncogene, although quantitative RT-PCR (Q-PCR) on case 5 RNA showed no overexpression of *MYB* compared with normal CD34$^+$ cells or total bone marrow (Supplemental Figure 1, D and E).

Relative expression of PRDM16 and sPRDM16 transcripts in AMLs. The relative expression of *PRDM16* and *sPRDM16* transcripts was analyzed by Q-PCR using primers from the 5′ PR domain to identify *PRDM16* only and from the 3′ region to measure both *PRDM16* and *sPRDM16* (Figure 2A). Expression was normalized to *GAPDH* and calibrated to the median level in 10 normal CD34$^+$ samples.

In all 5 cases with 1p36 rearrangements, *PRDM16* and/or *sPRDM16* were greatly overexpressed (19- to 74-fold; $P < 0.001$) with respect to levels in normal CD34$^+$ cells (Figure 2B). In 4 of 5 cases, combined expression of *PRDM16* and *sPRDM16* was higher than expression of *PRDM16* alone. Notably, only *sPRDM16* was expressed in case 3, and no expression of full-length *PRDM16* was seen from the nonrearranged allele, suggesting that the short isoform contributes to leukemogenesis.

Expression of *PRDM16* was also assessed in 40 AML cases and 22 AML cell lines without rearrangements of 1p36 (Figure 2C). With respect to levels in normal CD34$^+$ cells, expression of *PRDM16* was undetectable in cases carrying common translocations, as previously reported (14, 16). In contrast, high levels of *PRDM16* (defined as greater than the highest level observed among the CD34$^+$ samples) were observed in a significant subset (9 of 28; 32%) of AMLs with normal karyotype (AML-NK). In AML-NK patients with high *PRDM16* expression, 78% (7 of 9; $P < 0.001$) carried *NPM* mutations, 56% (5 of 9; $P < 0.001$) carried *FLT3-ITD*, and 44% (4 of 9; $P < 0.001$) were positive for both. Among the AML cell lines, only GDM1, derived from an AML-M4, was found to express *PRDM16* (Figure 2C). In all positive cases we found overexpression of both *PRDM16* and *sPRDM16*.

We then investigated whether gene amplification or promoter demethylation could account for the overexpression of *PRDM16* in AML-NK patients. No amplification of *PRDM16* was observed at the genomic level (Supplemental Figure 2A). Instead, CpG islands in the promoter of *PRDM16* were demethylated in patients showing high levels of expression (Supplemental Figure 2B), suggesting that differential promoter methylation may indeed control *PRDM16* expression.

Together, these findings suggest that 1p36 rearrangements lead to overexpression of *PRDM16*, and that *PRDM16* overexpression is a frequent event in AML-NK, in particular in cases with mutant *NPM*. However, with the exception of case 3, in which only the short isoform is expressed, this analysis is not conclusive with respect to which isoform (PRDM16 or sPRDM16) is associated with the leukemia phenotype. Thus, we investigated the leukemogenic potential of each isoform in vitro and in vivo.

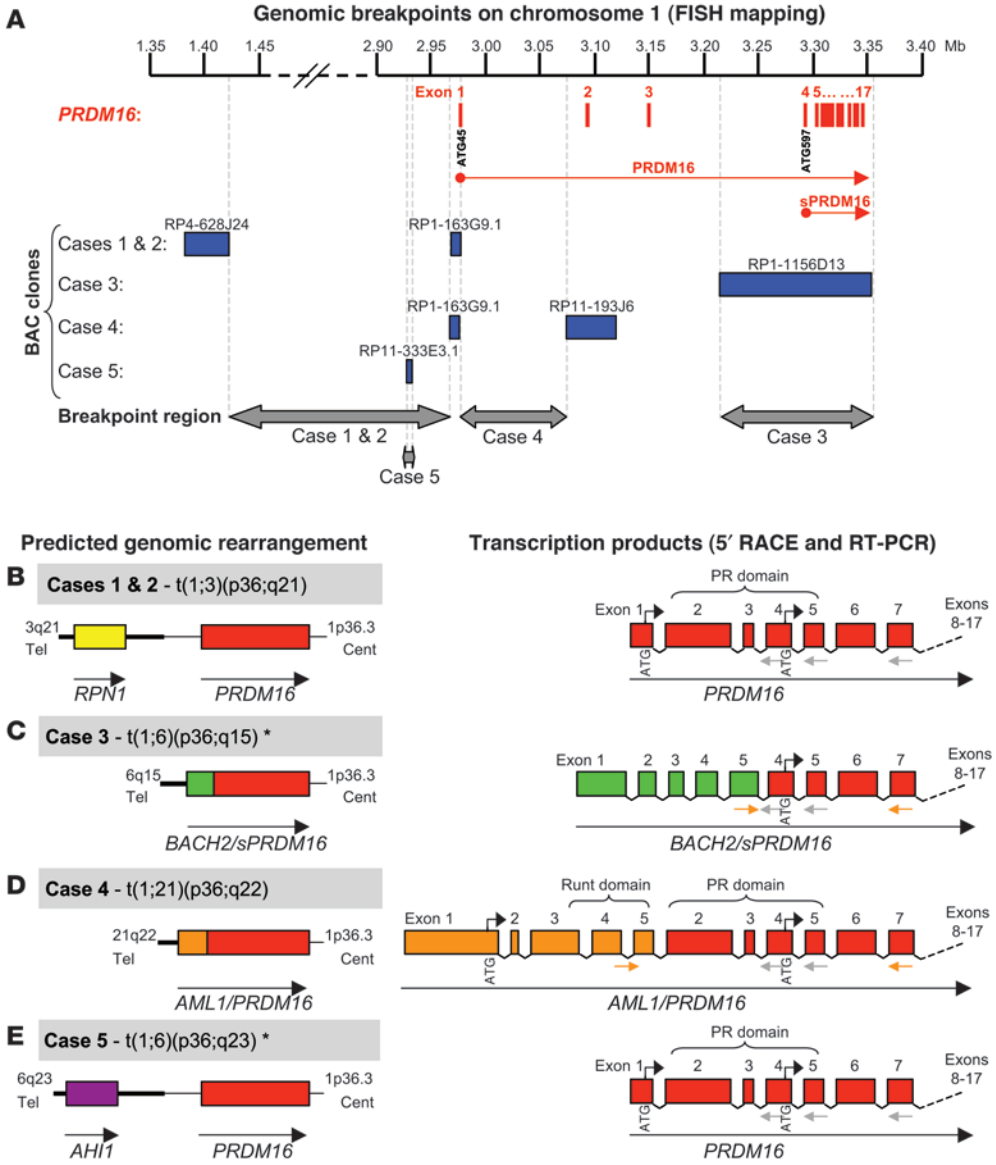

Figure 1

PRDM16 translocations in 5 leukemia cases. (**A**) Genomic breakpoint regions (double-headed arrows) at 1p36 of cases 1–5, defined by FISH mapping. *PRDM16* exons are shown in red, together with ATG start sites of PRDM16 and sPRDM16 in exons 1 and 4, respectively. The position of the bacterial artificial chromosome (BAC) clones (upstream of and spanning *PRDM16*) used as FISH probes to map the breakpoint regions of the 5 leukemias are shown in blue. (**B**–**E**) Predicted genomic rearrangement and transcription products for cases 1–5, defined by FISH, 5′-RACE, and RT-PCR. Grey and orange arrows indicate primer positions for 5′-RACE and RT-PCR, respectively. Asterisks indicate novel rearrangements.

Effects of PRDM16 and sPRDM16 on HSCs/progenitors. *PRDM16* and *sPRDM16* were expressed by retroviral gene transfer in a murine cell population enriched in HSCs and progenitors (lineage-negative [lin$^-$] cells; Figure 3A). Immunofluorescence confirmed expression of the proteins in the nucleoplasm of the transduced lin$^-$ cells (Figure 3B). The effects of PRDM16 and sPRDM16 on progenitor life span were assessed by serial replating of transduced lin$^-$ cells in methylcellulose, in the presence of cytokines G-CSF and GM-CSF. By the third and fourth plating, lin$^-$ cells transduced with empty vector (murine SC virus–internal ribosomal entry site–GFP [MSCV-IRES-GFP]; referred to herein as MSCV) were fully differentiated into either granulocytes or macrophages (Figure 3C) and were unable to grow in methylcellulose, as expected (Figure 3, D and E). In contrast, cells expressing sPRDM16, but not those expressing PRDM16, remained immature and continued to form colonies in methylcellulose upon serial replating (Figure 3, C–E, and Supplemental Figure 3A). Notably, overexpression of sPRDM16 increased both the number and the size of colonies, suggesting that it affects both clonogenicity and proliferation of progenitors (Figure 3, D and E). Similar results were obtained when cells where cultured with erythropoietin (EPO), IL-3, and SC factor (SCF; Figure 4A, Supplemental Figure 3B, and data not shown). Together, these findings suggest that sPRDM16 overexpression blocks differentiation and extends survival of hematopoietic progenitor cells.

