

UCSF

UC San Francisco Previously Published Works

Title

Identification of IRF8 as a potent tumor suppressor in murine acute promyelocytic leukemia

Permalink

<https://escholarship.org/uc/item/81p802dq>

Journal

Blood Advances, 2(19)

ISSN

2473-9529

Authors

Gaillard, Coline

Surianarayanan, Sangeetha

Bentley, Trevor

et al.

Publication Date

2018-10-09

DOI

10.1182/bloodadvances.2018018929

Peer reviewed

Identification of IRF8 as a potent tumor suppressor in murine acute promyelocytic leukemia

Coline Gaillard,¹ Sangeetha Surianarayanan,¹ Trevor Bentley,¹ Matthew R. Warr,² Briana Fitch,¹ Huimin Geng,¹ Emmanuelle Passegué,² Hugues de Thé,³ and Scott C. Kogan¹

¹Department of Laboratory Medicine and Helen Diller Family Comprehensive Cancer Center and ²Department of Medicine and Eli and Edythe Broad Center of Regeneration Medicine and Stem Cell Research, University of California, San Francisco, San Francisco, CA; and ³Institut Universitaire d'Hématologie, Université Paris Diderot, Unité mixte de recherche 944/7212, Paris, France

Key Points

- PML/RARA-mediated downregulation of *Irf8* accelerates APL initiation.
- *Irf8*^{-/-} mutants show expansion of promyelocyte compartment and PML/RARA *Irf8*^{-/-} mutants show reduced leukemia-free survival.

Although the role of promyelocytic leukemia/retinoic acid receptor α (PML/RARA) fusion protein is well recognized in acute promyelocytic leukemia (APL), its contribution to initiation and maintenance of leukemogenesis is not completely understood. Transcriptome analysis in the murine *MRP8-PML/RARA* APL model has demonstrated modest alterations in gene expression accompanied by expansion of the promyelocyte compartment. Of particular interest, mice expressing PML/RARA showed downregulation of the transcription factor *Irf8* mRNA. Interferon regulatory factor 8 (IRF8) is a known regulator of hematopoiesis. Previous research had implicated IRF8 as a tumor suppressor for myeloid neoplasia, and mice lacking IRF8 develop a well-differentiated myeloproliferative neoplasm characterized by expansion of neutrophilic lineage cells. We hypothesized that PML/RARA-mediated downregulation of *Irf8* transcript levels contributes to the initiation of APL. We observed significant downregulation of IRF8 protein levels in highly purified promyelocyte populations of PML/RARA transgenic mice. We also found that loss of IRF8 results in expansion of promyelocytes in vivo, partially phenocopying the impact of PML/RARA expression. Moreover, survival experiments showed that complete loss of IRF8 leads to acceleration of APL onset in our PML/RARA mice. Collectively, these data identify IRF8 downregulation as an important factor in APL initiation and highlight a tumor-suppressor role for IRF8 in this acute leukemia.

Introduction

Acute promyelocytic leukemia (APL) is marked by the accumulation of promyelocytes due to a blockade of differentiation coupled with unlimited expansion. Approximately 97% of patients with APL express the promyelocytic leukemia/retinoic acid receptor α (PML/RARA) fusion protein as a result of t(15;17) (q22;q11.2), and although the paradigm has long suggested that PML/RARA is a potent repressor of key myeloid maturation genes, we recently challenged this model,¹ reopening fundamental questions as to the precise contribution of the fusion protein to leukemic transformation.

IRF8 is a known regulator of hematopoiesis. IRF8 plays an important role in orchestrating specification and differentiation of B cells, dendritic cells, and monocytes.² Particularly in bipotential granulocyte/monocyte precursors, IRF8 expression is necessary to direct cells down the monocytic lineage.^{3,4} IRF8 is also a key mediator of innate immunity and will drive targeted transcriptional programs in response to interferon signaling, following binding to specific DNA elements and posttranslational modifications allowing association with partner proteins.^{5,6}

