ACUTE MYELOID LEUKEMIA

Acute promyelocytic leukemia: A model of molecular target based therapy

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Abstract
Leukemia, a group of hematological malignancies characterized by clonal expansion of hematopoietic cells with uncontrolled proliferation, decreased apoptosis and blocked differentiation, is one of the most notorious enemies of mankind which accounts for some 300,000 new cases and 222,000 deaths each year worldwide [1–4]. Leukemia can be divided into acute or chronic, lymphoid or myeloid types, based on the disease progression and hematopoietic lineages involved [5]. The responses of leukemia to therapies differ from one type or subtype to another. Hence, to improve the clinical outcome, the therapeutic strategies should be disease pathogenesis-based and individualized. The close collaboration between bench and bedside may not only shed new lights on leukemogenesis, gain insights into therapeutic mechanisms, but also provide opportunities for designing more rational therapies. The development of curative approaches for acute promyelocytic leukemia (APL) may serve as a paradigm [6].

Unveiling APL, a unique subtype of leukemia with ugly head

It took a long time for doctors to know and designate the disease, APL. In 1935, Risak [7] reported a patient with a ‘rapid down hill course and the coincident rise of myelocytes in the peripheral blood.’ In 1955, Cooperberg and Neiman [8] described a case of acute myelogenous leukemia (AML) with fibrinolytic purpura. A similar patient was also described by Pisciotta and Schultz [9]. In 1957, the Swedish author Leif Hillestad [10] reported three AML patients characterized by a very rapid fatal course of only a few weeks duration, a white blood cell picture dominated by promyelocytes, a severe bleeding tendency due to fibrinolysis and thrombocytopenia, and a normal erythrocyte sedimentation rate probably caused by the reduced fibrinogen concentration in the plasma. He mentioned that his cases were identical to those described by Risak [7], Cooperberg and Neiman [8] and Pisciotta and Schultz [9]. He designated this type of AML as acute promyelocytic leukemia (APL) (Box 1), and he concluded that APL ‘seems to be the most malignant form of acute leukaemia’. More detailed features of APL were then described by Bernard et al. [11] and Caen et al. [12].

In 1976, the well characterized morphology of the promyelocytes led the French–American–British (FAB) Nomenclature Committee to assign them the specific classification of M3 cells, and APL was named the M3 type AML (AML M3) [13]. Thereafter, two variants of APL, the hypogranular variant [14] and hyperbasophilic microgranular variant [15], were reported sequentially.

Also in 1976, a consistent chromosomal change, the balanced reciprocal translocation between the long arms of chromosomes 15 and 17 \[t(15;17)(q22;q21)\], was reported by Rowley et al. [16]. Intriguingly, 17q21 was shown to be involved in variants of t(15;17), the t(11;17)(q23;q21) [17], t(5;17)( q35;q21) [18], t(11;17)(q13;q21) [19], and dup(17)(q11;q21) [20] (Figure 1), suggesting 17q21 is important for normal hematopoiesis and disruption of 17q21 is crucial for disease pathogenesis of APL.

So far, APL has been shown to be characterized by three features [6]: the presence of an accumulation of...
abnormal promyelocytes in bone marrow; the occurrence of fibrinogenopenia and disseminated intravascular coagulation that is often worsened by chemotherapy; and the presence of the chromosomal translocation t(15;17)(q22;q21) or variants.

Development of curative therapeutic approaches for APL: From highly fatal to highly curable

The past half century has seen great advances in evolving therapeutic approaches for APL. In the first three decades after being recognized, APL was treated with chemotherapy and was once considered the most devastating subtype of AML. The introduction of all-trans retinoic acid (ATRA) and arsenic trioxide (ATO) by Chinese hematologists since mid 1980s opened a new page in the history of leukemia therapy. ATRA is a derivative of vitamin A which has dramatically improved the complete remission (CR) rate and long-term survival of APL patients [21]. On the other hand, the application of arsenic trioxide (ATO) further improved the clinical outcome of refractory or relapsed APL [22,23]. Intriguingly, a higher quality remission and survival in newly diagnosed APL were achieved when ATRA was combined with ATO as compared to either monotherapy, making APL a curable disease [24]. Thus, the history of APL could be subdivided into four periods: pre-ATRA period, incorporating ATRA, integrating ATO, and synergistic targeting periods (Box 1).