To investigate the effect of sPRDM16 on HSCs, we used the long-term culture–initiating cell (LTC-IC) assay that assesses the frequency of primitive HSCs in vitro (24, 25). Transduced lin$^-$ cells were seeded on a feeder-layer and cultured for 5 weeks. In this period, committed progenitors terminally differentiate, while HSCs self renew and their number can be assessed by the CFU assay. After long-term culture, cell morphology showed a higher proportion of immature myeloid cells upon expression of sPRDM16 (Figure 4B and Supplemental Figure 3C). Strikingly, this correlated with a marked (~70-fold) increase in the number of CFUs from the sPRDM16 LTC-IC, compared with control and PRDM16 plates (Figure 4, C and D).

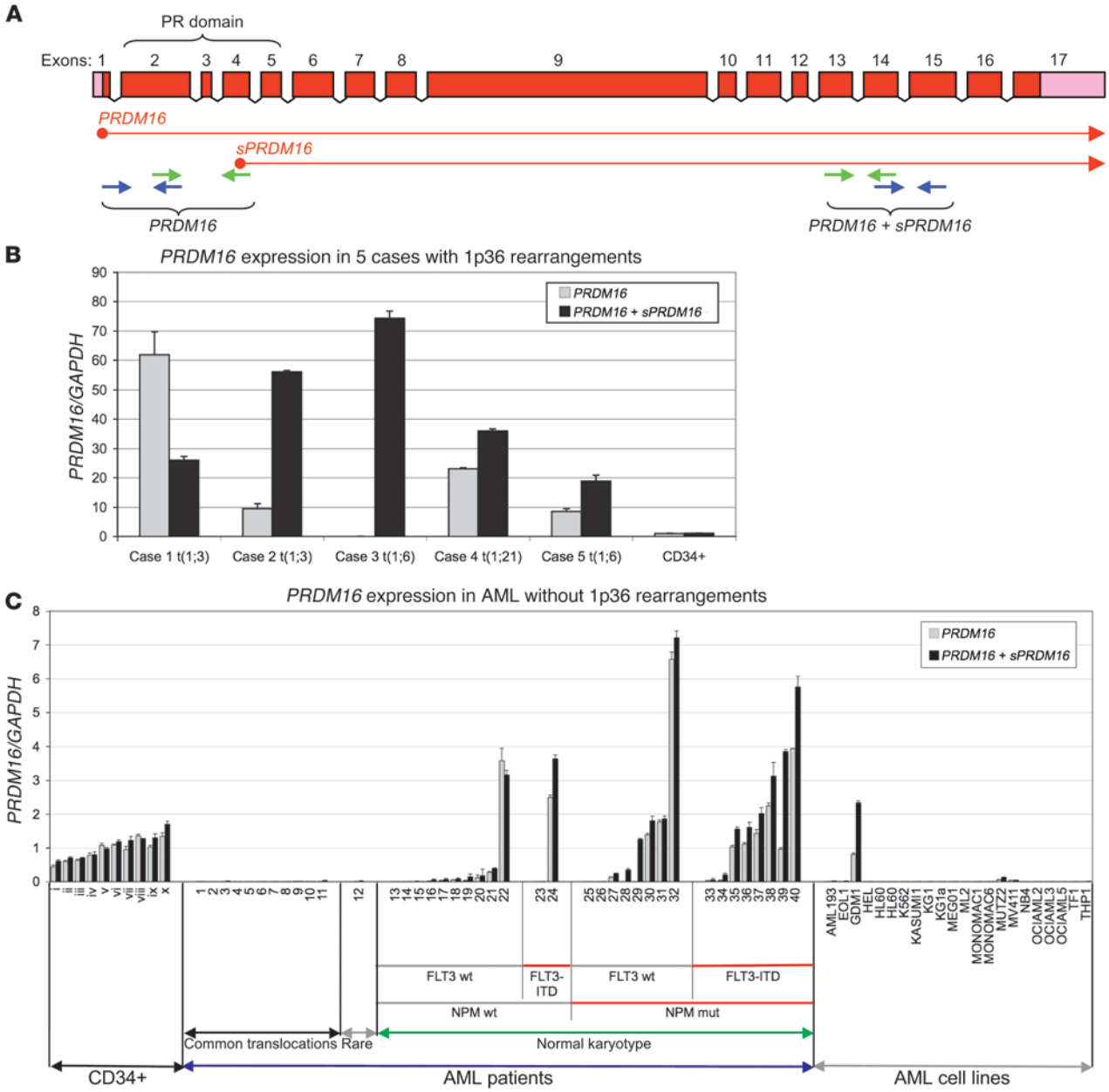

Figure 2

Expression patterns of *PRDM16*. (**A**) Positions of primers used to detect expression of *PRDM16* alone or both *PRDM16* and *sPRDM16* by SYBR Green (green arrows) and Taqman (blue arrows) Q-PCR along *PRDM16* and *sPRDM16* transcripts (red arrows). (**B** and **C**) Q-PCR of *PRDM16* expression in AMLs with (**B**) or without (**C**) 1p36 rearrangements. Expression was normalized to *GAPDH* and calibrated to the median level in 10 normal CD34$^+$ cells (shown individually in **C**). AMLs are grouped according to karyotype: common translocations [t(15;17), t(8;21), inv(16), 11q23, t(9;22)], rare translocation, or normal karyotype. AML-NK cases are grouped according to the presence of *NPM* or *FLT3-ITD* mutations.

To confirm this effect of sPRDM16 in vivo, we performed competitive BM transplantation experiments of lin$^-$ cells expressing, or not, sPRDM16. Transplanted cells were distinguished by expression of different CD45 allelic variants (CD45.1 and CD45.2). CD45.2$^+$ lin$^-$ cells were transduced with empty vector or sPRDM16, mixed with an equal number of CD45.1$^+$ competitor lin$^-$ cells, and injected into lethally irradiated CD45.1$^+$ mice (Figure 5A). At 4 and 8 weeks after transplantation, flow cytometry analysis of CD45.1 and CD45.2 expression in the peripheral blood revealed a significantly greater proportion of CD45.2$^+$ cells (~4-fold increase) in the mice reconstituted with sPRDM16-overexpressing lin$^-$ cells (Figure 5, B and C). Myeloid, B, and T cell lineages were efficiently reconstituted (Figure 5D). Together, these results demonstrate that sPRDM16 overexpression expands the pool of HSCs both in vitro and in vivo.

Leukemogenic potential of PRDM16 and sPRDM16. We transduced lin$^-$ cells with PRDM16, sPRDM16, or empty vector MSCV; sorted them for GFP; and reinoculated them into lethally irradiated WT recipi-

