Int J Hematol (2013) 97:717–725
DOI 10.1007/s12185-013-1354-4

Mechanisms of action and resistance to all-*trans* retinoic acid (ATRA) and arsenic trioxide (As_2O_3) in acute promyelocytic leukemia

Akihiro Tomita · Hitoshi Kiyoi · Tomoki Naoe

Received: 11 April 2013 / Revised: 26 April 2013 / Accepted: 1 May 2013 / Published online: 14 May 2013

Abstract Since the introduction of all-*trans* retinoic acid (ATRA) and arsenic trioxide (As_2O_3) for the treatment of acute promyelocytic leukemia (APL), the overall survival rate has improved dramatically. However, relapse/refractory patients showing resistance to ATRA and/or As_2O_3 are recognized as a clinically significant problem. Genetic mutations resulting in amino acid substitution in the retinoic acid receptor alpha (RARα) ligand binding domain (LBD) and the PML-B2 domain of PML-RARα, respectively, have been reported as molecular mechanisms underlying resistance to ATRA and As_2O_3. In the LBD mutation, ATRA binding with LBD is generally impaired, and ligand-dependent co-repressor dissociation and degradation of PML-RARα by the proteasome pathway, leading to cell differentiation, are inhibited. The PML-B2 mutation interferes with the direct binding of As_2O_3 with PML-B2, and PML-RARα SUMOylation with As_2O_3 followed by multimerization and degradation is impaired. To overcome ATRA resistance, utilization of As_2O_3 provides a preferable outcome, and recently, a synthetic retinoid Am80, which has a higher binding affinity with PML-RARα than ATRA, has been tested in the clinical setting. However, no strategy attempted to date has been successful in overcoming As_2O_3 resistance. Detailed genomic analyses using patient samples harvested repeatedly may help in predicting the prognosis, selecting the effective targeting drugs, and designing new sophisticated strategies for the treatment of APL.

Keywords APL · PML-RARα · ATRA · Arsenic trioxide (As_2O_3) · Drug resistance

A. Tomita (✉) · H. Kiyoi · T. Naoe
Department of Hematology and Oncology, Nagoya University Graduate School of Medicine, Tsurumai-cho 65, Showa-ku, Nagoya 466-8550, Japan
e-mail: atomita@med.nagoya-u.ac.jp

T. Naoe
Clinical Research Center, National Hospital Organization Nagoya Medical Center, Nagoya, Japan

Introduction

Almost two decades ago, the prognosis of acute promyelocytic leukemia (APL) was critically poor due to fatal coagulation disorders at diagnosis [1, 2]. Even with conventional chemotherapy using anthracyclines, more than 70 % of APL patients showed poor prognosis [3, 4]. After introduction of all-*trans* retinoic acid (ATRA) in the clinical setting in combination with conventional chemotherapy, the prognosis of APL has improved dramatically, with the result that more than 85 % of patients now achieve complete remission (CR) and nearly 70 % of patients can be cured [5–8]. Since 1994, the marked effectiveness of As_2O_3 in APL patients, even in relapsed patients after combination therapy with ATRA, has been confirmed [9–12]. When As_2O_3 is utilized as a single agent, ~70 % of patients can be cured, whereas nearly 90 % of patients can be cured if As_2O_3 is utilized in combination with ATRA [13, 14]. Although outcomes of APL treatment with ATRA and/or As_2O_3 in combination with conventional chemodrugs have improved, relapsed/refractory patients are still observed in the clinical setting and drug resistance to ATRA and As_2O_3 has been recognized as a critical problem.

More than 98 % of APL patients carry the t(15;17) translocation, which results in fusions of the retinoic acid

receptor alpha (RARα) gene with the promyelocytic leukemia (PML) gene, *PML-RARα* (Fig. 1) [15–17]. A very limited number of patients, showing APL phenotype without t(15;17), exhibit a variety of X-RARα fusions (Fig. 1) [18–25]. Interestingly, some patients expressing X-RARα show clinical resistance to ATRA and/or As_2O_3. Previous reports have indicated that both ATRA [26, 27] and As_2O_3 [28–30] have rigorously defined molecular targets, an improved understanding of their molecular mechanisms of action and resistance may be important to further improving clinical outcomes in APL treatment.

