ABSTRACT

Persistence of drug-resistant quiescent leukemic stem cells (LSC) and impaired natural killer (NK) cell immune response account for relapse of chronic myelogenous leukemia (CML). Inactivation of protein phosphatase 2A (PP2A) is essential for CML-quiescent LSC survival and NK cell antitumor activity. Here we show that MIR300 has antiproliferative and PP2A-activating functions that are dose dependently differentially induced by CCND2/CDK6 and SET inhibition, respectively. MIR300 is upregulated in CML LSCs and NK cells by bone marrow microenvironment (BMM) signals to induce quiescence and impair immune response, respectively. Conversely, BCR-ABL1 downregulates MIR300 in CML progenitors to prevent growth arrest and PP2A-mediated apoptosis. Quiescent LSCs escape apoptosis by upregulating TUG1 long noncoding RNA that uncouples and limits MIR300 function to cytostasis. Genetic and pharmacologic MIR300 modulation and/or PP2A-activating drug treatment restore NK cell activity, inhibit BMM-induced growth arrest, and selectively trigger LSC apoptosis in vitro and in patient-derived xenografts; hence, the importance of MIR300 and PP2A activity for CML development and therapy.

SIGNIFICANCE: Tumor-naïve microenvironment–induced MIR300 is the only tumor suppressor miRNA that induces CML LSC quiescence while inhibiting NK cell antitumor immune response, and CML LSC/progenitor cell apoptosis through its anti-proliferative and PP2A-activating functions, respectively. Thus, the importance of MIR300 and PP2A-activating drugs for formation/survival and eradication of drug-resistant CML LSCs.
INTRODUCTION

Chronic myeloid leukemia (CML) is a biphasic hematopoietic stem cell (HSC) myeloproliferative disorder driven by BCR-ABL1 oncogenic kinase activity (1). Despite being clinically manageable, CML is not a curable cancer and resistance to ABL tyrosine kinase inhibitors (TKI) remains a major therapeutic challenge (2). In fact, persistence of CML-initiating quiescent leukemic stem cells (qLSC) likely depends on their innate and acquired TKI resistance (3) and on impaired natural killer (NK) cell cytotoxicity against leukemic stem cells (LSC) (4), and accounts for disease relapse and dismal outcome (1, 5). Clinical trials, aimed at targeting intrinsic mechanisms of TKI resistance, failed to eradicate the TKI-resistant qLSC reservoir, likely because of bone marrow (BM) microenvironment (BMM) protective and inhibitory effects on LSCs and NK cells, respectively (5, 6).

Protein phosphatase 2A (PP2A) serine-threonine phosphatase is a druggable multimeric tumor suppressor inactivated in nearly all types of cancer, mostly by increased endogenous inhibitor (e.g., SET, CIP2A) or impaired subunit expression/function (7). PP2A loss-of-function is essential for cancer stem cell maintenance, tumor growth/progression, and activation of NK cell proliferation and antitumor cytotoxic activity (7). BCR-ABL1–independent and -dependent signals inhibit PP2A activity in CML [chronic (CP) and blastic (BC) phase] TKI-resistant qLSCs and TKI-sensitive and -resistant proliferating blasts, respectively, through activation of the SET-dependent PP2A inhibitory pathway (PIP; refs. 8, 9). Preclinical studies aimed at restoring physiologic PP2A activity with SET-sequestering PP2A-activating drugs (PADs; e.g., FDA-approved FTY720, OSU-2S, and OP449) have shown unprecedented antileukemia effects in TKI-sensitive and -resistant CP and BC phase CML qLSCs and progenitors with neither adverse effects on normal hematopoiesis.
nor organ toxicity (8–10). In contrast, PP2A inhibiting drugs (PIDs; e.g., LB100), alone and in combination with TKIs, arrest proliferation of TKI-resistant CML progenitors, but do not exert effects on qLSC survival, and enhance leukemogenesis when used alone (11, 12).

The mechanisms underlying CML LSC quiescence, survival and self-renewal, and reduced NK-cell number and cytotoxicity likely result from integration of CML cell-autonomous and BMM-generated signals (1, 6). The latter are triggered by BM niche–specific metabolic conditions (e.g., oxygen tension), cell-to-cell direct, and soluble and/or exosome-encapsulated factor [e.g., microRNA (miRNA)]-mediated interactions between leukemic, mesenchymal stromal (MSC), endothelial, and immune cells (13–15).

Several miRNAs have been associated with PP2A inactivation (16) and LSC expansion and maintenance (17, 18); however, a clear causal link between their altered expression, apoptosis (16) and LSC expansion and maintenance (17, 18); and immune cells (13–15).

Among the miRNAs predicted in silico to reactivate PP2A by targeting PIP factors (e.g., JAK2, hnRNPA1, and SET), we focused on hsa-miR-300 (MIR300), an intergenic miRNA that is inhibited in several stem cell–driven tumors and belongs to the 14q32.31 DLK1-DIO3 genomic–imprinted tumor suppressor miRNA cluster B (19). Here we report that MIR300 is a BMM-induced cell context–independent tumor suppressor with antiproliferative and PP2A-activating functions that are not only essential for induction and maintenance of LSC quiescence and impaired NK cell anticancer immunity, but they can also be exploited to selectively and efficiently induce PP2A-mediated cell death of CD34+ CML–quiescent LSCs and proliferating progenitors while sparing normal hematopoiesis.

### RESULTS

#### MIR300 Loss in Leukemic Progenitors and Differential Induction of Its Cell Context–Independent Tumor Suppressor Activities in TKI-Resistant Quiescent CML (CP and BC) LSCs

MIR300 levels were progressively and markedly reduced by BCR-ABL1 activity (imatinib treatment) in bulk and dividing BM CD34+ CML (CP and BC) progenitors compared with normal CD34+ BM (NBM) and umbilical cord blood (UCB) cells, and higher in HSC-enriched CD34+CD38+ than committed CD34+CD38+ CML (CP and BC) BM cells (Fig. 1A). Accordingly, MIR300 expression was up to 800-fold lower in dividing CD34+ progenitors than qLSCs (CD34+CFSEmax) from patients with CML (CP and BC), but similar in quiescent and proliferating CD34+ UCB (Fig. 1B) cells.

Restoring MIR300 expression at physiologic levels by CpG-based oligonucleotides (500 nmol/L CpG-miR-300) reduced ≥75% proliferation and clonogenic potential and enhanced apoptosis (spontaneous and TKI-induced) of CD34+ CML (CP and BC), but not UCB cells (Fig. 2A and B). Importantly, KI-67/DAPI and CFUCCBL-mediated cell-cycle analyses in CD34+ CML and LAMA-84 cells, respectively, indicated that MIR300 arrested cell cycle and markedly expanded qLSC (G0 ≥ 50%) and apoptotic sub-G1 cell fractions of CML, but not normal CD34+ cells (Fig. 2C).

Inhibition of MIR300 function (500 nmol/L CpG-anti-miR-300) did not reduce numbers [carboxyfluorescein diacetate succinimidyl diester (CFSE) assay] and clonogenic activity [colony forming cells (CFC) and/or long term culture-initiating cells (LTC-IC)] of CML and normal CD34+ stem and progenitor cells (Fig. 2D, Supplementary Fig. S1). In contrast,
Figure 2. MIR300 activity in quiescent leukemic stem and progenitor cells. A, Growth (48 hours) and clonogenic potential (CFC) of CpG-scramble- and CpG-miR-300-treated (500 nmol/L) CML-BC and UCB cells. B, Effect of CpG-miR-300 and CpG-scramble (500 nmol/L) on spontaneous and IM (18 hours)-induced apoptosis (Annexin V/7-AAD) in CD34+ CML-BC cells (n = 3). Data are reported as mean ± SE (P < 0.01) from three independent experiments inside representative Annexin V/7-AAD FACS pseudocolor plots. C, Ki-67/DAPI (left; G0: MIR300 ≅ 46% vs. scr ≅ 10%; G1: MIR300 ≅ 15% vs. scr ≅ 50%; S/G2–M: MIR300 ≅ 4% vs. scr ≅ 27.6%; and sub-G1: MIR300 ≅ 35% vs. scr ≅ 3%) and FUCCI-2BL (right; G1–G0: MIR300 ≅ 40.2% vs. scr ≅ 23.6%; G1–S: MIR300 ≅ 15% vs. scr ≅ 2.85%; S/G2–M: MIR300 ≅ 45% vs. scr ≅ 76.6%) cell-cycle analysis of UCB and Ph+ (primary CD34+ and synchronized LAMA-84) cells exposed to the indicated CpG-ONs. D, Dose-dependent differential regulation of MIR300 antiproliferative and proapoptotic activities on CML qLSC (CFSEmax) and progenitor (Div. 1–2) numbers. Vector transduced and 500 nmol/L CpG-scramble and CpG-anti-miR-300 served as controls. Inset, MIR300 levels in pCDH-MIR300 lentiviral–transduced and 250–500 nmol/L CpG-miR-300–treated Ph+ cells. Data are shown as mean ± SEM from at least three independent experiments; *, P < 0.05; **, P < 0.01; ***, P < 0.001; ****, P < 0.0001. Range values of controls are reported in Supplementary Table S1.
graded ectopic MIR300 expression (Fig. 2D, inset) differentially affected leukemic, but not UC-B-quesient stem cell activity and survival. In fact, low MIR300 levels (pCDH-MIR300 and 250 nmol/L CpG-miR-300) strongly inhibited CML (CP and BC) LTC-IC and/or CFC/replating activities without affecting qLSC numbers, whereas high MIR300 doses (500 nmol/L CpG-miR-300) also reduced by more than 80% CML qLSCs and dividing CD34+ progenitor cells (Fig. 2D; Supplementary Fig. S1). Thus, MIR300 functions as a cell-context–independent dual activity (antiproliferative and proapoptotic) tumor suppressor that inhibits LTC-IC–driven colony formation by impairing qLSC ability to enter cycle and undergo cytokine-induced differentiation without affecting their survival, which is halted at higher MIR300 expression levels.