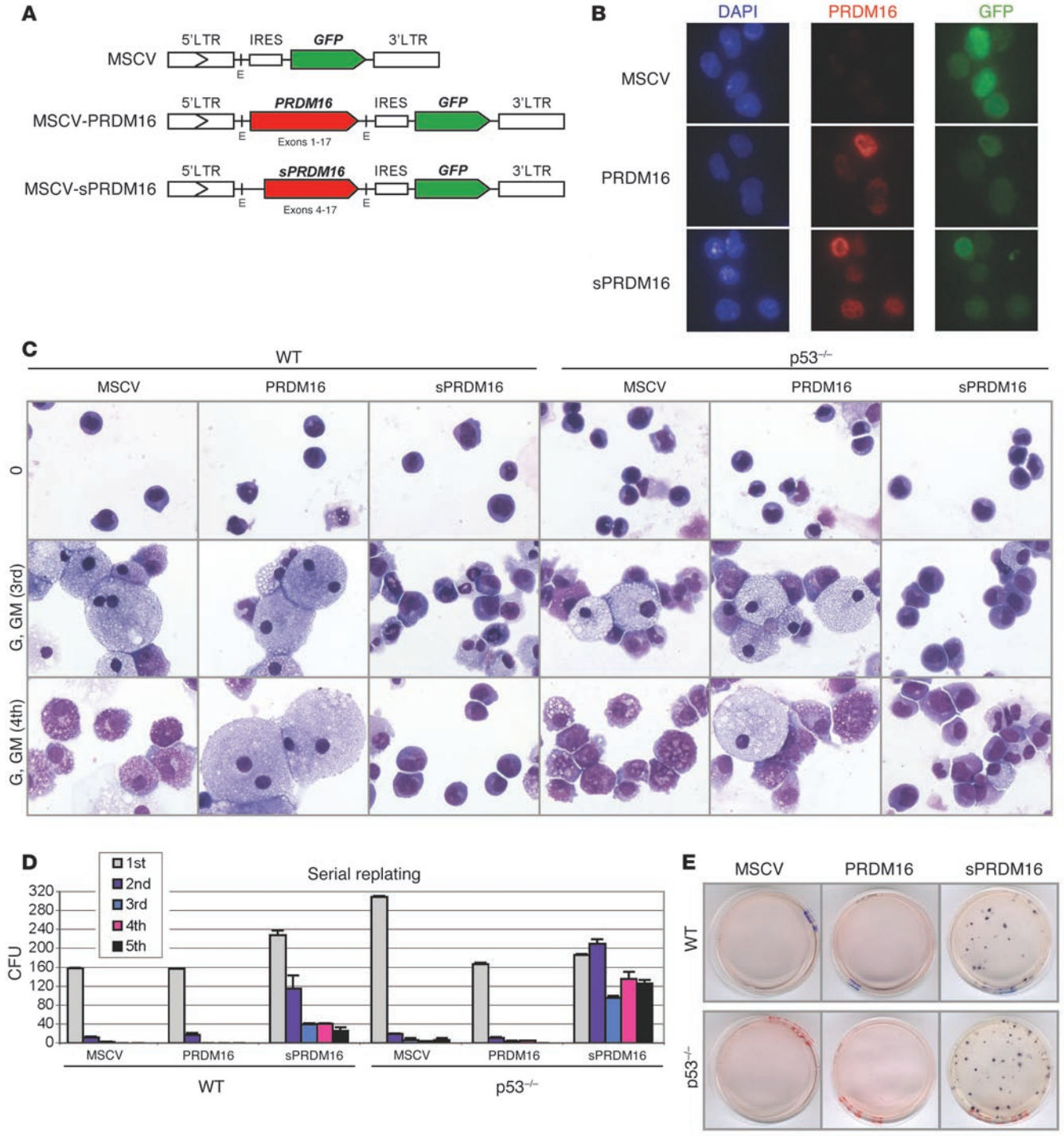

Figure 3

sPRDM16 overexpression in lin⁻ cells. (**A**) *PRDM16* and *sPRDM16* cDNAs were cloned into the MSCV retroviral vector at its EcoRI cloning site (E). (**B**) Anti-PRDM16 immunofluorescence of transduced lin⁻ cells. (**C**–**E**) Serial replating of transduced WT and p53$^{-/-}$ lin⁻ cells in methylcellulose, in the presence of cytokines G-CSF and GM-CSF (G, GM). Cell morphology before differentiation stimuli and at the third and fourth platings (MGG stain; **C**), CFUs upon serial replating (**D**), and micrographs at the third plating (**E**) are shown. Original magnification, ×1,000 (**B**); ×400 (**C**).

ent mice. None of the 10 MSCV or 6 PRDM16 mice developed leukemia or other hematological abnormalities. In contrast, 2 of 8 mice expressing sPRDM16 developed AML at 137 and 165 days, while of the remaining sPRDM16 mice, 3 were still alive at 11 months and 3 died of nonhematological diseases more than 1 year after transplant (Supplemental Figure 4A). Expression of sPRDM16 was confirmed in the spleen of 1 leukemic mouse (Supplemental Figure 4B). Both leukemic sPRDM16 mice presented with splenomegaly, leukocytosis,

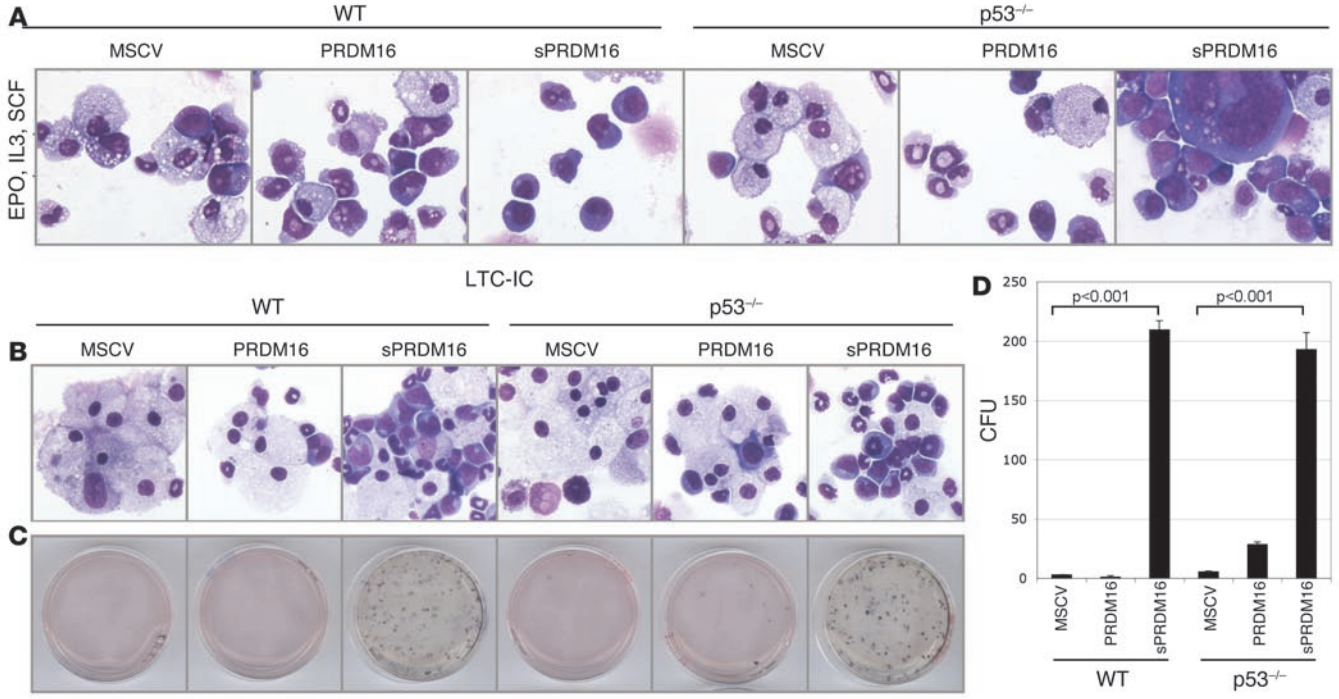

Figure 4
sPRDM16 overexpression in lin⁻ cells grown in methylcellulose and in LTC-IC. (**A**) Morphology of lin⁻ cells grown in the presence of cytokines EPO, IL-3, and SCF (MGG stain). (**B**) Morphology of lin⁻ cells after long-term culture in LTC-IC. (**C** and **D**) CFUs (micrographs, **C**; number, **D**) of transduced lin⁻ cells plated 5 weeks after LTC-IC. Original magnification, ×400.

and anemia (wbcs, 26 and >100 × 10^3/μl; hemoglobin [Hb], 3.7 and 2.9 g/dl; for the mice that died at 137 and 165 days, respectively; see Supplemental Figure 5 for differential counts) and approximately 90% infiltration of the bone marrow with myeloid elements showing varying degrees of maturation. Similarly, the splenic red pulp was markedly expanded and the white pulp partially replaced by myeloid cells (Supplemental Figure 4, C–R). Immunohistochemistry analysis performed in 1 mouse showed massive infiltration of the bone marrow, splenic red pulp, and liver with myeloperoxidase-positive blasts (Supplemental Figure 4, C–R). According to the Bethesda proposals for the classification of nonlymphoid hematopoietic neoplasms in mice, a diagnosis of "AML with maturation" was made for both mice (26). Therefore, sPRDM16 overexpression induces AML in a WT background, albeit with low penetrance.

Overexpression of sPRDM16 in p53⁻/⁻ lin⁻ cells induces AMLs with trilineage dysplasia. We then investigated the leukemogenic potential of PRDM16 and sPRDM16 in the presence of a putative cooperating mutation. We chose, as the first candidate, mutation of *TP53*. Analysis of *TP53* mutation status in cases 1–5 with 1p36 rearrangements revealed a heterozygous ACC to GCC mutation in case 2 that led to the amino acid substitution Thr253Ala (Figure 6A). *TP53* was also mutated in case 5, where a deletion of *TP53* exons 5–7 and retention of the rearranged introns 4–7 in the transcript resulted in premature termination of translation and formation of a truncated p53 protein lacking an intact DNA binding domain (Figure 6B).

We infected lin⁻ cells derived from p53⁻/⁻ mice as described above and transplanted them into WT mice so that only cells of the hematopoietic compartment would be p53 null. All the transplanted mice died or were sacrificed for moribund conditions 73–137 days after transplantation (Table 2 and Figure 7A). Expression of exogenous *PRDM16* or *sPRDM16* in the spleens and thymi of the mice was confirmed by RT-PCR (Figure 7B). While expression of PRDM16 and sPRDM16 had no significant effect on the overall survival of the mice, disease outcome was significantly different. The 12 MSCV mice developed thymic (n = 7) or nonthymic (n = 4) lymphomas and myeloproliferative disease–like myeloid leukemia (n = 1) (Table 2 and Supplemental Figure 6), as expected in a p53-null background (27). Conversely, the PRDM16 mice only developed thymic lymphomas (Table 2 and Supplemental Figure 6), suggesting that PRDM16 exerts tissue-specific effects on tumor development. In contrast, expression of sPRDM16 in the p53⁻/⁻ background induced AML in 13 of 13 mice. Mice presented with splenomegaly, hepatomegaly, elevated wbcs, and decreased Hb (Table 2). The majority (60%–80%) of nucleated cells in the peripheral blood were immature myeloid cells, metamyelocytes, and dysplastic granulocytes/neutrophils (Figure 7C and Supplemental Figure 5). Histopathological examination showed extensive infiltration of the bone marrow, spleen, and liver with myeloperoxidase-positive immature cells (Figure 7, D–I). Transplantation of spleen cells from sPRDM16 leukemias into nonirradiated secondary recipient mice induced AML with a significantly shorter latency (24–39 days after transplantation; Figure 7A).