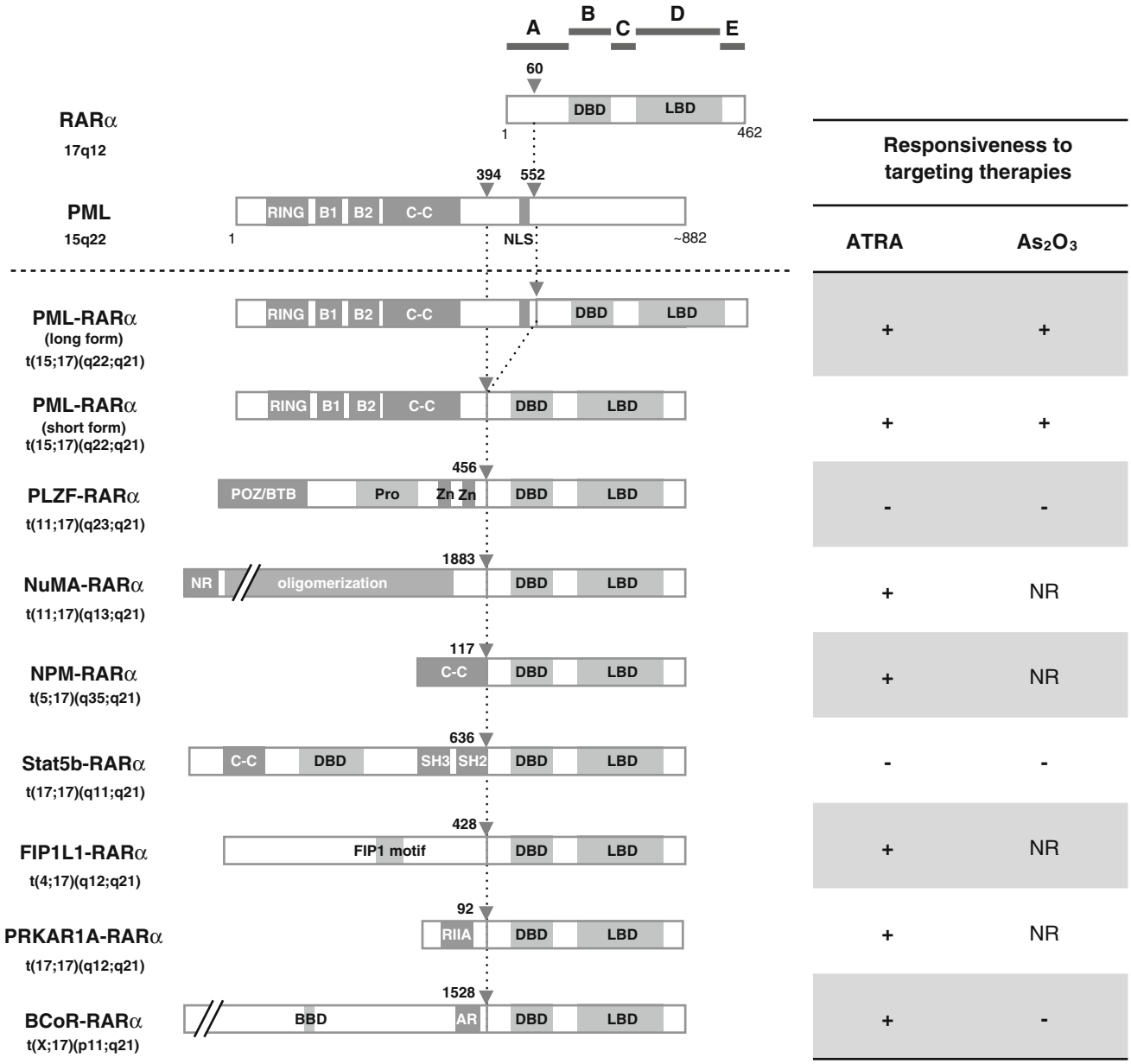

Fig. 1 Schematic representation of PML-RARα and X-RARα fusion protein confirmed in APL. Chromosomal translocations resulting in the fusion protein are also indicated under the name of fusion protein. Long and short forms of PML-RARα with or without nuclear localizing signal (NLS) are reported [86]. ATRA and As_2O_3 responsiveness in the clinical setting and/or in vitro analyses is indicated in the *right panel*. *Gray triangles* indicate break points of chimeric protein. *Numbers* indicate the amino acid positions. *A* to *E* functional domains in RARα, *DBD* DNA binding domain, *LBD* ligand binding domain, *RING* really interesting new gene finger domain, *B1 and B2* B-box motifs, *C–C* coiled-coil domain, *POZ/BTB* pox virus and zinc finger/BR–C, ttk and bab domain, *Pro* proline rich domain, *Zn* zinc finger domain, *NR* nuclear reassembly, *RIIA* dimerization domain, *BBD* BCL6-binding domain, *AR* ankyrin repeats, + sensitive, − resistant, *NR* not reported

Mechanisms of action of molecular targeting drugs to APL cells

ATRA

Wild-type RARα is a nuclear hormone receptor that binds to consensus sequence DR5 (five bases spaced between two AGGTCA motifs) in target gene promoters, normally as heterodimer with retinoid X receptor (RXR) [31–33]. Without ligands, ATRA and 9-*cis* retinoic acid, RAR-RXR heterodimer induces transcription repression throughout chromatin remodeling by recruiting transcription co-repressors, such as N-CoR/SMRT large protein complexes, that contain histone deacetylases (HDACs) [27, 34–37] and histone methyltransferases [38–40]. In the presence of ligand ($\sim 10^{-7}$ M), the co-repressor complexes dissociate from RAR-RXR, and transcriptional de-repression and activation are induced [34–37, 41]. PML-RARα binds to DR5 of target gene promoters primarily as a homodimer, but also as a heterodimer with RXR [42, 43], and induces transcription repression by recruiting N-CoR/SMRT complexes and polycomb group repressive complex 1 and 2 (PRC1/2) [39, 40], which contain histone methyl transferases, in the absence of ligands [27] (Fig. 1). PML-RARα can be SUMOylated at K160 of the PML protein to recruit death domain-associated protein (DAXX), resulting in the transcriptional repression of target genes [44]. Even in the presence of physiological concentration of ligand (10^{-7} M), the co-repressor complex still binds with PML-RARα and the transcriptional repression cannot be dissolved. In the presence of pharmacological concentration of ATRA (10^{-6} M), transcription activation can be induced by dissociation of co-repressor complexes from PML-RARα and proteasome-dependent PML-RARα degradation [45–47].

As_2O_3

The efficacy of As_2O_3 on APL cells was first reported by Chen et al. in 1996 [28], who showed the dual effect of apoptosis at relatively high concentrations (0.5–2 μM/L) and partial differentiation at low concentrations (0.1–0.5 μM/L) in both ATRA-responsive and ATRA-resistant APL cells. As_2O_3 induces the targeting of nucleoplasmic PML-RARα with a micro speckled pattern into nuclear bodies with a normal speckled pattern prior to degradation [30, 48–50]. As_2O_3 induces the formation of reactive oxygen species (ROS) [30], which induce multimerization of PML-RARα through intermolecular disulphide crosslinks at PML B1-domain (Fig. 2) and PML-RARα SUMOylation by ubiquitin-conjugating enzyme 9 (UBC9) [30]. A recent report indicated that As_2O_3 directly binds with PML at the C–C motif in the PML B2-domain, and that PML SUMOylation can be induced by enhancement of UBC9 binding at the PML RING domain [29, 30, 50]. SUMOylated PML recruits RING finger protein 4 (RNF4), which is known as a SUMO-dependent ubiquitin ligase [51], and polyubiquitylated PML-RARα can be degraded by ubiquitin–proteasome pathway [29, 49, 51].

Molecular mechanisms of drug resistance in APL cells

From the molecular mechanisms of ATRA and As_2O_3 effectiveness as indicated above, several mechanisms of drug resistance have been speculated [52]. In this section, we outline the molecular mechanisms of resistance that are thought to be significant from the clinical perspective.

RARα fusion proteins in APL

In very limited cases with APL phenotype, *RARα* translocations with *X*-genes other than *PML* (*PLZF* [18], *NuMA* [19], *NPM* [20], *STAT5b* [21, 53], *FIP1L1* [22], *PRKAR1A* [23, 24], and *BCOR* [25]) resulting in the production of X-RARα fusion protein have been reported (Fig. 1). PML-RARα forms mainly homodimers, and it has been reported that homodimerization of PML-RARα is critical for the pathogenesis of APL [42, 43]. Sternsdorf et al. [54] indicated that forced homodimerization of RARα induces ALP-like leukemia in a mouse model, indicating that the dimerization domain of the fusion protein may be critical to the induction of leukemogenesis by X-RARα. In fact, homodimerization through specific domains (coiled-coil; PML-, NPM-, and STAT5b-, POZ/BTB; PLZF-, RIIA; PRKAR1A-, and so on) has been confirmed in all X-RARα proteins. Interestingly, in PML-, PRKAR1A- [24], and BCOR-RARα [25], heterodimerization with RXR is also important for transformation and/or RARE binding.

Since those chimeric proteins all hold RARα DNA binding domain (DBD) and ligand binding domain (LBD), ATRA responsiveness is speculated in all cases. However, ATRA resistance has been confirmed clinically in cases showing *PLZF-RARα* [18, 34, 41] and *STAT5b-RARα* [21, 53, 55] fusions. One explanation for ATRA resistance is that the N-CoR/SMRT-corepressor complex interacts with PLZF, even in the presence of pharmacological concentration of ATRA, such that transcriptional de-repression cannot occur at RARα target gene promoters [34, 41]. The molecular mechanisms of ATRA resistance in STAT5b-RARα-expressing cells has not been fully explicated. Wild-type Stat5b is localized in cytoplasm, but STAT5b-RARα aberrantly localizes in nucleus [21]. STAT5b is a component of the janus kinase (JAK)-STAT signaling pathway, and phosphorylation of STAT5b by JAK causes homodimerization and translocation into the nucleus, where it acts as a transcription factor [56]. Aberrant transcription

