Acute promyelocytic leukemia and differentiation therapy: molecular mechanisms of differentiation, retinoic acid resistance and novel treatments

Akut promiyelositik lösemi ve diferansiyasyon tedavisi: diferansiyasyonun, retinoik asit direncinin ve yeni tedavilerin moleküler mekanizmaları

Bülent Özpolat
M. D. Anderson Cancer Center, University of Texas Department of Experimental Therapeutics, Houston, USA

Abstract

Incorporation of all-trans-retinoic acid (ATRA) into the treatment of acute promyelocytic leukemia (APL), a type of acute myeloid leukemia (AML), revolutionized the therapy of cancer in the last decade and introduced the concept of differentiation therapy. ATRA, a physiological metabolite of vitamin A (retinol), induces complete clinical remissions (CRs) in about 90% of patients with APL. In contrast to the cytotoxic chemotherapeutics, ATRA can selectively induce terminal differentiation of promyelocytic leukemic cells into normal granulocytes without causing bone marrow hypoplasia or exacerbation of the frequently occurring fatal hemorrhagic syndromes in patients with APL. However, remissions induced by ATRA alone are transient and the patients commonly become resistant to the therapy, leading to relapses in most patients and thus limiting the use of ATRA as a single agent. Therefore, ATRA is currently combined with anthracycline-based chemotherapy, and this regimen dramatically improves patient survival compared to chemotherapy alone, curing about 70% of the patients. However, 30% of APL patients still relapse and die in five years. Recently, arsenic trioxide (As_2O_3) was proven to be highly effective in inducing CRs not only in APL patients relapsed after ATRA treatment and conventional chemotherapy but also in primary APL patients. Despite the well-documented clinical efficacy of ATRA, molecular mechanisms responsible for development of ATRA resistance are not well understood. Based on in vitro and clinical observations, several mechanisms, including induction of accelerated metabolism of ATRA, decreased bioavailability and plasma drug levels, point mutations in the ATRA-binding domain of promyelocytic leukemia (PML)-retinoic acid receptor-alpha (RARα) and other molecular events have been proposed to explain ATRA resistance. In this review, the molecular mechanisms of ATRA-induced myeloid cell differentiation and resistance are discussed, together with novel clinical approaches to overcome ATRA resistance in APL. *(Turk J Hematol 2009; 26: 47-61)*

Key words: Acute promyelocytic leukemia, all-trans-retinoic acid, therapy, resistance, histone deacetylase, arsenic, metabolism

Received: June 5, 2008 *Accepted: November 11, 2008*

Özet

Son 10 yıl içerisinde, all-trans retinoik asidin (ATRA) bir akut miyeloid lösemi (AML) tipi olan akut promiyelositik lösemi (APL) tedavisinde kullanılmaya başlanması, kanser tedavisinde kökten değişiklik yapmış ve diferansiyasyon tedavisi kavramının ortaya çıkmasına neden olmuştur. A vitamininin (retinol) fizyolojik bir metaboliti olan ATRA, APL'li hastaların yaklaşık %90'ında tam klinik remisyonları (KR) indükler. Sitotoksik kemoterapötiklerin tersine, ATRA, APL'li hastalarda kemik iliği

Address for Correspondence: Bülent Özpolat, M.D., Ph.D., Anderson Cancer Center, Department of Experimental Therapeutics, Unit 422 1515 Holcombe Blvd. Houston, TX 77030 USA Phone: (713)563-0166 Fax: (713)796-1731 E-mail: bozpolat@mdanderson.org

hipoplazisi oluşumunu veya sık oluşan ölümcül hemorajik sendromların alevlenmesini önleyerek, seçici bir şekilde promiyelositik lösemik hücrelerin normal granülositlere terminal diferansiyasyonunu indükler. Buna rağmen, sadece ATRA tarafından indüklenen remisyonlar geçicidir ve çoğunlukla hastalar tedaviye direnç kazanırlar, bu da pek çok hastada hastalığın nüksüne neden olur; bu nedenle, ATRA' nın tek ajan olarak kullanımı sınırlı hale gelir. Bu nedenle, ATRA halen antrasiklin bazlı kemoterapi ile kombine haldedir ve bu rejim sadece kemoterapi kullanımı ile karşılaştırıldığında, hastaların yaklaşık %70'ini iyileştirerek, hasta sağkalımını önemli ölçüde arttırmaktadır. Buna rağmen, APL hastalarının halen %30'unda hastalık nüks etmekte ve 5 yıl içerisinde ölüm gerçekleşmektedir. Son zamanlarda, arsenik trioksit (As2O3)' in, sadece ATRA tedavisi ve klasik kemoterapiden sonra nükseden APL hastalarında değil, aynı zamanda, primer APL hastalarında da KR'leri indüklemede yüksek oranda etkili olduğu ispatlanmıştır. ATRA'nın yazılı kanıtlara dayanan klinik etkinliğine rağmen, ATRA direncinin gelişmesinden sorumlu olan moleküler mekanizmalar tam olarak anlaşılmamıştır. İnvitro ve klinik gözlemlere dayanarak, ATRA'nın hızlanmış metabolizmasının indüksiyonu, azalmış olan biyoyararlanım ve plazma ilaç düzeyleri dahil çeşitli mekanizmalar, PML-RARα'nın ATRA-bağlayan domain yapısında nokta mutasyonu ve diğer moleküler olaylar ATRA direncini açıklamak üzere öne sürülmüştür. Bu derlemede, APL'de ATRA direncinin üstesinden gelmek için, ATRA ile-indüklenmiş miyeloid hücre farklılaşmasının, direncin ve yeni klinik yaklaşımların moleküler mekanizmalarını ele alacağım. *(Turk J Hematol 2009; 26: 47-61)*

Anahtar kelimeler: Akut promiyelositik lösemi, all-trans retinoik asit, tedavi, direnç, histon deasetilaz, arsenik, metabolizma

Geliş tarihi: 05 Haziran 2008 Kabul tarihi: 11 Kasım 2008

Introduction

Undifferentiated phenotype is a common feature of cancer cells and is often associated with progressive disease and bad prognosis. Failure to terminally differentiate into mature blood cells or differentiation arrest at early steps of maturation is a major feature of acute myeloid leukemias (AML). Current standard chemotherapy cures only 30% of the AML patients, while about 70% of AML patients die to disease in five years, suggesting that alternative treatment strategies are required to cure these patients and increase patient survival.

Differentiation therapy is based on the concept that immature leukemia progenitor cells can be forced to differentiate into a more mature or terminally differentiated phenotype by using differentiation-inducing agents. Differentiation therapy holds promise as an alternative or complement to standard chemotherapy. This type of treatment has the advantage of being potentially less toxic than standard chemotherapy. Treatment of acute promyelocytic leukemia (APL) with retinoic acid (RA) is the first model of differentiation therapy, and it has proven extremely successful in inducing clinical remission (CR) in most patients. All-trans-retinoic acid (ATRA) can selectively induce terminal differentiation of promyelocytic leukemic cells into normal granulocytes without causing bone marrow hypoplasia or exacerbation of the frequently occurring fatal hemorrhagic syndromes associated with chemotherapy. Thus, ATRA-induced differentiation of promyelocytic cells provides an excellent in vitro model for studying myeloid cell differentiation. Although development of quick resistance to the differentiation therapy is commonly observed, when combined with chemotherapy, this therapy can dramatically increase patient survival by enhancing the efficacy of chemotherapy.

Acute Promyelocytic Leukemia and Differentiation Therapy

Acute promyelocytic leukemia (APL), a M3 type of AML based on French-American-British (FAB) classification, is uniquely sensitive to undergo terminal differentiation by differentiation-inducing agents, such as retinoids (i.e., ATRA, 9-cis-RA), phorbol ester, vitamin D, and dimethylsulfoxide As_2(subscript)O_3(subscript) (DMSO). Therefore, APL represents an excellent model for studying differentiation of normal and myeloid leukemia cells.

APL, which represents 10-15% of all AML, is characterized by chromosomal translocations fusing retinoic acid receptor-alpha (RARα) gene on chromosome 17 and one of four different genes, including promyelocytic leukemia (PML), promyelocytic zinc finger (PLZF), nucleophosmin (NPM), nuclear matrix associated (NuMA), or signal transducer and activator of transcription 5b (Stat5b) gene [1-5]. The most common form of translocations is t(15,17) (q22,q21) encoding PML-RARα (Figure 1) and t(11,17)(q23,q21) encoding PLZF-RARα fusion receptor proteins, found in 99% and >1% of APL patients, respectively [6,7]. The translocations are usually reciprocal chromosomal translocations, leading to creation of reciprocal hybrid receptor proteins (X-RARα and RARα-X). APLs expressing

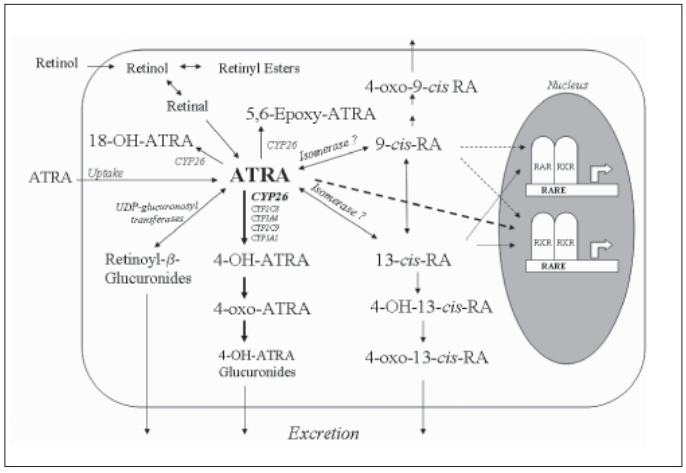


Figure 1. Oncogenic PML-RARα receptor proteins expressed in APL due to chromosomal translocation t(15;17). Chromosomal translocations involve retinoic acid receptor alpha (RARα) gene on chromosome 17 and either promyelocytic leukemia (PML) gene. Breakpoints may vary in the PML gene; however, it is always located in the same point in the RARα gene

PML-RARα, NPM-RARα or NuMA-RARα are responsive to ATRA-induced differentiation effects, with the exception of PLZF-RARα type APL, which is resistant to ATRA [8-11].

Retinoids and All-Trans-Retinoic Acid

Retinoids are a family of molecules that are structurally related to retinol (vitamin A), and are known to play a critical role in many physiological functions, such as cell proliferation, differentiation, apoptosis, homeostasis, reproduction, and fetal development [12]. Retinol is absorbed from the diet in the form of retinyl-esters or β-carotene and stored in the liver as retinyl palmitate.

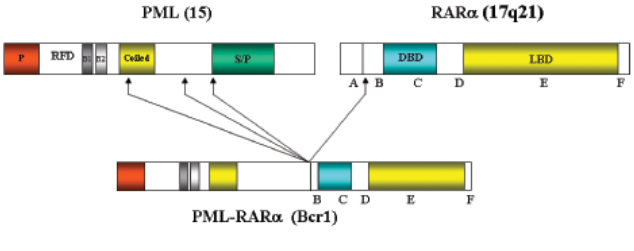
All-trans-retinoic acid (ATRA, tretinoin), 9-cis-RA, 13-cis-RA, isotretinoin), and retinal are physiologic or synthetic derivatives of retinol [13]. Even though only a small percent of retinol and β-carotene are converted to ATRA and 9-cis-RA, they are ~100- to ~1000-fold more potent than other natural retinoids. Retinol, ATRA and 13-cis-RA are found in the human plasma at levels of ~ 2 μM, ~8 nM and ~5 nM, respectively, and can induce differentiation of PML cells.

Modulation of Biologic Effects of Retinoids Through Nuclear Receptors

Retinoid receptors belong to a superfamily of ligand-inducible transcription factors including steroid, vitamin D, thyroid hormone, peroxisome proliferator-activated receptor, and orphan receptors with unknown functions [14]. Two classes of nuclear RARs and retinoid X receptor (RXR), each consisting of three isotypes (α, β and γ) encoded by separate genes and their isoforms (e.g., α1, α2, β1- β4, γ1 or γ2), have been identified and discussed in great detail in recent reviews [14,15].

