Retinoic acid (RA) and As$_2$O$_3$ treatment in transgenic models of acute promyelocytic leukemia (APL) unravel the distinct nature of the leukemogenic process induced by the PML-RAR$\alpha$ and PLZF-RAR$\alpha$ oncoproteins

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Acute promyelocytic leukemia (APL) is associated with chromosomal translocations always involving the RAR$\alpha$ gene, which variably fuses to one of several distinct loci, including PML or PLZF (X genes) in t(15;17) or t(11;17), respectively. APL in patients harboring t(15;17) responds well to retinoic acid (RA) treatment and chemotherapy, whereas t(11;17) APL responds poorly to both treatments, thus defining a distinct syndrome. Here, we show that RA, As$_2$O$_3$, and RA + As$_2$O$_3$ prolonged survival in either leukemic PML-RAR$\alpha$ transgenic mice or nude mice transplanted with PML-RAR$\alpha$ leukemic cells. RA + As$_2$O$_3$ prolonged survival compared with treatment with either drug alone. In contrast, neither in PLZF-RAR$\alpha$ transgenic mice nor in nude mice transplanted with PLZF-RAR$\alpha$ cells did any of the three regimens induce complete disease remission. Unexpectedly, therapeutic doses of RA and RA + As$_2$O$_3$ can induce, both in vivo and in vitro, the degradation of either PML-RAR$\alpha$ or PLZF-RAR$\alpha$ proteins, suggesting that the maintenance of the leukemic phenotype depends on the continuous presence of the former, but not the latter. Our findings lead to three major conclusions with relevant therapeutic implications: (i) the X-RAR$\alpha$ oncoprotein directly determines response to treatment and plays a distinct role in the maintenance of the malignant phenotype; (ii) As$_2$O$_3$ and/or As$_2$O$_3$ + RA combination may be beneficial for the treatment of t(15;17) APL but not for t(11;17) APL; and (iii) therapeutic strategies aimed solely at degrading the X-RAR$\alpha$ oncoprotein may not be effective in t(11;17) APL. The X-RAR$\alpha$ oncoprotein may not be effective in t(11;17) APL.

A distinctively worse prognosis with little or no response to treatment with RA, thus defining a distinct APL syndrome (8).

The X-RAR$\alpha$ fusion proteins form corepressor complexes with NCoR/SMRT-Sin3A-histone deacetylases, which are less sensitive to RA (9–12). PLZF-RAR$\alpha$, by means of its PLZF moiety, can form corepressor complexes that are insensitive to pharmacological doses of RA (9–12).

RA can also induce the caspase-mediated degradation of the PML-RAR$\alpha$ fusion protein (13, 14). Thus, the differential response to RA can be attributed to the differential repressible ability of the two X-RAR$\alpha$ fusion proteins.

As in human APL, leukemia in PML-RAR$\alpha$ transgenic mice (TM) responds well to RA, which can induce complete disease remission, whereas leukemia in PLZF-RAR$\alpha$ TM responds poorly to RA and complete disease remission is never attained (9).

Arsenic trioxide (As$_2$O$_3$) is extremely effective in the treatment of APL (7, 15). As$_2$O$_3$ may induce the degradation of the PML-RAR$\alpha$ protein through ubiquitination of the PML moiety (16–20). This event could be critical in mediating the biological effects of As$_2$O$_3$ in APL. However, NB4–306 cells, a RA-resistant cell line derived from NB4 that no longer expresses the PML-RAR$\alpha$ fusion protein, responded to As$_2$O$_3$ as the parental NB4 cells did (20, 21). In addition, in vitro, As$_2$O$_3$ shows antitumoral and proapoptotic activity in cancer cells that do not harbor t(15;17) (21–24).

