

REVIEW

Variations on a theme: the alternate translocations in APL

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The t(15;17)(q22;q21) translocation is tightly linked to the APL phenotype, and the resultant PML-RAR fusion can be demonstrated in 98% of APL cases. Rare variant translocations have been reported, the majority of which on detailed analysis represent cryptic PML-RAR fusions. However, a handful of APL cases have been described with different genotypes. These include the t(11;17)(q23;q21) that produces the PLZF-RAR fusion, t(5;17)(q35;q21) that forms NPM-RAR, t(11;17)(q13;q21) that generates NUMA-RAR, and der(17) that creates STAT5b-RAR. In this review we will discuss these variant translocations, and discuss the insights that we have gained from their study.

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Introduction

The vast majority of acute promyelocytic leukemia (APL) patients manifest the t(15;17)(q22;q12–23) translocation,¹ which results in expression of the PML-RAR fusion.^{2,3} Yet, there are a subset of patients with APL in which t(15;17) cannot be demonstrated, either on a cytogenetic or molecular level. These variants present themselves as 'experiments of nature' in which similar phenotypes are associated with different genotypes. They serve as potential tools to test hypotheses addressing the molecular basis for APL, and to identify common biologic pathways that truly underlie development of the APL phenotype.

Numerous cytogenetic variants have been reported in the literature, many of which have been shown to have compound or cryptic rearrangements of the PML and RARα (retinoic acid receptor alpha) loci. Indeed, the European Working Party found that the majority of cases without demonstrable t(15;17) have a PML-RAR rearrangement on molecular analysis.^{1,4} Cases without demonstrable PML-RAR rearrangement amounted to only 19 of the 611 cases compiled. These represent variants in which RARα was fused with a different locus (seven of the 611), leukemias lacking discernible RARα rearrangements (five of 611), and seven that were not fully characterized. None were found to have PML rearrangements without RARα, supporting the central role of the RARα in the pathogenesis of APL. In this review we will focus on the APL variants that have rearrangements of RARα (Table 1).

APL variant translocations

The first variant translocation in APL to be described, and the most intensively studied, is the t(11;17)(q23;q21). First

described by Chen *et al*⁵ in 1993, 16 cases of t(11;17) have been described in the world's literature.¹ The t(11;17) fuses the kruppel-like gene PLZF (promyelocytic leukemia zinc finger) with RARα.⁶ As in APL rearrangements with PML-RAR the breakpoint occurs within intron 2 of RARα,⁷ so that an in-frame fusion protein is expressed. Thus, both PML-RAR and PLZF-RAR fusion proteins contain the same B-F domains of RARα, which encode DNA binding, RXR heterodimerization, ligand binding, and co-repressor and co-activator interaction functions of RARα.⁸ A reciprocal RAR-PLZF fusion is also expressed.^{1,6,9} Though similar in phenotype to PML-RAR, t(11;17) leukemias fall into an unusual morphologic spectrum of APL,⁹ with features intermediate between M2 and M3. Clinically, the majority of t(11;17) patients fail to respond to ATRA, and indeed primary blasts of these patients do not differentiate when challenged with ATRA.⁹ Initial reports indicated that patients with t(11;17) carry a worse prognosis than t(15;17), with most failing to achieve remission with either conventional chemotherapy or ATRA differentiation therapy, though one case has been described in which treatment with ATRA plus G-CSF achieved remission status.¹⁰

The second variant chromosomal translocation to be characterized, and the second most plentiful, is the t(5;17)(q35;q21) translocation.¹¹ A total of four such patients have been identified. This variant translocates the nucleophosmin gene on 5q35 into the RARα locus on 17q21.¹² Again, the breakpoint occurs within the second intron of RARα, so that a fusion protein is expressed that encodes the B-F domains of RARα in the same reading frame as the N-terminal domains of nucleophosmin (NPM). Nucleophosmin is a nucleolar phosphoprotein that plays a role in ribosomal RNA assembly; it also has chaperoning activities, as well as nuclease activity. Recently it has been found to associate with centrosomes, and possibly play a role in regulation of centrosomal duplication.¹³ A reciprocal RAR-NPM fusion was described in three of the four cases. Though the original report found two NPM-RAR isoforms expressed, presumably as a result of splicing,¹² the three subsequent case reports indicated expression of only the shorter NPM-RAR transcript.^{1,14,15} Interestingly, NPM-RAR contains the same 118 N-terminal nucleophosmin sequences as is contained within the NPM-ALK fusion protein found in t(2;5) anaplastic lymphomas,¹⁶ in which nucleophosmin sequences are fused to the ALK kinase. Like the t(15;17) APL, the phenotype of t(5;17) is M3. There is compelling evidence to indicate that the t(5;17) blasts can respond *in vitro* to the differentiating effects of ATRA,¹⁷ and in the one evaluable case treated with ATRA, the patient attained a remission.¹⁴