RARs and RXRs contain different domains, A through F, with diverse functions (Figure 2A). A and B domains located at the amino terminal of each particular receptor contain isoform-specific, ligand-independent transactivation functions, AF-1 (14). These receptors bind to retinoic acid response elements (RARE) through a conserved DNA binding domain (C domain) containing ZF motifs [14]. Ligands (retinoids) bind to a ligand binding domain (LBD) or E domain at the C-terminus of the receptors that contain sequences involved in dimerization of the receptors, ligand-dependent transactivation (AF-2), and translocation to the nucleus [16]. The functions for F and D domains have not been clearly defined.

The complex diversity and pleiotropic effects in the retinoid signaling pathway are provided not only due to existence of multiple isoforms of RARs but also as a result of combinations of RAR-RXR heterodimers or homodimers and the presence of different ligands [14,17]. The RARs can be transcriptionally activated by binding to either ATRA or 9-cis-RA; however, RXRs can be activated only by 9-cis-RA and not by 13-cis-RA or ATRA. 13-cis-RA, a stereoisomer of ATRA, shows a lower affinity for RARs and RXRs (Figure 2) [18]. Upon ligand binding, activated nuclear receptors that bind to RAREs found in the upstream sequences (promoters) of RA responsive genes lead to transcription of the target genes. RARα plays a major role in ATRA-induced differentiation in HL-60 myeloid cells [19,20].



Translocation	Frequency	Fusion protein	Reciprocal translocation	ATRA response
t(15;17)	98%	PML-RARα	yes	yes
t(11;17)	>1%	PLZF-RARα	yes	no
t(5;17)	>1%	NPM-RARα	yes	yes
t(11;17)	>1%	NuMA-RARα	no	yes
t(17,17)	>1%	Stat1b-RAR α	yes	no

Figure 2. A. Retinoid nuclear receptors in normal cells. ATRA and its isomers (9-cis-RA and 13-cis-RA) bind ligand binding domain for trans-activation of the target genes. **B.** Receptor fusion proteins due to the translocation t(15;17). t(15:17) lead to expression of three different PML-RAR alpha isoforms

However, RXRα mediates induction of apoptosis in the same cell line by ATRA or 9-cis-RA [20]. ATRA treatment of APL cells induces expression of RARα mRNA, suggesting that ATRA can also modulate its own receptor, RARα, in addition to differentiation-related genes [21]. The availability of the retinoid ligands to its cognate receptors can be determined by the level of presence of certain non-receptor proteins, such as cytoplasmic RA-binding proteins and heat shock proteins [22]. Moreover, isoforms of PML-RAR may alter the retinoid signaling with or without ligand binding (Figure 2B).

Pathogenesis of Acute Promyelocytic Leukemia

PML-RARα fusion receptor protein is expressed at high levels in APL blasts and interferes with the physiologic functions of PML and RARα proteins, exerting a dominant negative effect [1,23]. Expression of the PML-RARα fusion receptor protein blocks differentiation of myeloid precursor cells at the promyelocytic stage, leading to accumulation of immature hematopoietic cells in the bone marrow [24-26]. It was also shown that overexpression of dominant negative or wild type RARα causes a differential block at the promyelocytic stage [27]. Recently, transgenic mice expressing PML-RARα had a block at the promyelocytic stage of myeloid maturation in blast cells, implicating the important role of PML-RARα abnormal receptor protein in leukemogenesis [26,28,29].

PML is involved in the regulation of proliferation and apoptosis [30,31]. Cells lacking PML are resistant to apoptosis by gamma irradiation, grow faster and have longer survival time, while cells overexpressing PML undergo apoptosis by the same stimulus [30,32]. It was shown that PML is located in the nucleus of normal cells in punctuate nuclear structures (PODs) or nuclear bodies associated with nuclear matrix; however, in PML-RARα-positive APL cells, localization and the normal pattern of nuclear bodies are disrupted [24,33,34]. Overall data suggest that disruption of PML function has been proposed to contribute to the APL pathogenesis [24,35].

Molecular Basis of ATRA Therapy in APL

ATRA induces differentiation of immature leukemic blasts into terminally differentiated granulocytic cells, which is associated with CRs [8,9,35]. ATRA-induced differentiation of APL blasts requires expression of PML-RARα receptor protein [11]. PML-RARα can heterodimerize with RXR or form homodimers and subsequently binds to RARE, located in the promoters of the ATRA-responsive target genes. ATRA can bind to PML-RARα with an affinity comparable to RARα. In the absence of ligand, RAR-RXR in normal blasts and PML-RARα-RXR heterodimers in APL cells recruit nuclear co-repressor proteins, NCoR or silencing mediator of retinoid and thyroid hormone receptor (SMRT), and Sin3A or Sin3B, which in turn form a complex with histone deacetylase enzymes (HDAC1 or HDAC2), resulting in transcriptional repression or silencing [36-38] (Figure 3A and B). The transcriptional suppression occurs because deacylation of histone protein creates conformational changes, limiting access and binding of transcription factors and RNA polymerase to related genes (Figure 3A) [39]. At physiologic concentrations of ATRA (10^{-9}-10^{-8} M), the NCoR protein and HDAC complex are dissociated from RARα in normal blasts, which in turn results in recruitment of co-activators with histone acetyltransferase (HAT) activity, such as steroid receptor coactivator-1 (SRC-1), PCAF, p300/CBP, ACTR, TIF2 or P/CIP [40-42]. Acetylation of lysine residues in the N-terminal of histones by HAT activity results in transactivation of responsive genes leading to differentiation. However, the physiologic concentration of ATRA does not cause dissociation of NCoR protein and HDAC complex from the PML-RARα fusion receptors in APL blasts, leading to differentiation block (Figure 3B). The CoR complex is dissociated from PML-RARα at only pharmacological concentrations (10^{-7} - 10^{-6} M) of ATRA, resulting in removal of transcriptional repression and transcription of genes related to differentiation [38-43].

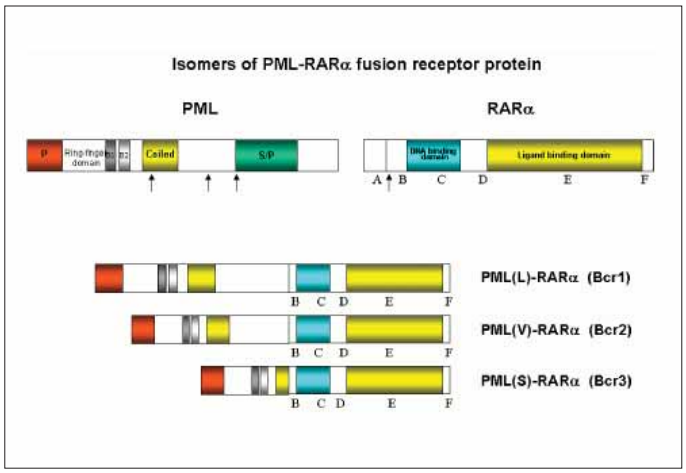


Figure 3. Molecular mechanisms causing transcriptional repression and differentiation block in APL. Nuclear co-repressor proteins, N-CoR or SMRT, and Sin3A or Sin3B, form a complex with histone deacetylase enzymes (HDAC1 or HDAC2), resulting in transcriptional repression or silencing. HDAC activity causes deacylation of histone protein, causing conformational changes, which in turn prevent transcription of target genes. Ac: Acetylated histones

In addition to release of transcriptional repression, the other possible mechanisms involved in ATRA effectiveness in myeloid cell differentiation include expression of different classes of genes including induction of expression of $p21^{WAF1/Cip1}$ cyclin-dependent kinase inhibitor [44], upregulation of C/EBP-γ,β, and ε [45], interferon regulatory factor-1 (IRF-1) [46], and regulation of the localization of PODs [47]. In APL cells isolated from patients, ATRA upregulates expression of RARα at mRNA and protein levels [48,49], whereas it causes the degradation of PML-RARα [50-52]. Therefore, the ratio of RAR/RXR to PML-RARα would be higher, which helps in overcoming the dominant negative effects of PML-RARα protein.

Resistance to Differentiation Therapy

ATRA therapy (45 mg/m^2/day) induces complete remission in 72%-95% of APL patients through induction of differentiation of immature promyelocytic blast cells into mature granulocytes, which subsequently undergo apoptosis [53-56]. The success of ATRA in the induction of complete remission in APL patients represents the first differentiation therapy in cancer and now constitutes a front-line treatment in combination with chemotherapy [54-55].

Unfortunately, resistance to ATRA treatment was encountered in the early clinical trials [56-59]. Later clinical studies demonstrated that ATRA as a single agent can not maintain remission and almost all APL patients routinely relapse within three months to one year [54,55,60-63]. The resistance is acquired rapidly in most cases within 1-3 months of ATRA [57]. Therefore, ATRA-induced CR is now combined with chemotherapy (i.e. anthracyclines) [54,55,62].

Pharmacokinetic studies showed that chronic oral administration of ATRA results in progressive decline in plasma drug concentrations, which associates with early relapses and resistance to ATRA in APL patients [61,64-66]. Plasma levels of ATRA, which usually start to decline as early as one week from the initiation of ATRA therapy, probably result in decreased intracellular ATRA levels below effective pharmacological concentration [67,68]. The higher ATRA plasma concentration correlates with lower peripheral blast count in APL patients [67]. The reduction in plasma levels after administration of ATRA has been observed in other species such as monkeys and mice [69,70]. However, this phenomenon is not seen with ATRA isomers such as 9-cis-RA and 13-cis-RA, suggesting that ATRA uptake and metabolism are different from its isomers [67]. Recently, it was shown that higher intracellular concentration of ATRA correlates with ATRA-induced differentiation of APL cells, indicating the importance of keeping ATRA at levels that support differentiation [71,72].

Relapsing patients were shown to be resistant to higher doses of ATRA, and doubling the initial ATRA dose failed to induce CR and to maintain stable plasma ATRA concentrations [61]. In addition, APL cells isolated from patients at the time of relapse were sensitive to ATRA (10^{-6} M) in vitro [61]. However, the response to ATRA was found to be decreased in vitro sensitivity in half of the cases in terms of induction of differentiation [57,65]. Interestingly, it was observed that acquired resistance to ATRA may be reversible after discontinuation of the ATRA therapy and patients may gain

sensitivity to ATRA, usually in 6 months to 24 months, suggesting that ATRA resistance is reversible [57,65,73,74].

After in vitro and clinical experiences with ATRA over a decade, the following mechanisms involved in development of the drug resistance have been proposed (Figure 4): 1) induction of accelerated metabolism of ATRA; 2) increased expression of cellular retinoic acid binding proteins (CRABPs); 3) constitutive degradation of PML-RARα; 4) point mutations in the LBD of RARα of PML-RARα; 5) P-glycoprotein expression; 6) transcriptional repression by HDAC activity; 7) isoforms of PML-RARα; 8) persistent telomerase activity; 9) expression of type II transglutaminase; and 10) topoisomerase II activity.

1. Accelerated ATRA metabolism

The major pathway for ATRA inactivation is the oxidative metabolism by microsomal cytochrome P450 isoenzyme system that is initiated by the 4-hydroxylation of ATRA to form 4-hydroxy-RA and 4-oxo-RA (Figure 5) [75-78]. Chronic oral administration of ATRA results in autoinduction of ATRA metabolism by cytochrome P450-dependent enzymes, leading to progressive reduction in plasma ATRA concentrations, which may be the most important mechanism for development of resistance to therapy. The decrease in peak plasma levels of ATRA is associated with urinary excretion of 4-oxo-ATRA, which is found to be increased about 10-fold during the continuous ATRA treatment, suggesting that decreased plasma levels of ATRA may not be due to impairment in the gastrointestinal uptake of the drug [61]. In vitro and in vivo studies with cytochrome P450 inhibitors (ketoconazole and liarozole), which suppress ATRA metabolism, resulted in increased plasma levels and delayed ATRA plasma clearance in animals and humans, thus further supporting this hypothesis [79-81].