RA and As$_2$O$_3$ are both very effective antileukemic drugs in t(15;17) APL. RA triggers differentiation whereas, As$_2$O$_3$ induces both apoptosis and partial differentiation of the leukemic blasts (7, 15–21, 25). However, it is still unclear whether combination of these drugs may be more effective in the treatment of t(15;17) APL, and whether therapy-resistant leukemia such as t(11;17) APL responds to As$_2$O$_3$ and/or As$_2$O$_3$ + RA. Furthermore, the mechanisms by which the X-RAR$\alpha$ oncoproteins mediate response or resistance to treatment still need to be established. To address these questions, we have used PML-RAR$\alpha$ and PLZF-RAR$\alpha$ mouse models of leukemia.

Abbreviations: RA, retinoic acid; RAR, RA receptor; APL, acute promyelocytic leukemia; PML, promyelocytic leukemia zinc-finger; PLZF, promyelocytic leukemia zinc-finger; TM, transgenic mice; NM, nude mice; PB, peripheral blood; BM, bone marrow; TUNEL, terminal deoxynucleotidyltransferase-mediated dUTP end labeling; UN, untreated; CI, confidence interval.

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Materials and Methods

TM and Diagnosis of Leukemia. We used leukemic hCG-PML-RARα and hCG-PLZF-RARα TM. The generation of these TM and the characterization of their hemophagocysis have been described (9, 26). Diagnosis of leukemia was made on the basis of the following concomitant criteria: (i) presence of blasts/ promyelocytes (>1%) in the peripheral blood (PB); (ii) leukocytosis (WBC > 30 × 10^9/μl); and (iii) anemia (hemoglobin < 10 g/dl) and/or thrombocytopenia (platelets < 500 × 10^9/μl).

Nude Mice (NM) Transplantation. NU/J Hff 11nu 4 to 8-week-old NM (The Jackson Laboratory) were injected with 5 × 10^7 leukemic cells i.p. The leukemic cells were obtained from the bone marrow (BM) and spleen of moribund leukemic PLZF-RARα or PML-RARα TM. For these experiments, three independent leukemias from each TM genotype were used. No differences in treatment response were observed between recipients of different donors. The morphology, immunophenotype, and expression of the oncoproteins by the transplanted leukemic cells were identical to those observed in leukemic TM. By sacrificing NM at various times after transplantation, we detected leukemic infiltration in the BM and spleen in 100% of the NM by day 25. Thus, the various treatments were started 25 days after transplant.

Treatment with Arsenic and RA. To determine the dose of As2O3 to be administered, wild-type (C57BL/6) and NM (n = 6, per group) were treated with 1, 2.5, 5, 7.5, and 10 μg/g of body weight per day As2O3 (Sigma) with or without 1.5 μg/g per day of RA (Sigma) daily i.p. for 4 weeks, after which three animals per group were killed and a thorough pathological analysis of all organs was performed. As2O3 (1 or 2.5 μg/g per day) with or without RA did not cause signs of toxicity, and the remaining animals remained healthy after more than 1 year of follow-up. As2O3 (7.5 and 10 μg/g per day) with or without RA resulted in lethality in both NM and wild-type mice. Lethality was probably caused by liver failure, because hepatic necrosis was constantly found. NM perfectly tolerated 5 μg/g per day of As2O3. In wild-type mice, however, this dose caused liver damage, but did not result in death. On the basis of these data, leukemic PLZF-RARα or PML-RARα TM were treated, at presentation, for 21 consecutive days with an i.p. daily injection of (i) RA at 1.5 μg/g per day; (ii) As2O3 at 2.5 μg/g per day; or (ii) As2O3 + RA at the same doses. NM were treated as above with (i) RA at 1.5 μg/g per day; (ii) As2O3 at 5 μg/g per day; or (iii) As2O3 + RA at the same doses. TM and NM were followed on a daily basis, and automatic and differential counts of PB were performed weekly. The animals were considered in hematological remission according to the following criteria: hemoglobin levels ≥ 12 g/dl; WBC counts ≤ 30,000/μl with ≤1% blasts, and platelets ≥ 500,000/μl. All these criteria were fulfilled in two consecutive weekly tests.