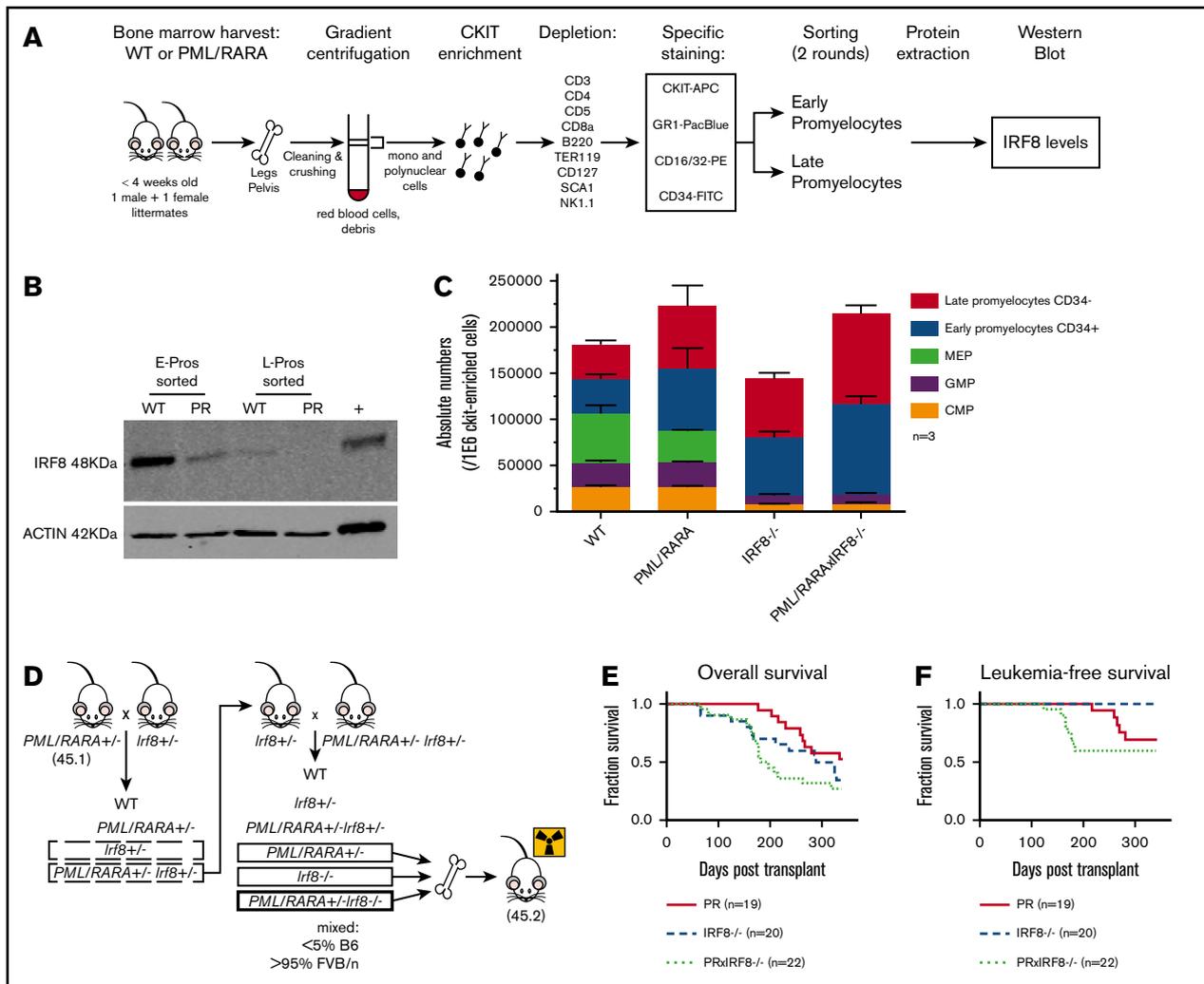


Figure 1. IRF8 is downregulated by PML/RARA in preleukemic promyelocytes and acts as a tumor suppressor in PML/RARA-driven APL. (A) Details of the experimental strategy used to investigate IRF8 protein levels in highly purified populations of sorted promyelocytes, in the presence or absence of PML/RARA. (B) Western blot analysis of IRF8 in sorted early and late promyelocytes of *PML/RARA* mice. Total spleen from WT animals was loaded as a positive control. IRF8 is detected at a size band of 48 kDa. Actin loading control is detected at a size band of 42 kDa. Images were obtained on LI-COR scanner. (C) Enumeration of c-Kit⁺ progenitor populations in the BM of *PML/RARA*, *Irf8*^{-/-}, and *PML/RARA Irf8*^{-/-} mice (n = 3 for each group). Error bar represents mean ± standard error of the mean. (D) Mating strategy used to investigate kinetics of APL initiation in *PML/RARA*, *Irf8*^{-/-}, and *PML/RARA Irf8*^{-/-} backgrounds. (E-F) Overall (E) and acute (F) leukemia-free survival of lethally irradiated recipients of BM from young *PML/RARA*, *Irf8*^{-/-}, and *PML/RARA Irf8*^{-/-} donor mice. Leukemia-free survival in the *PML/RARA Irf8*^{-/-} cohort was shorter than that of the *PML/RARA* cohort (P = .03, Gehan-Breslow-Wilcoxon test). APC, allophycocyanin; CMP, common myeloid progenitor; GMP, granulocyte/macrophage progenitor; FITC, fluorescein isothiocyanate; MEP, megakaryocyte-erythrocyte progenitor; PE, phycoerythrin; PR, PML/RARA.

We recently identified that *Irf8* is downregulated in murine PML/RARA preleukemic promyelocytes compared with their wild type (WT) counterparts.¹ Given the robustness of the *Irf8* downregulation that we observed in the absence of major alterations of other common myeloid transcription factors, we hypothesized that lower *Irf8* levels mediated by PML/RARA play a key role in the leukemogenic process. Further, we observed that *Irf8* is additionally downregulated in PML/RARA leukemic promyelocytes compared with their preleukemic counterparts,¹ suggesting that downregulation of *Irf8* levels could be important to maintain the leukemogenic program. Intrigued by these results, we investigated how IRF8 downregulation impacts the promyelocyte compartment in preleukemic mice as well as the kinetics of APL initiation in our *MRP8-PML/RARA* model. We show that young *Irf8*-null animals