The pre-ATRA period (1957–1986) [25]

APL was once considered “the most malignant form of acute leukemia” [10] and the clinical management of the disease at the first decade remained a nightmare for physicians as a result of the unpredictable onset of life-threatening bleeding disorders [25]. Chemotherapy was used unsuccessfully against APL in 1967. At that time proper supportive care was in shortage and standard cytotoxic chemotherapy as induction treatment exacerbated coagulopathy, with approximately 10–30% of patients with APL died of hemorrhage [26].

Particular sensitivity of APL to anthracyclines was reported by Bernard in 1973 [27], and the use of anthracyclines represented the first step forward in taming APL prognosis. Daunorubicin (DNR) and appropriate management of the APL-related coagulopathy improved the CR rate to between 55% and 80% during the 1980s [25,28,29]. However, even with consolidation and maintenance therapy, the median duration of CR was no more than 1–2 years, with only 20–35% of patients cured with chemotherapy alone and the remainder dying from hemorrhage or relapsed or refractory disease [30–33].
The second period: Incorporating ATRA gives APL patients new lease on life

APL as the first paradigm for cancer differentiation therapy

Accumulation of abnormal promyelocytes within bone marrow demonstrates a blockage of normal differentiation program in APL. Whether the induction of differentiation could be a treatment strategy for APL was an intriguing question.

In 1970s, Sachs et al. [34,35] reported that myeloid leukemic cells could be reprogrammed to resume normal differentiation and to become non-dividing mature granulocytes or macrophages as a result of stimulation by various cytokines. Based on this discovery, Leo Sachs hypothesized in 1978 [36,37] that treatment with agents that induce leukemic cells to complete differentiation could be a potential therapeutic option for leukemia patients. In the early 1980s, Breitman et al. [38,39] showed that retinoic acid (RA) could induce terminal differentiation of human APL cells in vitro. But the first clinical reports of using RA showed conflicting results. Some case reports showed beneficial effects of 13-cis RA in people with refractory or relapsed APL [40–42], while other reports showed that 13-cis RA was ineffective in treating APL [43,44].

Beginning in the early 1980s, the Shanghai Institute of Hematology (SIH) conducted a series of experiments on differentiation therapy for APL. These experiments showed that ATRA could induce terminal differentiation of HL-60, a cell line with promyelocytic features, and fresh leukemic cells from patients with APL. These intriguing results were the impetus for a clinical trial which was carried out in 1986. Twenty four patients with APL were treated with ATRA at a dose of 45 to 100 mg m² per day. The result was dramatic: 23 patients (95.8%) went into CR without developing bone marrow hypoplasia or abnormalities of clotting. The remaining one patient achieved CR when chemotherapy was added. The most striking feature was the gradual terminal differentiation of malignant cells in the bone marrow, sometimes combined with the presence of Auer rods in mature granulocytes. Hence, the hypothesis-oriented cancer differentiation therapy was brought for the first time into practice.

The efficacy of ATRA on APL was later confirmed by many randomized studies in centers around the world. Further trials showed improved rates of CR, a decrease in severe adverse effects, and lengthening of the duration of remission [6,44–47]. Trials combining ATRA with intensive chemotherapy were soon initiated and the results showed that ATRA combined with anthracycline-based chemotherapy could achieve CR in 90–95% of patients with APL and cure the disease in 70–75% of the cases [6,25,28,29,47]. These data suggest that treatment regimens incorporating agents with different performance can result in a better outcome, hence a global view at systems level should be consider when choosing a therapeutic protocol.

Mechanism of action at the cellular level

Treatment with retinoic acid appears to act on at least two stages of myeloid cell development, e.g. promyelocytes and earlier neoplastic progenitor cells that have retained a capacity for self-renewal but are nonetheless already committed along a myeloid differentiation pathway [44]. The absence of bone marrow hypoplasia during induction, the appearance of immunophenotypically unique “intermediate cells” that express both mature and immature cell-surface antigens and the persistence in morphologically mature granulocytes of both Auer rods and the t(15;17) until a late stage of induction [21,48–50], indicate that the induction of remission by ATRA is associated with the differentiation of immature neoplastic cells into mature granulocytes, followed by the emergence of normal hematopoietic cells as the patient achieves remission. After inducing an irreversible commitment to differentiation, ATRA may trigger apoptosis in the maturing cells [51–53].