In 8 of 13 sPRDM16 mice, we observed nucleated and immature erythroid forms in blood smears (Table 2, Figure 7C, and Supplemental Figure 5). In addition, 9 of 10 sPRDM16 mice had numerous dysplastic megakaryocytes present in the infiltrates of liver, spleen, bone marrow, and pancreatic/mesenteric lymph nodes, which stained positive with vWF (see Figure 7, J and K, for representative staining in the liver). Therefore, the leukemia induced by sPRDM16 in the absence of p53 can be described

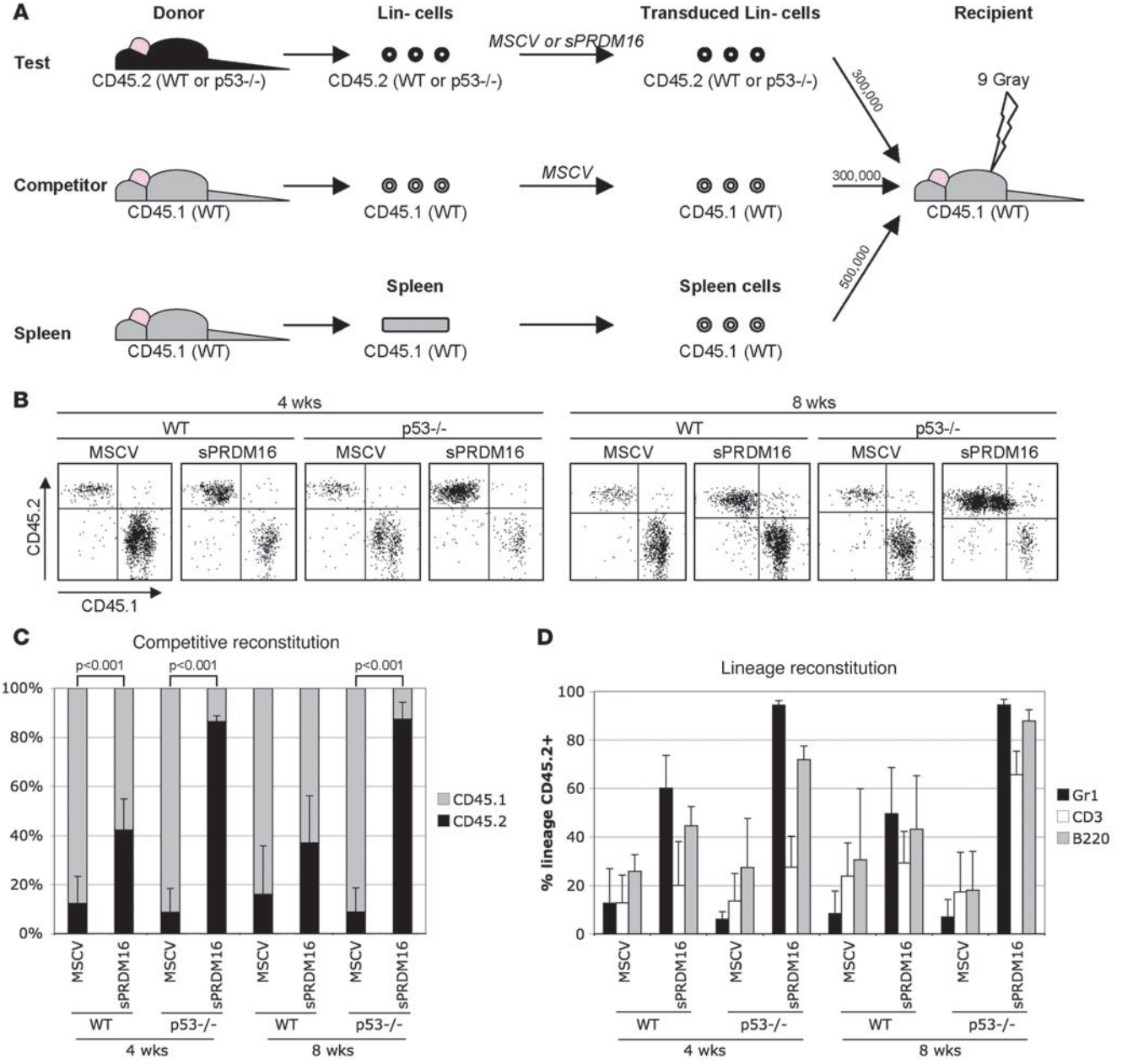

Figure 5

Competitive reconstitution assay. (**A**) Experimental approach. Lethally irradiated recipient mice (CD45.1) were competitively repopulated with 300,000 test lin$^-$ cells (CD45.2 WT or p53$^{-/-}$, transduced with MSCV or sPRDM16), 300,000 competitor lin$^-$ cells (CD45.1 WT, transduced with MSCV) and 500,000 spleen cells (CD45.1 WT). For each group, 4–5 mice were reinoculated, and peripheral blood was analyzed by FACS analysis at 4 and 8 weeks after transplantation. (**B** and **C**) Relative contributions of test (CD45.2) versus competitor (CD45.1) populations in peripheral blood of mice at 4 and 8 weeks after transplantation. Flow cytometry of representative mice (**B**) and mean contributions for each group of 4–5 mice (**C**) are shown. (**D**) The proportion of each lineage derived from donor test (CD45.2) cells is shown in the peripheral blood of mice at 4 and 8 weeks after transplantation.

as an "AML without maturation, with multilineage dysplasia," in accordance with the Bethesda proposals (26). Notably, in humans, the AML caused by abnormal expression of sPRDM16 in t(1;3)(p36;q21) cases is characterized by trilineage dysplasia and dysmegakaryocytopoiesis, strongly suggesting that the combination of sPRDM16 overexpression and p53 mutation recapitulate the corresponding human leukemia.

In vitro effects of sPRDM16 overexpression and loss of p53. We then investigated the biological basis of the sPRDM16-p53 cooperation by studying the effects of sPRDM16 in p53$^{-/-}$ HSCs/progenitors. P53$^{-/-}$ lin$^-$ cells behaved as WT lin$^-$ cells in terms of differentiation (although the granulocytic lineage was slightly favored) and self renewal upon serial replating (Figure 3, C–E) as well as kinetics of HSC growth in LTC-IC (Figure 4, B–D). The differentiation