Recently, a novel human p450 enzyme (CYP26) with specific RA 4-hydroxilase activity, was cloned from zebrafish, mouse and human [82-85]. CYP 26, which is rapidly inducible by ATRA, is expressed in tissues, including liver, kidney, lung, placenta, skin, and intestinal cells [82,84,86]. ATRA-induced expression of CYP26 was also shown in some human tumors such as hepatocellular carcinoma cell line, non-small cell lung carcinoma, breast cancer cells, as well as myeloblastic and PML cells [84,86-88]. The expression of full-length human cDNA for CYP26 in transfected cells closely correlated with the accumulation of 4-hydroxy-RA and 4-oxo-RA, the major metabolic products of ATRA [84]. CYP26 metabolizes ATRA into 4-hydroxy-ATRA, 4-oxo-ATRA, 18-hydroxy-ATRA and polar metabolites in F9 cells [89]. CYP26 was shown to be highly specific for the hydroxylation of ATRA but not for the hydroxylation of 13-cis RA or 9-cis-RA [87]. Several studies demonstrated that the expression of CYP26 is regulated by RARs and RXRs, suggesting a feedback loop mechanism for the regulation of ATRA levels [86,87,89,90]. We found that pharmacological doses of ATRA induce acute expression of CYP26 mRNA in myeloid (HL-60) and PML (NB4) cells. Its expression in these cells is regulated solely by RARα type receptor, indicating the existence of substrate-mediated control of ATRA metabolism [86]. The induction of CYP 26 expression in response to ATRA treatment is reversible and dependent on the continuous presence of ATRA, since the expression returned to baseline after withdrawal of the ATRA [86]. These studies suggested that ATRA-induced CYP26 expression might be responsible for the accelerated metabolism of ATRA, leading to decreased sensitivity and acquired resistance to ATRA in APL patients.

Intracellular levels of ATRA are strictly controlled through regulation of synthesis, metabolism and probably uptake. CYP26 is highly inducible and specific for hydroxylation of ATRA; thus, it might be the most important in the P450 enzyme system for the regulation of plasma and intracellular levels of ATRA. It has been shown that CYP1A1, CYP2C8, CYP2C9 and CYP3A4 in microsomes of human liver cells were able to hydroxylate ATRA, but none of these enzymes at protein and mRNA levels were inducible by ATRA and have low specificity for ATRA [91,92].

It is likely that the metabolic fate of ATRA after continuous administration is determined by the induction of CYP26 in

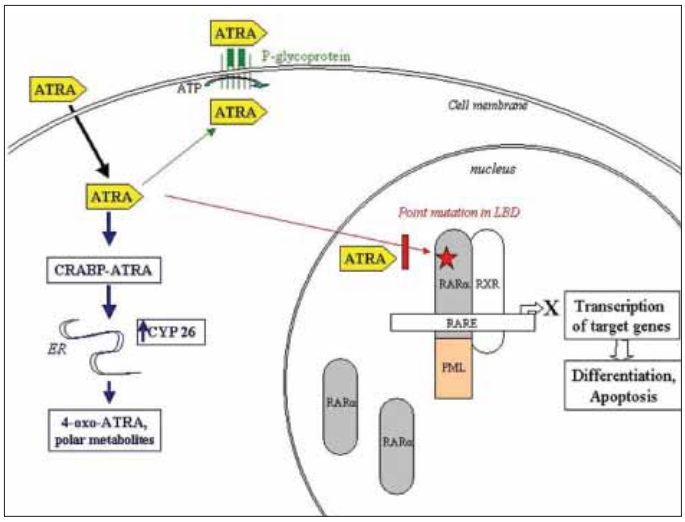


Figure 4. Metabolic pathways leading to inactivation of ATRA. P450-mediated metabolism is the major pathway for inactivation of ATRA

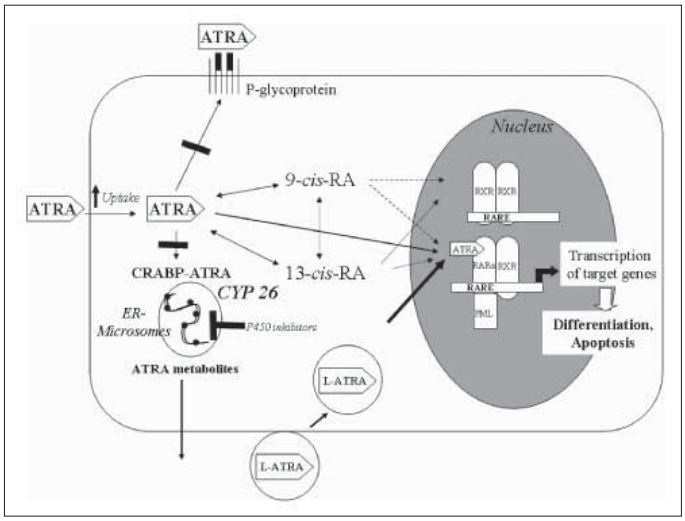


Figure 5. Possible mechanisms involved in development of ATRA resistance. Selective P450 inhibitors and liposomal ATRA may circumvent metabolic pathways and mechanisms involved in accelerated elimination of ATRA

leukemia and other metabolically active tissues such as the liver, intestine and skin. Following ATRA treatment, increased CYP26 activity in PML cells may reduce intracellular ATRA concentrations below the level that does not support differentiation, thus leading to ATRA resistance.

2. Increased cellular RA binding proteins (CRABPs)

In the cytoplasm, ATRA is bound by CRABPs I and II. CRABPs, which are conserved in vertebrates, are high affinity proteins for ATRA [93,94]. CRABP-I is expressed in almost all types of cells, whereas CRABP-II is expressed mainly in the skin. The difference in tissue expression patterns suggest that CRABP-I and CRABP-II have distinct functions in ATRA-mediated responses [95-97]. Early studies linked CRABPs with metabolism and regulation of cytoplasmic levels of ATRA. Therefore, the other possible explanation for progressive decline in the plasma levels of ATRA after continuous therapy with the drug may be the induction of CRABPs. It has been proposed that high levels of CRABP may sequester intracellular ATRA, resulting in decreased drug levels in plasma as well as in normal bone marrow and APL cells [57,65,98,99].

It was shown that the rate of ATRA metabolism in F9 teratocarcinoma but not in transfected CV-1 and COS-7 cells correlates with the expression levels of CRABP-I, suggesting that CRABP-I regulates the metabolism of ATRA depending on cell type [22,100]. Boylan et al. [100] also showed that increased CRABP-I expression resulted in decreased sensitivity of F9 cells to ATRA-induced differentiation, suggesting that this molecule functions as a regulator of intracellular ATRA levels by delivering ATRA to microsomes, facilitating catabolism, and/or by sequestering ATRA (Figure 4). CRABP molecules have been shown to be present not only in cytoplasm but also in the nucleus, suggesting that CRABPs may function to deliver ATRA to the nuclear retinoid receptors. It is also possible that CRABPs may be involved in transcriptional activation or inhibition of RARs [22,100]. Dong et al. [101] showed that expression of CRABP-II, but not CRABP-I, significantly induced RAR-mediated transcriptional activation of a reporter gene, indicating that CRABP-II indeed may be involved in transcriptional activity of ATRA. Recent studies in breast cancer and APL cell lines showed that CRABP-II associates with RARα and RXRα complex in a ligand-independent manner [102]. CRABP-II may function as a transcriptional regulator of ATRA signaling by binding RARE on the target genes as part of the receptor complex [101-103].

Increasing levels of CRABP-II were shown in normal and leukemia cells of APL patients undergoing ATRA treatment [57]. The investigators found that CRABP-II reached maximum levels after three months of continuous ATRA treatment and its levels decreased within a month after ATRA withdrawal. In relapsing patients, high levels of CRABP-II were detected in APL cells but not before ATRA therapy, suggesting that in a hypermetabolic state, excess CRABP might bind ATRA and prevent drug transport to the nucleus [57]. CRABP might also act as a transporter to the microsomes in the endoplasmic reticulum (ER) where ATRA is metabolized. Recently, Zhou et al. [104], however, found no difference between CRABP II levels in pretreatment and at the time of relapse in APL patients. Constitutive expression of CRABP II implicates that it may not be related to ATRA resistance in APL patients. Interestingly, CRABP-I/II knockout mice did not show significant phenotype difference or signs of toxicity, indicating that these proteins may not play an important role in the regulation of ATRA metabolism and signaling [105].

9-cis-RA and 13-cis-RA have stable plasma concentrations after continuous administration. This might be due to their lower affinity for CRABP compared with ATRA, which has progressive reduction of plasma levels with continuous treatment. The other possibility might be that these isomers do not induce specific p450 enzymes as ATRA does.

3. Mutations at the ligand binding domain of RARα

Leukemic blasts isolated from some ATRA-resistant APL patients are less sensitive or completely resistant to ATRA and 9-cis RA-mediated differentiations in vitro, suggesting that ATRA resistance mechanisms may involve selection of ATRA-resistant clones [106]. Point mutations in the LBD (E-domain) of RARα in HL-60 cells and LBD of RARα of PML-RARα fusion protein in NB4 cells can be induced by prolonged culture in the presence of ATRA; thus, this point mutation leads to ATRA resistance [19,107-110]. Shao et al. [108] identified a point mutation located at the amino acid 398, L398P (leucine replaced by proline), in LBD of PML-RARα in an ATRA-resistant NB4 clone (NB4-R4). The mutant receptor does not bind ATRA, but was able to bind RXRα and RARE, expressing dominant negative activity (Figure 4). They also found that pharmacological doses of ATRA could not dissociate the co-repressor SMRT from mutant PML-RARα, preventing expression of ATRA-responsive target genes.

Recently, point mutations leading to amino acid substitution in the E-domain (LBD) of RARα of PML-RARα fusion receptor protein were also detected in APL cells isolated from relapsing patients [111-113]. The mutations were absent before ATRA treatment. Imaizumi et al. [112] reported acquisition of missense mutations of G815A or A889 in a sequence of RARα cDNA, leading to amino acid replacement of R272Q (arginine to glutamine) and M297L (methionine to leucine) in RARα of PML-RARα. The mutations found in APLs isolated from two patients at the time of relapse, exhibiting ATRA resistance, were localized to the middle region of the E-domain. However, mutations detected in ATRA-resistant HL-60 and NB4 subclones are located at the carboxyl-terminal of the E-domain [108,114]. Furthermore, site-directed mutagenesis at A272 of RARα has been shown to inhibit binding of ATRA to RARα [115]. Recently, Marasca et al. [116], also reported that although no mutation could be detected before the onset of ATRA treatment, point mutations in LBD of PML-RARα in two relapsed patients were observed, confirming previous findings. Ding et al. [111] found mutations in PML-RARα in APL blasts of 3 of 12 patients following ATRA treatment. The mutations were located at codons 290 (L290V), 394 (R394W), and 413 (M413T).

These mutations interfere with ATRA binding activity and result in dominant negative function leading to resistant state and providing growth advantage of APL blasts carrying the mutation. Currently, the percent of ATRA-resistant patients having these mutations is not known. Therefore, studies with

larger numbers of patients are required to clarify the clinical importance of these mutations.

4. Constitutive degradation of PML-RARα

Expression of PML-RARα has been linked to initial ATRA sensitivity [24]. NB4 cells expressing dominant negative PML-RARα are resistant to ATRA and failed to upregulate tissue transglutaminase II expression [108]. Expression of the PML-RARα protein in U937 cells enhanced the sensitivity to RA-induced differentiation [24]. These results suggested the biological function for PML-RARα to transactivate differentiation-related genes that are critical for therapeutic response of ATRA in APL.

ATRA therapy was shown to induce degradation of PML-RARα through the action of proteosome, likely by caspases 3-like activity in APL cells isolated from patients and NB4 as well as U937 myeloid precursor cells expressing PML-RARα [50-52,117]. Fanelli et al. [52] demonstrated that ATRA-resistant NB4 subline, which was selected under the selective pressure of ATRA, expresses normal levels of PML-RARα mRNA, but does not express PML-RARα protein. They were able to partially restore ATRA sensitivity in the ATRA-resistant NB4 cells by proteosome inhibitors by blocking the degradation of the fusion receptor protein. Similarly, expression of PML-RARα by retrovirus-mediated transduction resulted in restoration of ATRA sensitivity in ATRA-resistant NB4 cells protein [52]. These results suggested that alterations in the proteosome pathway resulting in constitutive degradation of PML-RARα protein may lead to ATRA resistance, since previous data showed that expression of PML-RARα is critical for ATRA sensitivity in APL cells [52]. Downregulation of PML-RARα by ATRA probably results in reorganization of the PML nuclear bodies. Nervi et al. [117] found that prevention of ATRA-induced degradation of fusion protein by a member of the caspase 3 family did not abolish the ATRA-induced differentiation, suggesting that PML-RARα is involved in ATRA sensitivity of APL cells.

