Brief Report

MYELOID NEOPLASIA

Differential role of Id1 in MLL-AF9–driven leukemia based on cell of origin

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Key Points

- Loss of Id1 delays leukemogenesis in fetal MLL-AF9 leukemia model, but accelerates leukemogenesis in postnatal MLL-AF9 leukemia model.
- Deletion of Cdkn1a (p21) rescues the loss of Id1 in both MLL-AF9 mouse models.

Inhibitor of DNA binding 1 (Id1) functions as an E protein inhibitor, and overexpression of Id1 is seen in acute myeloid leukemia (AML) patients. To define the effects of Id1 on leukemogenesis, we expressed MLL-AF9 in fetal liver (FL) cells or bone marrow (BM) cells isolated from wild-type, *Id1*$^{-/-}$, *p21*$^{-/-}$, or *Id1*$^{-/-}$*p21*$^{-/-}$ mice, and transplanted them into syngeneic recipient mice. We found that although mice receiving MLL-AF9–transduced FL or BM cells develop AML, loss of Id1 significantly prolonged the median survival of mice receiving FL cells but accelerated leukemogenesis in recipients of BM cells. Deletion of Cdkn1a (p21), an Id1 target gene, can rescue the effect of Id1 loss in both models, suggesting that Cdkn1a is a critical target of Id1 in leukemogenesis. It has been suggested that the FL transplant model mimics human fetal-origin (infant) MLL fusion protein (FP)-driven leukemia, whereas the BM transplantation model resembles postnatal MLL leukemia; in fact, the analysis of clinical samples from patients with MLL-FP^{+} leukemia showed that Id1 expression is elevated in the former and reduced in the latter type of MLL-FP^{+} AML. Our findings suggest that Id1 could be a potential therapeutic target for infant *MLL-AF9*–driven leukemia. (*Blood*. 2016; 127(19):2322-2326)

Introduction

Inhibitor of DNA binding 1 (Id1) is a key transcriptional regulator of hematopoietic stem cell (HSC) lineage commitment, and the absence of Id1 compromises the self-renewal capacity of HSCs.[1-3] This functional defect is associated with transcriptional changes in Id1$^{-/-}$ HSCs, including the increased expression of p21, a well-established target of Id1 repression.[2,4] Id1 controls cancer-initiating cell self-renewal through cell-cycle restriction driven by p21,[5] and we demonstrated the opposing effects of Id1 and p21 on myeloid lineage differentiation.[4] We have recently identified that loss of Id1 inhibited t(8;21) leukemia initiation and progression by abrogating AKT1 activation.[6,7]

Many investigators have found that the MLL-AF9 fusion protein induces acute myeloid leukemia (AML) through effects on numerous transcriptional regulators (eg, Meis1, HoxA9, and Runx1) and epigenetic modulators (eg, Dot1L),[8-19] however, the role of Id1 in MLL-AF9–mediated leukemogenesis was unknown.[20] Overexpression of Id1 is seen in ~75% of patients with AML-M5, and recent clinical studies identified that high Id1 expression is associated with poor prognosis in AML-M5 patients, independently predicting for shorter disease-free survival and overall survival.[21] Id1 is highly expressed in the MLL-AF9^{+} leukemia cell lines THP1 and MOLM13 (supplemental Figure 1, available on the *Blood* Web site), which suggests that Id1 may have an important role in MLL-AF9–driven leukemia. To define the effects of Id1 on leukemogenesis, we used retroviral gene transfer to express MLL-AF9 in fetal liver (FL) or bone marrow (BM) cells isolated from wild-type (wt), *Id1*$^{-/-}$, *p21*$^{-/-}$, or *Id1*$^{-/-}$*p21*$^{-/-}$ mice, and then transplanted the transduced cells into lethally irradiated recipient mice. These studies have identified cell-type–specific effects of Id1.

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There is an Inside *Blood* Commentary on this article in this issue.