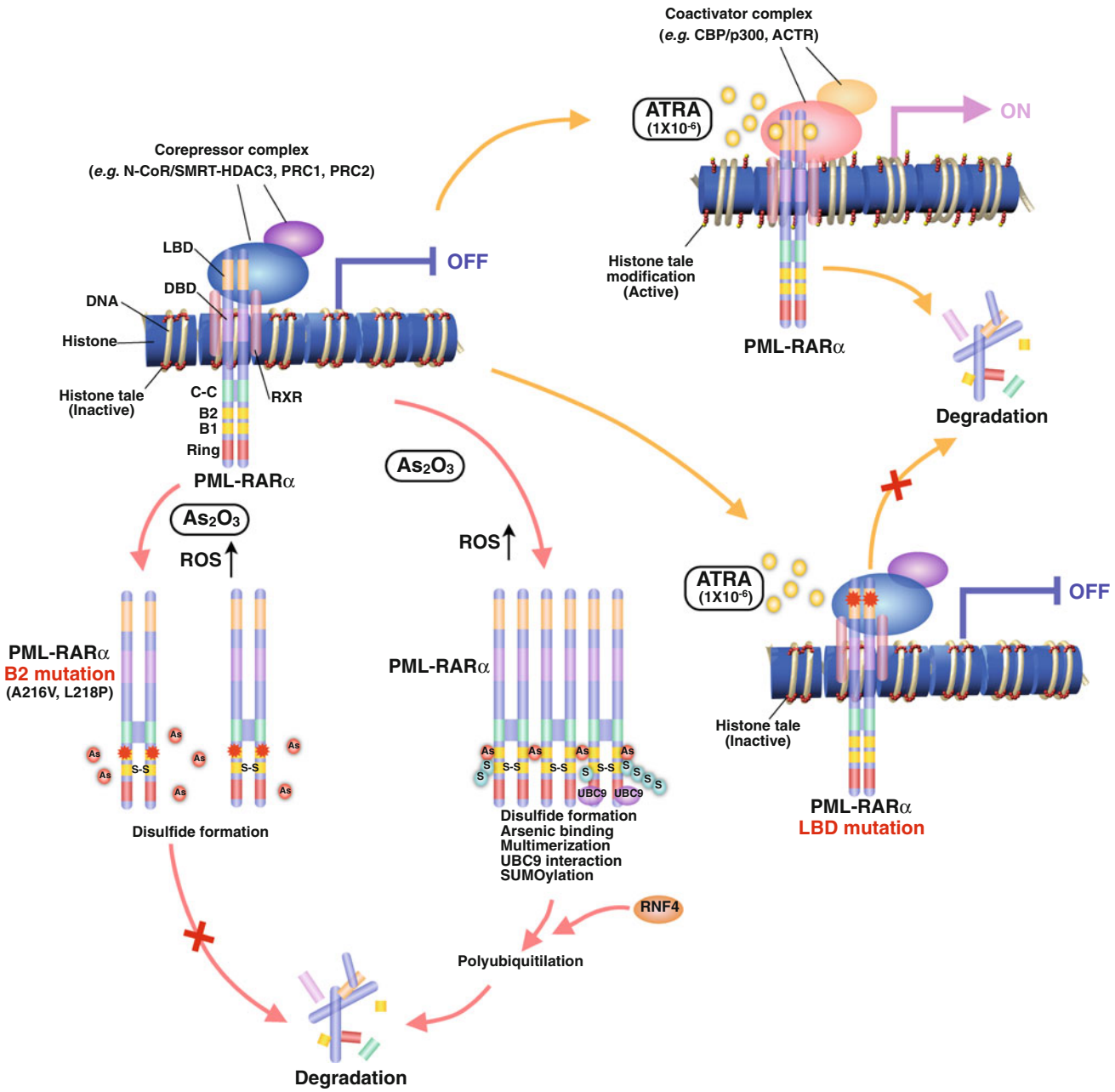

Fig. 2 Molecular mechanisms of action and resistance to ATRA and As_2O_3 in APL cells. PML-RARα are found mainly as homodimers through the C–C domain of PML, and partially as heterodimers with RXR. PML-RARα binds with target gene promoter in the absence of ligand, and recruits co-repressor complexes, such as N-CoR/SMRT complexes containing histone deacetylases (e.g. HDAC3) [34–37, 41] and PRC1/2 complex containing histone methyltransferases (e.g. EZH2) [39] to repress the gene expression. Histone tail deacetylation and/or methylation are related to transcription repression. In the presence of pharmacological concentration (1×10^6 μM) of ligand (ATRA), co-repressor complexes are dissociated from RARα, while co-activator complexes containing histone acetyltransferases (e.g. p300/CBP) are recruited, and transcription activation occurs. In the cases of PML-RARα with LBD mutations, ligand binding with LBD is interfered and co-repressor dissociation does not occur in the presence of pharmacological concentrations of ATRA. In the presence of As_2O_3, the formation of reactive oxygen species (ROS) is induced, and PML intermolecular disulfide crosslinks through B1 domain, that induce multimerization, and SUMOylation of PML by ubiquitin-conjugating enzyme 9 (UBC9) occur. As_2O_3 directly bind with PML-B2 domain and enhancing UBC9 binding and SUMOylation of PML. SUMOylated PML recruits RING finger protein 4 (RNF4), and is polyubiquitylated by RNF4, and proteasome-dependent degradation occurs. If PML-RARα has PML-B2 mutation, direct binding of As_2O_3 with PML is impaired, and polyubiquitylation and degradation are perturbed

regulation of STAT5b target genes in addition to RARα target genes by STAT5b-RARα may be related to ATRA resistance.

On the other hand, As_2O_3 resistance in clinical setting was observed in patients expressing PLZF- [57, 58], STAT5b- [55], and BCoR-RARα [25]. The As_2O_3-binding C–C motif is confirmed in PML-B2 domain, and As_2O_3 binding is critical for the multimerization followed by PML-RARα degradation [29, 30, 42]. Lack of As_2O_3 binding sites in X-RARα protein may be one explanation of loss of As_2O_3 responsiveness. However, no direct effect of As_2O_3 on RARα has been reported.

Mechanisms of resistance to ATRA

A number of mechanisms have been proposed to explain ATRA resistance in APL patients expressing PML-RARα, such as amino acid substitution in RARα LBD domain by genetic mutations, increased catabolism of ATRA, presence of cytoplasmic retinoic acid binding protein (CRABP), and abnormal ATRA delivery to the cell nucleus. Only genetic mutations on the RARα LBD domain in PML-RARα have been confirmed as an ATRA-resistant mechanism, from both clinical observations and in vitro molecular analyses [59–66]. Genetic mutations (missense, nonsense, and deletions) on RARα LBD domain