show an expansion of promyelocytes that phenocopies the expansion seen in *PML/RARA* mice. We also show that acute leukemia onset is strongly accelerated in *PML/RARA Irf8*^{-/-} recipients compared with *PML/RARA* mice. These data support the hypothesis that downregulation of IRF8 by PML/RARA is a key mechanism in the initiation of APL.

Methods

Murine model, BM harvest, and transplantation experiments

The *MRP8-PML/RARA*⁷ transgenic and *Irf8*^{-/-8} genetically modified models were previously described. Transplantation experiments (Figure 1D-F) were performed as described in Jones et al,⁹

Table 1. Alterations of *Irf8*/IRF8 described in the context of non-PML/RARA and PML/RARA hematological neoplasms

Setting	Phenotype described	Cell type of the phenotype (and rate if specified)	Reference
Non-PML/RARA leukemias/neoplasms	Myeloproliferative syndrome	<i>Irf8</i> ^{+/-} and <i>Irf8</i> ^{-/-} animals	8
	<i>IRF8</i> strongly downregulated or absent	Human CML (79%) and AML (66%)	10
	<i>IRF8</i> downregulated	Therapy-related AML with -5/del(5q)	11
	<i>Irf8</i> expression impairs BCR/ABL-mediated leukemogenesis in vivo	BCR/ABL-transfected 32D cells in murine recipients (48.5% death vs 89.8% in control)	12
	<i>IRF8</i> expression impairs AML in mice	Human AML cell lines (OCI-AML3 and U937) transplanted in NSG mice	13
	Loss of <i>Irf8</i> cooperates for leukemogenesis	NUP98-TOP1 leukemia in murine recipients (retro virally-induced)	14
	<i>IRF8</i> promoter hypermethylated	Human MDS (20%), de novo AML (25%), secondary AML (28%)	15
	<i>IRF8</i> downregulated		
PML/RARA expression APL	<i>IRF8</i> downregulated	Human t(8;21) AML and APL	15
	<i>IRF8</i> as a target gene of PML/RARA	Human APL and cell line (NB4)	16
	<i>IRF8</i> increased upon ATRA treatment	Human APL and cell line (NB4)	17
	<i>Irf8</i> downregulated	Murine CSG-PML/RARA preleukemic CMPs and GMPs (vs WT)	18
	<i>Irf8</i> downregulated	Murine <i>MRP8-PML/RARA</i> preleukemic promyelocytes (vs WT)	1
	<i>Irf8</i> downregulated	Murine <i>MRP8-PML/RARA</i> leukemia (vs preleukemic cells)	
	<i>Irf8</i> downregulated	Murine <i>PML/RARA</i> APL (vs <i>BXH2</i> AML)	S.C.K. and James R. Downing, unpublished observations, 15 November 2007
<i>Irf8</i> downregulated	Human PML compared with other human AMLs	TCGA data; supplemental Figure 1	

AML, acute myeloid leukemia; CML, chronic myeloid leukemia; MDS, myelodysplastic syndrome; TCGA, The Cancer Genome Atlas.

and each cohort included recipients of 6 individual bone marrow (BM) donors.