MIR300 Acts as Master PP2A Activator and Inhibitor of G1–S Transition in CML LSCs and Progenitors

Consistent with the absolute requirement of PP2A inhibition for CML, but not normal stem/progenitor cell proliferation and survival (9), gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) functional enrichment and clustering of MIR300-predicted and -validated (e.g., CTNNB1, CCND2, and Twist1) mRNA targets indicated that most of MIR300 targets are also validated PP2A targets (Supplementary Fig. S2) and that MIR300 antiproliferative and proapoptotic activities may result either from targeting SET that, in turn, induces PP2A-dependent inactivation of factors important for G1–S cell-cycle transition (e.g., CCND2, CDK6) and survival (e.g., CTNNB1, JAK2, Twist1, and MYC) of CML qLSCs and progenitors, or from their direct inhibition by MIR300 (Supplementary Figs. S2 and S3A). Accordingly, CpG-miR-300, but not CpG-scramble, reactivated PP2A and markedly reduced CCND2, CDK6, JAK2, hnRNP A1, SET, CTNNB1 (β-catenin), MYC, and Twist1 expression in primary CD34+ CML (CP and BC) progenitors and Philadelphia-positive (Ph+) cell lines, but not in normal CD34+ cells (Fig. 3A) in which PP2A has no proapoptotic activity (8, 20). Importantly, expression of MIR300-insensitive Flag-tagged SET mRNAs lacking the entire 3′UTR (Flag-SET) or just a region encompassing the high- and low-affinity MIR300-binding sites (Flag-Δ3′UTR-SET), but not that of full-length wild-type SET (Flag-wt3′UTR-SET), rescued Ph+ cells from MIR300-induced cell-cycle arrest and PP2A-dependent apoptosis (Annexin V+ cells; Fig. 3A, right).

MIR300 Dual Activity Is Regulated in a Dose-Dependent Differential Target-Selection Manner

Hierarchically clustering of MIR300 predicted and validated targets based on integration of different algorithms, some of which also take into account levels of MIR300 and of its targets in normal and leukemic BM cells (i.e., Comir, CSmirTar), positioned the G1–S cell-cycle regulators CCND2 and CDK6 within the top 2% and SET within the 5% of MIR300 targets, followed by JAK2, hnRNP A1, CTNNB1, and Twist1 clustering within the top 25%, and MYC in the lower 75% of MIR300-interacting mRNAs (Fig. 3C; Supplementary Fig. S3B). Notably, other regulators of G1–S transition (e.g., CNN2A2 and LSC cell-cycle reentry (e.g., Notch signaling) clustered together with CCND2 and CDK6, whereas MIR300 targets, belonging to JAK-STATs, PI3K-Akt, Wnt, MAPK signaling pathways, and regulating CML (CP and BC) progenitor cell survival and expansion, ranked below SET and within the bottom 75% of MIR300 target distribution (Supplementary Fig. S3B), suggesting that their MIR300-induced inactivation/downregulation is PP2A-mediated, occurs upon SET inhibition, and requires levels of MIR300 higher than those necessary to inhibit CCND2 and CDK6 and trigger cell-cycle arrest in a PP2A-independent fashion. This implies that the differential induction of MIR300 antiproliferative and proapoptotic activities (Fig. 2C and D) may depend on its ability to select targets in a dose-dependent manner. Indeed, low levels of ectopic MIR300 expression, achieved by exposing CD34+ CML-BC stem/progenitor cells to CpG-miR-300 concentrations (e.g., 100 nmol/L) not triggering qLSC apoptosis (Fig. 2D), strongly reduced CCND2 and CDK6, but not SET expression that, instead, became barely detectable at CpG-miR-300 doses (e.g., 500 nmol/L) inducing qLSC apoptosis (Fig. 3B, right). This is also consistent with the presence of four, six, and two MIR300-binding sites in CCND2, CDK6, and SET mRNA 3′UTRs, respectively (Fig. 3B, left).

Because CDK6/CCND2 downregulation is an essential feature of G1–G0–arrested myeloid qLSCs (21, 22) and SET inhibition is sufficient for inducing PP2A-dependent CML LSC and progenitor cell apoptosis (8, 9), the ability of MIR300 to sequentially trigger growth arrest and PP2A-mediated apoptosis of CD34+ CML (CP and BC) qLSCs and progenitors suggests that MIR300 expression in LSCs may account for their entry into quiescence, whereas its downregulation in leukemic progenitors likely occurs to prevent apoptosis (Fig. 3B, right).

MIR300 Antiproliferative Activity Accounts for BMM-Induced CML LSC Entry into Quiescence

MIR300 is under the control of an intergenic differentially methylated region (IG-DMR) preceding and controlling the expression of maternally expressed genomic-imprinted MEG3 lncRNA and other DLK1-DIO3 miRNAs (Supplementary Fig. S4A) that are strongly inhibited upon promoter methylation in several types of cancer, including myeloid leukemias (23). Treatment with 5-Aza-2′-deoxycytidine (5-Aza) augmented by 10−1−10−5-fold MIR300 expression in Ph+ cells (Supplementary Fig. S4B); however, nearly all cells underwent apoptosis after 24 hours, suggesting that MIR300 upregulation in CML qLSCs unlikely depends on IG-DMR demethylation and expression of all 74-cluster B tumor suppressor miRNAs (Supplementary Fig. S4A). Thus, MIR300 induction may depend on BM osteoigeic niche factors (e.g., MSCs, hypoxia, TGFβ1), known to inhibit growth and induce quiescence of leukemic cells (13, 14).

Indeed, expression of MIR300 increased at qLSC or higher levels (Fig. 1B) upon exposure of primary CD34+ CML-BC and/or LAMA-84 cells and BM-derived primary CD34+ CD45+ CD73+ CD105+ CD90+ CD44+ hMSCs and HS-5 MSCs to hypoxia, suggesting that MSCs may also contribute to enhanced MIR300 levels in qLSCs (Fig. 4A and B).
The MIR300 Tumor Suppressor Is Required for Leukemogenesis

Figure 3. MIR300 acts as master PP2A activator and inhibitor of G1–S transition through a dose-dependent target selection mechanism. A, Left, representative blots show effect of MIR300 on its targets and PP2A activity in UCB and CML-BC CD34+ cells and cell lines exposed to CpG-scramble and CpG-miR-300 (500 nmol/L; 48–72 hours). Right, (top) Dap/Ki67 cell-cycle analysis of CpG-scramble, -miR-300, and CpG-anti-miR-300 (500 nmol/L; 21 hours)-treated aphidicolin-synchronized K562 cells; (middle) Flag-SET lentiviral constructs with wild-type or a deleted miRNA 3’UTR; (bottom) MIR300-induced downregulation of Flag-SET proteins, and rescue of Ph+ cells from exogenous MIR300-induced growth inhibition (Trypan blue exclusion)/apoptosis (Annexin V) by Flag-SET cDNAs lacking MIR300-binding site. Similar results were obtained with LAMA-84 cells. B, Hierarchical clustering of (top 5%); (bottom 2/3) mRNA Targets (3’UTR) by Flag-SET cDNAs lacking MIR300-binding site. Similar results were obtained with LAMA-84 cells.}

Accordingly, MIR300 expression was strongly augmented in CD34+ CML cells exposed to hMSC and HS-S conditioned medium (CM) and/or MIR300-containing CD63+‘Alix+’ MSC exosomes (100 μg/mL; Fig. 4C).

Hypoxia- and MSC-induced MIR300 expression correlated with 40% to 60% reduced cell division and, importantly, with doubled numbers of cells in the CFSEmax CD34+ qLSC compartment (Fig. 4B and C; Supplementary Fig. S4C). Ph+ LAMA-84 cells also responded to MSC-CM exposure by ceasing proliferation and modifying gene expression (Supplementary Fig. S4D, left and right) in a manner similar to that of CML qLSCs (1). Importantly, exposure to MSCs (CM and/or exosomes) neither diminished SET and PP2A activity in UCB and CML-BC CD34+ cells and cell lines exposed to CpG-scramble and CpG-miR-300 (500 nmol/L; 48–72 hours). Right, (top) Dap/Ki67 cell-cycle analysis of CpG-scramble, -miR-300, and CpG-anti-miR-300 (500 nmol/L; 21 hours)-treated aphidicolin-synchronized K562 cells; (middle) Flag-SET lentiviral constructs with wild-type or a deleted miRNA 3’UTR; (bottom) MIR300-induced downregulation of Flag-SET proteins, and rescue of Ph+ cells from exogenous MIR300-induced growth inhibition (Trypan blue exclusion)/apoptosis (Annexin V) by Flag-SET cDNAs lacking MIR300-binding site. Similar results were obtained with LAMA-84 cells. B, Hierarchical clustering of (top 5%); (bottom 2/3) mRNA Targets (3’UTR) by Flag-SET cDNAs lacking MIR300-binding site. Similar results were obtained with LAMA-84 cells.
Figure 4. C/EBPβ-dependent MIR300 tumor suppressor antiproliferative activity accounts for BMM-induced LSC entry into quiescence. 

A. Effect of hypoxia on (i) MIR300 levels in CD34+ CML-BC, and BM-derived primary (hMSCs) and HS-5 MSCs (B); and (ii) MIR300 targets in untreated and Cpg-anti-miR-300–treated (500 nmol/L, 48 hours) and CML-BC cells. Inset, effect of hypoxia on CFSE+ CD34+ CML-BC proliferation.