Mechanism of action at the molecular level

The molecular mechanism underlying ATRA induced APL cell differentiation was uncovered nearly 10 years after the introduction of ATRA to the clinic. It is of the first important to understand the disease pathogenesis of APL.

Complex leukemogenesis with PML-RARα as a key player. The fact that t(15;17) was detected in 98% of patients with APL [54] suggested the key player of APL leukemogenesis might reside in this chromosomal abnormality. Great efforts had been made to unveil the gene generated by the reciprocal translocation. In 1990, Hugues de The et al. [55] reported that the retinoic acid receptor alpha (RARα) gene on 17q21 has been translocated to a locus, myl (renamed PML later), on chromosome 15, resulting in the synthesis of a myl/RARα fusion messenger RNA. In 1991, Kakizuka et al. [56] demonstrated that RARα was fused to promyelocytic leukemia (PML) gene on 15q22 with generation of a fusion gene, PML-RARα. PML-RARα was soon considered a molecular marker for diagnosis and monitoring the minimal residual disease. Fusion genes produced in t(11;17) and other variants were cloned subsequently [17–20,57].

The roles for PML-RARα to play in APL leukemogenesis were investigated. It has been well established that retinoids that are crucial for normal myeloid differentiation act via RA receptors (RARs)
and retinoid X receptors (RXRs). They belong to the steroid/thyroid/retinoid nuclear receptor superfamily of ligand-inducible transcription factors. Both RAR and RXR families consist of three subtypes: α, β, and γ [54]. RARα forms a heterodimer with RXR and binds to RA response element to control the expression of target genes in the presence of physiological concentrations (10⁻⁹–10⁻⁸ M) of retinoids. The PML-RARα chimeric protein acts as a dominant negative mutant over wild-type RARα. The chimeric protein prevents activation of key target genes required for normal myeloid differentiation by sequestering RXR and other RARα cofactors and inhibiting normal RARα functions. The PML-RARα oncoprotein binds to RAR target genes either on its own or with RXR and then recruits histone deacetylase complexes, which act as repressors of transcription [5,54]. PML-RARα may affect transcription in other pathways including those in which the transcription factor AP1 and interferon-responsive factors are involved. PML-RARα also binds to promyelocytic leukemia zinc finger (PLZF) protein and potentially affects its functions (e.g., growth suppression and transcription repression; control of developmental programs and differentiation) [5,54]. In addition, PML-RARα prevents apoptosis through delocalization of PML and antagonizes cytoplasmic PML function that is essential for TGFβ signaling [58]. PML-RARα may cooperate with activated mutations in protein tyrosine kinases, such as FLT3 [59], which confer proliferative and/or survival advantage to hematopoietic stem/progenitor cells. Recently Lane and Ley [60] reported that PML-RARα was cleaved in several positions by neutrophil elastase (NE) which was produced at maximal levels in promyelocytes. Interestingly, NE-mediated cleavage of PML-RARα may alter its activity and is important for the development of APL in mice. Zhu et al. [61] reported that the K160 sumoylation site in PML/RARα allowed the recruitment of a potent repressor, Daxx, and was absolutely required for PML-RARα transformation activity ex vivo. PML-RARα causes APL in transgenic mice [62–64], while PML-RARα K160R transgenic mice develop myeloproliferative syndromes, but never APL. The Daxx repressor no longer binds PML-RARα K160R, but fusion of these two proteins restores the differentiation block ex vivo. These results identify a repression domain in PML that controls the APL-specific differentiation block, which could explain why PML is the most common fusion partner of RARα in APL.