Staining of promyelocytes and flow cytometry

As previously described we identified early promyelocytes (c-Kit⁺GR1^{int}CD16/32⁺CD34⁺; “E-Pro”) and late promyelocytes (c-Kit⁺GR1^{int}CD16/32⁺CD34⁻; “L-Pro”) as well as granulocyte/macrophage progenitors (c-Kit⁺GR1⁻CD16/32⁺CD34⁺; GMPs), common myeloid progenitors (c-Kit⁺GR1⁻CD16/32⁻CD34⁺), and megakaryocyte-erythrocyte progenitors (c-Kit⁺GR1⁻CD16/32⁻CD34⁻).¹

Detection of IRF8 (interferon consensus sequence-binding protein) by western blot

Following 2 rounds of sorting, protein lysates of highly purified cells (65 000), were electrophoresed, transferred to nitrocellulose, and examined with anti-IRF8 (39-8800, Invitrogen) and goat anti-mouse IRDye (926-32210, LI-COR Biosciences). Additional details are provided in supplemental Methods.

Results and discussion

The role of IRF8 as a possible myeloid leukemia tumor suppressor has been documented by several groups in various hematologic

contexts (Table 1¹⁰⁻¹⁸). A few groups, including ours, have suggested that alteration of *Irf8* occurs in the context of PML/RARA (Table 1, bottom), and human APLs have lower levels of *IRF8* expression in comparison with a number of other subtypes of acute myeloid leukemia (supplemental Figure 1). Our gene expression studies showed that PML/RARA expression leads to lower *Irf8* transcript levels in preleukemic promyelocytes (~4.8-fold compared with WT), with further loss in fully leukemic cells (~90-fold compared with preleukemic),¹ suggesting that downregulation of this transcription factor mediated by and in association with PML/RARA could participate in both the emergence and maintenance of a leukemic clone. To interrogate if lower transcript abundance in our mice would be reflected as lower protein levels, we used the experimental strategy described in Figure 1A to investigate IRF8 protein levels in our highly purified promyelocyte populations from *MRP8-PML/RARA* transgenic animals or their WT littermates.

We confirmed a strong downregulation of IRF8 in our PML/RARA early promyelocyte population, with a similar trend in late promyelocytes (Figure 1B). IRF8 is normally downregulated with neutrophilic maturation, and the presence of PML/RARA results in precocious loss during granulocyte development. To delineate the impact of this early loss of IRF8, we assessed promyelocyte numbers in young healthy *Irf8*^{-/-} and *PML/RARA Irf8*^{-/-} mice

in comparison with WT *Irf8*^{+/+} mice and *Irf8*^{+/+} mice expressing the *PML/RARA* transgene (Figure 1C). As previously observed, young *PML/RARA Irf8*^{+/+} mice had a substantially increased number of marrow promyelocytes in comparison with WT mice.¹ Fascinatingly, loss of *Irf8* alone resulted in an essentially identical expansion of promyelocytes (as well as a loss of earlier myeloid progenitors in the BM, not seen in *PML/RARA Irf8*^{+/+} mice), and a combination of *PML/RARA* expression and *IRF8* loss did not result in a statistically significant further expansion of promyelocytes. These results suggest an epistatic relationship between *PML/RARA* and *IRF8*, compatible with downregulation of *IRF8* by *PML/RARA* as being a key mechanism by which t(15;17) expands promyelocytes in the initiation of APL. The prior observation that *PML/RARA* binds near the *IRF8* gene in the human NB4 cell line and primary human APL cells¹⁶ suggests that *PML/RARA* is able to downregulate *IRF8* through direct transcriptional repression.