One case of t(11;17)(q13;q21) has been published.¹⁸ This 6-month-old boy presented with leukocytosis and skin lesions. Blood smear, bone marrow, and skin biopsy specimens showed a predominance of promyelocytes and dysplastic maturing neutrophils. The patient was begun on ATRA, and achieved a complete remission. Southern blot analysis revealed

Table 1 Variant translocations

Translocation	Translocation partner	Product
t(11;17)(q23;q21)	PLZF (promyelocytic leukemia zinc-finger protein)	PLZF-RAR and RAR-PLZF
t(5;17)(q35;q21)	NPM (nucleophosmin)	NPM-RAR and RAR-NPM
t(11;17)(q13;q21)	NUMA (nuclear mitotic apparatus protein)	NUMA-RAR (no reciprocal product)
der(17)	Stat5b (signal transducer and activator of transcription)	Stat5b-RAR (no reciprocal product)

rearrangement of RARa within intron 2, the same breakpoint as in t(15;17) APL. Subsequent cloning of the breakpoint indicated that the fusion partner was NUMA, (nuclear mitotic apparatus protein). The fusion product was an in-frame NUMA-RAR protein. No reciprocal RAR-NUMA product was detected. The NUMA-RAR fusion contains the N-terminal 1883 amino-acids of NUMA, which encode domains responsible for NUMA nuclear reassembly, oligomerization and spindle association functionality. Linked to this are the same RARa C-terminal amino acids that encode the B-F domains expressed in the t(15;17), t(11;17), t(5;17) and der(17).

One case of der(17) has been reported.¹⁹ This was a 67-year-old man who was diagnosed with AML M1. A minority of marrow blasts showed morphologic evidence for the M3v microgranular variant of APL. Blasts did not respond to *in vitro* challenge with ATRA. Further chromosomal analysis revealed the der(17) to be the result of duplication of 17q21.3-q23.¹⁹ Rearrangement of the RARa locus was determined by Southern blotting. Cloning of the region indicated an insertion of the gene encoding the Stat5b member of the signal transducer and activator of transcription family, into the second intron of RARa, in the appropriate reading frame to produce a Stat5b-RARa fusion. Because of the nature of this interstitial duplication, no reciprocal product was produced. The predicted Stat5b-RARa fusion contains Stat5b sequences representing the N-terminal coiled-coil domain responsible for dimerization of Stat5b, the DNA binding and SH2 domains, and a portion of the SH3 domain. These sequences are fused to the same RARa sequences as in the other APL variants.

Several other potential APL variants have been reported in the literature that have rearrangements of 17q12–21, the locus for RARa. These include t(14;17)(q22;q21),²⁰ t(8;17)(p21;q21),²¹ t(1;17)(p36;q21),^{22,23} and t(7;17)(q36;q22).²³ Since the fusion partners have not been cloned it is possible that some or all may represent cryptic rearrangements of PML-RAR or the other APL variants. There are thus insufficient data to determine if these represent novel variants.

What have we learned

The PML-RAR fusion protein is a multifunctional protein that contains protein interaction domains of PML, dimerization domains of both PML and RARa, RARa DNA binding motifs, ligand binding domains, as well as transcriptional activating and repression domains of RARa. A variety of hypotheses have been proposed for the aberrant function of PML-RAR in APL, including its acting as a dominant negative for PML,²⁴ a dominant negative for RARa,^{3,25,26} or a rogue transcriptional activator or repressor.^{2,25} The study of the variant translocations holds promise for distinguishing between these competing hypotheses.

The importance of RARa

The obvious commonality of all the APL fusions is conservation of C-terminal RARa motifs. Indeed, all fusion proteins contain the exact same sequences of RARa, since all the translocations map to the second intron of the RARa gene. Interestingly, RARbeta and RARgamma translocations have not been reported, despite indications that all three RAR isoforms bind to the same consensus binding site and have similar biochemical function. This may purely be a result of the localization of a hot-spot for rearrangement in the sequences of the second intron of RARa, increasing the probability of RARa rearrangements.²⁷ RARa is not involved in translocations of other leukemias, though there has been one case report without molecular documentation suggesting that the RARa locus may have been the fusion partner to MLL in a novel 11q23 translocation with M5 phenotype.²⁸

All the known variants that have been studied bind the ligand ATRA. All bind to consensus retinoic acid response elements, though possibly with different sequence specificity.²⁹ All can interact with RXR, co-repressors and co-activators, though the roles of these interactions remain somewhat controversial (see below).

However, the European Working Party reported five cases of APL in which no rearrangement of RARa was detectable by Southern blotting, FISH, or RT-PCR.¹ Further detailed studies of these cases, including potential responses to ATRA, have not been reported. In a similar compilation of 284 APL cases from Shanghai,¹⁵ no such cases with unrearranged RARa were reported. The significance of these cases remains unclear. It is possible that they have undetected RARa mutations. It is interesting to speculate that they might have other genomic mutations that lead to alteration in the downstream pathways that are altered in the usual APL cases. Without further data, these cases remain a mystery, and potentially serve as evidence against the hypothesis that RARa rearrangements are fundamental to the biology of APL.