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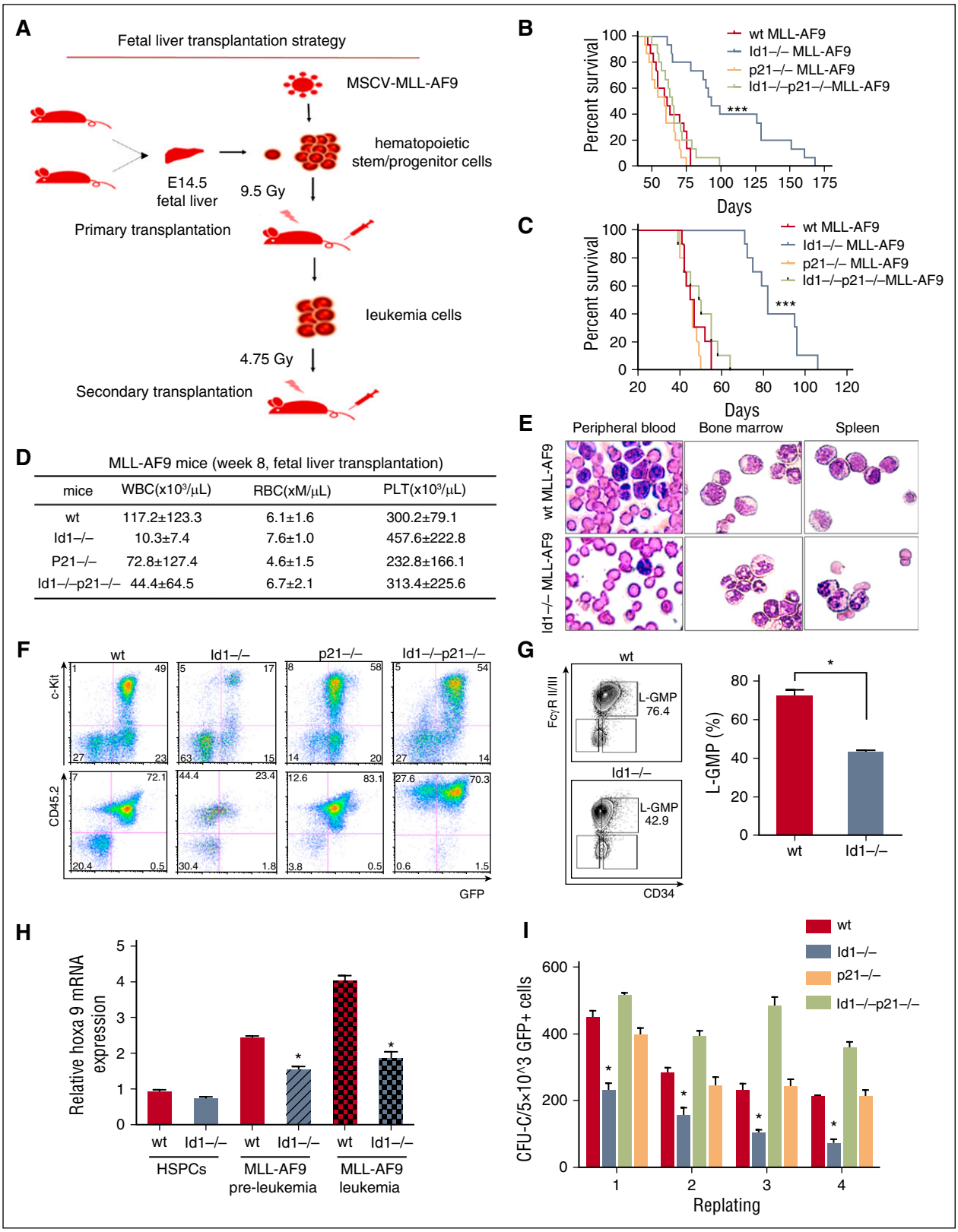

Figure 1. Loss of Id1 delays the development of MLL-AF9–driven leukemia from FL, and depletion of p21 can rescue Id1-deficiency–induced phenotype. (A) The strategy of FL transplantation model. (B) In the primary transplantation assay, loss of Id1 prolongs the survival time of recipient mice (61 days vs 93 days, n = 15/group, $P < .001$). Deletion of p21 alone or combined with Id1 deletion does not affect the survival time of recipient mice. (C) In the secondary transplantation assay, the mice in the Id1$^{-/-}$ group developed leukemia slower compared with the wt group (46 days vs 82 days, n = 15/group, $P < .001$). Deletion of p21 alone or combined with Id1 deletion does not affect the survival time of recipient mice. (D) The complete blood count analysis showed that, 8 weeks posttransplantation, the average WBC of the wt group is higher than the p21$^{-/-}$ group, the p21$^{-/-}$ group is higher than the Id1$^{-/-}$p21$^{-/-}$ group, and the Id1$^{-/-}$p21$^{-/-}$ group is higher than the Id1$^{-/-}$ group. The average RBC of the wt group is lower than the p21$^{-/-}$ group, the p21$^{-/-}$ group is lower than the Id1$^{-/-}$p21$^{-/-}$ group, and the Id1$^{-/-}$p21$^{-/-}$ group is lower than the Id1$^{-/-}$ group. Platelet (PLT) has no significant differences in these 4 groups. (E) The morphology