Catabolism of PML-RARα underlies ATRA-induced APL cell differentiation. PML-RARα is a direct target of ATRA [65]. ATRA triggers a caspases-mediated cleavage of the PML-RARα fusion protein at residue D522 within the α-helix region of the PML component of the fusion protein [66]. Further dissecting of the pathways involved in PML-RARα catabolism led to the discovery of ubiquitin/proteasome-mediated degradation of PML-RARα and RARα, which was dependent on the binding of SUG-1 in the AF2 transactivation domain of RARα with 19S proteasome [62,67]. Intriguingly, the drug ATO which induces a high CR rate in APL relapsed or refractory to ATRA and/or chemotherapy, also induces a degradation of PML-RARα oncoprotein (described below). The catabolism of PML-RARα results in restoration of normal retinoid signaling. RXR and PML sequestration is abrogated, and PML nuclear body is restored. The corepressor is released and the coactivator is recruited and bound with RARα, relieving the transcriptional repression of target genes. ATRA also induces cyclic AMP, a differentiation enhancer that boosts transcriptional activation, reverses the silencing of the transactivating function of RXR, and restores ATRA-triggered differentiation in mutant ATRA-resistant APL cells [68]. Additionally, ATRA induces the expression of RA-induced genes (RIGs, such as RIG-G, E, K and I) [69], and cyclooxygenase 1 [70], inhibits angiogenesis [71], downregulates the expression of tissue factor [72], and restores other signal pathways (e.g., the interferon pathway). Systems analysis of transcriptome and proteome in ATRA-induced APL cell differentiation reveals an array of transcription factors and cofactors, activation of calcium signaling, stimulation of the IFN pathway, activation of the proteasome system, degradation of the PML-RARα oncoprotein, restoration of the nuclear body, cell-cycle arrest, and gain of apoptotic potential [73]. Consequently, the abnormal promyelocytes differentiate and die through programmed cell death.

PLZF-RARα fusion transcript resulted from t(11;17)(q23;q21) [17,57] can also bind as homodimers to retinoic acid response elements (RAREs) [54] and acts as a dominant negative manner to inhibit the activity of wild-type RARα [54]. It is noteworthy that PLZF-RARα homodimers bound to a direct repeat of the sequence GGG TCA separated by 5 bp (DR5G) with equal avidity as PML-RARα but bound more strongly than PML-RARα to a repeat of the sequence GGT TCA (DR5T) [54,74]. Although it is possible that PLZF-RARα homodimers might display altered target gene specificity, in the presence of RXR, the PLZF-RARα/RXR heterodimer binds to RAREs in vitro with higher affinity than PLZF-RARα homodimers [75]. PLZF-RARα interacts aberrantly with the SMRT and NCoR corepressors, Sin3A and histone deacetylase 1 (HDAC1), both in vitro and in vivo. In the presence of 10⁻⁶ mol 1⁻¹ ATRA, PML-RARα was able to release the corepressors and HDAC1 whereas PLZF-RARα retained corepressors and HDAC1 even under these high ligand concentrations. As a result, ATRA alone can
not induce maturation of t(11;17)-harboring cells. HDAC inhibitor is required to cooperate with ATRA to induce t(11;17)-bearing cell differentiation [54].

**The third period: Incorporating ATO presents benefit to APL especially relapsed ones**

**Clinical outcome**

One of the limitations of ATRA in treating APL is its inefficiency for relapsed or refractory patients. Fortunately, great benefit was brought to patients of this proportion as well as those newly diagnosed by application of ATO which was also firstly reported in China. Arsenic is a common, naturally occurring substance that exists in organic and inorganic forms. The organic arsenicals consist of an arsenic atom in its trivalent or pentavalent state linked covalently to a carbon atom. There are three inorganic forms of arsenic: red arsenic (As₄S₄, also known as “realgar”), yellow arsenic (As₂S₃, also known as “orpiment”), and white arsenic, or arsenic trioxide (As₂O₃) which is made by burning realgar or orpiment [76].

Arsenic was used to treat chronic myelogenous leukemia (CML) in the 18th and 19th centuries, but was discarded as a treatment in the early 20th century because of its toxicity and the advent of radiation and cytotoxic chemotherapy. In the 1990s, Sun et al. [23] showed that intravenous infusions of Ailing-1, a crude solution composed of 1% arsenic trioxide with a trace amount of mercury chloride, induced CR in two-thirds of patients with APL. There was an impressive 30% survival rate after 10 years. In 1997, SIH reported [22,77,78] their striking results of pure ATO in treating relapsed APL. Fifteen APL patients at relapse after ATRA induced and chemotherapy maintained CR received intravenously administration of ATO at a dose of 0.16 mg kg⁻¹ per day for 28–54 days. Clinical CR was achieved in nine of 10 (90%) patients treated with ATO alone and in the remaining five patients treated by the combination of ATO and low-dose chemotherapeutic drugs or ATRA. During the treatment with ATO, there was no bone marrow depression and only limited side effects were encountered. These results showed that ATO is an effective and relatively safe drug for APL patients refractory to ATRA and conventional chemotherapy.