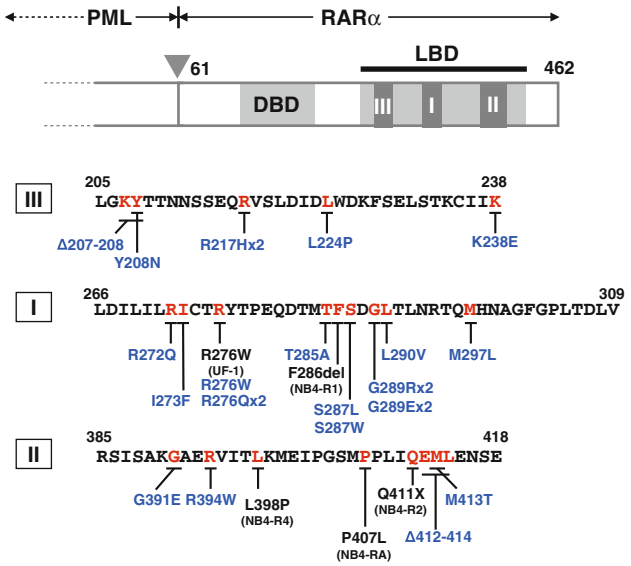

Fig. 3 Genetic mutations resulting in amino acid substitution in PML-RARα LBD confirmed in clinically ATRA-resistant patients and ATRA-resistant cell lines. Mutations are confirmed in 3 cluster regions (zones I to III) in RARα-LBD [66]. *Red letters* indicate amino acids substituted in specific patients and/or cells. Amino acid substitutions and deletions in ATRA-resistant patients are indicated in *blue letters*. Substitution in ATRA-resistant *cell lines* indicated in *black*. Names of *cell lines* are indicated in *brackets*. The position of the mutation is described with reference to normal amino acid sequence of RARα1 [31]

have been confirmed in ATRA-resistant patients and APL cell lines, which grow despite pharmacological concentrations of ATRA, as summarized in Fig. 3. These mutations accumulate in the three subregions (zones I, II, and III in Fig. 3) of the LBD domain [66]. Gallagher et al. [66] reported that *PML-RARα* LBD mutation was confirmed 18 of 45 (40 %) relapse patients treated with ATRA/chemotherapy. In vitro analyses using ATRA-resistant NB4 cells (NB4-R1, -R2 [67], -R4 [60], and -RA [61]) and mutated-*PML-RARα* expressing Cos-1 cells [65] indicated that ATRA binding affinity with mutated PML-RARα was generally lower than that with PML-RARα without mutations, due to conformational changes in LBD. Furthermore, ligand-dependent N-CoR/SMRT co-repressor release and co-activator recruitment (e.g. ACTR histone acetyltransferase), which are critical for the transcriptional activation of genes with RARE sites and morphological cell differentiation, was impaired under the therapeutic dose of ATRA [60, 65, 67].

To overcome ATRA resistance, a number of therapeutics has been tested in vitro and in vivo. Several clinical reports indicated that As_2O_3 rescue most of relapsed/refractory patients treated with ATRA/chemotherapy [9–12, 68]. Am80, a synthetic retinoid that shows higher binding affinity with PML-RARα than ATRA, is utilized in the clinical setting [69–71]. Am80 is approximately 10 times more potent than ATRA as an in vitro inducer of differentiation in NB-4 and HL60 cells, and is chemically more stable than ATRA [72, 73]. Histone deacetylase (HDAC) inhibitors [74], such as sodium butyrate (NaF), valproic acid (VPA), and trichostatin A (TSA), have been utilized with ATRA and are expected to transcriptionally activate PML-RARα target genes to inhibit co-repressors complexes that contain HDACs [75–77]. Another approach to overcoming the resistance uses other molecular targeting therapeutics, such as gemtuzumab ozogamicin (GO), an anti-CD33 monoclonal antibody linked with calicheamicins [78, 79].

Molecular mechanisms of resistance to As_2O_3

Even for relapsed/refractory patients following treatment with ATRA/chemotherapy, As_2O_3 therapy is highly effective, with a complete remission rate of more than 80 % [80–82]. Although the CR rate is high even in relapsed patients, resistance to As_2O_3 treatment has been recognized as a clinically critical problem. Information on As_2O_3 resistance remains limited compared with that on ATRA resistance.

Recently, we reported two cases showing clinical As_2O_3 resistance after treatment with ATRA/chemotherapy, which exhibited missense mutations leading to substitution of amino acids in the PML-B2 domain in PML-RARα [50, 68, 83]. One patient with the M3 variant, expressing PML-

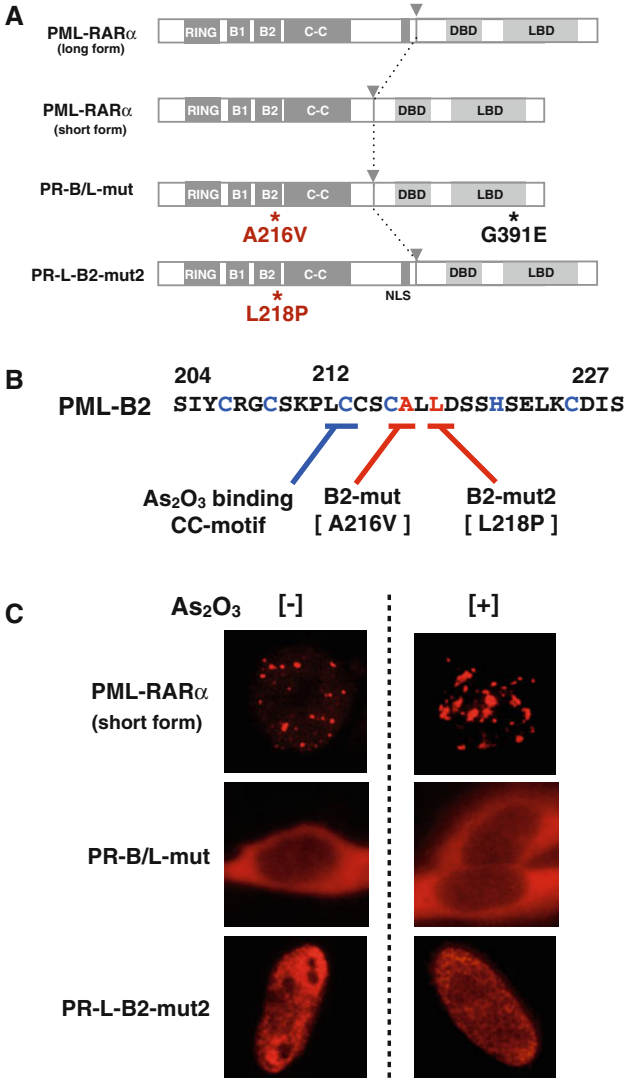

Fig. 4 Genetic mutations resulting in amino acid substitution in PML-B2 domain confirmed in clinically As_2O_3 resistant APL patients. **a** Schematic representation of PML-RARα chimeric protein with B2-domain mutation. One patient held PML-B2 mutation (A216V) and RARα-LBD mutation (G391E) on short form PML-RARα (PR-B/L-mut), and another patient held PML-B2 mutation (L218P) on long form PML-RARα [68]. **b** As_2O_3 direct binding dicysteine motif (C212/C213) [29, 30] and mutated positions in As_2O_3-resistant patients (C216 and L218) occur quite close to each other. **c** Flag-tagged PML-RARα short form, PR-B/L-mut, and PR-B2-mut2 were over expressed in HeLa cells with or without As_2O_3. Over expressed PML were detected by immunofluorescence staining using anti-Flag antibody. When using PML-RARα short form without As_2O_3, PML body was confirmed in the microspeckled pattern in cytoplasm. After incubation with As_2O_3, PML bodies showed macro granular patterns. When using PR-B/L-mut or PR-B2-mut2, the PML body showed diffuse pattern in cytoplasm or nucleus. No difference was seen with/without As_2O_3