Study design

For FL and BM transplantation, we used retroviral gene transfer to express the MLL-AF9 complementary DNA in the embryonic day 14.5 (E14.5) FL cells or BM cells of wt, *Id1*$^{-/-}$, *p21*$^{-/-}$, or *Id1*$^{-/-}$*p21*$^{-/-}$ mice. Hematopoietic stem/progenitor cells (HSPCs) were infected with retroviruses by spinoculation. The FL/BM HSPCs were cultured in X-VIVO medium with interleukin-3 (IL-3), IL-6, and stem cell factor.[22] The transduction efficiency was around 30%. The 1×10^5 green fluorescent protein–positive (GFP$^+$) MLL-AF9–transduced FL/BM cells were transplanted into lethally irradiated C57Bl/6.SJL recipient mice by tail-vein injection. For details and additional methods, see supplemental Methods.

Results and discussion

Id1 is more highly expressed in FL cells than in BM cells, suggesting that Id1 may have differential roles in these distinct types of HSPCs (supplemental Figure 2). The expression of MLL-AF9 in mouse FL cells leads to an increase in Id1 expression and the rapid development of AML with maturation. Loss of Id1 slowed the initiation of leukemia and markedly prolonged the median survival of the MLL-AF9 mice in the FL transplantation model, with deletion of p21 rescuing the effect of Id1 loss (Figure 1A-B). Splenomegaly and hepatomegaly were prominently observed in wt, *p21*$^{-/-}$, or *Id1*$^{-/-}$*p21*$^{-/-}$ MLL-AF9 mice, and less so in the *Id1*$^{-/-}$ MLL-AF9 mice (supplemental Figure 3). The white blood cell (WBC) counts of the mice in the MLL-AF9/*Id1*$^{-/-}$ group were significantly lower than the MLL-AF9/wt, *p21*$^{-/-}$, or *Id1*$^{-/-}$*p21*$^{-/-}$ groups and the red blood cell (RBC) counts were higher, reflecting impaired leukemogenesis (Figure 1D). Cytospins of the AMLs showed less circulating leukemic blast cells in the MLL-AF9/*Id1*$^{-/-}$ group than the MLL-AF9/wt group, and less infiltration of blast cells in the spleen and BM (Figure 1E). Furthermore, the peripheral blood (PB) of the MLL-AF9/*Id1*$^{-/-}$ group contained far fewer GFP$^+$C-Kit$^+$ leukemia blast cells (Figure 1F) than the MLL-AF9/wt, *p21*$^{-/-}$, or *Id1*$^{-/-}$*p21*$^{-/-}$ mice, and the frequencies of GMP-like leukemic cells (L-GMP) in the wt MLL-AF9 leukemic mice were higher than the Id1$^{-/-}$ MLL-AF9 leukemic mice (Figure 1G). To determine the effect of Id1 on the maintenance of MLL-AF9–driven AML, we transplanted MLL-AF9–expressing wt, *Id1*$^{-/-}$, *p21*$^{-/-}$, or *Id1*$^{-/-}$*p21*$^{-/-}$ cells into sublethally irradiated mice. Loss of Id1 significantly prolonged the median survival of the secondarily transplanted MLL-AF9 mice (Figure 1C). Interestingly, we found that both HoxA9 and Meis1 were downregulated in the Id1$^{-/-}$ MLL-AF9–expressing FL cells compared with wt MLL-AF9–expressing FL cells (Figure 1H; supplemental Figure 5).

We then examined the effect of Id1 on the self-renewal of the transformed FL cells by performing serial replating assays using MLL-AF9–transduced FL cells that were isolated from wt, *Id1*$^{-/-}$, *p21*$^{-/-}$, or *Id1*$^{-/-}$*p21*$^{-/-}$ mice. The Id1$^{-/-}$ MLL-AF9 cells had significantly less repopulating capacity than wt MLL-AF9 cells (Figure 1I; supplemental Figure 4), suggesting that Id1 controls the initiation of myeloid transformation by regulating the self-renewal of transformed HSPCs.