Interestingly, the short isoform of PML-RARα (bcr3-PML-RARα), which is found in about 35% of APL patients, does not contain the caspase cleavage site (Asp522, α-helix, located in PML part) and is not degraded after ATRA treatment [24,115,118,119]. However, these APL patients with the short isoform respond to ATRA, indicating degradation of PML-RARα may not be essential for ATRA-induced differentiation [120]. It is not known if ATRA treatment results in degradation of other ATRA-sensitive variants of APL with NPM-RARα or NuMA-RARα. Whether ATRA-induced degradation of PML-RARα is a cause or result of therapy needs to be clarified.

5. P-glycoprotein expression

P-glycoprotein (P-gp) is a membrane protein functioning as an ATP-dependent drug efflux pump that decreases intracellular accumulation of various lipophilic compounds (Figure 4) [121-124]. P-gp is the product of the multidrug resistance-1 (MDR1) gene that confers drug resistance to a variety of agents. P-gp is overexpressed in a variety of human tumor cells, leading to resistance to chemotherapy [121,123,125]. Therefore, it is possible that increased expression of P-gp results in resistance of APL cells to ATRA by decreasing intracellular ATRA concentrations. It has been shown that expression of P-gp is low in newly diagnosed APL patients, but higher in APL cells isolated from two relapsed ATRA-resistant patients [126]. It was also reported that expression of P-gp in HL-60 was lower when compared to ATRA-resistant HL-60 cells [126]. Moreover, treatment of HL-60 cells with P-gp antagonist (Verapamil) in the presence of ATRA partially restored ATRA resistance in resistant HL-60 and APL cells, implying that P-gp may play a role in ATRA resistance. More importantly, the direct evidence indicating that P-gp is responsible, in part, for acquisition of ATRA resistance in APL cells came from the experiment using ribozymes, which are able to target MDR1 RNA by a catalytic activity. HL-60-resistant cells stably transfected with 196 MDR1 ribozyme showed inhibition in the expression of MDR1 and were able to undergo differentiation and growth inhibition in a dose-dependent manner.

However, Takeshita et al. [127] recently reported that they did not find any difference in the intracellular levels of ATRA between parental (mock-transfected) and MDR1-transfected NB4 cells. They found similar results with APL cells isolated from patients relapsed after ATRA therapy, suggesting that P-gp may not be involved in the development of ATRA resistance [127]. P-gp expression is significantly lower in APL than in other AML cells [128]. This may be an important mechanism providing a biological basis for sensitivity of APL cells to chemotherapy and ATRA when compared to the AMLs.

6. Histone deacetylase (HDAC) activity

APL cells expressing PLZF-RARα receptor fusion protein are resistant to ATRA-induced differentiation [3,28]. Recent findings revealed that the RA-signaling pathway is constitutively repressed by HDAC activity at physiologic levels of ATRA in PLZF-RARα type APL blasts, leading to transcriptional repression/silencing and differentiation block [36,37]. The RARα part of PML-RARα fusion protein has one binding site for NCoR proteins and HDAC complex that is removed by binding of ATRA to PML-RARα/RXR dimer; thus, pharmacological concentrations of ATRA induce differentiation of PML-RARα-positive APL blasts in vitro and in vivo [36]. However, the same effect is not observed in PLZF-RARα-positive APL cells, since PLZF-RARα protein has two NCoR protein binding sites [38,43]. In order to transactivate responsive genes leading to cell differentiation, the removal of both of the CoR complexes from the PLZF-RARα is required. Even though ATRA is able to dissociate NCoR proteins and HDAC complex from RARα of PML-RARα protein, the second CoR proteins and HDAC complex can not be removed. Therefore, while ATRA induces differentiation of PML-RARα-positive APL blasts at pharmacological concentrations, PLZF-RARα-expressing blasts are resistant to ATRA-induced differentiation unless a HDAC inhibitor such as trichostatin A is used [36,43,129,130]. The presence of HDAC inhibitors and ATRA induces significant differentiation in most resistant APL cells with PLZF-RARα [36].

7. The role of PML/RARα isoforms in resistance

Variable breakpoints on the PML gene on chromosome 15 result in expression of distinct PML-RARα isoforms (Figure 1 and 2B) [54,131]. Short (S) isoform (bcr3) is created by a breakpoint

in intron 3, while long (L) isoform (bcr1) results from a breakpoint located in intron 6 of the PML gene, found in 35% and 60% of adult APL patients with t(15,17), respectively [6,132]. The rest of the patients have the other isoform called variable (V), having a breakpoint located in exon 6 of PML [133]. The S isoforms of PML-RARα associate with high white blood cell (WBC) count, M3v type morphology, CD34 and CD2 expression, and secondary cytogenetic abnormalities [118,120,134].

Although no significant correlation between type of PML-RARα isoform and ATRA-induced clinical response was found in most studies, in some studies, patients expressing S type PML-RARα had shorter remission time and poor prognosis with ATRA therapy [135-137]. In vitro treatment of APL blasts from patients with S and L type by ATRA was shown to induce differentiation of these blasts to a similar degree [138]. However, when compared to L isoform, the S isoform has lower binding affinity for ATRA but higher affinity and specificity for 9-cis-RA [6]. Gallagher et al. [138] showed that APL cells from patients with V (Bcr2) isotype have decreased in vitro response to ATRA.

A recently completed clinical study with liposomal ATRA at our institution reported that CR rates were 50% (4 of 8 patients) in the patients with S isoform and about 86% (6 of 7 patients) in patients with L isoform, suggesting that S isoform might play a role in resistance to ATRA [139]. Overall, based on the data available, it is hard to find a clear correlation between the type of PML-RARα isoform and outcome of ATRA therapy.

8. Telomerase Activity

It has been reported that there is a link between decreased telomerase activity and terminal differentiation of some tumor cells, including NB4 cells [140-143]. Nason-Burchenal et al. (144) showed that ATRA-resistant NB4 cells did not have repression in the activity of telomerase after ATRA treatment compared to ATRA-sensitive NB4 cells. However, when ATRA-sensitive and -resistant NB4 cells were treated by phorbol 12-myristate 13-acetate (PMA) and vitamin D3, all cells were induced to differentiate into monocytic cells and telomerase activity markedly declined, suggesting that persistent telomerase activity may be linked to ATRA resistance. This effect might be due to a defective signaling in ATRA-resistant cells, resulting in a block in decreasing telomerase activity.

9. Tissue Transglutaminase Expression

Transglutaminase II (Tgase-II) is a calcium-dependent enzyme that catalyzes an amine incorporation and a cross-linking of proteins. Intracellular Tgase-II was induced when human PML cells (NB4) and fresh leukemia cells were isolated from APL patients treated with RA. It was reported that ATRA induces Tgase-II mRNA in NB4 cells but not in ATRA-resistant NB4 cells or in APL patient cells lacking the t(15,17). This induction correlated with ATRA-induced growth arrest and granulocytic differentiation. ATRA did not induce growth arrest and differentiation and type II Tgase activity in an ATRA-resistant subclone of the NB4 cell line, or in leukemic cells derived from two patients morphologically defined as APL but lacking the t(15,17). ATRA induced expression of Tgase II in U937 cells transfected with PML-RARα but not in untransfected U937 cells, indicating that Tgase expression may be mediated by PML-RARα [145]. ATRA-induced expression of Tgase II in HL-60 cells is mediated by RXRα [20,146]. Induction and expression of Tgase II in HL-60 and other cell types are associated with apoptosis [146]. It is also suggested that Tgase II expression may be related to induction of differentiation, since its expression is an early event in response to ATRA treatment. Therefore, loss of Tgase II induction in resistant cells may be an important factor resulting in resistance to ATRA therapy.

10. Topoisomerase II Activity

Recently, McNamara et al. demonstrated that topoisomerase II beta associates with and negatively modulates RARalpha transcriptional activity and that increased levels of and association with TopoIIbeta cause resistance to RA in APL cell lines [147]. They showed that knockdown of TopoIIbeta could overcome resistance by permitting RA-induced differentiation and increased RA gene expression. Overexpression of TopoIIbeta in clones from an RA-sensitive cell line caused resistance by a reduction in RA-induced expression of target genes and differentiation. Using chromatin immunoprecipitation (CHIP) assays, they also demonstrated that TopoII-beta is bound to an RA response element and that inhibition of TopoII-beta causes hyperacetylation of histone 3 at lysine 9 and activation of transcription, suggesting a novel mechanism of resistance. However, this mechanism needs to be validated in samples from ATRA-resistant patients in terms of frequency and significance.

Potential Treatment Strategies to Overcome ATRA Resistance in APL

1. Liposomal ATRA: New treatment modalities are being investigated to overcome ATRA resistance and to further improve the disease outcome. To circumvent accelerated metabolism of ATRA, liposome incorporated-ATRA, inhibitors

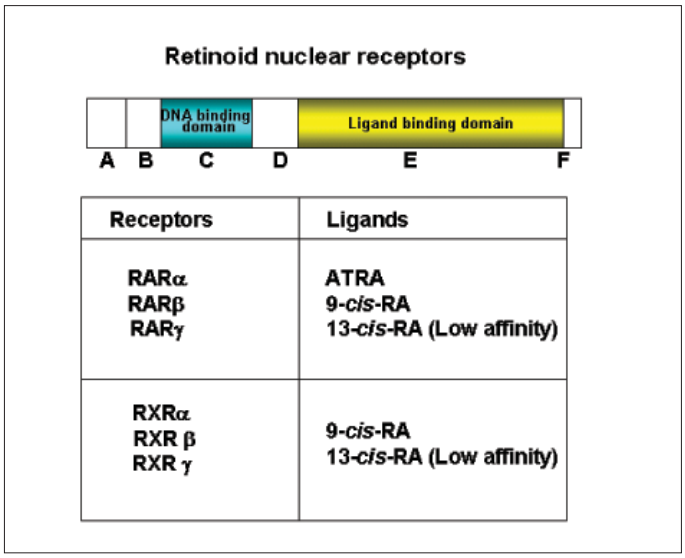


Figure 6. Possible strategies to prevent accelerated metabolism of ATRA. Selective P450 inhibitors and liposomal ATRA may circumvent metabolic pathways and mechanisms involved in accelerated elimination of ATRA

of the cytochrome p450 enzyme system, such as ketoconazole and liarozole, and lower or intermittent doses of ATRA administration have been tested [60,73,74,80,148].

Liposomal ATRA was developed to provide an intravenous (I.V.) formulation to generate potential pharmacological advantages over the oral formulation (Figure 6) [148]. An I.V. administration of liposomal-ATRA was shown to be superior to oral ATRA (non-liposomal) in terms of maintaining higher plasma levels in animal models and in humans [139,149-152]. I.V. administration of liposomal ATRA to rats over a prolonged period (7 weeks) did not cause a decrease in the levels of ATRA in plasma over time [149]. In contrast, chronic oral administration of ATRA (non-liposomal) in rats resulted in decreased drug plasma concentrations after the same period of time. In the same study, liver microsomes isolated from animals that were repeatedly treated with oral ATRA showed a significant increase in metabolism of the drug in vitro. However, microsomes isolated from animals that received I.V. liposomal ATRA the same number of times with the same doses showed that metabolism of the drug was not altered. Similarly, when F9 teratocarcinoma cells were treated with both liposomal and free ATRA, liposomal ATRA was metabolized at a slower rate than non-liposomal ATRA [150]. These results demonstrated that encapsulation of ATRA in liposomes and I.V. administration generate a better pharmacokinetic profile than oral ATRA by circumventing the hepatic metabolism of ATRA. In addition to bypassing the hepatic clearance, liposomal ATRA was shown to distribute in the skin to a lesser extent, which may contribute to maintaining steady and higher ATRA concentrations in the plasma [149].

Evaluation of liposomal ATRA in a phase I trial in patients with refractory hematological malignancies showed that in contrast to the decline in plasma AUC (area under the concentration time curve) of ATRA seen 3 to 4 days after initiation of oral ATRA, there were no differences between the AUC on day 1 and day 15 following liposomal ATRA treatment [151]. In the same study, liposomal ATRA was shown to be safe, and toxicity profiles were similar to those of oral ATRA, although liposomal ATRA produced much higher AUC. I.V. administration of liposomal ATRA (90 mg/m^2) monotherapy was shown to be effective in newly diagnosed APL patients, inducing polymerase chain reaction (PCR)-negative molecular CRs in a high proportion of patients [139,153]. These studies supported the hypothesis that I.V. liposomal administration may improve ATRA activity by altering its pharmacological profile, remaining elevated following extended treatment and providing a basis for long-term therapy in APL.