The role of co-repressors

Co-repressors mediate transcriptional repression through chromatin remodeling mechanisms.³⁰ PML-RAR binds to retinoic acid response elements (RAREs) and, like unliganded wild-type RARa, actively suppresses transcription by recruiting co-repressor complexes to the local environment of RAR-responsive promoters. Unlike RARa, PML-RAR fails to undergo the appropriate conformational changes that permit release of the co-repressors SMRT (silencing mediator of retinoid and thyroid hormones) or N-CoR (nuclear co-repressor) at physiological concentrations of ATRA.^{25,31,32} Rather, higher concentrations of ATRA are necessary for PML-RAR to release

the co-repressor complex, and bind co-activators to initiate transcription. This has led to the hypothesis that inappropriate repression of RA-target gene expression blocks myeloid differentiation to contribute to the APL phenotype; conversely, at high levels of retinoic acid, as achieved *in vivo* by pharmacologic treatment with ATRA, this transcriptional inhibition is released, to allow terminal differentiation of the APL blasts.

Study of the variant translocations has provided strong support for the hypothesis that these refractory interactions with co-repressors underlie the myeloid maturational arrest that characterizes APL, as well as the success of ATRA treatment for the retinoic acid responsive PML-RAR, NPM-RAR and NUMA-RAR variants. Like PML-RAR, both NPM-RAR^{33,34} and NUMA-RAR³⁵ have higher affinity for co-repressor molecules, and like PML-RAR, both activate transcription in the presence of high concentrations of ATRA. PLZF-RAR blasts fail to respond to even high concentrations of ATRA,⁹ as would be predicted by the observation that PLZF-RAR binds co-repressors through both ligand-dependent and ligand-independent interactions.²⁵ PLZF-RAR thus cannot fully release the co-repressor complex, and thereby fails to activate transcription of the appropriate target genes necessary for myeloid differentiation. Further supporting the model that co-repressor interaction is central to the maturational blockade of APL is the finding that PLZF-RAR blasts from transgenic mice respond to the combination of ATRA and a histone deacetylase inhibitor.²⁶ Such a combination would potentially ablate the activities of the PLZF-tethered co-repressor altogether.

Preliminary studies of the STAT5b-RAR fusion do not fully support this model, since STAT5b-RAR shows the same dependency on ligand concentration for interaction with co-repressors as PML-RAR.^{36,37} However, blasts from the index case were not sensitive to the differentiating effects of ATRA.¹⁹ Addressing this dilemma, Dong *et al*³⁶ have speculated that the STAT5b domains might bind an as yet unidentified co-repressor. It is also worth noting that the index case may have represented a chimera of two leukemic clones, with a sub-population being the microvariant APL clone:¹⁹ the apparent ATRA insensitivity that was reported may not be representative of the sub-population of APL blasts.

PML relocation

It has been hypothesized that PML-RAR induced disruption of wild-type PML protein interactions is critical for the APL phenotype.^{38,39} Wild-type PML associates with more than 20 proteins in nuclear structures known alternatively as PODS (for PML oncogenic domains) or NBs (nuclear bodies).⁴⁰ PML-RAR and PML form heterodimers, which serve to disrupt the nuclear bodies.³⁸ Indeed, in normal cells, PML can be found in 10–20 NBs throughout the nucleus; in cells expressing PML-RAR, immunofluorescence studies indicate that PML distributes into a microspiculated pattern, physically segregated from the other NB proteins. Upon treatment with ATRA, PML dissociates from PML-RAR, and reaggregates into the nuclear body structures. Since this occurs within 24–48 h after exposure to ATRA, before other signs of myeloid maturation are detectable, it has been suggested that relocation of PML into nuclear bodies is necessary for myeloid differentiation, and conversely that delocalization of PML from nuclear bodies underlies myeloid maturation arrest.³⁸

This scenario does not hold true for the variant translocations. In PLZF-RAR,¹ NPM-RAR,^{1,14,41} and NUMA-RAR blasts,¹⁸ PML is found within nuclear bodies. Treatment of

cells with ATRA at concentrations that induce differentiation do not alter PML localization in NPM-RAR expressing cells.⁴¹ Furthermore, pull-down experiments fail to indicate direct interaction between NPM-RAR and PML.⁴¹ Such studies have not been reported as yet with STAT5b-RAR fusions, but experiments to date with the other APL variants indicate that relocation of PML from nuclear bodies is not necessary for maturation blockade.

Interactions with RXR

RXR is the heterodimer partner for wild-type RAR α , and is necessary for RAR α binding to RARE.^{42–44} It also forms similar heterodimers with the VDR, TR and PPAR receptors, as well as acting as a ligand-responsive transcriptional activator itself. All of the APL fusion proteins are capable of interacting with RXR. Indeed, it has been formally demonstrated for PLZF-RAR,⁶ NPM-RAR,³³ NUMA-RAR,³⁵ and Stat5b-RAR,^{36,37} along with PML-RAR.^{2,3,39} Interaction with RXR has been shown to occur in solution phase as shown by co-precipitation experiments, as well as on target RARE oligonucleotides, as shown by electrophoretic mobility shift assay.