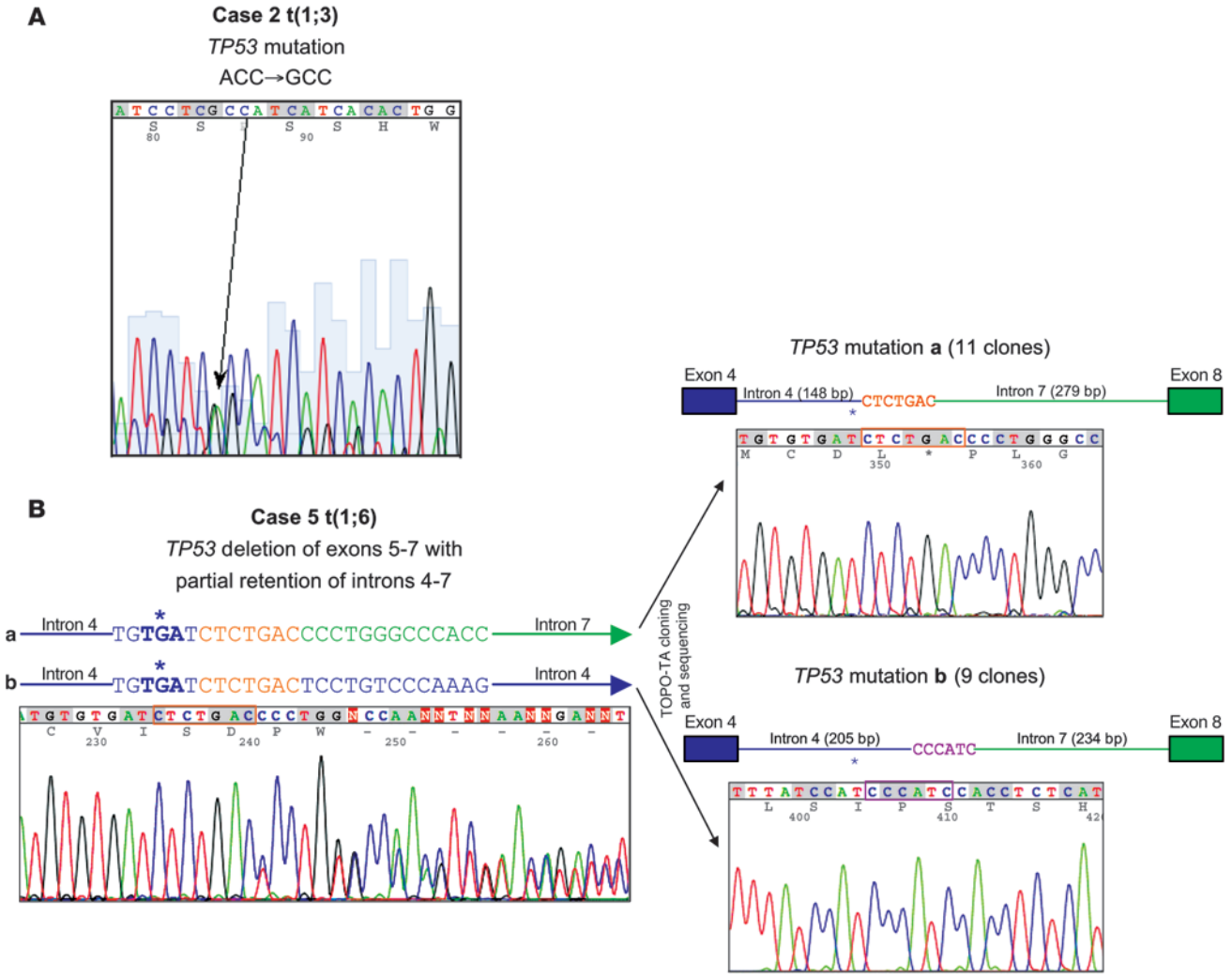

Figure 6

TP53 mutations in 2 AML patients with *PRDM16* overexpression. (**A**) Direct sequence analysis of the *TP53* transcript from case 2, showing a heterozygous point mutation. (**B**) Left: Direct sequence analysis of the *TP53* transcript from case 5, showing 2 different fusions of the 5′ and 3′ extremities of introns 4 and 7, respectively. Sequence from exons 5–7 was absent in both. Right: Nucleotide sequences of 20 clones, obtained after TOPO-TA cloning of the *TP53* transcript, revealed 2 classes of cDNAs lacking exons 5–7 and containing different portions of introns 4 and 7, as predicted by direct sequence analysis. The resulting 2 transcripts consist of *TP53* exons 1–4, rearranged introns 4 and 7, and exons 8–11. In both cases, premature TGA stop codon (asterisk) in intron 4 led to the formation of a 174–amino acid–truncated p53 protein lacking an intact DNA binding domain. No clones containing the WT *TP53* transcript were found.

block induced by sPRDM16 in the presence of G-CSF and GM-CSF was the same in $p53^{-/-}$ cells (Figure 3C and Supplemental Figure 3A). However, in the presence of EPO, sPRDM16 induced megakaryocyte dysplasia in the $p53^{-/-}$ background, a phenotype that was not observed upon expression in WT cells (Figure 4A and Supplemental Figure 3B). Notably, a greatly increased number of colonies was seen upon serial replating of sPRDM16-overexpressing $p53^{-/-}$ lin^- (Figure 3, D and E), suggesting that loss of p53 potentiates the effect of sPRDM16 on progenitor life span. In contrast, equal numbers of CFUs were observed in LTC-IC in WT and $p53^{-/-}$ backgrounds (Figure 4, C and D). Competitive repopulation experiments were performed to compare the effects of sPRDM16 expression on WT and $p53^{-/-}$ HSCs in vivo. We transduced lin^- cells of both WT and $p53^{-/-}$ origin ($CD45.2^+$) with empty vector or sPRDM16, mixed them with an equal number of WT competitor lin^- cells ($CD45.1^+$), and injected them into lethally irradiated mice ($CD45.1^+$) (Figure 5A). At both 4 and 8 weeks after transplantation, flow cytometry analysis of peripheral blood revealed that the ability of sPRDM16 to enhance reconstitution was increased in the $p53^{-/-}$ background (from 4- to 8-fold; Figure 5, B and C). Repopulation of myeloid, T, and B cell lineages was more effectively achieved when sPRDM16 was expressed in $p53^{-/-}$ cells (Figure 5D). Thus, sPRDM16 and p53 loss cooperate in prolonging progenitor life span and expanding the pool of HSCs.

Discussion

We describe here aberrant expression from the *PRDM16* gene in 5 leukemia cases with rearrangements of 1p36, of which 1 had

Table 2
Summary of disease in mice transplanted with p53$^{-/-}$ lin$^-$ cells transduced with the indicated expression vectors

Plasmid	Latency (d)	wbc ($10^3/\mu l$)	Hb (g/dl)	Diagnosis (*n*)						
				Thymic lymphoma	Nonthymic lymphoma	MPD-like leukemia	AML	E	MK	Total mice
MSCV	129 ± 14	20.1 ± 21.9	11.1 ± 3.9	7	4	1	0	0	0	12
PRDM16	131 ± 17	11.3 ± 15.3	13.8 ± 2.0	10	0	0	0	0	0	10
sPRDM16	108 ± 17	59.8 ± 47.8	6.5 ± 2.5	3[A]	0	0	13[A]	8	9	13

Mice were classified as having erythroid (E) or dysplastic megakaryocyte (MK) components when 2%–15% of nucleated cells in the peripheral blood were erythroid precursors or when numerous dysplastic megakaryocytes were present in liver infiltrates, respectively. MPD, myeloproliferative disease. [A]Three mice presented with both AML and thymic lymphoma.

exclusive expression of sPRDM16, and in the AML-NK subset. Furthermore, we present direct evidence that overexpression of the short isoform, sPRDM16, is leukemogenic. Penetrance of leukemias induced by sPRDM16 expression in WT hematopoietic cells was very low, while full penetrance was obtained in p53$^{-/-}$ cells, suggesting that disruption of the p53 pathway cooperates with aberrant sPRDM16 expression in inducing AML. Notably, no leukemias were obtained by overexpressing PRDM16, even in p53$^{-/-}$ cells, suggesting that the long isoform is not oncogenic. Because the 2 isoforms of PRDM16 only differ for the presence of the PR domain, careful scrutiny of its function should help to elucidate the oncogenic potential of *PRDM16*.

The cooperative effects of sPRDM16 and loss of p53 observed in the murine leukemias may reflect a similar cooperation in human AMLs. In fact, we have shown that *TP53* was mutated in AMLs with rearrangements of *PRDM16*. Indeed, the Thr253Ala point mutation we found in case 2 has been previously described in cases of breast carcinoma, colorectal cancer, and liposarcoma (28–30) and was found to have markedly reduced transactivating activity (31). The disruption of the DNA-binding domain and premature termination of translation in case 5, likely due to an intragenic deletion, would clearly disrupt p53 function. However, a more detailed study of *TP53* mutation status in rare cases of AML with *PRDM16* rearrangements is warranted to confirm our findings.

Importantly, in vitro overexpression of sPRDM16 in p53$^{-/-}$ lin$^-$ cells induced megakaryocytic dysplasia, while the murine AMLs induced by sPRDM16 in a p53$^{-/-}$ background were characterized by erythroid and megakaryocytic components. These features are reminiscent of human AMLs with rearrangements of *PRDM16* that are associated with trilineage dysplasia and dysmegakaryocytopoiesis (15). Therefore, disruption of the p53 pathway cooperates with aberrant sPRDM16 expression in inducing AMLs with trilineage dysplasia.

NPMc$^+$ AML, in which *NPM* is mutated, is also associated with the clinical presentation of multilineage dysplasia, high platelet counts, and dysplastic megakaryocytes (32–34). The association we found of *PRDM16* overexpression with AML-NK carrying mutant *NPM* could account for this association. Mutant NPM is believed to contribute to leukemogenesis by disrupting the ARF-p53 tumor suppressor pathway (35). In fact, it delocalizes ARF to the cytoplasm, rendering it more susceptible to degradation and hampering its ability to initiate a p53 response (35, 36). Because *NPM* and *TP53* mutations are rarely found together in AML (37), we speculate that *NPM* mutations in AML-NK may play a similar role to *TP53* mutations in AMLs with 1p36 rearrangements. Notably, expression of mutant NPM in WT or p53$^{-/-}$ lin$^-$ cells was not sufficient to induce AML in mouse transplantation models (our unpublished observations), reinforcing the hypothesis that mutant NPM requires cooperation with an oncogene, such as sPRDM16, to induce leukemias.

We demonstrated that sPRDM16 increased the pool of HSCs in vivo, and in vitro blocked myeloid differentiation and prolonged progenitor life span. All of these effects are likely to be critical for initiation of leukemogenesis and maintenance of the leukemic clone and are also exerted by several AML-associated fusion proteins, such as AML1-ETO, PML/RARα, MLL/ENL, MLL/AF9, and MOZ/TIF2 (38–41). Interestingly, EVI1, the homolog of sPRDM16, is predominantly expressed in HSCs and is implicated in the regulation of their self renewal, as suggested by the findings that HSCs in EVI1$^{-/-}$ mouse embryos are markedly decreased in number and are defective in their self-renewal and repopulating capacities (42).