RARα short form without nuclear localizing signal (NLS) [84], showed ATRA and As_2O_3 resistance at his terminal stage. Significant clonal expansion of *PML-RARα* mutant leading to A216V (PML-B2 domain mutation) and G391E (RARα-LBD mutation) was confirmed in leukemia cells harvested at the terminal stage (Fig. 4a, b). In vitro analysis using wild-type and mutant PML-RARα (PR-B/L-mut)-expressing HeLa and HL60 cells indicated that PML-RARα (short form) localized in cytoplasm as micro speckled pattern without As_2O_3, and as a macro granular pattern after adding As_2O_3 (Fig. 4c; PML-RARα). In contrast, PR-B/L-mut localized in cytoplasm with diffuse pattern without As_2O_3, and no change was confirmed in the presence of As_2O_3(Fig. 4c; PR-B/L-mut). Another case carried an L218P mutation, also in the PML-B2 domain (PR-B2-mut2), in PML-RARα long form with NLS. PML-RARα long form localized in nucleus, while PR-B2-mut2 was diffusely localized in the nucleus. No change was confirmed with or without As_2O_3 (Fig. 4c; PR-B2-mut2). Further in vitro analysis using PML-RARα overexpressed HeLa cells indicated that SUMOylation of PR-B/L-mut and PR-B2-mut2 after As_2O_3 treatment was strictly impaired. Recent reports have indicated that direct As_2O_3 binding to PML-B2 domain is critical for the serial reaction including SUMOylation, multimerization, and degradation [29, 30]. Jeanne et al. conclude that dicysteine C212/C213 in PML-B2 domain may be the direct As_2O_3 binding motif. From these results, genetic mutations identified in As_2O_3-resistant patients resulting in A216V and L218P may contribute to As_2O_3 resistance through impairment of direct As_2O_3 binding to PML-RARα due to conformational changes in As_2O_3 binding sites. Further accumulation of patients for genetic analyses is required for confirming the clinical significance of PML-B2 domain mutations in As_2O_3 resistance.

Conclusion

Although the overall survival of APL has been significantly prolonged since the introduction of ATRA and As_2O_3, relapse/refractory disease due to ATRA and/or As_2O_3 resistance remains a serious clinical problem. Additional genetic mutations in PML-RARα and another gene, such as FLT3-ITD or TP53 [66, 85], may contribute to disease progression and drug resistance in APL. Detailed genomic analyses using clinical samples harvested repeatedly from patients may help for predicting prognosis, selecting effective targeting drugs, understanding molecular backgrounds, and designing sophisticated new therapeutic strategies.

References

1. Warrell RP Jr, de The H, Wang ZY, Degos L. Acute promyelocytic leukemia. N Engl J Med. 1993;329:177–89.
2. Wang ZY, Chen Z. Acute promyelocytic leukemia: from highly fatal to highly curable. Blood. 2008;111:2505–15.

3. Cunningham I, Gee TS, Reich LM, Kempin SJ, Naval AN, Clarkson BD. Acute promyelocytic leukemia: treatment results during a decade at Memorial Hospital. Blood. 1989;73:1116–22.
4. Sanz MA, Jarque I, Martin G, Lorenzo I, Martinez J, Rafecas J, Pastor E, Sayas MJ, Sanz G, Gomis F. Acute promyelocytic leukemia. Therapy results and prognostic factors. Cancer. 1988;61:7–13.
5. Fenaux P, Castaigne S, Dombret H, Archimbaud E, Duarte M, Morel P, Lamy T, Tilly H, Guerci A, Maloisel F, et al. All-transretinoic acid followed by intensive chemotherapy gives a high complete remission rate and may prolong remissions in newly diagnosed acute promyelocytic leukemia: a pilot study on 26 cases. Blood. 1992;80:2176–81.
6. Fenaux P. Results of APL 91 European trial combining ATRA and chemotherapy: presentation of APL 1993 trial. Leukemia. 1994;8(Suppl 3):S70–2.
7. Ohno R, Ohnishi K, Takeshita A, Tanimoto M, Murakami H, Kanamaru A, Asou N, Kobayashi T, Kuriyama K, Ohmoto E, et al. All-trans retinoic acid therapy in relapsed/refractory or newly diagnosed acute promyelocytic leukemia (APL) in Japan. Leukemia. 1994;8(Suppl 3):S64–9.
8. Degos L, Dombret H, Chomienne C, Daniel MT, Miclea JM, Chastang C, Castaigne S, Fenaux P. All-trans-retinoic acid as a differentiating agent in the treatment of acute promyelocytic leukemia. Blood. 1995;85:2643–53.
9. Shen ZX, Chen GQ, Ni JH, Li XS, Xiong SM, Qiu QY, Zhu J, Tang W, Sun GL, Yang KQ, Chen Y, Zhou L, Fang ZW, Wang YT, Ma J, Zhang P, Zhang TD, Chen SJ, Chen Z, Wang ZY. Use of arsenic trioxide (As2O3) in the treatment of acute promyelocytic leukemia (APL): II. Clinical efficacy and pharmacokinetics in relapsed patients. Blood. 1997;89:3354–60.