Next, we transplanted lethally irradiated mice with MLL-AF9–transduced BM cells isolated from wt, *Id1*$^{-/-}$, *p21*$^{-/-}$, or *Id1*$^{-/-}$*p21*$^{-/-}$ mice (Figure 2A). In this case, loss of Id1 accelerated the initiation of leukemia and impaired the median survival of the MLL-AF9 mice (Figure 2B); this effect can also be rescued by deletion of p21 (supplemental Figure 6). Loss of Id1 also significantly accelerated death from MLL-AF9$^+$ leukemia in secondary BM transplantation models (Figure 2C), with far more circulating leukemic blast cells in the MLL-AF9/*Id1*$^{-/-}$ group than the MLL-AF9/wt group, and more extensive infiltration of blast cells in the spleen and BM (Figure 2D), leading to profound splenomegaly and hepatomegaly (supplemental Figure 7). The WBC counts of the mice that received MLL-AF9/*Id1*$^{-/-}$ BM cells were significantly higher than the MLL-AF9/wt group, reflecting accelerated leukemogenesis (supplemental Table 1). Indeed, the PB of the MLL-AF9/*Id1*$^{-/-}$ mice contained far more GFP$^+$, C-Kit$^+$, Mac1$^+$ leukemia cells than the MLL-AF9/wt mice (Figure 2E). The frequencies of L-GMP in the wt MLL-AF9–driven BM cell leukemias were lower than the Id1$^{-/-}$ MLL-AF9–driven BM cell leukemia (Figure 2F). In contrast to what we observed using FL cells, Id1 is downregulated in MLL-AF9–expressing BM cells and that HoxA9 and Meis1 are upregulated in the Id1$^{-/-}$MLL-AF9 BM cells, compared with wt MLL-AF9 BM cells (Figure 2G; supplemental Figure 7).

To examine the effect of Id1 on the self-renewal of the transformed BM cells, we performed limiting dilution assays, and found that loss of Id1 significantly increased the leukemia-initiating cell frequency in MLL-AF9–driven leukemia (1 in 286 vs 1 in 15 707) (supplemental Table 2). We also performed serial replating assays using GFP$^+$ MLL-AF9–transduced BM cells isolated from wt, *Id1*$^{-/-}$, or *Id1*$^{-/-}$*p21*$^{-/-}$ mice, and the *Id1*$^{-/-}$ MLL-AF9 cells had more repopulating capacity than the wt MLL-AF9 cells; this effect could also be rescued by deletion of p21 (Figure 2H; supplemental Figure 9). These results demonstrated that Id1 plays different roles in MLL-AF9–driven leukemias that develop from BM vs FL cells.

Gene expression studies suggest that human infant *MLL* fusion gene leukemia originates in cells that differ from MLL fusion gene-driven postnatal leukemia.[23] Interestingly, we found that loss of Id1 abrogated leukemogenesis in the mice transplanted with *MLL-AF9* FL cells but accelerated leukemogenesis in the mice transplanted with *MLL-AF9* BM cells. The AML that is generated from either source of HSPCs is the same in terms of its level of differentiation. These data describe for the first time a differential effect of a gene on leukemia initiation and development in vivo between MLL-AF9 infant leukemia, and postnatal MLL-FP–driven leukemia, which advances our understanding of the pathogenesis of MLL-FP–driven leukemia. Interestingly, an analysis of clinical samples showed higher Id1 messenger RNA (mRNA) levels in the MLL$^+$ AML patients who were younger than 3 years old, compared with the samples from patients who were older than 3 years (Figure 2I; supplemental Table 3).