C. Effect of hMSC and HS-5 conditioned medium (CM) and/or exosomes (50–100 μg/mL) from parental, vector (pZIP), and anti-MIR300 (pZIP-MIR300)–transduced primary hMSCs and/or HS-5 cells on: (i) proliferation (% growth inhibition); (ii) qLSC fraction (CFSE max CD34+); and (iii) MIR300 levels in CD34+ CML-BC and LAMA-84 cells. Insets, β-catenin and SET levels in anti-MIR300 (pZip-300)-transduced HS-5 (left); MIR300 in HS-5 Alix+CD63+ exosomes (right).

D. Effect of hypoxia on primary MIR300 transcripts (pri-miR-300), C/EBPβ (LAP1, LAP2, and LIP isoforms), BCR-ABL1 expression (αABL) and activity (αPY), and GRB2 levels in CD34+ CML-BC cells. (*) nonspecific band.

E. MIR300 promoter/enhancer activity in hypoxia (48 hours) and normoxia–cultured CML-BC CD34+ cells transfected with pGFP/Luc-based MIR300–reported constructs. p109mut is mutated in the -64 and -46 bp C/EBPβ-binding sites.

F. Effect of ectopic C/EBPβ (inset) on mature (MIR300) and primary (pri-miR-300) MIR300 levels in CD34+ CML-BC cells. Data are represented as mean ± SEM for at least three experiments. Range values of controls are reported in Supplementary Table S1.
β-catenin in HS-5 cells indicated functionality of lentivirally-transduced anti-MIR300 construct (Fig. 4C, inset).

**Hypoxia-Induced MIR300 Expression in CML-BC LSCs Requires C/EBPβ Activity**

The notion that hypoxia induces quiescence of CD34^+ CML LSCs and progenitors (24), and that hypoxia, but not MSCs, induces MIR300-dependent SET inhibition and increases levels of mature and primary (pri-miR-300) MIR300 transcripts (Fig. 4A and D; Supplementary Fig. S4D) suggests that the contribution of hypoxia to MIR300 expression in qLSCs is greater than that of MSC-derived exosomes, and that it may depend on increased MIR300 transcription. Indeed, luciferase (luc) activity (Fig. 4E), indicating that C/EBPβ binding to this 109 bp regulatory element is essential for MIR300 transcription in hypoxia-, but not normoxia-cultured CD34^+ CML-BC cells. Accordingly, increased pri-miR-300 expression correlated with markedly higher C/EBPβ LAP1 (transcriptionally active) levels in hypoxic CD34^+ CML-BC cells (Fig. 4D). In contrast, lack of C/EBPβ-driven p109-luc activity in normoxic CD34^+ CML-BC progenitors correlated with increased C/EBPβ LIP (inhibitory function) expression (Fig. 4D). Consistent with the notion that CEBPB translation is inhibited by BCR-ABL1 activity (25) and that hypoxia inactivates BCR-ABL1 to induce LSC quiescence (14), MIR300 induction by hypoxia was associated with decreased BCR-ABL1 activity, but not expression, (Fig. 4D) and ectopic C/EBPβ-ER TM expression rescued primary (pri-miR-300) and mature MIR300 expression in normoxic CD34^+ CML-BC cells (Fig. 4F).

Hypoxia also substantially increased by 10- to 20-fold MIR300 and C/EBPβ protein, but not mRNA levels, in BM-derived primary MSCs and/or HS-5 cells (Fig. 4B; Supplementary Fig. S4E), suggesting that the hypoxic conditions of the osteogenic BM niche may increase MIR300 levels in CML LSCs by simultaneously inducing C/EBPβ LAP1-dependent MIR300 transcription and increasing transfer of MSC-derived exosomal MIR300. Conversely, the evidence showing that MIR300 levels in C/EBPβ−responsive CD34^+ CML-BC cells and/or Ph^+ cell lines were not influenced by ectopic C/EBPβ expression or exposure to TGFβ1-blocking antibody (Supplementary Fig. S4F and S4G), indicated that these qLSC regulators do not contribute to increased MIR300 expression.

Notably, the notion that mouse c/ebpβ induces BCR-ABL^+ LSK exhaustion (26) does not argue against a role for human C/EBPβ as an inducer of LSC quiescence because: (i) LSKs are pushed into cycle by constitutively activated BCR-ABL1 that promotes C/EBPβ-dependent LSK maturation (26), (ii) the hypoxia-sensitive C/EBPβ-responsive MIR300 regulatory element is not conserved in mouse cells, and (iii) an A-to-G substitution in mmu-miR-300 seed sequence at +4 position is predicted (mirTar, MFE ≤ −10 kcal/mol; score: ≥136.5) to prevent mouse MIR300 targeting of ccnd2 and dk6 mRNAs.

**MIR300 Is Upregulated in CML NK Cells and Its BMM-Induced Antiproliferative and PP2A-Activating Functions Impair NK Cell Immune Response**

In CML, loss of NK cell antitumor immune response is causally linked to persistence of TKI-resistant LSCs (4, 27, 28). Cytotoxicity assays showed that cytokine-activated CD56^+CD3^− NK cells can kill CD34^+ CML-initiating qLSCs (Fig. 5A). Because cytokine-induced CCND2 expression and SET-dependent PP2A inhibition are essential for CD56^+CD3^− primary NK and clinically relevant NK-92 cell proliferation and antitumor cytotoxicity (refs. 29, 30; Supplementary Fig. S5A), and KEGG/GO analyses predicted that MIR300 regulates innate antitumor immunity (Supplementary Fig. S3A, right), we assessed whether reduced numbers and dysfunctional NK cells in patients with CML (28, 31) depend on increased MIR300 expression.

MIR300 levels were increased in CD56^+CD3^− NK cells from patients with CML at diagnosis, but not in NK cells, from healthy individuals (Fig. 5B), suggesting that loss of NK-cell proliferation and killing activity against CD34^+ CML qLSCs and progenitors may depend on BMM-generated and leukemia-sustained MIR300 antiproliferative and PP2A-activating functions. Indeed, exposure to hypoxia and BM MSC (primary hMSCs and HS-5 cells)-derived CM and/or Alix^CD63^ exosomes reduced by 45% to 75% IL2-dependent proliferation of CD56^+CD3^− primary NK and NK-92 cells (Fig. 5C and D; Supplementary Fig. S5B) and severely impaired NK-cell immunoregulatory (IFNγ production) and antitumor cytotoxic (K562 killing) activities (Fig. 5E). Importantly, lentiviral anti-miR-300 (pZIP-miR-300) transduction into HS-5 cells and pretreatment of NK-92 cells with CpG-anti-miR-300, but not with CpG-scramble, significantly suppressed MSC CM- and/or exosome-inhibitory effects on NK cell proliferation and cytotoxicity against CML cells (Fig. 5D and E).

Additionally, IL2-induced proliferation and cytotoxicity of CD56^+CD3^− NK and clinically relevant NK-92 cells was suppressed in CpG-mir-300-, but not CpG-scramble-treated cells (Fig. 5F), suggesting that impaired NK-cell growth and cytotoxicity against CML qLSCs and proliferating blasts occur in a MIR300-dependent manner through hypoxia- and MSC-CM and exosomes) generated signals, increasing levels of C/EBPβ and MIR300 that, in turn, reduces CCND2, CDK6, and SET expression (Fig. 5C, E, and F).

**Selective Suppression of MIR300 Proapoptotic, But Not Antiproliferative Activity by TUG1 IncRNA in CML Quiescent LSCs**

Myeloid qLSCs display low to undetectable CCND2 and CDK6 expression, but high SET levels (9, 22, 32), and survive despite the high expression levels of a functional (target downregulation) MIR300 (Figs. 1B and 4A), suggesting that a MIR300-interacting factor differentially regulates MIR300 activities to prevent CML qLSC apoptosis while allowing LSC cell-cycle exit.
Because the taurine upregulated gene 1 (TUG1) is a MIR300-interacting (33) long noncoding RNA (lncRNA) that prevents the inhibition of MIR300-regulated factors (e.g., CCND1, CTNNB1, and Twist1) described as important for tumor cell growth and survival (34), we investigated whether TUG1 may differentially regulate MIR300 tumor suppressor activities in CML qLSCs (Fig. 6A).

TUG1 levels are markedly increased in CD34+CFSEmax CML qLSCs compared with dividing CD34+ CML progenitors and to CD34+CFSEmax UCB cells, but not in either CD34+CFSEmax UCB HSCs, compared with dividing CD34+ UCB progenitors or CD34+ Lin− compared with CD34+ Lin+ BM cells from healthy individuals (Fig. 6B; Supplementary Fig. S6A and S6B). In CML, TUG1 is regulated in a manner similar to that of MIR300; in fact, TUG1 expression is imatinib-insensitive in qLSCs, but not in dividing CD34+ progenitors in which it is markedly suppressed by BCR-ABL1 activity (Fig. 6B).
The MIR300 Tumor Suppressor Is Required for Leukemogenesis

Because TUG1 expression correlates with that of TGFβ1 in tumor cells (35) and TGFβ1 is a known inducer of LSC quiescence and it is secreted by leukemic cells including CD34+ CML blasts (36), we investigated the role of TGFβ1 in the regulation of TUG1 expression and activity in CD34+ CML LSCs and progenitors. Exposure (48 hours) of CD34+ CML-BC cells to a TGFβ1-blocking antibody (anti-TGFβ Ab; green bars) halves TUG1 levels and also reduces levels of FoxM1 (Fig. 6C, left), a transcriptional factor that is essential for quiescence and maintenance of hematopoietic stem cells, including CML LSCs, and induces TUG1 expression in osteosarcoma cells (37–39). In contrast, FoxM1 and/or TUG1 expression is strongly induced in a TGFβ-dependent manner in hypoxia-cultured (48 hours, 1% O2) CD34+ CFSEmax and bulk CD34+ cells (Fig. 6C), suggesting that hypoxia-induced TGFβ1 (36) enhances TUG1 levels in a FoxM1-dependent manner to neutralize MIR300 proapoptotic activity in CML CD34+ LSCs.
Indeed, dosing TUG1 downregulation by exposure to low and high CpG-TUG1-shRNA concentrations mimicked the dose-dependent differential activation of MIR300 antiproliferative and proapoptotic functions exerted by different CpG-miR-300 doses (Fig. 2D); in fact, exposure of Ph+ cells (LAMA-84) to 100 nmol/L CpG-TUG1-shRNA efficiently induced growth arrest, but modestly affected survival (Annexin V+ cells; Fig. 6D and E, inset). Conversely, 500 nmol/L CpG-TUG1-shRNA decreased CD34+eFluormax CML-BC qLSC and LAMA-84 cell numbers in a MIR300-sensitive manner and further enhanced Cpg-miR-300–driven apoptosis resulting in nearly a complete loss of CD34+ eFluormax qLSCs and dividing CD34+ leukemic progenitors (Fig. 6D and E).