2. Arsenic trioxide (As_2O_3): Arsenic compounds, which have been used for more than 500 years in traditional Chinese medicine, have been shown to be highly effective in the treatment of APL. Arsenic alone induces CRs in about 90% of APL patients with t(15,17) [154,155]. More importantly, arsenic induces CRs not only in de novo APL patients but also in patients with relapses after ATRA/chemotherapy who have become resistant to these drugs [154-158]. Recently, arsenic trioxide was approved by the Food and Drug Administration (FDA) for APL patients who relapsed or failed to respond to standard therapy. Although arsenic is extremely effective especially in ATRA-resistant APL patients, its moderate toxic effects need to be further investigated.

In vitro and in vivo studies showed that arsenic triggers apoptosis at high concentrations (0.5-2.0 μM) and induces differentiation at low concentrations (0.1-0.5 μM) in APL cells [159,160]. No cross-resistance has been observed between ATRA and arsenic. Arsenic induces degradation of PML-RARα and endogenous PML and enhances acetylation of histones [160,161]. Arsenic- induced apoptosis might be mediated by down regulation of Bcl-2 and upregulation of death associated protein (DAP5/p86) that leads to activation of caspase 1 and 3 and PDCD4 [162-164] Arsenic has been effective in t(11,17) type APL expressing PLZF-RARα in a mouse model. Studies suggested that the mechanism of effect of As_2O_3 on PML is different from that of ATRA. As_2O_3 shows antitumoral activity in APL cells that do not harbor t(15;17), a variety of hematologic cancer cell lines including chronic myeloid leukemia (CML) (that is resistant to other agents), multiple myeloma, lymphoma, chronic lymphocytic leukemia (CLL), acute lymphoblastic leukemia (ALL), and megakaryocytic leukemia.

A recent trial using I.V. arsenic in patients with relapsed or refractory APL showed that 70% of patients achieved molecular remission and most of them stayed disease free CR in the 16-month follow-up [155]. Although toxicity and serious side effects of arsenic were reported in the same study, these effects were not permanent and did not cause interruption of therapy. Another study reported that the CR rate induced by arsenic was 90% in APL patients who relapsed after ATRA-based therapy [154]. More importantly, recent clinical studies suggested that combination therapy (ATRA and As_2O_3) was more effective at prolonging survival than either drug alone, suggesting that combination of ATRA and As_2O_3 acts synergistically [162].

3. Histone deacylase inhibitors: Induction of differentiation of ATRA-resistant APL with PLZF-RARα, using combination of pharmacological dose of ATRA and HDAC inhibitors (TSA or sodium phenylbutyrate) opened a new avenue in the treatment of not only APL but also AML1-ETO AML [36]. Although butyrate was the first identified HDAC inhibitor, it is not specific

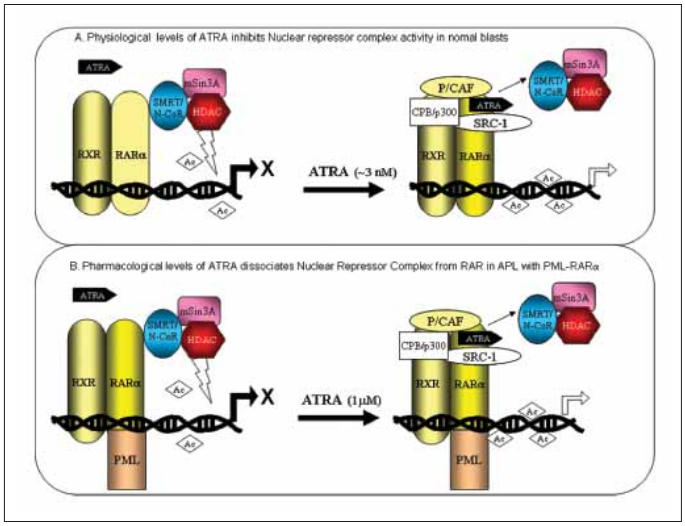


Figure 7. A. Physiological levels of ATRA inhibits nuclear repressor complex activity in normal blasts.
B. Pharmacological levels of ATRA dissociates nuclear repressor complex from RAR in APL with PML-RARα

for HDAC [165]. Trichostatin A and trapoxin are more specific and potent HDAC inhibitors [165,166]. The major problem regarding the use of these non-specific HDAC inhibitors might be side effects because of changing chromatin structure in cells other than leukemia.

4. Others

Am-80, a synthetic retinoid, has been successful in relapsed APL patients previously treated with ATRA, inducing CR in about 60% of patients [167,168]. However, in addition 4-HPR [169], 1,25-dihydroxyvitamin D3 [170,171] and K_2 in combination with ATRA [172] have been shown to be effective in ATRA-resistant APL cell lines inducing differentiation. Recently, 3-hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) were shown to have anti-leukemic activity against leukemia cells. Simvastatin was found to be the most active statin in the family and induced cytotoxic potency against HL-60 cells [173]. Combination of RA and tumor necrosis factor can overcome the maturation block in a variety of RA-resistant acute PML cells [174], suggesting that combination with RA can enhance the potency of the other drug or induce additional pathways that cannot be triggered in resistant cells by ATRA alone. In collaboration with Dr. Michael Danilenko, we demonstrated that combination with rosemary extract or its active compound carnosic acid can enhance ATRA-induced differentiation effects in NB4 and HL60 and resistant APL cells (Dr. Ozpolat-unpublished findings).

Conclusions/Future Prospects

Although the use of ATRA has greatly improved the treatment of APL, rapid development of ATRA resistance limits its use as a single agent. Therefore, understanding the mechanisms involved in acquired ATRA resistance and designing new therapeutic strategies would significantly improve the rate and long-term maintenance of CR in APL patients. Combination of ATRA with chemotherapy is currently the mainstay therapy in APL. In conclusion, designing drugs with favorable plasma pharmacokinetics and without exhibiting resistance and side effects will be the main goal of future studies for developing successful therapeutic strategies. New strategies based on our understanding of the fate of ATRA in patients with APL will facilitate the development of non-toxic and effective therapeutic modalities.

References

1. Kakizuka A, Miller WH, Umesono K, Warrell RP Jr, 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;66:663-74.
2. Larson RA, Kondo K, Vardiman JW, Butler AE, Golomb HM, Rowley JD. Evidence for a 15,17 translocation in every patient with acute promyelocytic leukemia. Am J Med 1984;76:827-41.
3. Lin RJ, Egan DA, Evans RM. Molecular genetics of acute promyelocytic leukemia. Trends Genet 1999;15:179-84.
4. 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.
5. 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.
6. Slack JL, Gallagher RE. The molecular biology of acute promyelocytic leukemia. Cancer Treat Res 1999;99:75-124.
7. Kalantry S, Delva L, Gaboli M, Gandini D, Giorgio M, Hawe N, He LZ, Peruzzi D, Rivi R, Tribioli C, Wang ZG, Zhang H, Pandolfi PP. Gene rearrangements in the molecular pathogenesis of acute promyelocytic leukemia. J Cell Physiol 1997;173:288-96.
8. Breitman TR, Collins SJ, Keene BR. Terminal differentiation of human promyelocytic leukemic cells in primary culture in response to retinoic acid. Blood 1981;57:1000-4.
9. Warrell RP, Frankel SR, Miller WH, 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). N Engl J Med 1991;324:1385-93.
10. Pandolfi PP. PML, PLZF and NPM genes in the molecular pathogenesis of acute promyelocytic leukemia. Haematologica 1996;81:472-82.
11. Slack JL. Biology and treatment of acute progranulocytic leukemia. Curr Opin Hematol 1999;6:236-40.
12. Lotan R. Effects of vitamin A and its analogs (retinoids) on normal and neoplastic cells. Biochim Biophys Acta 1980;605:33-91.
13. Lotan R. Vitamin A analogs (retinoids) as biological response modifiers. Prog Clin Biol Res 1988;259:261-71.
14. Chambon P. A decade of molecular biology of retinoic acid receptors. FASEB J 1996;10:940-54.
15. Pemrick SM, Lucas DA, Grippo JF. The retinoid receptors. Leukemia 1994;8 (Suppl 3):S1-10.
16. Nagpal S, Saunders M, Kastner P, Durand B, Nakshatri H, Chambon P. Promoter context- and response element-dependent specificity of the transcriptional activation and modulating functions of retinoic acid receptors. Cell 1992;70:1007-19.
17. Nagpal S, Friant S, Nakshatri H, Chambon P. RARs and RXRs. Evidence for two autonomous transactivation functions (AF-1 and AF-2) and heterodimerization in vivo. EMBO J 1993;12:2349-60.
18. Allenby G, Janocha R, Kazmer S, Speck J, Grippo JF, Levin AA. Binding of 9-cis-retinoic acid and all-trans-retinoic acid to retinoic acid receptors alpha, beta, and gamma. Retinoic acid receptor gamma binds all-trans-retinoic acid preferentially over 9-cis-retinoic acid. J Biol Chem 1994;269:16689-95.
19. Collins SJ, Robertson KA, Mueller L. Retinoic acid-induced granulocytic differentiation of HL-60 myeloid leukemia cells is mediated directly through the retinoic acid receptor (RAR-alpha). Mol Cell Biol 1990;10:2154-63.
20. Mehta K, McQueen T, Neamati N, Collins S, Andreeff M. Activation of retinoid receptors RAR alpha and RXR alpha induces differentiation and apoptosis, respectively, in HL-60 cells. Cell Growth Differ 1996;7:179-86.
21. Melnick A, Licht JD. Deconstructing a disease. RARalpha, its fusion partners, and their roles in the pathogenesis of acute promyelocytic leukemia. Blood 1999;93:3167-215.
22. Boylan JF, Gudas LJ. Overexpression of the cellular retinoic acid binding protein-I (CRABP- I) results in a reduction in differentiation-specific gene expression in F9 teratocarcinoma cells. J Cell Biol 1991;112:965-79.
23. Rousselot P, Hardas B, Patel A, Guidez, F., Gaken, J., Castaigne, S., Dejean, A., de The, H., Degos, L., and Farzaneh, F. The PML-RAR alpha gene product of the t(15,17) translocation inhibits retinoic acid-induced granulocytic differentiation and mediated transactivation in human myeloid cells. Oncogene 1994;9:545-51.