Since 1996, a large number of reports have shown that arsenic compounds induce a CR in 85% to 90% of patients with both newly diagnosed and relapsed APL [47]. Tetra-arsenic tetra-sulfide was also reported to be effective in APL treatment [79]. Furthermore, after CR is achieved by arsenic compounds, a molecular remission (i.e. negative for PML-RARα transcript detected by reverse transcriptase polymerase chain reaction) is obtainable either with arsenic compounds or with ATRA and chemotherapy as consolidation treatment. It seems likely that arsenic compounds appropriately used in post-remission therapy could prevent recurrence and achieve a longer survival time [47,79,80].

**Mechanism of action**

Unlike ATRA which induces terminal differentiation, ATO at cellular level exerts dose-dependent dual effects on APL cells including NB4 cell line and APL primary cells: inducing preferentially apoptosis at relatively high concentrations (0.5 to 2 μmol l⁻¹) and inducing partial differentiation at low concentrations (0.1 to 0.5 μmol l⁻¹) [78]. The clinical response of APL to ATO is also associated with incomplete cytodifferentiation and the induction of apoptosis with caspase activation in leukemic cells [80].

Sequence analysis of the PML gene has indicated the presence of a cysteine-rich region that may be a principal candidate for interaction with trivalent arsenic. Similar to ATRA, ATO at 0.1 to 2 μmol l⁻¹ induces a rapid modulation and degradation of PML-RARα proteins [77,78]. ATO targets the PML moiety of PML-RARα through a still unclear mechanism, and causes PML to localize to the nuclear matrix and become sumoylated. Sumoylation at K160 is necessary for 11S proteasome recruitment and ATO-induced degradation, whereas sumoylation at K490 is needed for nuclear localization [81,82]. Since ATRA-induced degradation of the PML-RARα leads to terminal differentiation, a question should be answered here: why ATO-triggered PML-RARα catalysis only results in partial differentiation? A two-step model in induction of APL cell differentiation was developed [83,84]. This model suggests that there are two discrete steps in the maturation process: an RA-dependent priming step that maintains proliferation while cells become competent to undergo maturation in response to retinoids and a cAMP-dependent step that triggers RA-primed cells to undergo terminal maturation. The first event, priming, corresponds to the derepression of a critical target gene repressed by PML-RARα. If the expression of this gene is high enough, differentiation will ensue. If this gene is expressed at intermediate levels, then additional signaling is required, for example by cAMP, G-CSF or histone desacytelase inhibitors. This model clearly accounts for the fact that either full dose arsenic or low dose RA triggered differentiations are non-terminal and that adding cAMP or G-CSF then promotes terminal differentiation, strongly supporting a model where derepression of a first set of genes has a permissive role on differentiation [83,84]. Indeed, ATO in combination with cAMP does fully induce APL cell differentiation [85].