Apoptosis and Differentiation. NM were transplanted as described with either PML-RARα or PLZF-RARα leukemic cells, and 25 days later they were treated for 3 days with As2O3 at 5 μg/g per day, RA at 1.5 μg/g per day, or As2O3 + RA at the same doses. Approximately 6 h after the last dose, NM were killed and the leukemic cells were then sorted from the liver by using a MoFlo flow cytometer (Cytomation, Fort Collins, CO). The cells were sorted on the basis of these immunophenotypic features: CD45+, CD3ε−, and B220− (all antibodies were purchased from PharMingen). On sorting, more than 90% myeloid cells were obtained as judged by morphological analysis (Fig. 4B). Apoptotic cells were scored by the terminal deoxynucleotidyltransferase-mediated dUTP nick end-labeling method (TUNEL) by using the In Situ Cell Death Detection Kit (Boehringer Mannheim). The TUNEL assay was performed according to the instructions of the supplier. 4′,6-Diamidino-2-phenylindole (DAPI; Sigma) was used to reveal nuclei. Myeloid differentiation on various treatments was evaluated: (i) by morphological analysis scoring the number of mature myeloid cells; and (ii) by measuring with flow cytometry the expression levels of the CD11b surface marker (clone M1/70, PharMingen) in the sorted leukemic cells.

Establishment of the Lee Cell Line from a Leukemic PLZF-RARα TM. BM cells were collected from PLZF-RARα TM with leukemia and cultured in DMEM supplemented with 30% FCS, 2% pokeweed mitogen-stimulated murine spleen cell-conditioned medium (StemCell Technologies, Vancouver), 2 mM L-glutamine, and 1% penicillinstreptomycin at 37°C in a 5% CO2 atmosphere. Without pokeweed mitogen-stimulated murine spleen cell-conditioned medium, cells died within 1 month. After six passages, the concentration of FCS was reduced to 20%, and recombinant stem cell factor and IL-3 (PeproTech, Rocky Hill, NJ) were added to the cultures at a final concentration of 10 and 5 ng/ml, respectively. Morphological and flow cytometric surface marker analyses as well as in vitro RA responses revealed features that paralleled those observed in vivo in leukemic blasts from PLZF-RARα TM.

Cell Cultures. NB4 cells were grown in RPMI medium 1640 supplemented with 10% FCS and 1% penicillin-streptomycin and maintained at 37°C in a 5% CO2 atmosphere. Lee cells were cultured as described. The cells were treated with As2O3 and/or RA at the indicated doses.

Immunocytofluorescence and Western Blot Analyses. Transplanted NM and leukemic TM were treated for 2 or 7 days with As2O3, RA, or As2O3 + RA. Leukemic cells were sorted from the liver of NM or harvested from the BM of leukemic TM, cytospun onto slides, and subjected to confocal indirect immunofluorescence analysis with a rabbit polyclonal anti-RARα antibody (a kind gift from P. Chambon, Institut de Génétique et de Biologie Moléculaire et Cellulaire, Strasbourg, France) as described (9). Staining with 4′,6-diamidino-2-phenylindole was performed to reveal nuclei. For Western blot analysis, whole-cell lysates were prepared by direct lysis in enzyme immunoassay buffer. Proteins were electrophoresed on an SDS/8% polyacrylamide gel, transferred to a nitrocellulose membrane, and immunostained with anti-RARα and anti-β-actin antibodies.

RNA Analysis. Total RNA was prepared by using Trizol reagents (GIBCO/BRL). For the Northern blot analysis, denatured total RNA (25 μg) was hybridized with the human PLZF cDNA probe 3′, following standard procedures.

Statistics. Statistical analysis was performed by using the spss 9.0 software (SPSS, Chicago). Survival analysis was based on Kaplan–Meyer estimation and groups were compared by the log-rank test. The comparison between the percentage of CD11b-positive and of TUNEL-positive cells in treated and untreated NM was performed by the Mann–Whitney u test. All quoted P values are two sided, and confidence interval (CI) refers to 95% boundaries.