24. Grignani F, Ferrucci PF, Testa U, Talamo G, Fagioli M, Alcalay M, Mencarelli A, Grignani F, Peschle C, Nicoletti I. The acute promyelocytic leukemia-specific PML-RAR alpha fusion protein inhibits differentiation and promotes survival of myeloid precursor cells. Cell 1993;74:423-31.
25. Perez A, Kastner P, Sethi S, Lutz Y, Reibel C, Chambon P. PMLRAR homodimers. Distinct DNA binding properties and heteromeric interactions with RXR. EMBO J 1993;12:3171-82.
26. Brown D, Kogan S, Lagasse E, Weissman I, Alcalay M, Pelicci PG, Atwater S, Bishop JM. A PMLRARalpha transgene initiates murine acute promyelocytic leukemia. Proc Natl Acad Sci U S A 1997;94:2551-6.
27. Tsai S, Bartelmez S, Heyman R, Damm K, Evans R, Collins SJ. A mutated retinoic acid receptor-alpha exhibiting dominant-negative activity alters the lineage development of a multipotent hematopoietic cell line. Genes Dev 1992;6:2258-69.
28. 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.
29. He LZ, Tribioli C, Rivi R, Peruzzi D, Pelicci PG, Soares V, Cattoretti G, Pandolfi PP. Acute leukemia with promyelocytic features in PML/RARalpha transgenic mice. Proc Natl Acad Sci U S A 1997;94:5302-7.
30. Wang ZG, Ruggero D, Ronchetti S, Zhong S, Gaboli M, Rivi R, Pandolfi PP. PML is essential for multiple apoptotic pathways. Nat Genet 1998;20:266-72.
31. Wang ZG, Delva L, Gaboli M, Rivi R, Giorgio M, Cordon-Cardo C, Grosveld F, Pandolfi PP. Role of PML in cell growth and the retinoic acid pathway. Science 1998;279:1547-51.
32. Quignon F, De Bels F, Koken M, Feunteun J, Ameisen JC, de Thé H. PML induces a novel caspase-independent death process. Nat Genet 1998;20:259-65.
33. Dyck JA, Warrell RP Jr, Evans RM, Miller WH Jr. Rapid diagnosis of acute promyelocytic leukemia by immunohistochemical localization of PML/RAR-alpha protein. Blood 1995;86:862-7.
34. Hodges M, Tissot C, Howe K, Grimwade D, Freemont PS. Structure, organization, and dynamics of promyelocytic leukemia protein nuclear bodies. Am J Hum Genet 1998;63:297-304.
35. Kogan SC, Bishop JM. Acute promyelocytic leukemia: from treatment to genetics and back. Oncogene 1999;18:5261-7.
36. 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.
37. Grunstein M. Histone acetylation in chromatin structure and transcription. Nature 1997;389:349-52.
38. Heinzel T, Lavinsky RM, Mullen TM, Söderstrom M, Laherty CD, Torchia J, Yang WM, Brard G, Ngo SD, Davie JR, Seto E, Eisenman RN, Rose DW, Glass CK, Rosenfeld MG. A complex containing N-CoR, mSin3 and histone deacetylase mediates transcriptional repression. Nature 1997;387:43-8.
39. Kouzarides T. Histone acetylases and deacetylases in cell proliferation. Curr Opin Genet Dev 1999;9:40-8.
40. Collingwood TN, Urnov FD, Wolffe AP. Nuclear receptors: coactivators, corepressors and chromatin remodeling in the control of transcription. J Mol Endocrinol 1999;23:255-75.
41. Glass CK, Rosenfeld MG, Rose DW, Kurokawa R, Kamei Y, Xu L, Torchia J, Ogliastro MH, Westin S. Mechanisms of transcriptional activation by retinoic acid receptors. Biochem Soc Trans 1997;25:602-5.
42. Glass CK, Rose DW, Rosenfeld MG. Nuclear receptor coactivators. Curr Opin Cell Biol 1997;9:222-32.
43. Nagy L, Kao HY, Chakravarti D, Lin RJ, Hassig CA, Ayer DE, Schreiber SL, Evans RM. Nuclear receptor repression mediated by a complex containing SMRT, mSin3A, and histone deacetylase. Cell 1997;89:373-80.
44. Casini T, Pelicci PG. A function of p21 during promyelocytic leukemia cell differentiation independent of CDK inhibition and cell cycle arrest. Oncogene 1999;18:3235-43.
45. Morosetti R, Park DJ, Chumakov AM, Grillier I, Shiohara M, Gombart AF, Nakamaki T, Weinberg K, Koeffler HP. (A novel, myeloid transcription factor, C/EBP epsilon, is upregulated during granulocytic, but not monocytic, differentiation. Blood 1997;90:2591-600.
46. Pelicano L, Li F, Schindler C, Chelbi-Alix MK. Retinoic acid enhances the expression of interferon-induced proteins. Evidence for multiple mechanisms of action. Oncogene 1997;15:2349-59.
47. Weis K, Rambaud S, Lavau C, Jansen J, Carvalho T, Carmo-Fonseca M, Lamond A, Dejean A. Retinoic acid regulates aberrant nuclear localization of PML-RAR alpha in acute promyelocytic leukemia cells. Cell 1994;76:345-56.
48. Agadir A, Cornic M, Jerome M, Menot ML, Cambier N, Gaub MP, Gourmel B, Lefebvre P, Degos L, Chomienne C. Characterization of nuclear retinoic acid binding activity in sensitive leukemic cell lines: cell specific uptake of ATRA and RAR alpha protein modulation. Biochem Biophys Res Commun 1995;213:112-22.
49. Chomienne C, Balitrand N, Ballerini P, Castaigne S, de Thé H, Degos L. All-trans retinoic acid modulates the retinoic acid receptor-alpha in promyelocytic cells. J Clin Invest 1991;88:2150-4.
50. Raelson JV, Nervi C, Rosenauer A, Benedetti L, Monczak Y, Pearson M, Pelicci PG, Miller WH. The PML/RAR alpha oncoprotein is a direct molecular target of retinoic acid in acute promyelocytic leukemia cells. Blood 1996;88:2826-32.
51. 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.
52. Fanelli M, Minucci S, Gelmetti V, Nervi C, Gambacorti-Passerini C, Pelicci PG. Constitutive degradation of PML/RARalpha through the proteasome pathway mediates retinoic acid resistance. Blood 1999;93:1477-81.
53. Huang ME, Ye YC, Chen SR, Zhao JC, Gu LJ, Cai JR, Zhao L, Xie JX, Shen ZX, Wang ZY. All-trans retinoic acid with or without low dose cytosine arabinoside in acute promyelocytic leukemia. Report of 6 cases. Chin Med J (Engl) 1987;100:949-53.
54. Lo CF, Nervi C, Avvisati G, Mandelli F. Acute promyelocytic leukemia: a curable disease. Leukemia 1998;12:1866-80.
55. Fenaux P, Chomienne C, Degos L. All-trans retinoic acid and chemotherapy in the treatment of acute promyelocytic leukemia. Semin Hematol 2001;38:13-25.
56. Warrell RP, Maslak P, Eardley A, Heller G, Miller WH, Frankel SR. Treatment of acute promyelocytic leukemia with all-trans retinoic acid: an update of the New York experience. Leukemia 1994;8:929-33.
57. Delva L, Cornic M, Balitrand N, Guidez F, Miclea JM, Delmer A, Treillet F, Fenaux P, Castaigne S, Degos L, Chomienne C. Resistance to all-trans retinoic acid (ATRA) therapy in relapsing acute promyelocytic leukemia: study of in vitro ATRA sensitivity and cellular retinoic acid binding protein levels in leukemic cells. Blood 1993;82:2175-81.
58. Castaigne S, Chomienne C, Daniel MT, Ballerini P, Berger R, Fenaux P, Degos L. All-trans retinoic acid as a differentiation therapy for acute promyelocytic leukemia. I. Clinical results. Blood 1990;76;1704-9.
59. Fenaux P, Le Deley MC, Castaigne S, Archimbaud E, Chomienne C, Link H, Guerci A, Duarte M, Daniel MT, Bowen D, Huebner

G, Bauters F, Fegueux N, Fey M, Sanz M, Lowenberg B, Maloisel F, Auzanneau G, Sadoun A, Gardin C, Bastion Y, Ganser A, Jacky E, Dombret H, Chastang C, Degos L. Effect of all transretinoic acid in newly diagnosed acute promyelocytic leukemia. Results of a multicenter randomized trial. European APL 91 Group. Blood 1993;82:3241-9.
60. Castaigne S, Degos L. Treatment of acute promyelocytic leukemia by all trans retinoic acid. C R Seances Soc Biol Fil 1995;189:515-20.
61. Muindi J, Frankel SR, Miller WH, Jakubowski A, Scheinberg DA, Young CW, Dmitrovsky E, Warrell RP Jr. Continuous treatment with all-trans retinoic acid causes a progressive reduction in plasma drug concentrations: implications for relapse and retinoid "resistance" in patients with acute promyelocytic leukemia. Blood 1992;79:299-303.
62. Tallman MS. Therapy of acute promyelocytic leukemia: all-trans retinoic acid and beyond. Leukemia 1998;12 (Suppl 1):S37-S40.
63. Warrell RP. Clinical and molecular aspects of retinoid therapy for acute promyelocytic leukemia. Int J Cancer 1997;70:496-7.
64. Muindi JR, Young CW, Warrell RP. Clinical pharmacology of all-trans retinoic acid. Leukemia 1994;8:1807-12.
65. Cornic M, Delva L, Castaigne S, Lefebvre P, Balitrand N, Degos L, Chomienne C. In vitro all-trans retinoic acid (ATRA) sensitivity and cellular retinoic acid binding protein (CRABP) levels in relapse leukemic cells after remission induction by ATRA in acute promyelocytic leukemia. Leukemia 1994;8:914-7.
66. Adamson PC. All-Trans-Retinoic Acid Pharmacology and Its Impact on the Treatment of Acute Promyelocytic Leukemia. Oncologist 1996;1:305-14.
67. Lefebvre P, Thomas G, Gourmel B, Agadir A, Castaigne S, Dreux C, Degos L, Chomienne C.. Pharmacokinetics of oral all-trans retinoic acid in patients with acute promyelocytic leukemia. Leukemia 1991;5:1054-8.
68. Lazzarino M, Regazzi MB, Corso A. Clinical relevance of all-trans retinoic acid pharmacokinetics and its modulation in acute promyelocytic leukemia. Leuk Lymphoma 1996;23:539-43.
69. Kalin JR, Starling ME, Hill DL. Disposition of all-trans-retinoic acid in mice following oral doses. Drug Metab Dispos 1981;9:196-201.
70. Kraft JC, Slikker W Jr, Bailey JR, Roberts LG, Fischer B, Wittfoht W, Nau H. Plasma pharmacokinetics and metabolism of 13-cis- and all-trans-retinoic acid in the cynomolgus monkey and the identification of 13-cis- and all-trans-retinoyl-beta-glucuronides. A comparison to one human case study with isotretinoin. Drug Metab Dispos 1991;19:317-24.
71. Agadir A, Cornic M, Lefebvre P, Gourmel B, Balitrand N, Degos L, Chomienne C. Differential uptake of all-trans retinoic acid by acute promyelocytic leukemic cells: evidence for its role in retinoic acid efficacy. Leukemia 1995;9:139-45.
72. Agadir A, Cornic M, Lefebvre P, Gourmel B, Jérôme M, Degos L, Fenaux P, Chomienne C.. All-trans retinoic acid pharmacokinetics and bioavailability in acute promyelocytic leukemia: intracellular concentrations and biologic response relationship. J Clin Oncol 1995;13:2517-23.
73. Adamson PC, Bailey J, Pluda J, Poplack DG, Bauza S, Murphy RF, Yarchoan R, Balis FM. Pharmacokinetics of all-trans-retinoic acid administered on an intermittent schedule. J Clin Oncol 1995;13:1238-41.
74. Atiba JO, Manzardo AM, Thiruvengadam R, Schell MJ, Meyskens FL. Restoration of oral all-trans retinoic acid bioavailability after a brief drug holiday. Am J Ther 1997;4:134-40.
75. Roberts AB, Nichols MD, Newton DL, Sporn MB. In vitro metabolism of retinoic acid in hamster intestine and liver. J Biol Chem 1979;254:6296-302.
76. Zile MH, Inhorn RC, DeLuca HF. Metabolism in vivo of all-trans-retinoic acid. J Biol Chem 1982;257:3544-50.
77. Roos TC, Jugert FK, Merk HF, Bickers DR. Retinoid metabolism in the skin. Pharmacol Rev 1998;50:315-33.
78. Sonneveld E, van der Saag PT. Metabolism of retinoic acid: implications for development and cancer. Int J Vitam Nutr Res 1998;68:404-10.
79. Miller VA, Rigas JR, Muindi JR, Tong WP, Venkatraman E, Kris MG, Warrell RP. Modulation of all-trans retinoic acid pharmacokinetics by liarozole. Cancer Chemother Pharmacol 1994;34:522-6.
80. Rigas JR, Francis PA, Muindi JR, Kris MG, Huselton C, DeGrazia F, Orazem JP, Young CW, Warrell RP Jr. Constitutive variability in the pharmacokinetics of the natural retinoid, all-trans-retinoic acid, and its modulation by ketoconazole. J Natl Cancer Inst 1993;85:1921-6.
81. Van Wauwe J, Van Nyen G, Coene MC, Stoppie P, Cools W, Goossens J, Borghgraef P, Janssen PA. Liarozole, an inhibitor of retinoic acid metabolism, exerts retinoid-mimetic effects in vivo. J Pharmacol Exp Ther 1992;261:773-9.
82. Ozpolat B, Tari AM, Lopez-Berestein G. Regulation of a highly specific retinoic Acid-Metabolizing Cytochrome P450RAI-1 (CYP26A1) and auto-induced metabolism of ATRA in human, Intestinal, Liver, Endothelial and Acute Promyelocytic Leukemia Cells. Leukemia & Lymphoma, 2005;46:1497-506
83. White JA, Guo YD, Baetz K, Beckett-Jones B, Bonasoro J, Hsu KE, Dilworth FJ, Jones G, Petkovich M. Identification of the retinoic acid-inducible all-trans-retinoic acid 4- hydroxylase. J Biol Chem 1996;271:29922-7.
84. White JA, Beckett-Jones B, Guo YD, Dilworth FJ, Bonasoro J, Jones G, Petkovich M. cDNA cloning of human retinoic acid-metabolizing enzyme (hP450RAI) identifies a novel family of cytochromes P450. J Biol Chem 1997;272:18538-41.
85. Fujii H, Sato T, Kaneko S, Gotoh O, Fujii-Kuriyama Y, Osawa K, Kato S, Hamada H.. Metabolic inactivation of retinoic acid by a novel P450 differentially expressed in developing mouse embryos. EMBO J 1997;16:4163-73.
86. Ozpolat B, Mehta K, Zapata-Benevides P, Tari AM, Lopez-Berestein G. All-trans-retinoic acid (ATRA) induces expression of cytochrome p450RAI (CYP26AI) in human intestinal, liver and acute promyelocytic cells. A potential mechanism contributing to increased ATRA metabolism. Blood 96: 722a-722a. 11-16-2000 (Abstract).
87. Sonneveld E, van den Brink CE, van der Leede BM, Schulkes RK, Petkovich M, van der BB, van der Saag PT. Human retinoic acid (RA) 4-hydroxylase (CYP26) is highly specific for all-trans-RA and can be induced through RA receptors in human breast and colon carcinoma cells. Cell Growth Differ 1998;9:629-37.
88. Lampen A, Meyer S, Arnhold T, Nau H. Metabolism of vitamin A and its active metabolite all-trans-retinoic acid in small intestinal enterocytes. J Pharmacol Exp Ther 2000;295:979-85.
89. Abu-Abed SS, Beckett BR, Chiba H, Chithalen JV, Jones G, Metzger D, Chambon P, Petkovich M. Mouse P450RAI (CYP26) expression and retinoic acid-inducible retinoic acid metabolism in F9 cells are regulated by retinoic acid receptor gamma and retinoid X receptor alpha. J Biol Chem 1998;273:2409-15.
90. Marikar Y, Wang Z, Duell EA, Petkovich M, Voorhees JJ, Fisher GJ. Retinoic acid receptors regulate expression of retinoic acid 4-hydroxylase that specifically inactivates all-trans retinoic acid in human keratinocyte HaCaT cells. J Invest Dermatol 1998;111:434-9.
91. Martini R, Murray M. Participation of P450 3A enzymes in rat hepatic microsomal retinoic acid 4-hydroxylation. Arch Biochem Biophys 1993;303:57-66.
92. McSorley LC, Daly AK. Identification of human cytochrome P450 isoforms that contribute to all-trans-retinoic acid 4-hydroxylation. Biochem Pharmacol 2000;60:517-26.
93. Harrison EH, Blaner WS, Goodman DS, Ross AC. Subcellular localization of retinoids, retinoid-binding proteins, and acyl-CoA:retinol acyltransferase in rat liver. J Lipid Res 1987;28:973-81.