10. Niu C, Yan H, Yu T, Sun HP, Liu JX, Li XS, Wu W, Zhang FQ, Chen Y, Zhou L, Li JM, Zeng XY, Yang RR, Yuan MM, Ren MY, Gu FY, Cao Q, Gu BW, Su XY, Chen GQ, Xiong SM, Zhang TD, Waxman S, Wang ZY, Chen Z, Hu J, Shen ZX, Chen SJ. Studies on treatment of acute promyelocytic leukemia with arsenic trioxide: remission induction, follow-up, and molecular monitoring in 11 newly diagnosed and 47 relapsed acute promyelocytic leukemia patients. Blood. 1999;94:3315–24.
11. Ohnishi K, Yoshida H, Shigeno K, Nakamura S, Fujisawa S, Naito K, Shinjo K, Fujita Y, Matsui H, Sahara N, Takeshita A, Satoh H, Terada H, Ohno R. Arsenic trioxide therapy for relapsed or refractory Japanese patients with acute promyelocytic leukemia: need for careful electrocardiogram monitoring. Leukemia. 2002;16:617–22.
12. Shigeno K, Naito K, Sahara N, Kobayashi M, Nakamura S, Fujisawa S, Shinjo K, Takeshita A, Ohno R, Ohnishi K. Arsenic trioxide therapy in relapsed or refractory Japanese patients with acute promyelocytic leukemia: updated outcomes of the phase II study and postremission therapies. Int J Hematol. 2005;82:224–9.
13. Shen ZX, Shi ZZ, Fang J, Gu BW, Li JM, Zhu YM, Shi JY, Zheng PZ, Yan H, Liu YF, Chen Y, Shen Y, Wu W, Tang W, Waxman S, De The H, Wang ZY, Chen SJ, Chen Z. All-trans retinoic acid/As_2O_3 combination yields a high quality remission and survival in newly diagnosed acute promyelocytic leukemia. Proc Natl Acad Sci USA. 2004;101:5328–35.
14. Tallman MS, Altman JK. How I treat acute promyelocytic leukemia. Blood. 2009;114:5126–35.
15. Rowley JD. Mapping of human chromosomal regions related to neoplasia: evidence from chromosomes 1 and 17. Proc Natl Acad Sci USA. 1977;74:5729–33.
16. de The 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;347:558–61.
17. de The 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;66:675–84.
18. 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;91:1178–82.
19. 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;17:109–13.
20. 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;87:882–6.
21. Arnould C, Philippe C, Bourdon V, Gr goire 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;8:1741–9.
22. Kondo T, Mori A, Darmanin S, Hashino S, Tanaka J, Asaka M. The seventh pathogenic fusion gene FIP1L1-RARA was isolated from a t(4;17)-positive acute promyelocytic leukemia. Haematologica. 2008;93:1414–6.
23. Catalano A, Dawson MA, Somana K, Opat S, Schwarer A, Campbell LJ, Iland H. The PRKAR1A gene is fused to RARA in a new variant acute promyelocytic leukemia. Blood. 2007;110:4073–6.
24. Qiu JJ, Lu X, Zeisig BB, Ma Z, Cai X, Chen S, Gronemeyer H, Tweardy DJ, So CW, Dong S. Leukemic transformation by the APL fusion protein PRKAR1A-RAR{alpha} critically depends on recruitment of RXR{alpha}. Blood. 2010;115:643–52.
25. Yamamoto Y, Tsuzuki S, Tsuzuki M, Handa K, Inaguma Y, Emi N. BCOR as a novel fusion partner of retinoic acid receptor alpha in a t(X;17)(p11;q12) variant of acute promyelocytic leukemia. Blood. 2010;116:4274–83.
26. Huang ME, Ye YC, Chen SR, Chai JR, Lu JX, Zhoa L, Gu LJ, Wang ZY. Use of all-trans retinoic acid in the treatment of acute promyelocytic leukemia. Blood. 1988;72:567–72.
27. Grignani F, De Matteis S, Nervi C, Tomassoni L, Gelmetti V, Cioce M, Fanelli M, Ruthardt M, Ferrara FF, Zamir I, Seiser C, Lazar MA, Minucci S, Pelicci PG. Fusion proteins of the retinoic acid receptor-alpha recruit histone deacetylase in promyelocytic leukaemia. Nature. 1998;391:815–8.
28. Chen GQ, Zhu J, Shi XG, Ni JH, Zhong HJ, Si GY, Jin XL, Tang W, Li XS, Xong SM, Shen ZX, Sun GL, Ma J, Zhang P, Zhang TD, Gazin C, Naoe T, Chen SJ, Wang ZY, Chen Z. In vitro studies on cellular and molecular mechanisms of arsenic trioxide (As_2O_3) in the treatment of acute promyelocytic leukemia: As_2O_3 induces NB4 cell apoptosis with downregulation of Bcl-2 expression and modulation of PML-RAR alpha/PML proteins. Blood. 1996;88:1052–61.
29. Zhang XW, Yan XJ, Zhou ZR, Yang FF, Wu ZY, Sun HB, Liang WX, Song AX, Lallemand-Breitenbach V, Jeanne M, Zhang QY, Yang HY, Huang QH, Zhou GB, Tong JH, Zhang Y, Wu JH, Hu HY, de The H, Chen SJ, Chen Z. Arsenic trioxide controls the fate of the PML-RARalpha oncoprotein by directly binding PML. Science. 2010;328:240–3.
30. Jeanne M, Lallemand-Breitenbach V, Ferhi O, Koken M, Le Bras M, Duffort S, Peres L, Berthier C, Soilihi H, Raught B, de The H. PML/RARA oxidation and arsenic binding initiate the antileukemia response of As_2O_3. Cancer Cell. 2010;18:88–98.
31. Giguere V, Ong ES, Segui P, Evans RM. Identification of a receptor for the morphogen retinoic acid. Nature. 1987;330:624–9.
32. Zechel C, Shen XQ, Chambon P, Gronemeyer H. Dimerization interfaces formed between the DNA binding domains determine