Results

The Combination of As2O3 and RA Is More Effective in the Treatment of Leukemia in PML-RARα TM Than Either Drug Alone. PML-RARα leukemic TM were treated with a daily i.p. dose of As2O3, RA, or As2O3 + RA. In any of the three therapeutic arms, survival was significantly longer than in untreated (UN) animals (P < 0.0001) (mean survival time of UN: 9.3 days, 95% C.I. = 6.6–12 days; RA: 44.3 days, 95% C.I. = 36.7–51.9 days; As2O3: 36.7 days, 95% C.I. = 29.5–43.9 days; As2O3 + RA: 72 days, 95% C.I. = 47.2–96.8 days) (Fig. 1A). Although in mice treated with RA alone the mean survival was longer than in those treated with As2O3 alone, this difference was not statistically significant (P = 0.26), whereas the mean survival of leukemic PML-RARα TM
treated with As$_2$O$_3$ + RA was significantly longer than the one observed with RA alone ($P = 0.04$) or As$_2$O$_3$ alone ($P = 0.03$).

RA and As$_2$O$_3$, alone or in combination, induced disease remission in 100% of leukemic PML-RAR$\alpha$ TM as demonstrated by the disappearance of the leukemic cells from the PB (Fig. 1 B–E) and the return of the WBC, hemoglobin, and platelet counts to normal (data not shown). Disease remission was also confirmed by killing mice and performing differential bone marrow counts in each therapeutic arm at the time when the blasts disappeared from the PB (not shown). Spontaneous disease remission was never observed among untreated mice. After relapse, leukemic infiltration of spleen, liver, and BM was consistently detected in the necropsy.

**RA, As$_2$O$_3$, or As$_2$O$_3$ + RA Are Ineffective in Leukemic PLZF-RAR$\alpha$ TM.**

PLZF-RAR$\alpha$ leukemic TM were treated with a daily i.p dose of As$_2$O$_3$, RA, or As$_2$O$_3$ + RA. Leukemic mice survived longer when treated. However, the mean survival time did not differ statistically between untreated and treated mice ($P = 0.1$) (mean survival time of UN: 14.6 days, 95% C.I. = 11.1–18 days; RA: 30.3 days, 95% C.I. = 17.9–42.6 days; As$_2$O$_3$: 21 days, 95% C.I. = 11.7–30.3 days; As$_2$O$_3$ + RA: 29.67 days, 95% C.I. = 13.4–45.9 days) (Fig. 1A). Moreover, RA, As$_2$O$_3$, or their combination never induced disease remission, as shown by the constant presence of leukemic cells in the peripheral blood (Fig. 1 B–E).

**As$_2$O$_3$ + RA Prolongs the Survival of NM Transplanted with PML-RAR$\alpha$ Leukemic Cells.** In NM transplanted with PML-RAR$\alpha$ leukemic cells, either RA or As$_2$O$_3$ treatments resulted in a significantly ($P < 0.001$) longer mean survival than that observed in UN mice (Fig. 2A). The treatment with RA or As$_2$O$_3$ alone resulted in comparable mean survival time (UN: 60.6 days, 95% C.I. = 55.5–65.7 days; As$_2$O$_3$: 75.6 days, 95% C.I. = 68.3–82.8 days; RA: 83.6 days, 95% C.I. = 75.6–91.6 days). When treated with the As$_2$O$_3$ + RA combination, transplanted NM survived longer than those treated with either drug alone (105 days, 95% C.I. = 98.6–111.4 days; $P = 0.004$ for the comparison between As$_2$O$_3$ + RA and RA; $P < 0.001$ for As$_2$O$_3$ + RA versus As$_2$O$_3$). Prolonged survival induced by the various drugs was accompanied by a longer interval between the transplantation and the appearance of blasts in the PB (Fig. 2 B–E).