Mechanisms underlying ATO-triggered APL cell apoptosis have been broadly studied. The apoptosis-inducing effect is associated with the downregulation of Bcl-2 [77] which cooperates with PML-RARα to
block neutrophil differentiation and initiate APL [86], collapse of mitochondrial transmembrane potentials (MTP) in a thiol-dependent manner [87,88], activation of caspases [89,90], and modulation of tumor suppressor PML [91] (Figure 2). PML has drawn intense attention recently for its role in growth suppression and apoptosis. PML is a tumor suppressor which normally epitomizes a multiprotein nuclear structure, the PML-nuclear body (PML-NB) that is a macromolecular structure of doughnut shape and approximately 0.2–1.0 micrometer in size. Cells typically contain 10–30 of these macromolecular structures. Cytoplasmic PML is a critical TGFβ regulator [58]. It is becoming apparent that PML and the PML-NB act as molecular hubs for controlling apoptosis [92–94]. At lower levels, PML is essential for the proper function of proapoptotic transcription factors, ultimately leading to caspase activation, while at higher levels PML might trigger apoptosis independently of transcription or caspase activation through protein sequestration into the PML-NB [94,95]. PML also regulates cell proliferation and senescence. In the APL blasts, PML-RARα causes the delocalization of PML into microspeckled nuclear structures through physical association leading to disruption of the PML-NB [54], and antagonizes cytoplasmic PML function [58]. ATO induces the reaggregation of NB antigens, recruits PML proteins onto NBs and induces degradation of PML and PML-RARα [91]. Recently Hayakawa and Privalsky [96] reported that ATO treatment induced phosphorylation of the PML protein through a mitogen-activated protein (MAP) kinase pathway. Increased PML phosphorylation is associated with increased sumoylation of PML and increased PML-mediated apoptosis. Conversely, MAP kinase cascade inhibitors, or the introduction of phosphorylation or sumoylation-defective mutations of PML, impair As2O3-mediated apoptosis by PML. Thus phosphorylation by MAP kinase cascades potentiates the antiproliferative functions of PML and helps mediate the proapoptotic effects of ATO.

Recently systems analysis of transcriptome and proteome in ATO-induced APL cell apoptosis [73] showed that at transcriptome level many ATO-regulated genes were also regulated by RA. Unlike RA, ATO mainly induces the degradation rather than the activation of PML-RARα. ATO may target or interact with many other proteins which may underlie the synergistic effect with RA. For example, several ubiquitin/proteasome genes appear to be specifically regulated by ATO, which may consequently contribute to a more effective and efficient protein-degradation system in RA plus ATO-treated cells than in RA-alone- or ATO-alone-treated cells, suggesting a possible synergistic effect for ATRA plus ATO in treating APL. At proteome level, ATO may particularly enhance mechanisms of post-transcriptional/translational modifications.

**The fourth period: Systems biology-based synergistic targeting makes APL a curable disease**

As mentioned above, APL pathogenesis is complex with PML-RARα acting as a key player, suggesting the treatment regimen containing drugs against different targets or mechanisms might confer a superior outcome. Moreover, the two step model for differentiation induction (Figure 2) suggests that combined use of cAMP/cytokine with ATO or low dose ATRA may cause APL terminal differentiation. Indeed,
ATRA plus chemotherapy yield a higher CR rate and a longer overall survival [25,28,29,47]. Hence, understanding the complexity of APL and drug mechanism at systems level may be of the first importance for proficient option of therapeutic protocol.

**ATRA combined with ATO: Two hits on one molecule accelerate APL clearance**

A striking similarity in the effect of the two otherwise unrelated agents, ATRA and ATO, is the degradation of PML-RAR results in accelerated differentiation and/or a dramatic induction of apoptosis. Using syngenic grafts of RA showed accelerated differentiation and/or a greater rate of cell death, as determined by means of clinical examinations. Although RA or arsenic alone only marginally decreases PML-RAR fusion transcripts, and side effects were evaluated by means of clinical examinations. Although CR rates in these groups were all high (>90%), the time to achieve CR differed significantly, with that of the combination group being the shortest one. Earlier recovery of platelet count was also found in this group. The disease burden as reflected by fold change of PML-RAR transcripts at CR decreased more significantly in combined therapy as compared with ATRA or ATO mono-therapy (P<0.01). This difference persisted after consolidation (P<0.05). Importantly, all 20 cases in the combination group remained in CR whereas 7 of 37 cases treated with mono-therapy relapsed (P<0.05) after a follow-up of 8–30 months (median: 18 months). Synergism of ATRA and ATO on apoptosis and degradation of PML-RAR results in apoptosis and nuclear receptor signaling molecules, interferon pathway members, and factors involved in other cascades. At the time point of 12–24 h, ATRA/ATO regulated genes/proteins seemed to be an amplification of RA signaling and a strong activation of the ubiquitin/proteasome system which might facilitate degradation of PML-RAR. After 48–72 h of treatment with RA/ATO, the expression of differentiation markers and functional molecules reached a maximum, while genes/proteins promoting cell cycle or enhancing cell proliferation were significantly repressed. As the cells approached terminal differentiation, the expression of apoptosis agonists increased gradually. These might contribute to the mechanisms of ATRA/ATO-induced differentiation/apoptosis of APL cells.