The ability of sPRDM16 to interfere with the myeloid differentiation program in vitro was similar in WT and p53$^{-/-}$ lin$^-$ cells. Instead, the effects of sPRDM16 and loss of p53 were cumulative in the serial replating assay, where, most notably, sPRDM16-overexpressing p53$^{-/-}$ lin$^-$ cells became virtually immortal. Similarly, in the competitive reconstitution assay, the effect of sPRDM16 on HSCs was more pronounced in the p53$^{-/-}$ background, suggesting that overexpression of sPRDM16 and loss of p53 cooperate in inducing expansion of the pool of HSCs. It appears, therefore, that loss of p53 augments the effects of sPRDM16 on HSC number and progenitor life span.

The cellular basis for the sPRDM16/p53-loss cooperation is not clear. p53 is a potent tumor suppressor protein. Individuals carrying 1 altered *TP53* gene in their germline have a high probability of developing a tumor, and most spontaneous human cancers contain either mutations in the *TP53* gene or altered expression of other gene products that disrupt p53 function (43). Functionally, p53 exerts 2 main activities: it senses and reacts to DNA damage through the ATM/ATR and Chk1/Chk2 kinases, thus ensuring genome stability and integrity, and limits oncogene-induced hyperproliferation in response to upregulation of the p53-stabilizing protein ARF. Recent genetic evidence in mice indicates that the response of p53 to DNA damage has little impact on cancer protection, while the ARF-dependent activation of p53 is critical for p53-mediated tumor suppression (44). Thus, loss of p53 might potentiate the effects of sPRDM16 on HSC and progenitor growth by abrogating or reducing the ARF-dependent cellular response to hyperproliferation induced by the sPRDM16 oncogene. Because the function of p53 in HSCs and progenitors has not been fully investigated, further investigations are needed to address this issue.

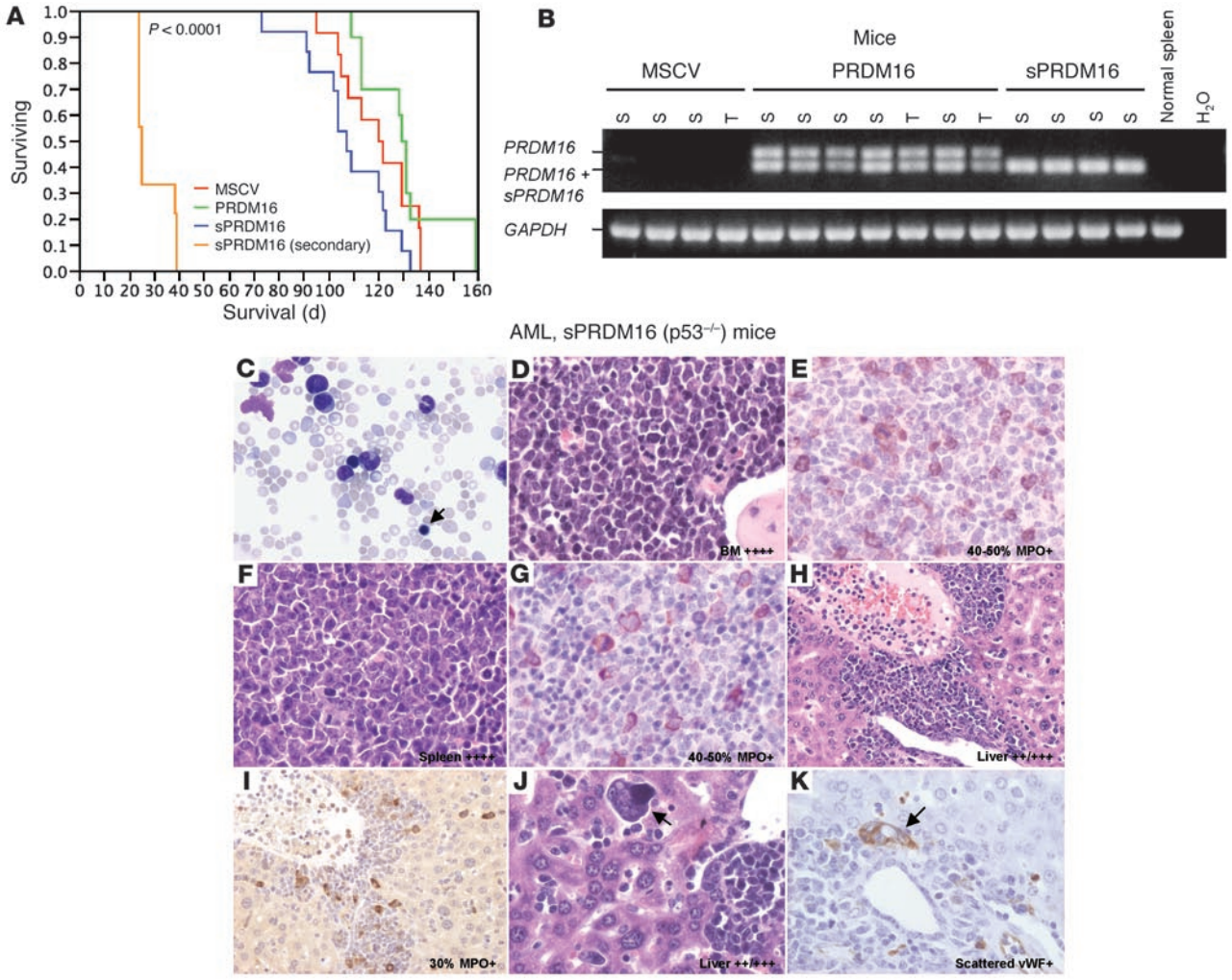

Figure 7

AML induced by sPRDM16 in the p53$^{-/-}$ background. (**A**) Overall survival of mice transplanted with transduced p53$^{-/-}$ lin^{-} cells and of secondary recipients transplanted with spleen cells of sPRDM16 (p53$^{-/-}$) primary leukemia. (**B**) Expression by RT-PCR of *PRDM16*, *PRDM16* and *sPRDM16*, and *GAPDH* in spleens (S) and thymi (T) of transplanted mice. (**C**) Blood smear of 1 AML with erythroid component (arrow; MGG stain). (**D**–**I**) H&E and myeloperoxidase (MPO) staining of tissue sections from bone marrow (**D** and **E**), spleen (**F** and **G**), and liver (**H** and **I**). (**J** and **K**) Liver sections showing dysplastic megakaryocytes (arrows; H&E stain; **J**) and stained for vWF (**K**). Neoplastic infiltration for each organ is denoted as follows: –, absent; +, minimum (<10%); ++, moderate (10%–30%); +++, extensive (30%–60%); ++++, heavy/diffuse (60%–100%). The percentage of cells staining positive for myeloperoxidase or vWF is indicated within the context of the neoplastic infiltrations. Original magnification, ×400 (**C**–**G**, **J**, and **K**); ×200 (**H** and **I**).

In conclusion, we demonstrate that overexpression of sPRDM16 and disruption of the p53 tumor suppressor pathway cooperate in leukemogenesis, both in human patients with AML and in our murine model of leukemia. We provide further evidence that accumulation of mutations that deregulate self renewal of SCs and growth of progenitors is critical during leukemogenesis. Finally, our data suggest that inhibition of sPRDM16 is a potentially relevant antileukemogenic strategy.

Methods

Mapping of translocation breakpoints. The use of all patient samples in this study was approved by the European Institute of Oncology's institutional review board, and all patients gave informed consent according to the Declaration of Helsinki. Clinical presentations of 5 leukemia patients (cases 1–5) with 1p36 rearrangements are shown in Supplemental Table 1. FISH mapping of translocation breakpoints as well as 5′-RACE and RT-PCR to confirm the presence of fusion genes were performed as described previously (45). Details of the bacterial artificial chromosome clones and positions of primers used are shown in Figure 1, and primer sequences are shown in Supplemental Table 2.

Q-PCR. RNA was obtained from normal CD34^{+} cells, AML cell lines, or bone marrow aspirates of de novo AML patients enrolled in the Italian GIMEMA trial. These cases were of various FAB subtypes, had been previously karyotyped and assessed for common fusion transcripts (*PML/RARα*, *AML1/ETO*, *CBFB/MYH11*, and *BCR/ABL*), *MLL* status, and *FLT3* and *NPM* mutations. RNA was extracted using the RNeasy kit with RNase-Free DNase treatment (Qiagen) and reverse transcribed to cDNA using SuperScript II RNase H-RT (Invitrogen) with random hexamers. With the exception of cell line OCI-AML2,

none of the AML patients or AML cell lines had rearrangements of 1p36.