**RA, As$_2$O$_3$, or As$_2$O$_3$ + RA Are Ineffective in the Treatment of NM Transplanted with PLZF-RAR$\alpha$ Leukemic Cells.** As observed in PLZF-RAR$\alpha$ TM, neither of the treatments prolonged survival in...
transplanted NM in a significant manner (UN: 66.2 days, 95% C.I. = 61.7–70.7 days; As2O3: 69.4, 95% C.I. = 65.9–72.8 days; RA: 70.2 days, 95% C.I. = 65.9–74.5 days; As2O3 + RA: 73.5 days, 95% C.I. = 69.7–77.3 days). The differences between the three arms were not significant (P = 0.15). In addition, no significant difference was observed among the various treatments in the interval time between transplantation and the detection of blasts in peripheral blood (Fig. 2 B–E).

**Distinct Biological Response to As2O3, RA, or As2O3 + RA Treatments in PML-RARα or PLZF-RARα Leukemia Models.** In NM transplanted with either PML-RARα or PLZF-RARα leukemic cells, both As2O3 and As2O3 + RA treatments resulted in increased apoptosis of the leukemic blasts (Fig. 3). However, whereas in the PML-RARα model As2O3 and As2O3 + RA caused a marked increase in apoptosis (>5-fold), in the PLZF-RARα model, the increase was modest (up to 2-fold) (Fig. 3).

The differential response of the PML-RARα and PLZF-RARα leukemic cells to the various treatments was even more pronounced when we evaluated the differentiating ability of the various drugs. In the PML-RARα NM model, we observed a significant increase in the number of mature myeloid cells (UN: 28.1% ± 8%; RA: 54.5% ± 12.3%; As2O3: 35.7% ± 8.3%; As2O3 + RA: 57.7% ± 13.2%) as well as in the levels of CD11b expression on treatment with RA or with As2O3 + RA, compared with that detected in mice untreated or treated with As2O3 alone (Fig. 4). In contrast, in the PLZF-RARα NM model, none of the treatments induced a significant increase in the number of mature myeloid cells (UN: 37.1% ± 12.1%; RA: 41.1% ± 8.1%; As2O3: 39% ± 11.7%; As2O3 + RA: 43.9% ± 8%) or in the CD11b expression in the leukemic cells, as compared with cells from untreated NM (Fig. 4).

**RA or RA + As2O3 Can Induce, Both in Vivo and in Vitro, the Complete Degradation of the PML-RARα or PLZF-RARα Oncoproteins.** We next studied the effects of RA, As2O3, or their combination on the stability of the PML-RARα or PLZF-RARα oncoproteins in our leukemia models. Immunofluorescence analysis was performed on the cells from the BM of leukemic TM and on the leukemic cells sorted from transplanted NM on various treatments. By using an anti-RARα antibody that recognizes the X-RARα fusion proteins, a characteristic nuclear microspeckled staining pattern was detected in leukemic cells from both PML-RARα and PLZF-RARα TM (ref. 9 and Fig. 5A). In the PML-RARα TM and NM models, RA, As2O3, or RA + As2O3 treatments resulted in the disappearance of the nuclear microspeckled signal after 2 or 7 days of treatment, in agreement with the notion that these drugs can induce the degradation of the PML-RARα protein (Fig. 5A and not shown). Surprisingly, however, in both PLZF-RARα TM and NM models of leukemia, RA + As2O3 or RA treatments also induced the disappearance of the nuclear microspeckled signal, whereas As2O3 alone did not (Fig. 5A and not shown), suggesting that RA can induce the degradation of the PLZF-RARα fusion protein as well. Western blot analysis demonstrated that the PLZF-RARα oncoprotein is persistently absent in actively growing leukemic cells treated for 7 days with RA + As2O3 (Fig. 5A). This result also rules out the possibility that a novel subpopulation of leukemic blasts resistant to the RA + As2O3-induced degradation of the PLZF-RARα protein is selected throughout treatment.