As expected, exposure (48 hours) to a TGFβ1-blocking antibody markedly decreases qLSC numbers, but very modestly affects dividing leukemic progenitors (Fig. 6E). In contrast, TUG1 overexpression (TUG1 RNA) antagonized Cpg-miR-300–induced qLSC and progenitor cell apoptosis (Fig. 6E). Cpg-anti-miR-300 fully prevented TUG1-shRNA-induced apoptosis of CML CD34+ eFluor qLSCs (Fig. 6E, inset), suggesting that CML LSC entry into quiescence and qLSC survival are MIR300-driven TUG1-regulated effects. Thus, hypoxia-induced TGFβ1-FoxM1–mediated signals increase TUG1 expression to allow CD34+ CML LSC entry into quiescence and qLSC survival by maintaining the amount of free functional MIR300 at levels sufficient for arresting cell cycle, but not for triggering PP2A-dependent apoptosis (Fig. 6A; Supplementary Fig. S6C). Conversely, Cpg-anti-miR-300 did not counteract TUG1-shRNA–induced apoptosis in CD34+ CML progenitors (Fig. 6E). Moreover, the nearly complete killing of CD34+ CML dividing progenitors induced by Cpg-miR-300 and -TUG1-shRNA combined treatment (Fig. 6E) suggests that the BCR-ABL–regulated low TUG1 expression in CD34+ CML blasts (Fig. 6B) is required for inhibiting the activity of other miRNAs negatively regulating cell survival.

**Disruption of MIR300-TUG1 Interplay and PAD Treatment Abrogate the BMM-Protective Effect on Survival of CML qLSCs and BCR-ABL1+ Leukemia-Initiating Cells**

By using a previously described patient-derived xenograft (PDX)-based approach suitable for determining changes in CML LT-HSC survival (40), we assessed the effects of disrupting the MIR300-TUG1 interplay on BM-repopulating CML qLSCs. NRG-SGM3 mice (n = 4/group) were transplanted with >95% Ph+ CD34+ CML-CP, -AP (accelerated phase) and -BC cells previously exposed for 48 hours to 500 nmol/L CpG scramble (control), Cpg-miR-300, and Cpg-TUG1-shRNA used as single agents or in combination (Fig. 7A). At 4 and 8 weeks after engraftment, BM hCD45+CD34+ progenitors and LSC-enriched hCD45+CD34+CD38− cells were reduced by 75% to 97% in all arms (Fig. 7B and C). Inhibition and/or saturation of TUG1 MIR300–sponging activity with Cpg-TUG1-shRNA and Cpg-miR-300 resulted in the killing of approximately 100% of leukemia-initiating (hCD45+CD34+CD38−CD90+) quiescent HSCs (Fig. 7C), barely detectable BCR-ABL1 transcripts, and 3.6-fold increased numbers of normal (Ph−) 1 BM cells (Fig. 7D), and strongly reduced numbers of PB and BM CML (CP, AP, and BC) hCD45+ cells (Fig. 7E). Thus, CpG-oligonucleotide-mediated pharmacologic disruption of MIR300-TUG1 balance allows PP2A-mediated MIR300 proapoptotic activity to suppress chronic and blastic CML development by selectively and efficiently eliminating nearly all TKI-resistant leukemic, but not normal quiescent HSCs and progenitors. Note that the extremely low numbers (0%-0.0015% of total BM cells recovered/12 mice/arm) of Cpg-miR-300, Cpg-TUG1-shRNA, and Cpg-miR-300/TUG1-shRNA–treated CD34+CD38−CD900 (CP, AP, and BC) BM-repopulating cells (Fig. 7C) did not justify serial BM transplantation into secondary and tertiary recipients.

Consistent with the role of MIR300 as a BMM-induced tumor suppressor capable of triggering CML CD34+ qLSC apoptosis through suppression of SET-mediated PP2A inhibition, and with the notion that SET expression is increased in CD34+CD38−CD900 CML LSCs (9) and that SET-sequestering PADS selectively induce apoptosis of TKI-resistant serially transplanted leukemic LT-HSCs and CD34+ CFSEmax quiescent CML, but not normal HSCs (9), PAD treatment (2 μmol/L FTY720) of HS-5-cocultured CFSE+CD34+ CML-BC cells markedly reduced the cobblestone area–forming cell (CAF) activity (Fig. 7F, left) and numbers of adherent CFSE+AnnexinV+CD34+ CML qLSCs (~91.1% inhibition relative to DMSO-treated controls; Fig. 7F, right), suggesting that FTY720-mediated PP2A activation (20) circumvents the MSC-mediated protective effect on CML qLSC survival. Likewise, HS-5 CM did not protect LAMA-84 CML progenitors from FTY720-induced apoptosis (Supplementary Fig. S4D, middle). As expected (41), TKI treatment (imatinib) did not reduce, but augmented CAF and qLSC numbers (Fig. 7F). Accordingly, FTY720, but not imatinib, strongly decreased CAF activity (red arrows) derived from Lin-Sca1+ CD45+CD31+ mouse MSC (mMSC)-induced (inset) CFSE+AnnexinV+32D-BCR-ABL cells (Fig. 7G), further indicating that pharmacologic PP2A activation by SET-sequestering PADS can lead to CML eradication at qLSC level.

**Figure 7.** Disruption of MIR300-TUG1 interplay and PAD treatment abrogate the BMM-protective effect on survival of CML qLSCs and BCR-ABL1+ leukemia-initiating cells. A, Xenotransplantation protocol of ex vivo–treated CD34+ chronic (CP), accelerated (AP) and blastic phase (BC) CML cells in NRG-SGM3 mice (n = 4 mice/treatment/patient sample). B and C, Analysis of Cpg-MIR300, Cpg-TUG1-shRNA, Cpg-TUG1+shRNA+Cpg-MIR300, and Cpg-scramble–treated CML cells from BM aspirates at 2–12 (3D plots) and 10 to 20 weeks posttransplant quantitative analysis of CML cells stained with the indicated antibodies. D, Evaluation at 10 to 20 weeks posttransplant of BCR-ABL1 transcripts by qRT-PCR (left) and of % Ph-positive (Ph+) cells by FISH (right) in total and FACS-sorted hCD45+BM cells, respectively. E, Analyses of BMM-cocultured CML cells at 10 to 20 weeks posttransplant: hCD45+ cells (% of BM) (left) and Ph+ (right) of mice transplanted with CML (CP AP and BC) and treated with the indicated Cpg-ODNs. Age-matched mice served as controls. Error bars, mean ± SEM. F, Effect of 2.5 μmol/L FTY720 or 1 μmol/L imatinib (IM) on CAF activity (left) and numbers of CFSE+AnnexinV+CD34+CD38− CML qLSCs derived from CFSE-labeled CD34+ CML-BC cells cocultured for 7 days on BM-derived HS-5 MSCs cells (right). Inset, FACS plot shows gating of CFSE+ CML qLSCs. G, Relative number and representative images of CAF (red arrows) of CFSE-labeled 32D-BCR-ABL cells cocultured with primary mMSCs in the absence or presence of IM (1 μmol/L, 48 hours) or FTY720 (2 μmol/L, 48 hours; n = 5). Inset, CFSE+ fraction of adherent 32D-BCR-ABL cells in medium and cocultured for 4 days with mMSCs. Range values of controls are reported in Supplementary Table S1.
The MIR300 Tumor Suppressor Is Required for Leukemogenesis

CD34

FACS Analysis in BM and PB:
- % hCD45+
- % hCD45+CD34+ (bulk progenitors)
- % hCD45+CD34+CD38− (primitive progenitors)
- % hCD45+CD34+CD38− (HSCs)

BM Engraftment (2 weeks):
Detectable hCD45 in all groups

Euthanasia 8 weeks after detection of hCD45 in all groups

qRT-BCR-ABL1 in hCD45+ BM cells

Interphase FISH for Ph+ hCD45+ BM cells

miR-300

CpG-scramble; CpG-miR-300

CpG-TUG1 shRNA

CpG-miR-300 + TUG1 shRNA

CML-BC (10 weeks)
CML-CP (20 weeks)
CML-AP (12 weeks)

CML-CP (n = 3)
CML-AP (n = 3)
CML-BC (n = 3)

CML (n = 3)