Immunofluorescence data have been published for PML-RAR only: PML-RAR and RXR co-localize in both APL and transfected cell lines.³⁹ In preliminary experiments, Redner *et al*⁴⁵ have shown that mutants of NPM-RAR that fail to bind RXR lose their ability to inhibit U937 cell differentiation. These observations lend credence to the hypothesis that the fusions might sequester RXR to act as dominant negatives for wild-type RAR, as well as other RXR-binding nuclear hormone receptors. Whether the fusions bind DNA as heterodimers with RXR remains controversial (see below).

Dimerization

Grigani *et al*⁴⁶ first indicated the importance of the PML dimerization motif for PML-RAR function. This led to the hypothesis that dimerization of fusion proteins was critical for function, and indeed it has been shown that an exogenous dimerization domain linked to RAR α sequences could reproduce PML-RAR-induced maturation arrest.^{47,48} Further supporting this hypothesis is the observation that all of the variant fusion proteins form homodimers by virtue of domains contributed by the N-terminal fusion partner. Whether the biologic function of the variant proteins depends upon dimerization remains controversial (see below).

What we are still learning

There is much that we do not know concerning the function of the APL fusion proteins. Unfortunately, study of the variants has not yet resolved many of the controversial hypotheses concerning the function of PML-RAR.

Do the fusion proteins bind to DNA as homodimers or heterodimers?

All the fusion proteins contain the appropriate motifs to form homodimers. All of the fusion proteins have the appropriate motifs to form heterodimers with RXR. All interact with RARE target sequences, and there is much evidence to support the

hypothesis that alteration of transcription of target genes is a major mechanism underlying the maturational blockade. What remains unclear is whether the fusions bind to DNA as homodimers or heterodimers. PML-RAR and PLZF-RAR preferentially bind as homodimers.^{29,49} Yet, for NPM-RAR,³³ NUMA-RAR,³⁵ and STAT5b-RAR,^{36,37} the RXR-heterodimeric complex seems to be more stable. Such a difference in homodimer vs heterodimer could alter target DNA sequence recognition.⁴⁹

What are the target genes?

Numerous studies have shown that all fusions are capable of binding to RAREs. In cells, however, it is not yet clear which target genes are most important for the APL phenotype. It has been shown that PML-RAR and PLZF-RAR may preferentially bind to different target sequences than wild-type RARa.^{29,50} In both EMSA and transcriptional activation assays NPM-RAR³³ and NUMA-RAR³⁵ also seem to have different target gene specificity from wild-type RARa. However, direct comparison of all the variants has not yet been performed, nor have gene array studies, which might potentially identify the all-important target genes themselves.

What is the role of ATRA-induced degradation of the fusion proteins?

ATRA induces degradation of PML-RAR,^{51,52} which may account for amelioration of its dominant-negative effects on PML or RARa. ATRA similarly leads to degradation of PLZF-RAR,³⁶ yet ATRA does not lead to differentiation of PLZF-RAR blasts. ATRA does not affect the stability of STAT5b-RAR.³⁶ Studies on the stability of NPM-RAR and NUMA-RAR have not yet been reported, but to date the significance of ATRA-induced degradation of the fusion protein remains unclear.

How applicable is arsenic to non-PML-RAR leukemias?

Arsenic has been used since the days of Aristotle for medicinal uses, both good and bad. With the pioneering studies of the group at Harbin China, it has re-emerged as a potential agent for relapsed APL. Arsenic trioxide produces partial differentiation and apoptosis of APL cells.^{52,53} It was originally proposed that arsenic brings about degradation of PML-RAR through increased binding of the proteosomal-targeting modifier SUMO to PML domains of the fusion. Consistent with this hypothesis is the finding of relative arsenic insensitivity of PLZF-RAR cells.⁵³ However, arsenic has many other effects on cells, including induction of apoptosis through PML-RAR independent mechanisms.⁵³ The sensitivities of NPM-RAR, NUMA-RAR and STAT5b-RAR to arsenic-induced degradation have not been determined. Therefore, the mechanism and potential applicability of arsenic remains unproven.

What is the role of the reciprocal translocation product?

It remains unclear whether there is a consistent role for the reciprocal translocation products in APL. The reciprocal product potentially contains the N-terminal transactivation

domain of RARa linked to C-terminal domains of the fusion partner. Transgenic animals expressing the RAR-PML have no phenotype of their own, but double RAR-PML/PML-RAR transgenics develop an APL phenotype with greater penetrance than PML-RAR single transgenics, suggesting a function for the RAR-PML protein.⁵⁴ RAR-PLZF links the transactivating domain of RARa to seven zinc fingers of the DNA binding domain of PLZF: not surprisingly, it functions as a transcriptional activator for PLZF-target genes.⁵⁵ Transgenic RAR-PLZF animals have abnormal myeloid development, and double RAR-PLZF/PLZF-RAR animals manifest a phenotype closer to human t(11;17) APL than PLZF-RAR single transgenics.⁵⁶ RAR-NPM has as yet no phenotype: RAR-NPM expressing U937 cells grow and differentiate normally.⁵⁷ RAR-NUMA was not detected in the report describing this translocation,¹⁸ and RAR-STAT5b is not created by the der17 rearrangement. Thus, the variant translocations have not yet elucidated a common role for the reciprocal fusion products.