the cooperative binding of RXR/RAR and RXR/TR heterodimers to DR5 and DR4 elements. EMBO J. 1994;13:1414–24.
33. Kurokawa R, DiRenzo J, Boehm M, Sugarman J, Gloss B, Rosenfeld MG, Heyman RA, Glass CK. Regulation of retinoid signalling by receptor polarity and allosteric control of ligand binding. Nature. 1994;371:528–31.
34. He LZ, Guidez F, Tribioli C, Peruzzi D, Ruthardt M, Zelent A, Pandolfi PP. Distinct interactions of PML-RARalpha and PLZF-RARalpha with co-repressors determine differential responses to RA in APL. Nat Genet. 1998;18:126–35.
35. Tomita A, Buchholz DR, Obata K, Shi YB. Fusion protein of retinoic acid receptor alpha with promyelocytic leukemia protein or promyelocytic leukemia zinc finger protein recruits N-CoR-TBLR1 corepressor complex to repress transcription in vivo. J Biol Chem. 2003;278:30788–95.
36. Atsumi A, Tomita A, Kiyoi H, Naoe T. Histone deacetylase 3 (HDAC3) is recruited to target promoters by PML-RARalpha as a component of the N-CoR co-repressor complex to repress transcription in vivo. Biochem Biophys Res Commun. 2006;345:1471–80.
37. Villa R, Morey L, Raker VA, Buschbeck M, Gutierrez A, De Santis F, Corsaro M, Varas F, Bossi D, Minucci S, Pelicci PG, Di Croce L. The methyl-CpG binding protein MBD1 is required for PML-RARalpha function. Proc Natl Acad Sci USA. 2006;103:1400–5.
38. Carbone R, Botrugno OA, Ronzoni S, Insinga A, Di Croce L, Pelicci PG, Minucci S. Recruitment of the histone methyltransferase SUV39H1 and its role in the oncogenic properties of the leukemia-associated PML-retinoic acid receptor fusion protein. Mol Cell Biol. 2006;26:1288–96.
39. Villa R, Pasini D, Gutierrez A, Morey L, Occhionorelli M, Vire E, Nomdedeu JF, Jenuwein T, Pelicci PG, Minucci S, Fuks F, Helin K, Di Croce L. Role of the polycomb repressive complex 2 in acute promyelocytic leukemia. Cancer Cell. 2007;11:513–25.
40. Boukarabila H, Saurin AJ, Batsche E, Mossadegh N, van Lohuizen M, Otte AP, Pradel J, Muchardt C, Sieweke M, Duprez E. The PRC1 Polycomb group complex interacts with PLZF/RARA to mediate leukemic transformation. Genes Dev. 2009;23:1195–206.
41. Grignani F, De Matteis 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;391:815–8.
42. de The H, Chen Z. Acute promyelocytic leukaemia: novel insights into the mechanisms of cure. Nat Rev Cancer. 2010;10:775–83.
43. Martens JH, Brinkman AB, Simmer F, Francoijs KJ, Nebbioso A, Ferrara F, Altucci L, Stunnenberg HG. PML-RARalpha/RXR alters the epigenetic landscape in acute promyelocytic leukemia. Cancer Cell. 2010;17:173–85.
44. Zhu J, Zhou J, Peres L, Riaucoux F, Honore N, Kogan S, de The H. A sumoylation site in PML/RARA is essential for leukemic transformation. Cancer Cell. 2005;7:143–53.
45. Yoshida H, Kitamura K, Tanaka K, Omura S, Miyazaki T, Hachiya T, Ohno R, Naoe T. Accelerated degradation of PML-retinoic acid receptor alpha (PML-RARA) oncoprotein by all-trans-retinoic acid in acute promyelocytic leukemia: possible role of the proteasome pathway. Cancer Res. 1996;56:2945–8.
46. Duprez E, Saurin AJ, Desterro JM, Lallemand-Breitenbach V, Howe K, Boddy MN, Solomon E, de The H, Hay RT, Freemont PS. SUMO-1 modification of the acute promyelocytic leukaemia protein PML: implications for nuclear localisation. J Cell Sci. 1999;112(Pt 3):381–93.
47. Zhu J, Gianni M, Kopf E, Honore N, Chelbi-Alix M, Koken M, Quignon F, Rochette-Egly C, de The H. Retinoic acid induces proteasome-dependent degradation of retinoic acid receptor alpha (RARalpha) and oncogenic RARalpha fusion proteins. Proc Natl Acad Sci USA. 1999;96:14807–12.
48. Muller S, Matunis MJ, Dejean A. Conjugation with the ubiquitin-related modifier SUMO-1 regulates the partitioning of PML within the nucleus. EMBO J. 1998;17:61–70.
49. Lallemand-Breitenbach V, Zhu J, Puvion F, Koken M, Honore N, Doubeikovsky A, Duprez E, Pandolfi PP, Puvion E, Freemont P, de The H. Role of promyelocytic leukemia (PML) sumolation in nuclear body formation, 11S proteasome recruitment, and As2O3-induced PML or PML/retinoic acid receptor alpha degradation. J Exp Med. 2001;193:1361–71.
50. Lang E, Grudic A, Pankiv S, Bruserud O, Simonsen A, Bjerkvig R, Bjoras M, Boe SO. The arsenic-based cure of acute promyelocytic leukemia promotes cytoplasmic sequestration of PML and PML/RARA through inhibition of PML body recycling. Blood. 2012;120:847–57.
51. Maroui MA, Kheddache-Atmane S, El Asmi F, Dianoux L, Aubry M, Chelbi-Alix MK. Requirement of PML SUMO interacting motif for RNF4- or arsenic trioxide-induced degradation of nuclear PML isoforms. PLoS ONE. 2012;7:e44949.
52. Gallagher RE. Retinoic acid resistance in acute promyelocytic leukemia. Leukemia. 2002;16:1940–58.
53. Kusakabe M, Suzukawa K, Nanmoku T, Obara N, Okoshi Y, Mukai HY, Hasegawa Y, Kojima H, Kawakami Y, Ninomiya H, Nagasawa T. Detection of the STAT5B-RARA fusion transcript in acute promyelocytic leukemia with the normal chromosome 17 on G-banding. Eur J Haematol. 2008;80:444–7.
54. Sternsdorf T, Phan VT, Maunakea ML, Ocampo CB, Sohal J, Silletto A, Galimi F, Le Beau MM, Evans RM, Kogan SC. Forced retinoic acid receptor alpha homodimers prime mice for APL-like leukemia. Cancer Cell. 2006;9:81–94.
55. Strehl S, Konig M, Boztug H, Cooper BW, Suzukawa K, Zhang SJ, Chen HY, Attarbaschi A, Dworzak MN. All-trans retinoic acid and arsenic trioxide resistance of acute promyelocytic leukemia with the variant STAT5B-RARA fusion gene. Leukemia 2013;1–4.
56. LaFave LM, Levine RL. JAK2 the future: therapeutic strategies for JAK-dependent malignancies. Trends Pharmacol Sci. 2012;33:574–82.
57. Wang ZY, Chen Z, Huang W, Li XS, Lu JX, Huang LA, Zhang FQ, Gu LJ, Ouyang RR, Chen SJ, et al. Problems existing in differentiation therapy of acute promyelocytic leukemia (APL) with all-trans retinoic acid (ATRA). Blood Cells. 1993;19:633–41 discussion 642–637.
58. Jansen JH, Lowenberg B. Acute promyelocytic leukemia with a PLZF-RARalpha fusion protein. Semin Hematol. 2001;38:37–41.
59. Duprez E, Ruchaud S, Houge G, Martin-Thouvenin V, Valensi F, Kastner P, Berger R, Lanotte M. A retinoid acid 'resistant' t(15;17) acute promyelocytic leukemia cell line: isolation, morphological, immunological, and molecular features. Leukemia. 1992;6:1281–7.
60. Shao W, Benedetti L, Lamph WW, Nervi C, Miller WH Jr. A retinoid-resistant acute promyelocytic leukemia subclone expresses a dominant negative PML-RAR alpha mutation. Blood. 1997;89:4282–9.
61. Kitamura K, Kiyoi H, Yoshida H, Saito H, Ohno R, Naoe T. Mutant AF-2 domain of PML-RARalpha in retinoic acid-resistant NB4 cells: differentiation induced by RA is triggered directly through PML-RARalpha and its down-regulation in acute promyelocytic leukemia. Leukemia. 1997;11:1950–6.
62. Nason-Burchenal K, Allopenna J, Begue A, Stehelin D, Dmitrovsky E, Martin P. Targeting of PML/RARalpha is lethal to retinoic acid-resistant promyelocytic leukemia cells. Blood. 1998;92:1758–67.