CML-CP (20 weeks)
CML-AP (12 weeks)
CML-BC (10 weeks)

n = 3/group

BM from 12 mice/group

RNA from 12 mice/group

59
DISCUSSION

Altered miRNA expression and PP2A tumor suppressor activity are tightly linked to leukemogenesis and impaired NK-cell-mediated anticancer immunity. The notion that PADs, but not TKIs, kill CML qLSCs (42) implies that BCR-ABL1 kinase-independent signals, likely arising from the endo stance hypoxic BMM (5), regulate CML LSC activity and survival through inhibition of PP2A. We previously reported that BCR-ABL1 expression, but not activity, is essential for recruitment of Jak2-driven hnRNPA1-mediated signals inducing SET-dependent PP2A inhibition that, in turn, allows CTNNB1 (β-catenin)-dependent regulation of CML (CP and BC) LSC proliferation and survival (9). We also demonstrated that inhibition of cytokine-induced SET upregulation or PAD treatment impair NK-cell cytotoxicity against Ph+ cells through activation of PP2A (30). Here we showed that posttranscriptional signals, which are initiated by the tumor-naïve BMM, are strongly maintained by the tumor-reshaped BMM, control CML LSC entry/maintenance into quiescence and impair NK-cell immunity. This occurs through the induction of MIR300, a tumor suppressor miRNA with dose-dependent antiproliferative and PP2A-activating functions, which are uncoupled and differentially regulated by TUG1 IncRNA decay activity in CML LSCs. Importantly, a dose-dependent target selection mechanism (43) allows the sequential activation of MIR300 antiproliferative and PP2A-activating functions through the induction of CDK6/CCND2 and SET, respectively, in CML qLSCs and progenitors and in NK cells.

MIR300 Role in CML LSCs and Progenitors

Expression studies revealed that MIR300 levels are downregulated in CML (CP and BC) CD34+ progenitors, but not in the CML-initiating (1) qLSC (CD34+CFSE™) fraction. Restoration of MIR300 expression arrested proliferation, expanded the G0–G1 quiescent stem cell fraction, strongly impaired survival of dividing CD34+ CML stem/progenitor cells, and was associated with downregulation of CCND2/CDK6, SET, and other PP2A-regulated CML growth- and survival-promoting factors (e.g., JAK2, CTNNB1, hnRNPA1 and MYC; ref. 1). Because CCND2/CDK6 inhibition characterizes quiescent long-term HSCs (LT-HSCs) and is sufficient to arrest CD34+ leukemic progenitors in G0–G1 (21, 22), MIR300-induced loss of CCND2/CDK6, which occurs at low levels of MIR300 expression, likely represents the mechanism by which MIR300 antiproliferative activity contributes to CML stemness. Likewise, SET inhibition, which occurs when MIR300 is highly expressed and is sufficient for triggering PP2A-mediated cell death of CML (CP and BC) qLSC and progenitors (8, 9), likely account for MIR300-induced apoptosis. In fact, we showed that loss of MIR300 binding to SET impaired MIR300-induced PP2A-mediated Ph+ cell apoptosis. This also suggests that inactivation and/or downregulation of JAK2, CTNNB1, Twist1, and MYC may result from MIR300-induced PP2A activation. Indeed, bioinformatics analysis that integrates several algorithms and also takes into account miRNA and mRNA targets’ expression levels in normal and myeloid leukemia BM cells indicated that MIR300-induced inhibition of other PP2A-regulated survival factors (e.g., Twist1, CTNNB1, JAK2, and MYC) requires levels of MIR300 significantly higher than those suppressing SET (Fig. 2B; Supplementary Fig. S3B). Thus, downregulation of these MIR300 targets unlikely represent the primary mechanism of MIR300-induced apoptosis of CML qLSCs and progenitors. Strengthening the importance of CCND2/CDK6 and SET as key MIR300 effectors is the notion that MIR300-induced CCND2/CDK6 and SET inhibition may not be limited to MIR300-induced posttranscriptional downregulation. In fact, MIR300 may also impair SET, CCND2 and/or CDK6 transcription, mRNA nuclear export, translation and/or protein stability/activation upon inhibition of other PIP factors (e.g., hnRNPA1, JAK2, and SETBP1), and the associated XPO1 (Supplementary Fig. S3A, left; refs. 8, 20, 44–46).

The evidence that high levels of MIR300 expression does not induce apoptosis of CML qLSCs, which exhibit inactivation of PP2A and activation of JAK2, SET, and CTNNB1 (1, 9), raises the questions of whether MIR300 is required for LSC quiescence and survival; how MIR300 is regulated in qLSCs and leukemic progenitors; and how qLSCs elude MIR300-induced apoptosis.

The requirement of MIR300 for induction and maintenance of CML LSC quiescence is clearly demonstrated (Figs. 2D, 3B, and 4C, Supplementary Fig. S4D) by: (i) the ability of anti-MIR300 molecules to antagonize MSC-induced inhibition of leukemic cell proliferation, (ii) impaired LTC-IC-driven colony formation in the absence of qLSC apoptosis in CD34+ cells exposed to CpG-miR-300 concentrations inhibiting CCND2/CDK6 but not SET expression, and by the (iii) inability of MSCs to induce SET downregulation in CML cells. Importantly, the evidence that MIR300-dependent SET inhibition is induced by hypoxia, but not MSCs, in CD34+ CML stem/progenitor cells (Fig. 4A; Supplementary Fig. S4D) and that SET, JAK2, and CTNNB1 are active in CML qLSCs (1, 46), suggests that LSC entrance into quiescence is initiated by MSCs prior to LSC niching into the BM endosteal area with the lowest O2 tension in which the MIR300 PP2A-activating proapoptotic function is likely inhibited by the hypoxia-induced TUG1 sponging activity. Moreover, the evidence that anti-MIR300–expressing MSCs fail to suppress leukemic CD34+ cell proliferation, and that exposure of CD34+ CML stem/progenitor cells to MSC CM and/or exosomes increases MIR300 expression and double the number of CD34+ CML cells in the quiescent LSC compartment (Fig. 4), suggests that the MSC-induced transition into quiescence of CD34+ CML LSCs/progenitors may depend on CDK6/CCND2 downregulation induced by MSC-derived exosomal MIR300. In this scenario, hypoxia-induced MIR300 will sustain, but not promote, CML LSC quiescence. However, MSCs may contribute to the hypoxia-dependent regulation of MIR300-induced LSC quiescence and qLSC survival by increasing TUG1 expression through the release of TGFβ1 (36). Mechanistically, we showed that hypoxia induces MIR300 transcription in CD34+ CML cells and MSCs through reduced LIP (inhibitory) and increased LAP1 (activatory) C/EBPβ, which binds/transactivates a hypoxia-sensitive regulatory element located 109 bp upstream the MIR300 gene. Accordingly, it was found that C/EBPβ was found expressed in CML LSCs (26), induced by hypoxia, and to negatively regulate G1–S transition and SET expression (47, 48).

Although other miRNAs regulating LSC survival or qLSC reentry into cycle have been associated with CML development (18, 49, 50), to our knowledge, MIR300 is the only cell
context–independent (same activity in LSCs and progenitors) miRNA capable of both supporting CML leukemogenesis by inducing LSC quiescence and triggering CML qLSC and progenitor cell apoptosis.

**MIR300 Role in NK Cells**

*MIR300* is also the only tumor-naive–induced tumor suppressor miRNA that inhibits NK cell–mediated innate anticancer immunity while promoting LSC quiescence. NK cells preferentially kill cancer stem cells (4, 51), including BM-repopulating TKI-resistant BCR-ABL1+ qLSCs (Fig. 5B), and NK-cell quantitative and functional impairment is a feature of patients with untreated and TKI-treated CML (6, 52).

Impaired NK-cell immunity also associates with CML qLSC persistence in patients with TKI-treated CML in deep molecular remission (28, 31), whereas normal levels of activated NK cells characterize patients in sustained treatment-free remission (28) and account for increased disease-free survival after T-cell–depleted stem cell transplant (4), suggesting that NK-cell–based therapies may lead to qLSC eradication.

Despite RNA sequencing (RNAseq) of MSC exosomal RNA suggesting that other 14 MSC-derived miRNAs may contribute to impaired NK-cell activity (Supplementary Fig. 5S), we showed that *MIR300* levels are increased in circulating NK cells from patients with CML at diagnosis and that BM-derived inhibition of NK cell proliferation and antitumor activity require *MIR300* induction. In fact, BMN-induced NK cell inhibition was recapitulated by *MIR300* mimics and abrogated by *MIR300* RNAi. Furthermore, our data suggests that impaired NK-cell proliferation and cytotoxicity may depend on C/EBPβ-MIR300 signals leading to CCND2/CDK6 and SET downregulation. Because the tumor-naïve BMN-induced loss of CCND2 and SET expression (PP2A inhibition) account for suppression of NK cell proliferation (29) and antitumor cytotoxicity (30), the NK cell quantitative and qualitative defects observed in CML may arise from *MIR300*-mediated signals initiated by the naïve BMN (53), sustained and/or exacerbated by the leukemia-reshaped BMN (54) and over-riding cytokine-driven NK cell activation (55). Interestingly, treatment with MSC exosomes also reduced pre-miR-155 levels (BIC) in IL12/IL18-stimulated and resting NK-92 cells (Supplementary Fig. SSD). Because, miR-155 not only inhibits SHIP1 and PP2A to allow MAPK- and AKT-dependent NK cell proliferation and cytotoxic activity (16, 55), but also suppresses C/EBPβ expression (ref. 56; Supplementary Fig. SSD), MSC-induced BIC downregulation likely contributes to *MIR300*-dependent NK cell inhibition by antagonizing miR155-induced C/EBPβ downregulation. Furthermore, consistent with the notion that *TUG1* acts as a *MIR300* decoy and in contrast with the mechanism regulating *MIR300* activities in leukemic cells, BMN-induced NK cell inhibition occurs in a *MIR300*-dependent, but not *TUG1*-independent manner; in fact, hypoxia did not increase, but decreased *TUG1* expression in NK cells, and *TUG1*-shRNAs did not alter NK cell number (Supplementary Fig. SSE and SSF).