Given the concordance of our experimental results with prior studies of *IRF8* in myeloid neoplasia, we hypothesized that downregulated *Irf8* levels mediated by *PML/RARA* could play a critical role in disease initiation in our mouse model. If so, then further depletion of *Irf8* should cooperate in APL development. To test this hypothesis, we compared the development of leukemia in mice expressing *PML/RARA* and lacking *IRF8* in their BM with that seen in mice having only the single genetic changes. The experimental design is depicted in Figure 1D, and representative pathology of ill animals in these cohorts is presented in supplemental Figure 2. As discussed above, *Irf8*^{-/-} recipients develop a myeloproliferative neoplasm, requiring euthanasia of most animals within one year. These non-acute leukemia deaths are reflected as similar overall survival curves observed in the *Irf8*^{-/-} and *PML/RARA Irf8*^{-/-} cohorts (Figure 1E). When looking specifically at deaths due to acute leukemias, loss of *Irf8* strongly accelerated *PML/RARA*-mediated leukemia initiation (Figure 1F). In the *PML/RARA*-only cohort, the leukemias that developed arose at 215 to 279 days. In the *PML/RARA Irf8*^{-/-} cohort, the leukemias that developed arose at 122 to 182 days. It was notable that all the acute leukemias in the *PML/RARA Irf8*^{-/-} cohort occurred prior to the first appearance of acute leukemia in the *PML/RARA* cohorts. These data demonstrate that further amplifying the aberrant *PML/RARA*-mediated downregulation of *IRF8* cooperates in leukemic transformation. Nevertheless, even in the absence of *IRF8*, there is still substantial disease latency. One limitation of the present study is a lack of *in vivo* data herein on the effects of *IRF8* overexpression in APL cells. To bridge this gap, a recently published study demonstrated that overexpression of *IRF8* in leukemic cells

from our *MRP8-PML/RARA* mouse model⁷ inhibits transplantation of established leukemia.¹⁹

Our work complements existing data to indicate an important role for *IRF8* suppression in APL pathogenesis. Altogether, our data support a model of APL leukemogenesis in which the translocation of chromosomes 15 and 17 initiates leukemia development, in part by downregulating *IRF8*, and in which the resulting expansion of the promyelocyte compartment contributes to acquisition of additional cooperating events (eg, trisomy of chromosome 8,²⁰ mutation of *FLT3*²¹) that complete leukemic transformation.

Acknowledgments

The authors thank the UCSF Comprehensive Cancer Center Shared Resources in Mouse Pathology and Cell Analysis, the UCSF Flow Cytometry Core, Keiko Ozato for kindly providing authorization to use the *Irf4/Irf8* knockout model, and Damien Reynaud for helping to acquire this line.

This work was funded by the National Cancer Institute, National Institutes of Health (R01-CA95274).

Authorship

Contribution: C.G. cowrote the manuscript, designed and executed experiments, analyzed data, and prepared figures; S.S. designed and executed experiments, cowrote the manuscript, analyzed data, and prepared figures; T.B. and M.R.W. executed experiments and analyzed data; B.F. and H.G. analyzed data; E.P. and H.d.T. provided project direction; and S.C.K. cowrote the manuscript, provided project direction, designed experiments, and analyzed data.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

The current affiliation for C.G. is Genentech, Inc., South San Francisco, CA.

The current affiliation for T.B. is Allogene Therapeutics, South San Francisco, CA.

The current affiliation for M.R.W. is Gilead Sciences, Inc., Foster City, CA.

The current affiliation for E.P. is Columbia Stem Cell Initiative, Columbia University Medical Center, New York, NY.

Correspondence: Scott C. Kogan, Department of Laboratory Medicine and Helen Diller Family Comprehensive Cancer Center, 513 Parnassus Ave, Room S-561, Box 0451, University of California, San Francisco, San Francisco, CA 94143-0451; e-mail: scott.kogan@ucsf.edu.

References

1. Gaillard C, Tokuyasu TA, Rosen G, et al. Transcription and methylation analyses of preleukemic promyelocytes indicate a dual role for *PML/RARA* in leukemia initiation. *Haematologica*. 2015;100(8):1064-1075.
2. Wang H, Lee CH, Qi C, et al. *IRF8* regulates B-cell lineage specification, commitment, and differentiation. *Blood*. 2008;112(10):4028-4038.
3. Li L, Jin H, Xu J, Shi Y, Wen Z. *Irf8* regulates macrophage versus neutrophil fate during zebrafish primitive myelopoiesis. *Blood*. 2011;117(4):1359-1369.
4. Tamura T, Nagamura-Inoue T, Shmeltzer Z, Kuwata T, Ozato K. ICSBP directs bipotential myeloid progenitor cells to differentiate into mature macrophages. *Immunity*. 2000;13(2):155-165.
5. Kautz B, Kakar R, David E, Eklund EA. SHP1 protein-tyrosine phosphatase inhibits gp91PHOX and p67PHOX expression by inhibiting interaction of PU.1, IRF1, interferon consensus sequence-binding protein, and CREB-binding protein with homologous Cis elements in the *CYBB* and *NCF2* genes. *J Biol Chem*. 2001;276(41):37868-37878.