Is there a set of proteins with which APL fusions interact?

PML-RAR has direct protein interactions with C/EBPalpha,⁵⁸ as well as Dnmt3.⁵⁹ Either or both of these interactions could have significant effects on myeloid differentiation: by altering function of a transcription factor necessary for myeloid development, or by altering DNA methylation of target promoters. Interactions have not been described between C/EBPalpha and Dnmt3 and the variant fusions as yet, and so the overall significance of these findings for the APL phenotype is still unclear.

PML-RAR and wild-type RARa interact with the AP-1 family of transcription factors to create transcriptional 'cross-talk'. Whereas RARa acts as an inhibitor of AP-1, PML-RAR acts as an activator.⁶⁰ However, preliminary experiments with NPM-RAR have failed to show a similar AP-1 enhancing activity.⁶¹ Analyses of the interactions of other variants with AP-1 have yet to be reported, leaving the question of the role of aberrant AP-1 signaling in the APL phenotype unanswered.

There is still much to be learned about the molecular mechanisms underlying APL. Fortunately, nature has provided us with reagents that have allowed us to gain insights into the biology of APL, and with further study, should allow us to identify the elusive common pathways that lead to the APL phenotype.

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References

- 1 Grimwade D, Biondi A, Mozziconacci MJ, Hagemeijer A, Berger R, Neat M, Howe K, Dastugue N, Jansen J, Radford-Weiss I, Lo Coco F, Lessard M, Hernandez JM, Delabesse E, Head D, Liso V, Sainy D, Flandrin G, Solomon E, Birg F, Lafage-Pochitaloff M. Characterization of acute promyelocytic leukemia cases lacking the classic t(15;17): results of the European Working Party. Groupe Français de Cytogénétique Hematologique, Groupe de Français