**Biologic and Therapeutic Relevance of the MIR300-TUG1 Interplay in CML Cells**

*TUG1* is an oncogenic IncRNA upregulated in different types of cancer in which it has strong diagnostic, prognostic and therapeutic relevance (34). In CML-BC qLSCs, *TUG1* is induced by hypoxia and uncouples *MIR300* functions and dose-dependently suppresses only *MIR300* PP2A-mediated proapoptotic function (Fig. 6). This unprecedented lncRNA function allows *TUG1* to maintain unbound *MIR300* at levels sufficient for inducing CML LSC growth arrest but not for triggering PP2A-mediated apoptosis, which does not depend on loss of *TUG1* survival signals, but on the effect of freed *MIR300* on SET mRNA. Accordingly, *TUG1* loss in solid tumors was associated with G2–G1 arrest and apoptosis, whereas its overexpression with induction of proliferation and upregulation of mitogenic and survival factors (e.g., CCND1/2, CTNNB1, and Twist1) also described as *MIR300* targets (34). In addition, we showed that *TUG1* activity is *MIR300*-restricted in CD34+ CML qLSCs, but not in leukemic progenitors in which *TUG1*-shRNAs induced apoptosis of anti-*MIR300*-treated CD34+ CML cells. Because *TUG1* interacts with several tumor suppressor miRNAs (34, 57), this suggests that *TUG1* may function in CML LSCs and progenitors as a hub for specific subsets of functionally related tumor suppressor miRNAs. Indeed, 56 experimentally validated *TUG1*-interacting miRNAs that possess growth-suppressive and/or proapoptotic functions are differentially expressed in CD34+/CD38− LSC-enriched and CD34+/CD38−–committed progenitor CML (CP and BC) cells (Supplementary Fig. S7A). Notably, 96.4% and 44.3% of these miRNAs are predicted to shut down BCR-ABL1–dependent signals in CML blasts and, like *MIR300*, to act as inhibitors of cell-cycle progression and PP2A activators (Supplementary Fig. S7A and S7B). Thus, it is conceivable that balanced *TUG1-MIR300* levels are essential for CML qLSC induction/maintenance, whereas insufficient *TUG1* expression will lead to CML qLSC and progenitor cell apoptosis by freeing *MIR300* and other miRNAs with similar tumor suppressor activities. Conversely, high *TUG1* expression will likely promote CML cell proliferation, survival, and qLSC cell-cycle reentry, although an aberrant *TUG1* increase that also inhibits *MIR300* antiproliferative activity may induce LSC exhaustion by impairing entry into quiescence and forcing reentry into cycle.

Mechanistically, we showed that increased *TUG1* expression in qLSCs depends on hypoxia-induced TGFβ1 secretion by CD34+ CML stem/progenitor cells. In fact, exposure to a TGFβ1 blocking antibody markedly impaired *TUG1* expression in CML progenitors and in hypoxia-cultured CML qLSCs. However, hypoxia-induced TGFβ1/2 upregulation in CML LSCs (36) may also contribute to increased *TUG1* expression and regulation of *MIR300* functions. Hypoxia-induced *TUG1* expression in CML qLSCs may also depend on Notch activity (58); however, blocking TGFβ1 signaling in CD34+ CML progenitors and hypoxia-exposed qLSCs also strongly suppressed expression of FoxM1, a *TUG1* transcriptional inducer and regulator of CML LSC quiescence and cycling activity (37, 39, 50). Thus, hypoxia–TGFβ–FoxM1, but not Notch-induced signals, increases *TUG1* expression in CML LSCs to selectively inhibit *MIR300* PP2A-mediated proapoptotic function while allowing *MIR300*-dependent entry into quiescence. However, Notch signaling may contribute to the *MIR300-TUG1*-dependent regulation of LSC quiescence and survival through the RBPJ-mediated inhibition of miR-155 that may induce *TUG1* and *MIR300* expression by...
preventing FoxM1 and C/EBPβ downregulation, respectively (Supplementary Fig. S6C).

In conclusion, tumor-naïve BMM-induced MIR300 tumor suppressor antiproliferative and PP2A-activating functions support CML development through induction of LSC quiescence and inhibition of NK cell-mediated qLSC killing, respectively. This may represent the initial step leading to formation and expansion of the TKI-resistant CML qLSC pool. Once established, the CML clone will reshape the BMM to further support disease development and progression. TUG1–MIR300 interaction plays a central role in this process because altering its ratio leads to the nearly complete and selective P2A-dependent eradication of chronic and blastic CML qLSCs in vitro and in PDXs. This, together with the ability of SET-sequestering PADs to bypass the MSC-induced protective effect on CML qLSC survival, not only highlights the therapeutic importance of pharmacologically modulating PP2A activity in anti-LSC and NK cell–based therapeutic approaches for CML eradication, but also indicates that the activity of a tumor suppressor (i.e., MIR300) can be exploited by LSCs to preserve their ability to induce and maintain leukemia.

**METHODS**

**Cell Culture and Treatments**

**Cell Lines** Ph+ CML-BC K562 and LAMA-84, human BM MSC–derived HS-5 (59), mouse BM–derived 293T and medium. NK-92 cultures were supplemented with 150 IU/mL rhIL2 derived HS-5 (59), mouse BM–derived 32D-BCR/ABL, and the clini-Cell Culture and Treatmentscal CML-BC K562 and LAMA-84.

**Primary Cells** Human hematopoietic stem and progenitor cell fractions from healthy and leukemic individuals were isolated from BM, PB, or UCB. Prior to their use, cells were kept (18 hours) in MesenCult Proliferation Kit medium and used for CM and exosome purification (Supplementary Methods). Human CD56–/CD3+ NK cells (purity >95%) from healthy (UCB or PB), CML (CP, BC and AP) individuals were isolated from BM cells by Ficoll-Hypaque density-gradient centrifugation followed by culture in complete human MesenCult Proliferation Kit medium and used for CM and exosome purification (Supplementary Methods). Human CD56–/CD3+ NK cells from healthy (UCB or PB), CML (CP, BC and AP) individuals were isolated from BM cells by Ficoll-Hypaque density-gradient centrifugation followed by culture in complete human MesenCult Proliferation Kit medium and used for CM and exosome purification (Supplementary Methods).

**Flow Cytometry and Cell Sorting**

CD34+, CD34*CD38+, CD34*CD38* fractions were magnetic (CD34 MicroBead Kit; Miltenyi Biotec) and/or FACS (αCD34 APC/PE and αCD38 PE/Cy7 Abs, BD Biosciences) purified (purity: >90%–100%). Primary human NK cells were sorted using Alexa Fluor 488 NHS-5, PE-Cy5 NHS-CD19c and PE-Cy7 NHS-CD3 Abs (BD Biosciences) and their purity assessed by PE αCD56 (Beckman Coulter, Life Sciences) and APC-eFluor780 αCD3 (eBioscience) antibody staining. hMSC purity (>99%) was assessed by flow cytometry using anti-CD34 and APC FITC, CD73 PE/Cy7, CD105 Alexa 647, CD44 PerCP-Cy5.5, and CD90 PE antibodies (BD Biosciences). Apoposis was quantified by FACS upon staining cells with PE Annexin V and 7-AAD. Cells were sorted using the FACsaria II (BD Biosciences). Data acquisition and analyses were performed at the UMBCCC Flow Cytometry Facility. Data from LSRII or CANTO II flow cytometers (BD Biosciences) were analyzed by using either the FlowJo v8.8.7 or Diva v6.1.2 software.

**LTC-IC and CFC/Replating Assays**

CD34+ CML (CP and BC) and UCB cells were lentivirally transduced/GFP-sorted and/or treated (500 nmol/L, 3 days) with αCD34 MicroBead Kit; Miltenyi Biotec) and/or FACS (αCD34 APC/PE and αCD38 PE/Cy7 Abs, BD Biosciences) purified (purity: >90%–100%). Primary human NK cells were sorted using Alexa Fluor 488 NHS-5, PE-Cy5 NHS-CD19c and PE-Cy7 NHS-CD3 Abs (BD Biosciences) and their purity assessed by PE αCD56 (Beckman Coulter, Life Sciences) and APC-eFluor780 αCD3 (eBioscience) antibody staining. hMSC purity (>99%) was assessed by flow cytometry using anti-CD34 and APC FITC, CD73 PE/Cy7, CD105 Alexa 647, CD44 PerCP-Cy5.5, and CD90 PE antibodies (BD Biosciences). Apoposis was quantified by FACS upon staining cells with PE Annexin V and 7-AAD. Cells were sorted using the FACsaria II (BD Biosciences). Data acquisition and analyses were performed at the UMBCCC Flow Cytometry Facility. Data from LSRII or CANTO II flow cytometers (BD Biosciences) were analyzed by using either the FlowJo v8.8.7 or Diva v6.1.2 software.