The effects of Id1 in leukemogenesis appear to be largely dependent on p21. The expression of p21 is extremely low in human fetal HSPCs, but it increases as the cells differentiate into myeloid cells; this is consistent with our observation that inhibition of Id1 can promote the myeloid differentiation of leukemia cells originating from FL.[24] Id1

Figure 1 (continued) results showed that there were less leukemia blast cells in the PB, BM, and spleen of the Id1$^{-/-}$ group mice compared with the wt group. (F) The flow analysis showed that the PB cells of the mice in the Id1$^{-/-}$ group express less GFP$^+$c-Kit$^+$ cells compared with the wt, p21$^{-/-}$, and Id1$^{-/-}$p21$^{-/-}$ group. (G) The flow analysis of L-GMP (IL-7R-Lin-Sca-1-c-Kit$^+$CD34-FcγRII/III$^+$ GMP-like leukemic cells) in the BM cells from wt and Id1$^{-/-}$ MLL-AF9 leukemia mice (left panel). The frequency of L-GMP in the BM cells from Id1$^{-/-}$ MLL-AF9 leukemia mice is lower than that from wt MLL-AF9 leukemia mice (right panel). (H) The mRNA expression level of Hoxa 9 in wt and Id1$^{-/-}$ fetal liver HSPCs (marked as wt and Id1$^{-/-}$), E14.5 FL HSPCs transduced with MLL-AF9 (marked as MLL-AF9 preleukemia wt and Id1$^{-/-}$) and leukemia cells in resulting leukemia (marked as MLL-AF9 leukemia wt and Id1$^{-/-}$). (I) The columns represent the numbers of total colonies in each plating of the sorted GFP$^+$ wt or Id1$^{-/-}$ E14.5 FL HSPCs transduced with MLL-AF9 ($\pm$ standard error of the mean [SEM]; n = 3). CFU-C, colony-forming unit in culture; MSCV, murine stem cell virus.