- d'Hematologie Cellulaire, UK Cancer Cytogenetics Group and BIOMED 1 European Community-Concerted Action 'Molecular Cytogenetic Diagnosis in Haematological Malignancies'. *Blood* 2000; **96**: 1297–1308.
- 2 de Thé H, Lavau C, Marchio A, Chomienne C, Degos L, Dejean A. The PML-RAR alpha fusion mRNA generated by the t(15;17) translocation in acute promyelocytic leukemia encodes a functionally altered RAR. *Cell* 1991; **66**: 675–684.
 - 3 Kakizuka A, Miller WJ, Umesono K, Warrell RJ, Frankel SR, Murty VV, Dmitrovsky E, Evans RM. Chromosomal translocation t(15;17) in human acute promyelocytic leukemia fuses RAR alpha with a novel putative transcription factor, PML. *Cell* 1991; **66**: 663–674.
 - 4 Grimwade D, Gorman P, Duprez E, Howe K, Langabeer S, Oliver F, Walker H, Culligan D, Waters J, Pomfret M, Goldstone A, Burnett A, Freemont P, Sheer D, Solomon E. Characterization of cryptic rearrangements and variant translocations in acute promyelocytic leukemia. *Blood* 1997; **90**: 4876–4885.
 - 5 Chen Z, Brand NJ, Chen A, Chen SJ, Tong JH, Wang ZY, Waxman S, Zelent A. Fusion between a novel Kruppel-like zinc finger gene and the retinoic acid receptor-alpha locus due to a variant t(11;17) translocation associated with acute promyelocytic leukaemia. *Embo J* 1993; **12**: 1161–1167.
 - 6 Chen Z, Guidez F, Rousselot P, Agadir A, Chen SJ, Wang ZY, Degos L, Zelent A, Waxman S, Chomienne C. PLZF-RAR alpha fusion proteins generated from the variant t(11;17)(q23;q21) translocation in acute promyelocytic leukemia inhibit ligand-dependent transactivation of wild-type retinoic acid receptors. *Proc Natl Acad Sci USA* 1994; **91**: 1178–1182.
 - 7 de Thé H, Chomienne C, Lanotte M, Degos L, Dejean A. The t(15;17) translocation of acute promyelocytic leukaemia fuses the retinoic acid receptor alpha gene to a novel transcribed locus. *Nature* 1990; **347**: 558–561.
 - 8 Evans R. The steroid and thyroid hormone receptor superfamily. *Nature* 1988; **240**: 889–895.
 - 9 Licht JD, Chomienne C, Goy A, Chen A, Scott AA, Head DR, Michaux JL, Wu Y, DeBlasio A, Miller WH Jr. Clinical and molecular characterization of a rare syndrome of acute promyelocytic leukemia associated with translocation (11;17). *Blood* 1995; **85**: 1083–1094.
 - 10 Jansen JH, Lowenberg B. Acute promyelocytic leukemia with a PLZF-RARalpha fusion protein. *Semin Hematol* 2001; **38**: 37–41.
 - 11 Corey SJ, Locker J, Oliveri DR, Shekhter-Levin S, Redner RL, Panchansky L, Gollin SM. A non-classical translocation involving 17q12 (retinoic acid receptor alpha) in acute promyelocytic leukemia with atypical features. *Leukemia* 1994; **8**: 1350–1353.
 - 12 Redner RL, Rush EA, Faas S, Rudert WA, Corey SJ. The t(5;17) variant of acute promyelocytic leukemia expresses a nucleophosmin-retinoic acid receptor fusion. *Blood* 1996; **87**: 882–886.
 - 13 Okuda M, Horn HF, Tarapore P, Tokuyama Y, Smulian AG, Chan PK, Knudsen ES, Hofmann IA, Snyder JD, Bove KE, Fukasawa K. Nucleophosmin/B23 is a target of CDK2/cyclin E in centrosome duplication. *Cell* 2000; **103**: 127–140.
 - 14 Hummel JL, Wells RA, Dube ID, Licht JD, Kamel-Reid S. Deregulation of NPM and PLZF in a variant t(5;17) case of acute promyelocytic leukemia. *Oncogene* 1999; **18**: 633–641.
 - 15 Xu L, Zhao WL, Xiong SM, Su XY, Zhao M, Wang C, Gao YR, Niu C, Cao Q, Gu BW, Zhu YM, Gu J, Hu J, Yan H, Shen ZX, Chen Z, Chen SJ. Molecular cytogenetic characterization and clinical relevance of additional, complex and/or variant chromosome abnormalities in acute promyelocytic leukemia. *Leukemia* 2001; **15**: 1359–1368.
 - 16 Morris SW, Kirstein MN, Valentine MB, Dittmer KG, Shapiro DN, Saltman DL, Look AT. Fusion of a kinase gene, ALK, to a nuclear protein gene, NPM, in non-Hodgkin's lymphoma. *Science* 1994; **263**: 1281–1284.
 - 17 Redner RL, Corey SJ, Rush EA. Differentiation of t(5;17) variant acute promyelocytic leukemic blasts by all-trans retinoic acid. *Leukemia* 1997; **11**: 1014–1016.
 - 18 Wells RA, Catzavelos C, Kamel-Reid S. Fusion of retinoic acid receptor alpha to NuMA, the nuclear mitotic apparatus protein, by a variant translocation in acute promyelocytic leukaemia. *Nat Genet* 1997; **17**: 109–113.