94. Donovan M, Olofsson B, Gustafson AL, Dencker L, Eriksson U. The cellular retinoic acid binding proteins. J Steroid Biochem Mol Biol 1995;53:459-65.
95. Zheng WL, Ong DE. Spatial and temporal patterns of expression of cellular retinol-binding protein and cellular retinoic acid-binding proteins in rat uterus during early pregnancy. Biol Reprod 1998;58:963-70.
96. Yamamoto M, Drager UC, Ong DE, McCaffery P. Retinoid-binding proteins in the cerebellum and choroid plexus and their relationship to regionalized retinoic acid synthesis and degradation. Eur J Biochem 1998;257:344-50.
97. Wardlaw SA, Bucco RA, Zheng WL, Ong DE. Variable expression of cellular retinoic acid binding proteins. Biol Reprod 1997;56:125-32.
98. Adamson PC, Boylan JF, Balis FM, Murphy RF, Godwin KA, Gudas LJ, Poplack DG. Time course of induction of metabolism of all-trans-retinoic acid and the up-regulation of cellular retinoic acid-binding protein. Cancer Res 1993;53:472-6.
99. Cornic M, Delva L, Guidez F, Balitrand N, Degos L, Chomienne C. Induction of retinoic acid-binding protein in normal and malignant human myeloid cells by retinoic acid in acute promyelocytic leukemia patients. Cancer Res 1992;52:3329-34.
100. Boylan JF, Gudas LJ. The level of CRABP-I expression influences the amounts and types of all-trans-retinoic acid metabolites in F9 teratocarcinoma stem cells. J Biol Chem 1992;267:21486-91.
101. Dong D, Ruuska SE, Levinthal DJ, Noy N. Distinct roles for cellular retinoic acid-binding proteins I and II in regulating signaling by retinoic acid. J Biol Chem 1999;274:23695-8.
102. Delva L, Bastie JN, Rochette-Egly C, Kraïba R, Balitrand N, Despouy G, Chambon P, Chomienne C. Physical and functional interactions between cellular retinoic acid binding protein II and the retinoic acid-dependent nuclear complex. Mol Cell Biol 1999;19:7158-67.
103. Jing Y, Waxman S, Lopez R. The cellular retinoic acid binding protein II is a positive regulator of retinoic acid signaling in breast cancer cells. Cancer Res 1997;57:1668-72.
104. Zhou DC, Hallam SJ, Lee SJ, Klein RS, Wiernik PH, Tallman MS, Gallagher RE. Constitutive expression of cellular retinoic acid binding protein II and lack of correlation with sensitivity to all-trans retinoic acid in acute promyelocytic leukemia cells. Cancer Res 1998;58:5770-6.
105. Lampron C, Rochette-Egly C, Gorry P, Dollé P, Mark M, Lufkin T, LeMeur M, Chambon P. Mice deficient in cellular retinoic acid binding protein II (CRABPII) or in both CRABPI and CRABPII are essentially normal. Development 1995;121:539-48.
106. Miller WH, Jakubowski A, Tong WP, Miller VA, Rigas JR, Benedetti F, Gill GM, Truglia JA, Ulm E, Shirley M, Warren RP Jr. 9-cis retinoic acid induces complete remission but does not reverse clinically acquired retinoid resistance in acute promyelocytic leukemia. Blood 1995;85:3021-7.
107. Robertson KA, Emami B, Collins SJ. Retinoic acid-resistant HL-60R cells harbor a point mutation in the retinoic acid receptor ligand-binding domain that confers dominant negative activity. Blood 1992;80:1885-9.
108. Shao W, Benedetti L, Lamph WW, Nervi C, Miller WH. A retinoid-resistant acute promyelocytic leukemia subclone expresses a dominant negative PML-RAR alpha mutation. Blood 1997;89:4282-9.
109. Rosenauer A, Raelson JV, Nervi C, Eydoux P, DeBlasio A, Miller WH Jr. Alterations in expression, binding to ligand and DNA, and transcriptional activity of rearranged and wild-type retinoid receptors in retinoid-resistant acute promyelocytic leukemia cell lines. Blood 1996;88:2671-82.
110. 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.
111. Ding W, Li YP, Nobile LM, Grills G, Carrera I, Paietta E, Tallman MS, Wiernik PH, Gallagher RE. Leukemic cellular retinoic acid resistance and missense mutations in the PML-RARalpha fusion gene after relapse of acute promyelocytic leukemia from treatment with all-trans retinoic acid and intensive chemotherapy. Blood 1998;92:1172-83.
112. Imaizumi M, Suzuki H, Yoshinari M, Sato A, Saito T, Sugawara A, Tsuchiya S, Hatae Y, Fujimoto T, Kakizuka A, Konno T, Iinuma K. Mutations in the E-domain of RAR portion of the PML/RAR chimeric gene may confer clinical resistance to all-trans retinoic acid in acute promyelocytic leukemia. Blood 1998;92:374-82.
113. 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.
114. Li YP, Said F, Gallagher RE. Retinoic acid-resistant HL-60 cells exclusively contain mutant retinoic acid receptor-alpha. Blood 1994;83:3298-302.
115. Lamour FP, Lardelli P, Apfel CM. Analysis of the ligand-binding domain of human retinoic acid receptor alpha by site-directed mutagenesis. Mol Cell Biol 1996;16:5386-92.
116. 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.
117. Nervi C, Ferrara FF, Fanelli M, Rippo MR, Tomassini B, Ferrucci PF, Ruthardt M, Gelmetti V, Gambacorti-Passerini C, Diverio D, Grignani F, Pelicci PG, Testi R. Caspases mediate retinoic acid-induced degradation of the acute promyelocytic leukemia PML/RARalpha fusion protein. Blood 1998;92:2244-51.
118. Gallagher RE, Willman CL, Slack JL, Andersen JW, Li YP, Viswanatha D, Bloomfield CD, Appelbaum FR, Schiffer CA, Tallman MS, Wiernik PH. Association of PML-RAR alpha fusion mRNA type with pretreatment hematologic characteristics but not treatment outcome in acute promyelocytic leukemia: an intergroup molecular study. Blood 1997;90:1656-63.
119. Mandelli F. New strategies for the treatment of acute promyelocytic leukaemia. J Intern Med Suppl 1997;740:23-7.
120. Slack JL, Yu M. Constitutive expression of the promyelocytic leukemia-associated oncogene PML-RARalpha in TF1 cells: isoform-specific and retinoic acid- dependent effects on growth, bcl-2 expression, and apoptosis. Blood 1998;91:3347-56.
121. Hamada H, Tsuruo T. Functional role for the 170- to 180-kDa glycoprotein specific to drug-resistant tumor cells as revealed by monoclonal antibodies. Proc Natl Acad Sci U S A 1986;83:7785-9.
122. Ueda K, Cornwell MM, Gottesman MM, Pastan I, Roninson IB, Ling V, Riordan JR. The mdr1 gene, responsible for multidrug-resistance, codes for P-glycoprotein. Biochem Biophys Res Commun 1986;141:956-62.
123. Chen CJ, Chin JE, Ueda K, Clark DP, Pastan I, Gottesman MM, Roninson IB. Internal duplication and homology with bacterial transport proteins in the mdr1 (P-glycoprotein) gene from multidrug-resistant human cells. Cell 1986;47:381-9.
124. Gerlach JH, Kartner N, Bell DR, Ling V. Multidrug resistance. Cancer Surv 1986;5:25-46.
125. Chauncey TR. Drug resistance mechanisms in acute leukemia. Curr Opin Oncol 2001;13:21-6.
126. Kizaki M, Ueno H, Yamazoe Y, Shimada M, Takayama N, Muto A, Matsushita H, Nakajima H, Morikawa M, Koeffler HP, Ikeda Y. Mechanisms of retinoid resistance in leukemic cells: possible role of cytochrome P450 and P-glycoprotein. Blood 1996;87:725-33.