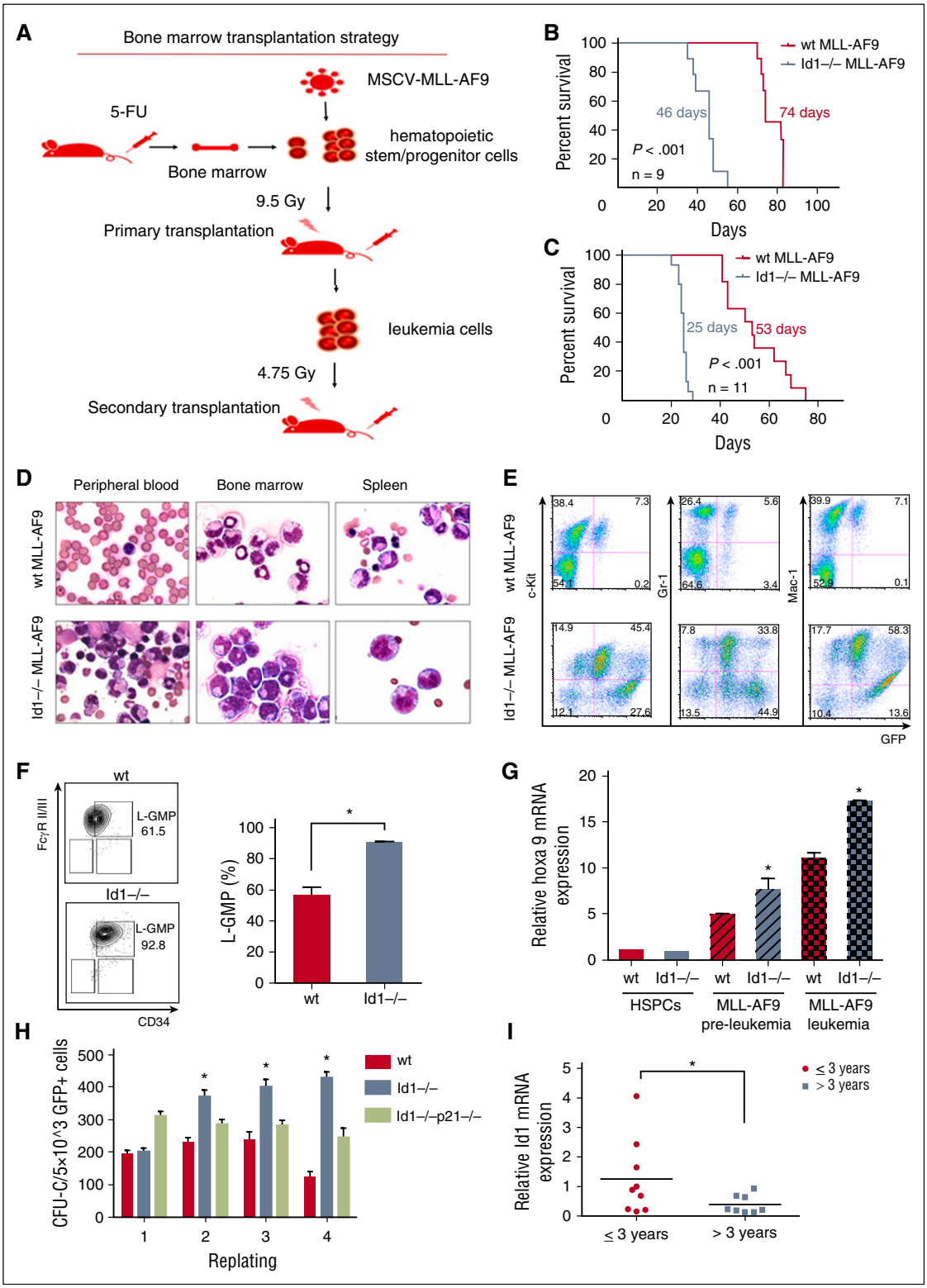

Figure 2. Loss of Id1 accelerates the development of MLL-AF9–driven leukemia in the BM transplantation model. (A) The strategy of BM transplantation model. (B) In the primary transplantation assay, loss of Id1 shortens the survival time of recipient mice (46 days vs 74 days, n = 10 per group, $P < .001$). (C) In the secondary transplantation assay, the mice in Id1$^{-/-}$ group developed leukemia faster compared with the wt group. (D) The morphology results show that, 6 weeks after transplantation, there were more leukemia blast cells in the PB, BM, and spleen of Id1$^{-/-}$ group mice compared with the wt group. (E) The flow analysis shows that, 6 weeks after transplantation, the PB cells of the mice in Id1$^{-/-}$ group express less GFP^{+}c-Kit^{+} cells compared with the wt group. (F) The flow analysis of L-GMP (FcγR II/III^{hi}CD34^{+}) (left panel) in the BM cells from wt and Id1$^{-/-}$ MLL-AF9 leukemia mice. The frequency of L-GMP in the BM cells from Id1$^{-/-}$ MLL-AF9 leukemia mice is higher than that from wt MLL-AF9 leukemia mice (right panel). (G) The mRNA expression level of Hoxa 9 in wt and Id1$^{-/-}$ BM HSPCs (marked as wt and Id1$^{-/-}$), BM HSPCs transduced with MLL-AF9 (marked as MLL-AF9 preleukemia wt and Id1$^{-/-}$), and leukemia cells in resulting leukemia (marked as MLL-AF9 leukemia wt and Id1$^{-/-}$). (H) The columns represent the numbers of colonies in each plating of wt, Id1$^{-/-}$, or Id1$^{-/-}$p21$^{-/-}$ BM cells transduced with MLL-AF9 (±SEM; n = 3). (I) The quantitative polymerase chain reaction (Q-PCR) analysis shows the Id1 expression in the MLL^{+} patient BM samples (age ≤3 group [n = 9] vs age >3 group [n = 8]). 5-FU, fluorouracil.

expression is higher in FL HSPCs than in BM HSPCs, which could account for the differences in the effects of its absence. The frequency of L-GMP is increased in the $Id1^{-/-}$ MLL-AF9 BM cells but decreased in the $Id1^{-/-}$ MLL-AF9 fetal liver cells, suggesting that leukemia-initiating cell frequency depends on Id1 expression. Loss of Id1 increased the expression levels of HoxA9 and Meis1, the indicators of MLL-AF9–driven leukemogenesis, in MLL-AF9–transformed HSPCs from FL, but decreased the expression levels of HoxA9 and Meis1 in MLL-AF9–transformed HSPCs from BM. Although these changes may contribute to the observed differences in leukemogenicity, they could also be the result of cellular transformation. Defining the differential roles of Id1 in fetal vs postnatal MLL-$AF9^{+}$ leukemia will allow for further elucidation of the various cell origins implicated in AML and may provide a potential target for *MLL-AF9*–driven infant leukemia.

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Authorship

Contribution: L.W., S.D.N., N.M., and X.-J.S. designed the research, analyzed data, and wrote the paper; M.G.-C., R.B., Y.Z., and F.-C.Y. contributed new reagents and analyzed data; and L.W., N.M., X.-J.S., Y.T., M.G.-C., F.L., G.C., M.H., H.X., R.S., N.L., G.H., N.C., J.S., and M.S. performed research and analyzed data.

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