 - 19 Arnould C, Philippe C, Bourdon V, Gregoire MJ, Berger R, Jonveaux P. The signal transducer and activator of transcription STAT5b gene is a new partner of retinoic acid receptor alpha in acute promyelocytic-like leukaemia. *Hum Mol Genet* 1999; **8**: 1741–1749.
 - 20 Cigudosa JC, Calasanz MJ, Otero MD, Marin J, Bengoechea E, Gullon A. A variant t(14;17) in acute promyelocytic leukemia. Positive response to retinoic acid treatment. *Cancer Genet Cytogenet* 1995; **80**: 160–161.
 - 21 Miura I, Nishinari T, Hashimoto K, Nimura T, Miura S, Miura AB. Translocation (8;17)(p21;q21), a possible variant of t(15;17), in acute promyelocytic leukemia. *Cancer Genet Cytogenet* 1994; **72**: 75–77.
 - 22 Schwartz JG, Clare N, Hansen K, Britton H, Manhoff L. Acute promyelocytic leukemia: report of a variant translocation, t(1;17). *Cancer Genet Cytogenet* 1986; **20**: 89–93.
 - 23 Yamada K, Sugimoto E, Amano M, Imamura Y, Kubota T, Matsumoto M. Two cases of acute promyelocytic leukemia with variant translocations: the importance of chromosome No. 17 abnormality. *Cancer Genet Cytogenet* 1983; **9**: 93–99.
 - 24 Salomoni P, Pandolfi PP. The role of PML in tumor suppression. *Cell* 2002; **108**: 165–170.
 - 25 Guidez F, Ivins S, Zhu J, Soderstrom M, Waxman S, Zelent A. Reduced retinoic acid-sensitivities of nuclear receptor corepressor binding to PML- and PLZF-RARalpha underlie molecular pathogenesis and treatment of acute promyelocytic leukemia. *Blood* 1998; **91**: 2634–2642.
 - 26 He LZ, Guidez F, Tribioli C, Peruzzi D, Ruthardt M, Zelent A, Pandolfi PP. Distinct interactions of PML-RAR-alpha and PLZF-RAR-alpha with co-repressors determine differential responses to RA in APL. *Nature Genet* 1998; **18**: 126–135.
 - 27 Chen SJ, Zhu YJ, Tong JH, Dong S, Huang W, Chen Y, Xiang WM, Zhang L, Li XS, Qian GQ, Wang GD, Chen Z, Larsen CJ, Berger R. Rearrangements in the second intron of the RARA gene are present in a large majority of patients with acute promyelocytic leukemia and are used as molecular marker for retinoic acid-induced leukemic cell differentiation. *Blood* 1991; **78**: 2696–2701.
 - 28 Shekhter-Levin S, Gollin SM, Kaplan SS, Redner RL. Involvement of the MLL and RARalpha genes in a patient with acute monocytic leukemia with t(11;17)(q23;q12). *Leukemia* 2000; **14**: 520–522.
 - 29 Hauksdottir H, Privalsky ML. DNA recognition by the aberrant retinoic acid receptors implicated in human acute promyelocytic leukemia. *Cell Growth Differ* 2001; **12**: 85–98.
 - 30 Redner RL, Wang J, Liu JM. Chromatin remodeling and leukemia: new therapeutic paradigms. *Blood* 1999; **94**: 417–428.
 - 31 Grignani F, Dematteis S, Nervi C, Tomassoni L, Gelmetti V, Cioce M, Fanelli M, Ruthardt M, Ferrara FF, Zamir I, Seiser C, Grignani F, Lazar MA, Minucci S, Pelicci PG. Fusion proteins of the retinoic acid receptor-alpha recruit histone deacetylase in promyelocytic leukaemia. *Nature* 1998; **391**: 815–818.
 - 32 Lin RJ, Nagy L, Inoue S, Shao WL, Miller WH, Evans RM. Role of the histone deacetylase complex in acute promyelocytic leukaemia. *Nature* 1998; **391**: 811–814.
 - 33 Redner RL, Chen JD, Rush EA, Li H, Pollock SL. The t(5;17) acute promyelocytic leukemia fusion protein NPM-RAR interacts with co-repressor and co-activator proteins and exhibits both positive and negative transcriptional properties. *Blood* 2000; **95**: 2683–2690.
 - 34 So CW, Dong S, So CK, Cheng GX, Huang QH, Chen SJ, Chan LC. The impact of differential binding of wild-type RARalpha, PML-, PLZF- and NPM-RARalpha fusion proteins towards transcriptional co-activator, RIP-140, on retinoic acid responses in acute promyelocytic leukemia. *Leukemia* 2000; **14**: 77–83.
 - 35 Dong S, Qiu J, Patel K, Brinkley WR, Mancini MA, Twardy DA. The coiled-coil domain of NuMA-RAR alpha is essential for its oncogenic activities and localization to NuMA sites within the nucleus. *Blood* 2001; **98**: 559a.
 - 36 Dong S, Twardy DJ. Interactions of STAT5b-RARalpha, a novel acute promyelocytic leukemia fusion protein, with retinoic acid receptor and STAT3 signaling pathways. *Blood* 2002; **99**: 2637–2646.
 - 37 Maurer AB, Wichmann C, Gross A, Kunkel H, Heinzel T, Ruthardt M, Groner B, Grez M. The Stat5-RARalpha fusion protein represses transcription and differentiation through interaction with a corepressor complex. *Blood* 2002; **99**: 2647–2652.
 - 38 Dyck JA, Gerd GG, Miller WH, Chen JD, Kakizuka A, Evans RM.