**LTC-ICs and CFC/Replating Assays**

LTC-ICs A total of 2 × 10⁶ CML and 2 × 10⁶ UCB CD34+ cells were cultured with a 1:1 mixture of irradiated (80 Gy) ILY/G-CSF–producing M2-10B4 and ILY/KL–producing SI/SI murine fibroblasts in MyeloCult H5100 (Stemcell Technologies) supplemented with hydrocortisone. Medium was replaced after 7 days, followed by weekly half-medium changes, and fresh 500 nmol/L αCD34 MicroBead Kit; Miltenyi Biotec) and/or FACS (αCD34 APC/PE and αCD38 PE/Cy7 Abs, BD Biosciences) purified (purity: >90%–100%). Primary human NK cells were sorted using Alexa Fluor 488 NHS-5, PE-Cy5 NHS-CD19c and PE-Cy7 NHS-CD3 Abs (BD Biosciences) and their purity assessed by PE αCD56 (Beckman Coulter, Life Sciences) and APC-eFluor780 αCD3 (eBioscience) antibody staining. hMSC purity (>99%) was assessed by flow cytometry using anti-CD34 and APC FITC, CD73 PE/Cy7, CD105 Alexa 647, CD44 PerCP-Cy5.5, and CD90 PE antibodies (BD Biosciences). Apoposis was quantified by FACS upon staining cells with PE Annexin V and 7-AAD. Cells were sorted using the FACsaria II (BD Biosciences). Data acquisition and analyses were performed at the UMBCCC Flow Cytometry Facility. Data from LSRII or CANTO II flow cytometers (BD Biosciences) were analyzed by using either the FlowJo v8.8.7 or Diva v6.1.2 software.
CFCs and CFC/replating assays CD34+ and CD34+ CD38− CML (5 × 10⁴) and/or UCB (2 × 10⁴) cells were seeded in MethoCult H4435 supplemented with Epo, KL, G-CSF, GM-CSF, IL3, and IL6. Fourteen-day colonies were mixed and the same number of cells replated twice (first and second replating) and scored 2 weeks later.

**CFSE (or eFluor670)-Mediated Tracking of Cell Division**

CFSE (CellTrace CFSE Proliferation Kit, Invitrogen) or eFluor670 (Cell Proliferation Dye eFluor670; ebioScience)-stained cells were FACS sorted to isolate the highest fluorescent peak, treated as indicated, harvested after 4 to 5 days, and counterstained with Near-IR Fluorescent Dye (Live/Dead Cell Stain Kit, Invitrogen) to determine the number of viable dividing (CFSE/^NearIR−) and quiescent cells (CFSE%^NearIR−). Where indicated, cells were stained with anti-CD34 APC and sorted into dividing and quiescent subpopulations. Quiescent stem (CFSE%^CD34^) and dividing progenitor cells in each peak were reported as a fraction of the initial number of CD34+ cells (46). For NK cells, 4- and 7-day-cultured CFSE^−/NearIR− and primary NK cells, respectively, were CFSE^−/NearIR− and Aquafluorescent Dye (Live/Dead Cell Stain Kit, Invitrogen) stained. NK cells were used in cytotoxicity assays and proliferation was quantitated as fold changes of CFSE mean fluorescence intensity in living CD56^+ NK cells.

**Plasmids**

pCDH-miR-300 (hsa-miR-300) pCDH-MIR300 was generated by subcloning a 490 bp Nhel/BamHI PCR fragment encompassing the mature hsa-MIR300 into the pCDH-CMV-MCS-EF1-copGFP-puro (SBI) vector. Sequence was confirmed by sequencing.

pSIH-H1-Zip-MIR300 (pZip-MIR300) To knockdown MIR300, a 5′-EcoRI and 3′-BamHI-flanked MIR300 antisense dsODN was directionally cloned into the pSIH-H1-copGFP vector (SBI).

pSIH-H1-copGFP-shTUG1 (TUG1 shRNA) A shRNA cassette containing the targeted nt 4571 to 4589 of hTUG1 RNA was subcloned into pSIH-H1-copGFP vector (SBI). A nonfunctional scrambled TUG1 shRNA was used as a control.

pLenti-TUG1 The hTUG1 into the pLenti-GII-III-CMV-GFP-2A-Puro-based vector was from Applied Biological Materials, Inc.

pGFP/Luc-based MIR300 Promoter Constructs p513-GFP/Luc, p245-GFP/Luc, and p109-GFP/Luc were generated by cloning the -522 bp, -245 bp, and -109 bp regions upstream the hsa-MIR300 gene locus (Sequence ID: NC_000014.9) into pGreenFire1 (pGFP/Luc, pGFluc, SBI) reporter vector. The region upstream the hsa-MIR300 gene were PCR amplified from K562 DNA and cloned into the pGFluc EcoRI-blunted site.

p109-C/EBPβmut-GFP/Luc An EcoRI-containing double-stranded synthetic oligonucleotide spanning the -109 bp MIR300 regulatory (region and containing the T/G and C/G mutated C/EBPβ consensus binding sites located at positions -64 and -46, respectively, was subcloned into EcoRI-digested pGFluc vector. pCDH-Flag-SET-FUCCI, fluorescent ubiquitinization-based cell-cycle indicator reporter pCDH-FUCCI2BL, MigrK1-auORF-C/EBPβ-ER^TM1, and MigrK1-auORF-C/EBPβ-ER^TM3 were constructed as described previously (25, 62–64).

pCDH-Flag-SET 3′UTR_uORF-GFP and pCDH-Flag-SET 3′UTR_uORF-GFP Wild-type (627 bp) or 3′-deleted (513 bp) SET 3′UTR were PCR amplified from K562 cDNA using a common EcoRI-linked 5′-primer and specific BamHI-linked 3′-primers for the wild-type and deleted SET mRNA 3′UTR. The PCR products were cloned into the pCDH-Flag-SET plasmid and sequenced.

**MSC CM and Exosome Purification**

HS-5- and hMSC-derived CM was obtained by culturing cells in complete RPMI (24 hours) and StemSpan SFMII (48 hours) medium, respectively. Alix^CD63^ exosomes were purified by differential centrifugation (3,000 × g, 15 minutes; 10,000 × g, 10 minutes; and 100,000 × g, 70 minutes) from HS-5 CM cultured in exosome-free FBS-supplemented medium (65, 66). When required, exosomes were precipitated (Exo-Quick; SBI) prior to isolation by ultracentrifugation.

**Lentiviral and Retroviral Transduction**

Lentiviral or retroviral particles were produced by transient calcium phosphate transfection (Profection Mammalian Transfection System, Promega) of 293T and Phoenix cells, respectively (8, 9, 67). Briefly, viral supernatant was collected 24 and 48 hours after transfection and directly used or concentrated by PEG precipitation. Viral titer was determined by flow cytometry by calculating number of GFP^+ 293T cells exposed (48 hours) to different viral dilutions. One to three spinoculation rounds were used for cell line infection, whereas a single spinoculation with diluted viral supernatants (MOI = 6) was used for primary cells. Viral supernatants were supplemented with polybrene (4 μg/ml). FACS sorting or puromycin selection was initiated 48 hours after transduction.

**Cell-cycle Analysis**

Cell-cycle analysis was performed by 4′,6′-diamidino-2-phenylindole (DAPI)/Ki-67 staining of CpG-scramble- or CpG-miR-300-treated (500 nmol/L, 72 hours) primary CD34+ CML (CP and BC) and UCB cells as described previously (64). Briefly, cells were fixed, permeabilized (Cytofix/Cytoperm kit, BD Biosciences), and Ki-67 PE (BioLegend) and DAPI (Sigma) stained. Alternatively, cell-cycle analysis was performed on pCDH-Fucci2BL-infected cells and subjected to flow cytometric analysis. LAMA-84-FUCCI2BL CML-BC cells were exposed to 500 nmol/L CpG-scramble and CpG-miR-300 oligonucleotides after being G1–S synchronized (+4 μmol/L aphidicolin, 6 hours). CpG-oligo–treated (48 hours) Fucci2BL+ cells were subjected to live-cell imaging for 48 hours and analyzed as described previously (64). Briefly, CpG-scramble- or CpG-miR-300-treated cells in G0–G1 were mVenus^−/PE-TexasRed^+, G0–S were mVenus^−/PE-TexasRed^+, and S–G2–M cells were mVenus^+/PE-TexasRed^-.

**Live-Cell Imaging and Confocal Microscopy**

Wide-field fluorescence imaging of CpG-ODN–treated pCDH-FUCCI2BL–transduced LAMA-84 cells was executed with an Olympus Vivaview digital camera, maintained at humidified atmosphere 37°C with 5% CO₂ mounted into a microscope-equipped incubator with green and red fluorescence filters. Emission spectral ranges were already (490–540 nm) and red-narrow (570–620 nm). Imaging was carried out for 24 or 48 hours. Confocal fluorescence images were acquired using a LEISS LSM510-inverted confocal microscope equipped with 40×/1.2-NA water immersion objective with 0.55-μm pixel size. Cell-cycle phases were determined by averaging red and green fluorescent intensity over time using Prisim 6.0 d software. The Vivaview raw images were reconstructed using ImageJ v5.2.5 and Adobe CS6 software.

**Real-time RT-PCR**

Total RNA was extracted using mirNeasy Micro Kit (Qiagen Inc.) or TRIzol (Invitrogen) and reverse transcribed using a standard cDNA synthesis or the TaqMan MicroRNA Reverse Transcription Kit.
with mouse and/or human-specific sets of primers/probe for BCR-ABL1, CEBPB, FOXM1, TUG1, IFNg, pre-MIR300, MIR300, pre-miR-155 (BIC), IHS, RN44, RN66b, and snoRNA202 (Applied Biosystems). PCR reactions were performed using a StepOnePlus Real Time PCR System (Applied Biosystems). Data were analyzed according to the comparative C, method using RN44, RN66b, IHS, and snoRNA202 transcripts as an internal control. Results are expressed as fold change of mean ± SEM.