Q-PCR was performed to determine the relative expression of the long isoform (*PRDM16*) or both long and short isoforms (*PRDM16* and *sPRDM16*) with respect to *GAPDH* (for primer positions and sequences, see Figure 2A and Supplemental Table 2). SYBR Green was used for cases 1–5 and Taqman for the other samples (Applied Biosystems). Cases 3 and 5 were assayed using both methods with similar results. Threshold cycle (Ct) values of *PRDM16* were normalized to those of *GAPDH* and calibrated to the median level of expression in $CD34^+$ samples (defined as 1.0), using the ΔΔCt method. High levels of *PRDM16* expression were defined as levels higher than the highest level of combined *PRDM16* and *sPRDM16* expression observed in $CD34^+$ cells (>1.7-fold that of baseline).

TP53 mutations in cases 1–5 were assessed by PCR amplification of the full-length transcript using HotStar HiFidelity Polymerase (Qiagen) and primers TP53_F and TP53_R (Supplemental Table 2). Direct sequencing of the PCR product was performed using internal primers TP53_F_int and TP53_R_int. For case 5, *TP53* cDNA was also amplified using primers mapping to exons 4 and 8 (TP53_F_ex4 and TP53_R_ex8; Supplemental Table 2) and the PCR products were TOPO-TA cloned (Invitrogen) and sequenced.

Expression of the *MYB* transcript was analyzed by Q-PCR (SYBR Green) in case 5 using primers MYB_F and MYB_R (Supplemental Table 2). Expression was normalized to *GAPDH* and calibrated to the level in $CD34^+$ samples as described above.

Q-PCR (SYBR Green) was performed on genomic DNA (gDNA) from selected patients to investigate the possibility of genomic amplification of the *PRDM16* locus. Primers PRDM16_gF and PRDM16_gR, spanning an intron-exon boundary, were used to specifically amplify genomic *PRDM16* (Supplemental Table 2). *PRDM16* copy number was normalized to that of genomic *HOXA9* and calibrated to the level in normal human gDNA derived from peripheral blood lymphocytes (Roche) using the ΔΔCt method.

Bisulfite sequencing method. CpG islands upstream and within the *PRDM16* gene were chosen to investigate the methylation status of the promoter of the long isoform (CpG 406) and additional potential transcription start sites upstream of exons 1, 2, 3 and 4. Bisulfite sequencing was performed on gDNA from 3 patients with low levels of *PRDM16* expression and 3 patients with high levels of expression, as previously described (46). Positions of the CpG islands and primer sequences are shown in Supplemental Table 3.

Transduction and transplantation of lin^- cells. *PRDM16* (exons 1–17) and *sPRDM16* (exons 4–17) were cloned into the MSCV retroviral vector (Figure 3A). The empty vector was used as a negative control in all experiments.

All mouse work was performed in accordance with national guidelines and was approved by the European Institute of Oncology's Institutional Review Board. Lin^- cells from WT or $p53^{-/-}$ mice (129SvJ/Ev-IEO/IFOM) were infected and sorted for GFP as previously described (5). Sorting and infection efficiencies are shown in Supplemental Table 4. WT, syngeneic recipient mice were lethally irradiated with 9 Gy and reinoculated intravenously with 300,000 WT or $p53^{-/-}$ donor lin^- cells, plus 500,000 spleen cells obtained from an untreated WT mouse (5). Animals were checked periodically for clinical signs of disease. Peripheral blood smears were stained with May-Grünwald-Giemsa (MGG), and blood counts were performed on a COULTER A^C.T 5diff hematology cell counter (Beckman Coulter).

Immunofluorescence. Immunofluorescence was used to check expression of PRDM16 and sPRDM16 in cytospins of lin^- cells, as previously described (35). A rabbit polyclonal anti-PRDM16 antibody raised against the C-terminal peptide sequence consisting of amino acids 1,016–1,277 (Eurogentec s.a.) was used, followed by detection with a Cy3-conjugated anti-rabbit IgG secondary antibody.

Biological assays. To assess self renewal, 5,000 lin^- cells were serially plated in methylcellulose supplemented with G-CSF and GM-CSF as previously described (5), or in M3334 methylcellulose containing EPO supplemented with IL-3 (2 ng/ml) and SCF (50 ng/ml) (Stem Cell Technologies). Differentiation was assessed by MGG staining of cytospins at each plating.

For LTC-IC, stromal cells were prepared from the bone marrow of WT mice and grown for 3 weeks in Iscove's modified Dulbecco's medium supplemented with 12.5% FBS, 12.5% horse serum, 276 ng/ml hydrocortisone, and 50 μM 2-mercaptoethanol, prior to irradiation with 15 Gy. Transduced lin^- cells (15,000 cells) were seeded on the irradiated stromal layer, grown for 5 weeks, and plated in methylcellulose. CFUs were counted after 7–10 days.

Competitive reconstitution assay. Test lin^- cells from WT or $p53^{-/-}$ mice (C57BL/6, $CD45.2^+$; Charles River Laboratories) were infected with MSCV or MSCV-sPRDM16 and sorted for GFP as described above. In parallel, competitor lin^- cells from WT mice (C57BL/6, $CD45.1^+$; Charles River Laboratories) were transduced with MSCV alone. Test lin^- cells (300,000 cells; CD45.2) and competitor lin^- cells (300,000 cells; CD45.1) were reinoculated into lethally irradiated syngeneic recipient mice (CD45.1) together with spleen cells (500,000 cells; CD45.1), derived from a healthy mouse. Double FACS staining was performed on peripheral blood taken at 4 and 8 weeks after transplantation, using the antibodies PE-conjugated anti-mouse CD45.1, PE-conjugated anti-mouse Ly-6G (Gr1), PE-conjugated anti-mouse CD3, PE-conjugated anti-mouse CD45R (B220), and biotin-conjugated anti-mouse CD45.2, followed by detection with streptavidin-Cy5 (eBioscience). Cells were analyzed using a BD FACScan equipped with Cell Quest software.

Histopathology and immunohistochemistry of mouse tissues. Diseased mice were sacrificed humanely by CO_2 asphyxiation and underwent necropsy. Main organs were fixed in 10% buffered formalin, embedded in paraffin, and sectioned at 4 μm. Tissue sections were stained with H&E or subjected to immunohistochemical analysis as standard using anti-myeloperoxidase, anti-CD3, or anti-vWF rabbit polyclonal antibodies (DakoCytomation). Detection was performed using anti-rabbit Envision$^+$ System, HRP (DakoCytomation) and the DAB Substrate Kit, 3,3′ diaminobenzidine (Vector Laboratories). Tissues were counterstained with Mayer's hemalum solution.

RT-PCR of mouse tissues. Expression of exogenous *PRDM16* and *sPRDM16* was checked in the spleens and thymuses of sacrificed mice using RT-PCR and primers recognizing *PRDM16*, both *PRDM16* and *sPRDM16*, and *GAPDH* (Supplemental Table 2; ref. 47).

Statistics. Statistical analysis was performed using JMP software. Chi-squared test was used to determine association of *FLT3-ITD* or *NPM* mutations with *PRDM16* expression, Kaplan-Meier analysis was used for mouse survival, and 2-tailed Student's *t* test was used to compare means in LTC-IC and competitive reconstitution assays. *P* values of less than 0.05 were considered significant.

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Address correspondence to: Pier Giuseppe Pelicci, Department of Experimental Oncology, Via Ripamonti 435, 20141 Milan, Italy. Phone: 39-02-57489868; Fax: 39-02-57489851; E-mail: piergiuseppe.pelicci@ifom-ieo-campus.it.