- A novel macromolecular structure is a target of the promyelocytic retinoic acid receptor oncoprotein. *Cell* 1994; **76**: 333–343.
- 39 Weis K, Rambaud S, Lavau C, Jansen J, Carvalho T, Carmo-Fonseca M, Lammond A, Dejean A. Retinoic acid regulates aberrant nuclear localization of PML-RAR α in acute promyelocytic leukemia cells. *Cell* 1994; **76**: 345–356.
 - 40 Negorev D, Maul GG. Cellular proteins localized at and interacting within ND10/PML nuclear bodies/PODs suggest functions of a nuclear depot. *Oncogene* 2001; **20**: 7234–7242.
 - 41 Redner RL, Schlesinger KW, Pollock SL, Watkins SC. The t(5;17) APL fusion protein NPM-RAR does not alter PML localization. *Blood* 1997; **90** (Suppl.): 321a.
 - 42 Leid M, Kastner P, Lyons R, Nakshatri H, Saunders M, Zacharewski T, Chen JY, Staub A, Garnier JM, Mader S, Chambon P. Purification, cloning, and RXR identity of the HeLa cell factor with which RAR or TR heterodimerizes to bind target sequences efficiently. *Cell* 1992; **68**: 377–395.
 - 43 Mader S, Chen JY, Chen Z, White J, Chambon P, Gronemeyer H. The patterns of binding of RAR, RXR and TR homo- and heterodimers to direct repeats are dictated by the binding specificities of the DNA binding domains. *EMBO J* 1993; **12**: 5029–5041.
 - 44 Yu VC, Delsert C, Andersen B, Holloway JM, Devary OV, Naar AM, Kim SY, Boutin JM, Glass CK, Rosenfeld MG. RXR β : a coregulator that enhances binding of retinoic acid, thyroid hormone, and vitamin D receptors to their cognate response elements. *Cell* 1991; **67**: 1251–1266.
 - 45 Redner RL, Rush EA, Pollack SA. RXR binding is essential for NPM-RAR inhibition of U937 differentiation. *Blood* 1998; **92** (Suppl. 1): 213a.
 - 46 Grignani F, Testa U, Rogaia D, Ferrucci PF, Samoggia P, Pinto A, Aldinucci D, Gelmetti V, Fagioli M, Alcalay M, Seeler J, Grignani F, Nicoletti I, Peschle C, Pelicci PG. Effects on differentiation by the promyelocytic leukemia PML/RAR α protein depend on the fusion of the PML protein dimerization and RAR α DNA binding domains. *EMBO J* 1996; **15**: 4949–4958.
 - 47 Grignani F, Gelmetti V, Fanelli M, Rogaia D, De Matteis S, Ferrara FF, Bonci D, Nervi C, Pelicci PG. Formation of PML/RAR α high molecular weight nuclear complexes through the PML coiled-coil region is essential for the PML/RAR α -mediated retinoic acid response. *Oncogene* 1999; **18**: 6313–6321.
 - 48 Lin RJ, Evans RM. Acquisition of oncogenic potential by RAR chimeras in acute promyelocytic leukemia through formation of homodimers. *Mol Cell* 2000; **5**: 821–830.
 - 49 Perez A, Kastner P, Sethi S, Lutz Y, Reibel C, Chambon P. PML/RAR homodimers: distinct DNA binding properties and heterodimeric interactions with RXR. *EMBO J* 1993; **12**: 3171–3182.
 - 50 Licht JD, Shaknovich R, English MA, Melnick A, Li JY, Reddy JC, Dong S, Chen SJ, Zelent A, Waxman S. Reduced and altered DNA-binding and transcriptional properties of the PLZF-retinoic acid receptor- α chimera generated in t(11;17)-associated acute promyelocytic leukemia. *Oncogene* 1996; **12**: 323–336.
 - 51 Warrell RJ, Frankel SR, Miller WJ, Scheinberg DA, Itri LM, Hittelman WN, Vyas R, Andreeff M, Tafuri A, Jakubowski A, Gabrilove J, Gordon MS, Dmitrovsky E. Differentiation therapy of acute promyelocytic leukemia with tretinoin (all-*trans*-retinoic acid). *New Engl J Med* 1991; **324**: 1385–1393.
 - 52 Zhu J, Lallemand-Breitenbach V, de Thé H. Pathways of retinoic acid- or arsenic trioxide-induced PML/RAR α catabolism, role of oncogene degradation in disease remission. *Oncogene* 2001; **20**: 7257–7265.
 - 53 Zhang TD, Chen GQ, Wang ZG, Wang ZY, Chen SJ, Chen Z. Arsenic trioxide, a therapeutic agent for APL. *Oncogene* 2001; **20**: 7146–7153.
 - 54 Pollock JL, Westervelt P, Kurichety AK, Pelicci PG, Grisolan JL, Ley TJ. A bcr-3 isoform of RAR α -PML potentiates the development of PML-RAR α -driven acute promyelocytic leukemia. *Proc Natl Acad Sci USA* 1999; **96**: 15103–15108.
 - 55 Sitterlin D, Tiollais P, Transy C. The RAR α -PLZF chimera associated with acute promyelocytic leukemia has retained a sequence-specific DNA-binding domain. *Oncogene* 1997; **14**: 1067–1074.
 - 56 He LZ, Bhaumik M, Tribioli C, Rego EM, Ivins S, Zelent A, Pandolfi PP. Two critical hits for promyelocytic leukemia. *Mol Cell* 2000; **6**: 1131–1141.
 - 57 Redner RL, Rush EA, Wang AS. Divergent roles for the NPM-RAR and RAR-NPM t(5;17) APL fusion proteins in inhibition of myeloid differentiation. *Blood* 2001; **98** (Suppl. 2): 154b.
 - 58 Tenen DG. Abnormalities of the CEBP α transcription factor: a major target in acute myeloid leukemia. *Leukemia* 2001; **15**: 688–689.
 - 59 Di Croce L, Raker VA, Corsaro M, Fazi F, Fanelli M, Faretta M, Fuks F, Lo Coco F, Kouzarides T, Nervi C, Minucci S, Pelicci PG. Methyltransferase recruitment and DNA hypermethylation of target promoters by an oncogenic transcription factor. *Science* 2002; **295**: 1079–1082.
 - 60 Doucas V, Brockes JP, Yaniv M, de Thé H, Dejean A. The PML-retinoic acid receptor α translocation converts the receptor from an inhibitor to a retinoic acid-dependent activator of transcription factor AP-1. *Proc Natl Acad Sci USA* 1993; **90**: 9345–9349.
 - 61 Fakhri MG, Redner RL. Acute promyelocytic leukemia t(5;17) fusion product NPM-RAR down-regulates AP-1 activity in the presence of ATRA. *Proc AACR* 2001.