To confirm that the disappearance of the microspeckled signal observed in the PLZF-RARα leukemic cells on RA or RA +
As$_2$O$_3$ is not caused by a subcellular redistribution of the oncprotein, but is the result of its degradation, and to compare the effects of the various drugs on PML-RAR$\alpha$ and PLZF-RAR$\alpha$, we took advantage of a cell line established from a leukemic PLZF-RAR$\alpha$ TM (Lee) and the APL NB4 cell line, which express the PML-RAR$\alpha$ oncprotein (27). Lee cells express PLZF-RAR$\alpha$, which is detected in its classical nuclear microspeckled distribution (Fig. 5B). RA was used at subpharmacological doses (10$^{-7}$ M), which could induce only a partial degradation of PML-RAR$\alpha$ in NB4 (Fig. 5B and refs. 13 and 14). As observed in vivo, however, at this dose of RA, in Lee cells either this drug or RA + As$_2$O$_3$, but not As$_2$O$_3$, induced the disappearance of the nuclear microspeckled staining and the PLZF-RAR$\alpha$ fusion protein as demonstrated by immunofluorescence and Western blot analysis, respectively (Fig. 5B). On treatment with RA + As$_2$O$_3$, the complete disappearance of the microspeckled staining pattern could already be demonstrated after 3 h (not shown). At this time point, Northern blot analysis showed identical amounts of PLZF-RAR$\alpha$ RNA in treated and untreated Lee cells (Fig. 5B), suggesting that the disappearance is likely caused by protein degradation. In NB4 cells, in concordance with what was observed in cells from PML-RAR$\alpha$ NM and TM, As$_2$O$_3$ and RA + As$_2$O$_3$ induced the complete degradation of the PML-RAR$\alpha$ fusion protein, whereas a subpharmacological dose of RA only partially degraded PML-RAR$\alpha$ (Fig. 5B). Immunofluorescence analysis in NB4 cells revealed the disappearance of the microspeckled nuclear signal on As$_2$O$_3$ and RA + As$_2$O$_3$, and its weakening on RA, corroborating what was observed by Western blot analysis (not shown). Thus, despite the unresponsiveness to RA and RA + As$_2$O$_3$ observed in the PLZF-RAR$\alpha$ TM and NM models of leukemia, both regimens could induce the degradation of the PLZF-RAR$\alpha$ oncprotein.

**Discussion**

The first conclusion that can be drawn from the data presented here is that RA and As$_2$O$_3$ in combination might be effective in the treatment of t(15;17) APL, which represents the most frequent genetic subtype of APL. This is clearly demonstrated by the fact that in vivo, in both PML-RAR$\alpha$ leukemic TM and NM transplantation models, the two drugs are effective when administered together. These data are in agreement with the notion that RA and As$_2$O$_3$ can both induce the degradation of the PML-RAR$\alpha$ fusion protein (13, 14, 17–20, 28–30), as we have confirmed in vivo in our mouse models. As$_2$O$_3$ targets PML-RAR$\alpha$ for degradation through the PML moiety (17, 18), whereas RA targets through both the PML and the RAR$\alpha$ moieties (13, 14, 28–30). Synergy between RA and As$_2$O$_3$ was also reported in a murine transplantation model of PML-RAR$\alpha$ leukemia (31). Furthermore, in our PML-RAR$\alpha$ animal models, RA acts as an inducer of differentiation, whereas As$_2$O$_3$ acts as a proapoptotic agent. In combination, the two drugs do not antagonize their respective activities, thus providing a rationale for combining these drugs in APL treatment. Thus, although caution should be taken in extrapolating from murine models, an important clinical implication of our findings is that RA + As$_2$O$_3$ may be effective for the treatment of t(15;17) APL, even at presentation. NM seemed to respond to treatment less efficiently than the leukemic TM, which may be caused by differences in immune response and drug metabolism between NM and TM. However, despite these differences, similar conclusions can be drawn from trials in both TM and NM models.

RA and As$_2$O$_3$ activities converge toward the functional and physical inactivation of PML-RAR$\alpha$, which correlates with the efficacy of this treatment. An etiopathogenetic implication of these findings is that the PML-RAR$\alpha$ oncogenic function is critical for the maintenance of the malignant phenotype. It is, of course, possible that other biological effects of RA and As$_2$O$_3$ may also contribute to the efficacy of the combined treatment. Furthermore, because the animal models studied here do not harbor the reciprocal product of the t(15;17), RA-RAR$\alpha$-PML, we cannot exclude that this fusion protein may also play a critical role in the maintenance of the leukemic phenotype in human APL, as suggested by the fact that one APL case has been reported in which only the reciprocal RA-RAR$\alpha$-PML product was expressed (32).