**Immunoblot Analysis**

Lysate from cell lines and primary cells were subjected to SDS-PAGE and Western blotting as described previously (46). The antibodies used were anti-actin, anti-Myc, anti-C/EBPβ, anti-ALix, anti-Twist, and anti-CD63 (Santa Cruz Biotechnology); anti-GRB2 (Transduction Laboratories), anti-JAK2, anti-Flag (Sigma), anti-FoxM1, anti-CDK6, anti-pJAK2 (1007/1008), and anti-CCND2 (Cell Signaling Technology); anti-hnRNPA1 (Abcam); and anti-SET (Globozymes); anti-ABL (Ab-3), anti-CCND1/2, anti-PY (4G10), and anti-PP2Ac (EMD); and anti-Flag (M2; Sigma).

**PP2A Phosphatase Assay**

PP2A assays were performed using the malachite green-based PP2A Immunoprecipitation Phosphatase Assay Kit (Millipore) as described previously (46). The antibody used was anti-PP2A (1:1000). The reaction was incubated in a shaking water bath at 30°C for 2 hours and used in the phosphatase reaction according to the manufacturer’s protocol.

**Luciferase Assay**

Transcriptional activity of the intergenic 513 bp region upstream the MIR300 gene was investigated by luciferase assay using the pGreenFire-Lenti-Reporter system (pGF1; SBI) in cell lines and primary cells. The empty vector (pGFP-Luc; pGF1) was used as a negative control. After 48 hours, cells were lysed and luciferase activity was determined by Pierce Firefly Luciferase Flash Assay Kit (Thermo Scientific).

**Cytotoxicity Assays**

A flow cytometry-based killing assay was performed using K562 cells as targets (68). Briefly, cells were transduced with constructs containing wild-type full-length 513 bp (p513-GFP/Luc) and deleted 245 bp (p245-GFP/Luc) base pair-long region upstream the MIR300 gene locus, or with the p109-GFP/Luc construct mutated in the two C/EBPβ-binding sites (p109-CEBPβmut-GFP/Luc). The empty vector (pGFP-Luc; pGF1) was used as a negative control. After 48 hours, cells were lysed and luciferase activity was determined by Pierce Firefly Luciferase Flash Assay Kit (Thermo Scientific).

**LSC Engraftment and Disease Development in NRG-SGM3 Mice**

In vivo evaluation of changes in BM-repopulating LSCs was performed as described previously (40). A total of 10^5 BM CD34+ CML (n = 3; >95% Ph'; CP, AP, and BC) cells, treated (500 nmol/L, 48 hours) ex vivo with Cpg-ODNs, were intravenously injected (4 mice/treatment group/patient sample) into sublethally irradiated (2.6 Gy) 6 to 8-week-old NOD.Cg-Rag1tm1Mom Il2rgtm1Wj tg(CMV-IL3, CSF2, KitLG)1Eav/J mice (NRG-SGM3; The Jackson Laboratory). Engraftment was assessed by anti-human CD45 (BD Biosciences) flow staining of intrafemur BM aspirates (46) at 2 weeks in Cpg scramble control groups and at 2, 4, and 12 weeks posttransplant in Cpg-MIR300, -TUG1RNA, and MIR300+TUG1RNA CML-BC, AP, and CP, respectively. Animal studies were performed according to IRB- and Institutional Animal Care and Use Committee–approved protocols.

**Oligonucleotides and Primers**

The partially phosphorylated (*) 2'-O-Methyl ODNs were linked using 5 units of C3 carbon chain linker, (CH2)n, (X). The ODNs were synthesized by the DNA/RNA Synthesis Laboratory, Beckman Research Institute of the City of Hope (Duarte, CA).

**Cpg-oligonucleotides and Probes**

Cpg-miR-300: 5'-g*g*tgcatctgacgg*g*g*g*xgxxlxxaacagggcaga cuxccuuc-3'

Cpg-anti-miR-300: 5'-g*g*tgcatctgacgg*g*g*xgxxlxxagagacu cgeuuccuuaa-3'

Cpg-Tug1-3RNA: 5'-g*g*tgcatctgacgg*g*g*xgxxlxxauucugge gucucacac-3'

Cpg-scramble: 5'-g*g*tgcatctgacgg*g*g*xgxxlxxguacaaacuc gacucuaa-3'

**pCDH-CMV-MCS-1FF-copGFP-puro miRNA Constructs**

MIR300(F): 5'-gtgctagctgtgactagttgtaccttag-3'

pS1H1-1-copGFP Constructs

Zip-MIR300 ODN: 5'-tgtgtgttgttgcatctgacgg*g*g*xgxxlxxagagacu cgeuuccuuaa-3'

shTUG1(+) : 5'-gtgctagctgacgg*g*g*xgxxlxxagacugcuacacac gacucuaa-3'

shTUG1(−): 5'-aattcagctgacgg*g*g*xgxxlxxagacugcuacac gacucuaa-3'

**pCDH-Flag-SET Constructs**

SET3'UTRwt-GFP(F): 5'-gaattctgtagctgactagttgtaccttag-3'

SET3'UTR-RFP(F): 5'-ccgattgacatgacagctgactagttgtaccttag-3'

SET3'UTR-GFP(F): 5'-gaattctgtagctgactagttgtaccttag-3'

**pGreenFire1**

pS22-pGF1(F): 5'-cgggatttccggcagtcctcagttcatcaagtc-3'

p245-pGF1(F): 5'-cgggatttccggcagtcctcagttcatcaagtc-3'

p109-pGF1(F): 5'-cgggatttccggcagtcctcagttcatcaagtc-3'

Common(0): 5'-gtgctagctgacgg*g*g*xgxxlxxagacugcuacac gacucuaa-3'

**p109/C/EBPβ Mutant Construct**

(+) strand ODN: 5'-aatgcgtctggtctcattgctctgacttcatcaagtc-3'

(+) strand ODN: 5'-aatgcgtctggtctcattgctctgacttcatcaagtc-3'

Bioinformatics Tools

Statistically significant (P < 0.05 with FDR correction) predicted and validated hsa-miR-300 mRNA targets according to mRNA target
function and MIR300 doses were identified using DIANAmirT-CD5 (diana.imis.athena-innovation.gr), ComiR (http://www.benoslab.pitt.edu/comir/), CSmirTar (http://cosbi4.ee.ncu.edu.tw/CSmirTar/), and mirDIP 4.1 (ophid.utoronto.ca/mirDIP/; refs. 69–71). KEGG and GO analyses were used to define the biological pathways and the functional roles of miRNA-300 and TUG1 targets were performed using mirPath v.3 (snf-35788.vm.okeanos.grnet.gr). MIR300 and TUG1 individual gene expression profiles were obtained from curated datasets in the Gene Expression Omnibus (GEO) repository (ncbi.nlm.nih.gov/geo/profiles/). TUG1 levels in normal and leukemic myelopoesis were analyzed from BloodSpot database (servers.binf.ku.dk/bloodspot/) of healthy and malignant hematopoiesis. Integration of Starbase v2.0 (starbase.sysu.edu.cn/starbase2/index.php) database with the RNaseq Mirbase data from CD34+CD38+, CD34+/CD38+ CML and NBM cells was used to identify TUG1-sponged miRNAs.

Statistical Analysis

P values were calculated by Student t test (GraphPad Prism v6.0). Results are shown as mean ± SEM. A P value less than 0.05 was considered significant (∗, P < 0.05; ∗∗, P < 0.01; ∗∗∗, P < 0.001; ∗∗∗∗, P < 0.0001). Mixed models’ approach, the split-plot design, was used to assess differences in percent cell across three treatment groups. The percent cell change was estimated using a model with two main effects for treatment and stage of a cell cycle, as well as their interaction. Tests of fixed effects revealed that interaction of treatment and stage of a cell cycle is highly significant (P = 0.01) and the two main effects, treatment and stage, should be interpreted with caution (P values are 1.0 and <0.0001, respectively). The differences in average percent cell were tested across groups and sliced at a particular stage of cell cycle.

Disclosure of Potential Conflicts of Interest

D. Perrotti has ownership interest in patents 8,633,161, 9,220,706 and 8,318,812. P. Neviani has ownership interest in patents 9,220,706, 8,633,161. C.J. Walker is a consultant at Karyopharm Therapeutics, Inc. F. Stagno has received speakers bureau honoraria from Novartis, Bristol-Myers Squibb, Pfizer, and Incyte. P. Vigneri reports receiving a commercial research grant from Novartis, has received speakers bureau honoraria from Incyte, is a consultant/advisory board member for Incyte. M.W. Deininger is a consultant/advisory board member at Blueprint, Fusion Pharma, Novartis, Sangamo, Takeda, Medscape, Incyte, Ascenage Pharma, Humana, TRM and reports receiving commercial research grants from Blueprint, Takeda, Novartis, Incyte, SPARC, Leukemia & Lymphoma Society, and Pfizer. D. Milojkovic has received speakers bureau honoraria from Novartis, Bristol-Myers Squibb, Incyte, and Pfizer. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions


Acknowledgments

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Abbreviations: 5-Aza-2′-deoxycytidine (5-Aza); blast crisis (BC); bone marrow (BM) microenvironment (BMIM); carboxylfluorescein diacetate succinimidyl diester (CFSE); CCAAT enhancer binding protein (C/EBP); colony forming cells (CFC); chronic myelogenous leukemia (CML); chronic phase (CP); cobblestone area forming cell (CAFC); conditioned medium (CM); hematopoietic stem cell (HSC); imatinib (IM); leukemic stem cells (LSC); long term culture–initiating cells (LTC-LC); luciferase (luc); mesenchymal stromal cells (MSC); natural killer (NK); normal BM (NBM); peripheral blood (PB); PP2A activating drugs (PAD); PP2A inhibiting drugs (PID); PP2A inhibitory pathway (PIP); protein phosphatase 2A (PP2A); quiescent leukemic stem cells (qLSCs); taurine upregulated gene 1 (TUG1); tyrosine kinase inhibitors (TKI); umbilical cord blood (UCB).

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