6. Kuwata T, Gongora C, Kanno Y, et al. Gamma interferon triggers interaction between ICSBP (IRF-8) and TEL, recruiting the histone deacetylase HDAC3 to the interferon-responsive element. *Mol Cell Biol.* 2002;22(21):7439-7448.
7. Brown D, Kogan S, Lagasse E, et al. A PMLRARAalpha transgene initiates murine acute promyelocytic leukemia. *Proc Natl Acad Sci USA.* 1997;94(6):2551-2556.
8. Holtschke T, Löhler J, Kanno Y, et al. Immunodeficiency and chronic myelogenous leukemia-like syndrome in mice with a targeted mutation of the ICSBP gene. *Cell.* 1996;87(2):307-317.
9. Jones L, Wei G, Sevcikova S, et al. Gain of MYC underlies recurrent trisomy of the MYC chromosome in acute promyelocytic leukemia. *J Exp Med.* 2010;207(12):2581-2594.
10. Schmidt M, Nagel S, Proba J, et al. Lack of interferon consensus sequence binding protein (ICSBP) transcripts in human myeloid leukemias. *Blood.* 1998;91(1):22-29.
11. Qian Z, Fernald AA, Godley LA, Larson RA, Le Beau MM. Expression profiling of CD34+ hematopoietic stem/progenitor cells reveals distinct subtypes of therapy-related acute myeloid leukemia. *Proc Natl Acad Sci USA.* 2002;99(23):14925-14930.
12. Burchert A, Cai D, Hofbauer LC, et al. Interferon consensus sequence binding protein (ICSBP; IRF-8) antagonizes BCR/ABL and down-regulates bcl-2. *Blood.* 2004;103(9):3480-3489.
13. Sharma A, Yun H, Jyotsana N, et al. Constitutive IRF8 expression inhibits AML by activation of repressed immune response signaling. *Leukemia.* 2015;29(1):157-168.
14. Gurevich RM, Rosten PM, Schwieger M, Stocking C, Humphries RK. Retroviral integration site analysis identifies ICSBP as a collaborating tumor suppressor gene in NUP98-TOP1-induced leukemia. *Exp Hematol.* 2006;34(9):1192-1201.
15. Otto N, Manukjan G, Göhring G, et al. ICSBP promoter methylation in myelodysplastic syndromes and acute myeloid leukaemia. *Leukemia.* 2011;25(7):1202-1207.
16. Martens JHA, Brinkman AB, Simmer F, et al. PML-RARAalpha/RXR alters the epigenetic landscape in acute promyelocytic leukemia. *Cancer Cell.* 2010;17(2):173-185.
17. Meani N, Minardi S, Licciulli S, et al. Molecular signature of retinoic acid treatment in acute promyelocytic leukemia. *Oncogene.* 2005;24(20):3358-3368.
18. Wartman LD, Welch JS, Uy GL, et al. Expression and function of PML-RARA in the hematopoietic progenitor cells of Ctsg-PML-RARA mice. *PLoS One.* 2012;7(10):e46529.
19. Liu X, Chen J, Yu S, et al. All-trans retinoic acid and arsenic trioxide fail to derepress the monocytic differentiation driver *Irf8* in acute promyelocytic leukemia cells. *Cell Death Dis.* 2017;8(5):e2782.
20. Le Beau MM, Bitts S, Davis EM, Kogan SC. Recurring chromosomal abnormalities in leukemia in PML-RARA transgenic mice parallel human acute promyelocytic leukemia. *Blood.* 2002;99(8):2985-2991.
21. Sohal J, Phan VT, Chan PV, et al. A model of APL with FLT3 mutation is responsive to retinoic acid and a receptor tyrosine kinase inhibitor, SU11657. *Blood.* 2003;101(8):3188-3197.