In contrast, RA, As$_2$O$_3$, or their combination does not induce disease remission in leukemic PLZF-RAR$\alpha$ TM. These regimens are also ineffective in the PLZF-RAR$\alpha$ NM model. Not only do PLZF-RAR$\alpha$ leukemias respond poorly to RA, As$_2$O$_3$, or RA + As$_2$O$_3$, but the leukemic blasts harboring the PLZF-

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RARs display a marked protection from the differentiating and proapoptotic effects of these treatments. Thus, the different NH2-terminal moiety of the two X-RARα oncoproteins directly mediates differential response to both RA and As2O3. The unresponsiveness to As2O3 might be explained by the inability of the drug to induce the degradation of the PLZF-RARα oncoprotein. Surprisingly, however, RA with or without As2O3 is effective, both in vivo and in vitro, in inducing the degradation of PLZF-RARα. Effective degradation of PLZF-RARα by RA has also been reported in vitro in cells from one t(11;17) human APL patient (33). These findings unravel a striking difference between leukemias associated with PML-RARα or PLZF-RARα. Whereas PML-RARα leukemia appears to depend on the presence of the oncoprotein, in PLZF-RARα leukemia, the malignant phenotype does not appear to depend entirely on the presence of the oncoprotein, although it cannot be excluded that undetectable amounts of the PLZF-RARα oncoprotein may suffice to exert its leukemogenic effects.

The absence of the PLZF-RARα oncoprotein in treated leukemic cells originates a paradox: how can PLZF-RARα dictate unresponsiveness to treatment, and in its absence, these leukemic cells originate a paradox: how can PLZF-RARα oncoprotein before its degradation. This mechanism is not effective in the case of the PLZF-RARα because this molecule acts as a potent transcriptional repressor even in the presence of RA (9–12). (ii) In a multistep model of leukemogenesis, it is formally possible that the additional genetic events which co-occur with the two X-RARα fusion proteins are distinct. These events might render PLZF-RARα, but not PML-RARα, redundant for maintenance of the malignant phenotype. (iii) PLZF-RARα might affect transcription through epigenetic mechanisms. Abrupt transcriptional regulation would be, therefore, maintained throughout cell replication even in the absence of the PLZF-RARα oncoprotein. This is supported by the fact that PLZF-RARα through the PLZF moiety can directly interact with nuclear corepressors and NCoR/SMRT-Sin3A-histone deacetylase, thus leading to chromatin remodeling (9–12). In this regard, it is also important to notice that methylation has been associated with epigenetic transcriptional inactivation and cancer pathogenesis (34). A link between gene methylation and histone deacetylation has now been established by a protein known as MeCP2, which binds the mSin-3/NCoR/SMRT-Sin3A-histone deacetylase complex to methylated cytosines (35). Thus, aberrant reorganization of chromatin and/or gene methylation might be propagated through cell division in the promyelocytic blasts. Irrespective of the mechanisms, these findings predict that therapeutic strategies aiming solely at targeting the expression or the stability the PLZF-RARα oncoprotein, such as approaches with hammerhead ribozyme (36), might be ineffective in t(11;17) APL, and that the inactivation/degradation of the fusion protein should be accompanied by a proper stimulus. The complete remission obtained in a t(11;17) APL patient with RA administered in combination with granulocyte colony-stimulating factor (37) might represent a promising precedent. In summary, our data provide strong support to the tenet that cancer treatment has to be tailored on the basis of the specific molecular mechanisms underlying the pathogenesis of each cancer subtype. To this end, mouse models are invaluable tools to unravel these mechanisms as well as to test in vivo the efficacy of novel therapeutic strategies.

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