63. Marasca R, Zucchini P, Galimberti S, Leonardi G, Vaccari P, Donelli A, Luppi M, Petrini M, Torelli G. Missense mutations in the PML/RARalpha ligand binding domain in ATRA-resistant As(2)O(3) sensitive relapsed acute promyelocytic leukemia. Haematologica. 1999;84:963–8.
64. Duprez E, Benoit G, Flexor M, Lillehaug JR, Lanotte M. A mutated PML/RARA found in the retinoid maturation resistant NB4 subclone, NB4-R2, blocks RARA and wild-type PML/RARA transcriptional activities. Leukemia. 2000;14:255–61.
65. Cote S, Zhou D, Bianchini A, Nervi C, Gallagher RE, Miller WH Jr. Altered ligand binding and transcriptional regulation by mutations in the PML/RARalpha ligand-binding domain arising in retinoic acid-resistant patients with acute promyelocytic leukemia. Blood. 2000;96:3200–8.
66. Gallagher RE, Moser BK, Racevskis J, Poire X, Bloomfield CD, Carroll AJ, Ketterling RP, Roulston D, Schachter-Tokarz E, Zhou DC, Chen IM, Harvey R, Koval G, Sher DA, Feusner JH, Tallman MS, Larson RA, Powell BL, Appelbaum FR, Paietta E, Willman CL, Stock W. Treatment-influenced associations of PML-RARalpha mutations, FLT3 mutations, and additional chromosome abnormalities in relapsed acute promyelocytic leukemia. Blood. 2012;120:2098–108.
67. Ruchaud S, Duprez E, Gendron MC, Houge G, Genieser HG, Jastorff B, Doskeland SO, Lanotte M. Two distinctly regulated events, priming and triggering, during retinoid-induced maturation and resistance of NB4 promyelocytic leukemia cell line. Proc Natl Acad Sci USA. 1994;91:8428–32.
68. Goto E, Tomita A, Hayakawa F, Atsumi A, Kiyoi H, Naoe T. Missense mutations in PML-RARA are critical for the lack of responsiveness to arsenic trioxide treatment. Blood. 2011;118:1600–9.
69. Takeuchi M, Yano T, Omoto E, Takahashi K, Kibata M, Shudo K, Harada M, Ueda R, Ohno R. Relapsed acute promyelocytic leukemia previously treated with all-trans retinoic acid: clinical experience with a new synthetic retinoid, Am-80. Leuk Lymphoma. 1998;31:441–51.
70. Ohnishi K. PML-RARalpha inhibitors (ATRA, tamibaroten, arsenic troxide) for acute promyelocytic leukemia. Int J Clin Oncol. 2007;12:313–7.
71. S.O. Katsuji Shinagawa, Toru Sakura, Yasunori Ueda, Masashi Sawa, Jun-ichi Miyatake, Noriko Usui, Makoto Onitsuka, Yoshihiro Hatta, Nobuhiko Emi, Shigehisa Tamaki, Yoshikazu Ito, Toru Murayama, Hiroyuki Fujita, Katsumichi Fujimaki, Norio Asou, Akihiro Takeshita, Yasushi Miyazaki, Shuichi Miyawaki, Kazunori Ohnishi, Tomoki Naoe, Ryuzo Ohno, A Phase III Study of New Synthetic Retinoid Tamibarotene (Am80) Compared with ATRA in Maintenance Therapy for Newly Diagnosed Acute Promyelocytic Leukemia (APL): Japan Adult Leukemia Study Group (JALSG) APL204 Study, 54th American Society Of Hematology Annual Meeting, Atlanta, GA, 2012, pp. December 10, 2012.
72. Kagechika H, Kawachi E, Hashimoto Y, Himi T, Shudo K. Retinobenzoic acids. 1. Structure-activity relationships of aromatic amides with retinoidal activity. J Med Chem. 1988;31:2182–92.
73. Hashimoto Y, Kagechika H, Kawachi E, Fukasawa H, Saito G, Shudo K. Correlation of differentiation-inducing activity of retinoids on human leukemia cell lines HL-60 and NB4. J Cancer Res Clin Oncol. 1995;121:696–8.
74. Ungewickell A, Medeiros BC. Novel agents in acute myeloid leukemia. Int J Hematol. 2012;96:178–85.
75. Zhou DC, Kim SH, Ding W, Schultz C, Warrell RP Jr, Gallagher RE. Frequent mutations in the ligand-binding domain of PML-RARalpha after multiple relapses of acute promyelocytic leukemia: analysis for functional relationship to response to all-trans retinoic acid and histone deacetylase inhibitors in vitro and in vivo. Blood. 2002;99:1356–63.
76. Jing Y, Xia L, Waxman S. Targeted removal of PML-RARalpha protein is required prior to inhibition of histone deacetylase for overcoming all-trans retinoic acid differentiation resistance in acute promyelocytic leukemia. Blood. 2002;100:1008–13.
77. Kosugi H, Towatari M, Hatano S, Kitamura K, Kiyoi H, Kinoshita T, Tanimoto M, Murate T, Kawashima K, Saito H, Naoe T. Histone deacetylase inhibitors are the potent inducer/enhancer of differentiation in acute myeloid leukemia: a new approach to anti-leukemia therapy. Leukemia. 1999;13:1316–24.
78. Takeshita A, Shinjo K, Naito K, Matsui H, Sahara N, Shigeno K, Suzumura T, Horii T, Shirai N, Maekawa M, Yada Y, Teshima H, Takeuchi J, Ohnishi K, Ohno R. Two patients with all-trans retinoic acid-resistant acute promyelocytic leukemia treated successfully with gemtuzumab ozogamicin as a single agent. Int J Hematol. 2005;82:445–8.
79. Ito Y, Wakita A, Takada S, Mihara M, Gotoh M, Ohyashiki K, Ohtake S, Miyawaki S, Ohnishi K, Naoe T. Phase 1 trial of gemtuzumab ozogamicin in combination with enocitabine and daunorubicin for elderly patients with relapsed or refractory acute myeloid leukemia: Japan Adult Leukemia Study Group (JALSG)-GML208 study. Int J Hematol. 2012;96:485–91.
80. Mi JQ, Li JM, Shen ZX, Chen SJ, Chen Z. How to manage acute promyelocytic leukemia. Leukemia. 2012;26:1743–51.
81. Fox E, Razzouk BI, Widemann BC, Xiao S, O'Brien M, Goodspeed W, Reaman GH, Blaney SM, Murgo AJ, Balis FM, Adamson PC. Phase 1 trial and pharmacokinetic study of arsenic trioxide in children and adolescents with refractory or relapsed acute leukemia, including acute promyelocytic leukemia or lymphoma. Blood. 2008;111:566–73.
82. Soignet SL, Frankel SR, Douer D, Tallman MS, Kantarjian H, Calleja E, Stone RM, Kalaycio M, Scheinberg DA, Steinherz P, Sievers EL, Coutre S, Dahlberg S, Ellison R, Warrell RP Jr. United States multicenter study of arsenic trioxide in relapsed acute promyelocytic leukemia. J Clin Oncol. 2001;19:3852–60.
83. Gallagher RE. Mutants strike again in APL. Blood. 2011;118:1432–4.
84. Avantaggiato V, Pandolfi PP, Ruthardt M, Hawe N, Acampora D, Pelicci PG, Simeone A. Developmental analysis of murine Promyelocyte Leukemia Zinc Finger (PLZF) gene expression: implications for the neuromeric model of the forebrain organization. J Neurosci. 1995;15:4927–42.
85. Welch JS, Ley TJ, Link DC, Miller CA, Larson DE, Koboldt DC, Wartman LD, Lamprecht TL, Liu F, Xia J, Kandoth C, Fulton RS, McLellan MD, Dooling DJ, Wallis JW, Chen K, Harris CC, Schmidt HK, Kalicki-Veizer JM, Lu C, Zhang Q, Lin L, O'Laughlin MD, McMichael JF, Delehaunty KD, Fulton LA, Magrini VJ, McGrath SD, Demeter RT, Vickery TL, Hundal J, Cook LL, Swift GW, Reed JP, Alldredge PA, Wylie TN, Walker JR, Watson MA, Heath SE, Shannon WD, Varghese N, Nagarajan R, Payton JE, Baty JD, Kulkarni S, Klco JM, Tomasson MH, Westervelt P, Walter MJ, Graubert TA, DiPersio JF, Ding L, Mardis ER, Wilson RK. The origin and evolution of mutations in acute myeloid leukemia. Cell. 2012;150:264–78.
86. Pandolfi PP, Alcalay M, Fagioli M, Zangrilli D, Mencarelli A, Diverio D, Biondi A, Lo Coco F, Rambaldi A, Grignani F, et al. Genomic variability and alternative splicing generate multiple PML/RAR alpha transcripts that encode aberrant PML proteins and PML/RAR alpha isoforms in acute promyelocytic leukaemia. EMBO J. 1992;11:1397–407.