1. Passegue, E., Jamieson, C.H., Ailles, L.E., and Weissman, I.L. 2003. Normal and leukemic hematopoiesis: are leukemias a stem cell disorder or a reacquisition of stem cell characteristics? *Proc. Natl. Acad. Sci. U. S. A.* **100**(Suppl. 1):11842–11849.
2. Hoffman, R., et al. 2000. *Hematology: basic principles and practice.* Churchill Livingstone. Philadelphia, Pennsylvania, USA. 2896 pp.
3. Look, A.T. 1997. Oncogenic transcription factors in the human acute leukemias. *Science.* **278**:1059–1064.
4. Falini, B., et al. 2005. Cytoplasmic nucleophosmin in acute myelogenous leukemia with a normal karyotype. *N. Engl. J. Med.* **352**:254–266.
5. Minucci, S., et al. 2002. PML-RAR induces promyelocytic leukemias with high efficiency following retroviral gene transfer into purified murine hematopoietic progenitors. *Blood.* **100**:2989–2995.
6. Higuchi, M., et al. 2002. Expression of a conditional AML1-ETO oncogene bypasses embryonic lethality and establishes a murine model of human t(8;21) acute myeloid leukemia. *Cancer Cell.* **1**:63–74.
7. Wattel, E., et al. 1994. p53 mutations are associated with resistance to chemotherapy and short survival in hematologic malignancies. *Blood.* **84**:3148–3157.
8. Schnittger, S., et al. 2002. Analysis of FLT3 length mutations in 1003 patients with acute myeloid leukemia: correlation to cytogenetics, FAB subtype, and prognosis in the AMLCG study and usefulness as a marker for the detection of minimal residual disease. *Blood.* **100**:59–66.
9. Bacher, U., Haferlach, T., Schoch, C., Kern, W., and Schnittger, S. 2006. Implications of NRAS mutations in AML: a study of 2502 patients. *Blood.* **107**:3847–3853.
10. Shimizu, S., et al. 2000. Identification of breakpoint cluster regions at 1p36.3 and 3q21 in hematologic malignancies with t(1;3)(p36;q21). *Genes Chromosomes Cancer.* **27**:229–238.
11. Mochizuki, N., et al. 2000. A novel gene, MEL1, mapped to 1p36.3 is highly homologous to the MDS1/EVI1 gene and is transcriptionally activated in t(1;3)(p36;q21)-positive leukemia cells. *Blood.* **96**:3209–3214.
12. Sakai, I., et al. 2005. Novel RUNX1-PRDM16 fusion transcripts in a patient with acute myeloid leukemia showing t(1;21)(p36;q22). *Genes Chromosomes Cancer.* **44**:265–270.
13. Stevens-Kroef, M.J., et al. 2006. Identification of truncated RUNX1 and RUNX1-PRDM16 fusion transcripts in a case of t(1;21)(p36;q22)-positive therapy-related AML. *Leukemia.* **20**:1187–1189.
14. Hazourli, S., et al. 2006. Overexpression of PRDM16 in the presence and absence of the RUNX1/PRDM16 fusion gene in myeloid leukemias. *Genes Chromosomes Cancer.* **45**:1072–1076.
15. Bloomfield, C.D., Garson, O.M., Volin, L., Knuutila, S., and de la Chapelle, A. 1985. t(1;3)(p36;q21) in acute nonlymphocytic leukemia: a new cytogenetic-clinicopathologic association. *Blood.* **66**:1409–1413.
16. Barjesteh van Waalwijk van Doorn-Khosrovani, S., Erpelinck, C., Lowenberg, B., and Delwel, R. 2003. Low expression of MDS1-EVI1-like-1 (MEL1) and EVI1-like-1 (EL1) genes in favorable-risk acute myeloid leukemia. *Exp. Hematol.* **31**:1066–1072.
17. Nishikata, I., et al. 2003. A novel EVI1 gene family, MEL1, lacking a PR domain (MEL1S) is expressed mainly in t(1;3)(p36;q21)-positive AML and blocks G-CSF-induced myeloid differentiation. *Blood.* **102**:3323–3332.
18. Jiang, G.L., and Huang, S. 2000. The yin-yang of PR-domain family genes in tumorigenesis. *Histol. Histopathol.* **15**:109–117.
19. Nucifora, G., et al. 1994. Consistent intergenic splicing and production of multiple transcripts between AML1 at 21q22 and unrelated genes at 3q26 in (3;21)(q26;q22) translocations. *Proc. Natl. Acad. Sci. U. S. A.* **91**:4004–4008.
20. Morishita, K., et al. 1992. Activation of EVI1 gene expression in human acute myelogenous leukemias by translocations spanning 300-400 kilobases on chromosome band 3q26. *Proc. Natl. Acad. Sci. U. S. A.* **89**:3937–3941.
21. Xiao, Z., et al. 2006. MEL1S, not MEL1, is overexpressed in myelodysplastic syndromes patients with t(1;3)(p36;q21). *Leuk. Res.* **30**:332–334.
22. Yoshida, M., et al. 2004. Aberrant expression of the MEL1S gene identified in association with hypomethylation in adult T-cell leukemia cells. *Blood.* **103**:2753–2760.
23. Du, Y., Jenkins, N.A., and Copeland, N.G. 2005. Insertional mutagenesis identifies genes that promote the immortalization of primary bone marrow progenitor cells. *Blood.* **106**:3932–3939.
24. Sutherland, H.J., Lansdorp, P.M., Henkelman, D.H., Eaves, A.C., and Eaves, C.J. 1990. Functional characterization of individual human hematopoietic stem cells cultured at limiting dilution on supportive marrow stromal layers. *Proc. Natl. Acad. Sci. U. S. A.* **87**:3584–3588.
25. Ploemacher, R.E., van der Sluijs, J.P., Voerman, J.S., and Brons, N.H. 1989. An in vitro limiting-dilution assay of long-term repopulating hematopoietic stem cells in the mouse. *Blood.* **74**:2755–2763.
26. Kogan, S.C., et al. 2002. Bethesda proposals for classification of nonlymphoid hematopoietic neoplasms in mice. *Blood.* **100**:238–245.
27. Harvey, M., McArthur, M.J., Montgomery, C.A., Jr., Bradley, A., and Donehower, L.A. 1993. Genetic background alters the spectrum of tumors that develop in p53-deficient mice. *FASEB J.* **7**:938–943.
28. Nawa, G., Miyoshi, Y., Yoshikawa, H., Ochi, T., and Nakamura, Y. 1999. Frequent loss of expression or aberrant alternative splicing of P2XM, a p53-inducible gene, in soft-tissue tumours. *Br. J. Cancer.* **80**:1185–1189.
29. Hamelin, R., et al. 1994. Association of p53 mutations with short survival in colorectal cancer. *Gastroenterology.* **106**:42–48.
30. Gentile, M., Bergman Jungestrom, M., Olsen, K.E., Soderkvist, P., and Wingren, S. 1999. p53 and survival in early onset breast cancer: analysis of gene mutations, loss of heterozygosity and protein accumulation. *Eur. J. Cancer.* **35**:1202–1207.
31. Kato, S., et al. 2003. Understanding the function-structure and function-mutation relationships of p53 tumor suppressor protein by high-resolution missense mutation analysis. *Proc. Natl. Acad. Sci. U. S. A.* **100**:8424–8429.
32. Thiede, C., et al. 2006. Prevalence and prognostic impact of NPM1 mutations in 1485 adult patients with acute myeloid leukemia (AML). *Blood.* **107**:4011–4020.
33. Pasqualucci, L., et al. 2006. Mutated nucleophosmin detects clonal multilineage involvement in acute myeloid leukemia: Impact on WHO classification. *Blood.* **108**:4146–4155.
34. Dohner, K., et al. 2005. Mutant nucleophosmin (NPM1) predicts favorable prognosis in younger adults with acute myeloid leukemia and normal cytogenetics: interaction with other gene mutations. *Blood.* **106**:3740–3746.
35. Colombo, E., et al. 2006. Delocalization and destabilization of the Arf tumor suppressor by the leukemia-associated NPM mutant. *Cancer Res.* **66**:3044–3050.
36. den Besten, W., Kuo, M.L., Williams, R.T., and Sherr, C.J. 2005. Myeloid leukemia-associated nucleophosmin mutants perturb p53-dependent and independent activities of the Arf tumor suppressor protein. *Cell Cycle.* **4**:1593–1598.
37. Suzuki, T., et al. 2005. Clinical characteristics and prognostic implications of NPM1 mutations in acute myeloid leukemia. *Blood.* **106**:2854–2861.
38. Krivtsov, A.V., et al. 2006. Transformation from committed progenitor to leukaemia stem cell initiated by MLL-AF9. *Nature.* **442**:818–822.
39. Huntly, B.J., et al. 2004. MOZ-TIF2, but not BCR-ABL, confers properties of leukemic stem cells to committed murine hematopoietic progenitors. *Cancer Cell.* **6**:587–596.
40. Cozzio, A., et al. 2003. Similar MLL-associated leukemias arising from self-renewing stem cells and short-lived myeloid progenitors. *Genes Dev.* **17**:3029–3035.
41. Alcalay, M., et al. 2005. Acute myeloid leukemia bearing cytoplasmic nucleophosmin (NPMc+ AML) shows a distinct gene expression profile characterized by up-regulation of genes involved in stem-cell maintenance. *Blood.* **106**:899–902.
42. Yuasa, H., et al. 2005. Oncogenic transcription factor Evi1 regulates hematopoietic stem cell proliferation through GATA-2 expression. *EMBO J.* **24**:1976–1987.
43. Vousden, K.H., and Prives, C. 2005. P53 and prognosis: new insights and further complexity. *Cell.* **120**:7–10.
44. Efeyan, A., and Serrano, M. 2007. p53: guardian of the genome and policeman of the oncogenes. *Cell Cycle.* **6**:1006–1010.
45. Belloni, E., et al. 2005. 8p11 myeloproliferative syndrome with a novel t(7;8) translocation leading to fusion of the FGFR1 and TIF1 genes. *Genes Chromosomes Cancer.* **42**:320–325.
46. Zardo, G., et al. 2002. Integrated genomic and epigenomic analyses pinpoint biallelic gene inactivation in tumors. *Nat. Genet.* **32**:453–458.
47. Lahortiga, I., et al. 2004. Molecular characterization of a t(1;3)(p36;q21) in a patient with MDS. MEL1 is widely expressed in normal tissues, including bone marrow, and it is not overexpressed in the t(1;3) cells. *Oncogene.* **23**:311–316.