127. Takeshita A, Shinjo K, Naito K, Ohnishi K, Sugimoto Y, Yamakawa Y, Tanimoto M, Kitamura K, Naoe T, Ohno R. Role of P-glycoprotein in all-trans retinoic acid (ATRA) resistance in acute promyelocytic leukaemia cells: analysis of intracellular concentration of ATRA. Br J Haematol 2000;108:90-2.
128. Paietta E, Andersen J, Racevskis J, Gallagher R, Bennett J, Yunis J, Cassileth P, Wiernik PH. Significantly lower P-glycoprotein expression in acute promyelocytic leukemia than in other types of acute myeloid leukemia: immunological, molecular and functional analyses. Leukemia 1994;8:968-73.
129. He LZ, Merghoub T, Pandolfi PP. In vivo analysis of the molecular pathogenesis of acute promyelocytic leukemia in the mouse and its therapeutic implications. Oncogene 1999;18:5278-92.
130. Lin RJ, Nagy L, Inoue S, Shao W, Miller WH Jr, Evans RM. Role of the histone deacetylase complex in acute promyelocytic leukaemia. Nature 1998;391:811-4.
131. Grignani F, Fagioli M, Alcalay M, Longo L, Pandolfi PP, Donti E, Biondi A, Lo Coco F, Grignani F, Pelicci PG. Acute promyelocytic leukemia: from genetics to treatment. Blood 1994;83:10-25.
132. Slack JL, Arthur DC, Lawrence D, Mrózek K, Mayer RJ, Davey FR, Tantravahi R, Pettenati MJ, Bigner S, Carroll AJ, Rao KW, Schiffer CA, Bloomfield CD. Secondary cytogenetic changes in acute promyelocytic leukemia-prognostic importance in patients treated with chemotherapy alone and association with the intron 3 breakpoint of the PML gene: a Cancer and Leukemia Group B study. J Clin Oncol 1997;15:1786-95.
133. Slack JL, Willman CL, Andersen JW, Li YP, Viswanatha DS, Bloomfield CD, Tallman MS, Gallagher RE. Molecular analysis and clinical outcome of adult APL patients with the type V PML-RARalpha isoform: results from intergroup protocol 0129. Blood 2000;95:398-403.
134. Guglielmi C, Martelli MP, Diverio D, Fenu S, Vegna ML, Cantù-Rajnoldi A, Biondi A, Cocito MG, Del Vecchio L, Tabilio A, Avvisati G, Basso G, Lo Coco F. Immunophenotype of adult and childhood acute promyelocytic leukaemia: correlation with morphology, type of PML gene breakpoint and clinical outcome. A cooperative Italian study on 196 cases. Br J Haematol 1998;102:1035-41.
135. Vahdat L, Maslak P, Miller WH, Eardley A, Heller G, Scheinberg DA, Warrell RP. Early mortality and the retinoic acid syndrome in acute promyelocytic leukemia: impact of leukocytosis, low-dose chemotherapy, PMN/RAR-alpha isoform, and CD13 expression in patients treated with all-trans retinoic acid. Blood 1994;84:3843-9.
136. Borrow J, Goddard AD, Gibbons B, Katz F, Swirsky D, Fioretos T, Dubé I, Winfield DA, Kingston J, Hagemeijer A, Rees JKH, Lister A and Solomon E. Diagnosis of acute promyelocytic leukaemia by RT-PCR: detection of PML-RARA and RARA-PML fusion transcripts. Br J Haematol 1992;82:529-40.
137. Huang W, Sun GL, Li XS, Cao Q, Lu Y, Jang GS, Zhang FQ, Chai JR, Wang ZY. Waxman S, Chen Z, Chen SJ. Acute promyelocytic leukemia: clinical relevance of two major PML-RAR alpha isoforms and detection of minimal residual disease by retrotranscriptase/polymerase chain reaction to predict relapse. Blood 1993;82:1264-9.
138. Gallagher RE, Li YP, Rao S, Paietta E, Andersen J, Etkind P, Bennett JM, Tallman MS, Wiernik PH. Characterization of acute promyelocytic leukemia cases with PML-RAR alpha break/fusion sites in PML exon 6: identification of a subgroup with decreased in vitro responsiveness to all-trans retinoic acid. Blood 1995;86:1540-7.
139. Estey EH, Giles FJ, Kantarjian H, O'Brien S, Freireich EJ, Lopez-Berestein G, Keating M. Molecular remissions induced by liposomal-encapsulated all-trans retinoic acid in newly diagnosed acute promyelocytic leukemia. Blood 1999;94:2230-5.
140. Reichman TW, Albanell J, Wang X, Moore MA, Studzinski GP. Downregulation of telomerase activity in HL60 cells by differentiating agents is accompanied by increased expression of telomerase-associated protein. J Cell Biochem 1997;67:13-23.
141. Albanell J, Han W, Mellado B, Gunawardane R, Scher HI, Dmitrovsky E, Moore MA. Telomerase activity is repressed during differentiation of maturation - sensitive but not resistant human tumor cell lines. Cancer Res 1996;56:1503-8.
142. Zhang W, Piatyszek MA, Kobayashi T, Estey E, Andreeff M, Deisseroth AB, Wright WE, Shay JW. Telomerase activity in human acute myelogenous leukemia: inhibition of telomerase activity by differentiation-inducing agents. Clin Cancer Res 1996;2:799-803.
143. Zhang W, Kapusta LR, Slingerland JM, Klotz LH. Telomerase activity in prostate cancer, prostatic intraepithelial neoplasia, and benign prostatic epithelium. Cancer Res 1998;58:619-21.
144. Nason-Burchenal K, Maerz W, Albanell J, Allopenna J, Martin P, Moore MA, Dmitrovsky E. Common defects of different retinoic acid resistant promyelocytic leukemia cells are persistent telomerase activity and nuclear body disorganization. Differentiation 1997;61:321-31.
145. Benedetti L, Grignani F, Scicchitano BM, Jetten AM, Diverio D, Lo Coco F, Avvisati G, Gambacorti-Passerini C, Adamo S, Levin AA, Pelicci PG, Nervi C.Retinoid-induced differentiation of acute promyelocytic leukemia involves PML-RARalpha-mediated increase of type II transglutaminase. Blood 1996;87:1939-50.
146. Nagy L, Thomazy VA, Shipley GL, Fésüs L, Lamph W, Heyman RA, Chandraratna RA, Davies PJ. Activation of retinoid X receptors induces apoptosis in HL-60 cell lines. Mol Cell Biol 1995;15:3540-51.
147. McNamara S, Wang H, Hanna N, Miller WH Jr. Topoisomerase IIbeta negatively modulates retinoic acid receptor alpha function: a novel mechanism of retinoic acid resistance. Mol Cell Biol. 2008;28:2066-77.
148. Ozpolat B, Lopez-Berestein G, Adamson P, Fu CJ, Williams AH.J Pharmacokinetics of intravenously administered Liposomal all-trans-retinoic acid (RA) and orally administered RA in healthy volunteers. J Pharm and Pharmaceutical Sci. 2003;6:292-301.
149. Mehta K, Sadeghi T, McQueen T, Lopez-Berestein G. Liposome encapsulation circumvents the hepatic clearance mechanisms of all-trans-retinoic acid. Leuk Res 1994;18:587-96.
150. Parthasarathy R, Mehta K. Altered metabolism of all-trans-retinoic acid in liposome-encapsulated form. Cancer Lett 1998;134:121-8.
151. Estey E, Thall PF, Mehta K, Rosenblum M, Brewer T Jr, Simmons V, Cabanillas F, Kurzrock R,Lopez-Berestein G. Alterations in tretinoin pharmacokinetics following administration of liposomal all-trans retinoic acid. Blood 1996;87:3650-4.
152. Lopez-Berestein G, Rosenfeld M, Taraneh S, Mehta K. Pharmakinetics, tissue distribution, and toxicology of tretinoin incorporated in liposomes. J Liposome Res 1994;4:689-700.
153. Douer D, Estey E, Santillana S, Bennett JM, Lopez-Bernstein G, Boehm K, Williams T. Treatment of newly diagnosed and relapsed acute promyelocytic leukemia with intravenous liposomal all-trans retinoic acid. Blood 2001;97:73-80.
154. 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.
155. Soignet SL, Maslak P, Wang ZG, Jhanwar S, Calleja E, Dardashti LJ, Corso D, DeBlasio A, Gabrilove J, Scheinberg DA, Pandolfi PP, Warrell RP Jr. Complete remission after treatment of acute promyelocytic leukemia with arsenic trioxide. N Engl J Med 1998;339:1341-8.

156. 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 (As2O3) in the treatment of acute promyelocytic leukemia. As2O3 induces NB4 cell apoptosis with downregulation of Bcl-2 expression and modulation of PML-RAR alpha/PML proteins. Blood 1996;88:1052-61.
157. Gallagher RE. Arsenic--new life for an old potion. N Engl J Med 1998;339:1389-91.
158. Cai X, Shen YL, Zhu Q, Jia PM, Yu Y, Zhou L, Huang Y, Zhang JW, Xiong SM, Chen SJ, Wang ZY, Chen Z, Chen GQ. Arsenic trioxide-induced apoptosis and differentiation are associated respectively with mitochondrial transmembrane potential collapse and retinoic acid signaling pathways in acute promyelocytic leukemia. Leukemia 2000;14:262-70.
159. Huang XJ, Wiernik PH, Klein RS, Gallagher RE. Arsenic trioxide induces apoptosis of myeloid leukemia cells by activation of caspases. Med Oncol 1999;16:58-64.
160. Niu C, Yan H, Yu T, Sun HP, Liu JX, Li XS, Wu W, Zhang FQ, Chen Y, Zhou L, 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.
161. Chen Z, Chen GQ, Shen ZX, Chen SJ, Wang ZY. Treatment of acute promyelocytic leukemia with arsenic compounds: in vitro and in vivo studies. Semin Hematol 2001;38:26-36.
162. 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/As2O3 combination yields a high quality remission and survival in newly diagnosed acute promyelocytic leukemia. Proc Natl Acad Sci U S A. 2004;101:5328-35
163. Ozpolat B, Harris M, Tirado-Gomez M, Bradbury EM, Chen X, Lopez-Berestein G. All-trans-retinoic acid and arsenic trioxide-induced suppression of translational initiation involves death associated protein 5 (DAP5/p97/NAT1) in leukemia cell differentiation. Apoptosis 2008;4:1-11.
164. Ozpolat B, Akar, U Steiner M, Zorrilla-Calancha, I , SanguinoA, Tirado-Gomez M, Nancy Colburn,Danilenko M, and Lopez Berestein G. Programmed Cell Death 4 (PDCD4) Tumor Suppressor Protein is Involved in Granulocytic Differentiation of Myeloid Leukemia Cells:Mol Cancer Res. 2007;5:95-108
165. Yoshida M, Kijima M, Akita M, Beppu T. Potent and specific inhibition of mammalian histone deacetylase both in vivo and in vitro by trichostatin A. J Biol Chem 1990;265:17174-9.
166. Kijima M, Yoshida M, Sugita K, Horinouchi S, Beppu T. Trapoxin, an antitumor cyclic tetrapeptide, is an irreversible inhibitor of mammalian histone deacetylase. J Biol Chem 1993;268:22429-35.
167. Tobita T, Takeshita A, Kitamura K, Ohnishi K, Yanagi M, Hiraoka A, Karasuno T, Takeuchi M, Miyawaki S, Ueda R, Naoe T, Ohno R. Treatment with a new synthetic retinoid, Am80, of acute promyelocytic leukemia relapsed from complete remission induced by all-trans retinoic acid. Blood 1997;90:967-73.
168. Takeuchi M, Yano T, Omoto E, Takahashi K, Kibata M, Shudo K, Ueda R, Ohno R, Harada M. Re-induction of complete remission with a new synthetic retinoid, Am-80, for relapse of acute promyelocytic leukaemia previously treated with all-trans retinoic acid. Br J Haematol 1997;97:137-40.
169. Taimi M, Breitman TR. N-4-hydroxyphenylretinamide enhances retinoic acid-induced differentiation and retinoylation of proteins in the human acute promyelocytic leukemia cell line, NB4, by a mechanism that may involve inhibition of retinoic acid catabolism. Biochem Biophys Res Commun 1997;232:432-6.
170. Muto A, Kizaki M, Yamato K, Kawai Y, Kamata-Matsushita M, Ueno H, Ohguchi M, Nishihara T, Koeffler HP, Ikeda Y. 1,25-Dihydroxyvitamin D3 induces differentiation of a retinoic acid-resistant acute promyelocytic leukemia cell line (UF-1) associated with expression of p21(WAF1/CIP1) and p27(KIP1). Blood 1999;93:2225-33.
171. Nakajima H, Kizaki M, Ueno H, Muto A, Takayama N, Matsushita H, Sonoda A, Ikeda Y. All-trans and 9-cis retinoic acid enhance 1,25-dihydroxyvitamin D3- induced monocytic differentiation of U937 cells. Leuk Res 1996;20:665-76.
172. Yaguchi M, Miyazawa K, Katagiri T, Nishimaki J, Kizaki M, Tohyama K, Toyama K. Vitamin K2 and its derivatives induce apoptosis in leukemia cells and enhance the effect of all-trans retinoic acid. Leukemia 1997;11:779-87.
173. Tomiyama N, Matzno S, Kitada C, Nishiguchi E, Okamura N, Matsuyama K. The possibility of simvastatin as a chemotherapeutic agent for all-trans retinoic acid-resistant promyelocytic leukemia. Biolog Pharm Bull. 2008;31:369-74.
174. Withcher M, Shiu HY, Guo O, Miller WH Jr. Combination of retinoic acid and tumor necrosis factor overcomes the maturation block in a variety of retinoic acid-resistant acute promyelocytic leukemia cells. Blood. 2004;104:3335-42.