**Synergistic effects of ATRA/ATO and cAMP signaling in APL cell differentiation: Crosstalk promise**

Cyclic AMP was shown to be capable of inducing differentiation in AML cell lines [102]. cAMP boosts transcriptional activation by RA and activates PML-RARA targets [68]. In ATRA-resistant NB4-R1 cells, cAMP-elevating agents or stable agonistic cAMP analogs can induce maturation of these cells at the endogenous level of cAMP reduces the ATRA concentration required for APL cell maturation to near physiological levels [103]. Furthermore, cAMP also strongly synergizes with low concentration of ATO (0.25 μM) to fully induce differentiation of NB4, NB4-R1, and fresh APL cells, and facilitates the ATO-mediated PML-RAR degradation. cAMP significantly inhibits cell growth by modulating several major players in G1/S transition regulation. An antagonist of protein kinase A, H89, could block the differentiation-inducing effect of ATO potentiated by cAMP. In RA-sensitive or RA-resistant mouse models of APL, continuous infusions of 8-chloro-cAMP triggers major growth arrest, greatly enhanced both spontaneous and RA- or ATO-induced differentiation and accelerated the restoration of normal hematopoiesis. Theophylline, a well-tolerated phosphodiesterase inhibitor which stabilizes endogenous cAMP, also impairs APL growth and enhanced spontaneous or ATO-triggered cell differentiation in vivo. Remark-
ably, in an APL patient resistant to combined ATRA-ATO therapy, theophylline induced blast clearance and restored normal hematopoiesis [104]. These results suggest that cAMP signaling is essential for the intricate cell differentiation process, activation of cAMP pathway provides an alternative option not only for APL synergistic differentiation therapy, but also for other subtypes of myeloid leukemias.

The 50-year history of APL has seen tremendous advances in developing curative approaches, turning APL from once considered “the most malignant form” to currently the most curable form of AML. Of note, the introduction of ATRA in initial therapy represents one of the most spectacular advances in the treatment of human cancer [105] and the first example for oncoprotein-targeting therapy [25]. Benefits gained from ATO which also targeting PML-RARα confirm the philosophy of an old Chinese saying “treating an evil with a toxic” in modern medicine. Moreover, systems biology-based synergistic targeting therapies bring a superior clinical outcome as compared with monotherapy. The experience in taming APL indicates that understanding the disease pathogenesis of other subtypes of leukemia at systems level may be helpful in developing curative approaches for these leukemias.

Possible synergistic targeting treatment cocktail for other leukemias

APL is the first model of a malignant disease that can be treated by drugs targeting an oncogenic event which alters the biological process of the diseased cells [25]. Recently Imatinib Mesylate (Gleevec, STI-571), which competes the adenosine triphosphate (ATP) binding site of the kinase domain of ABL [106], has been shown to have significant antileukemic activity in patients with CML [107–110], establishing another paradigm for leukemia targeted therapy. However, its effects on patients with accelerated phase or blast phase (advanced phase, AP) are unsatisfactory [111–113]. In addition, even among patients at chronic phase (CP), Imatinib seems unable to eradicate the malignant progenitors and a significant portion of patients develops drug resistance after long-time use [114–117]. Li et al. [118] at Shanghai Institute of Hematology showed that arsenic sulfide (As₄S₄) induced apoptosis of CML cells, then the synergic effects of Imatinib and As₄S₄ on CML cells were investigated. The results showed that As₄S₄ induced G2/M arrest whereas imatinib induced G1 arrest. Imatinib plus As₄S₄ induced a much higher ratio of apoptotic cells than that triggered by either Imatinib or As₄S₄ alone. Moreover, the 2 drugs exhibited a synergistic effect in targeting BCR-ABL protein. While As₄S₄ triggered its degradation and imatinib inhibited its tyrosine kinase activity, combined use of the two led to lower protein/enzymatic activity levels of BCR-ABL [119]. Clinical trial using Imatinib plus As₄S₄ to treat CML is undergoing in Shanghai Institute of Hematology and the phase I results in a group of CML AP patients confirmed the safety of the combination protocol.

In AML M2 with t(8;21), the resultant AML1-ETO fusion protein plays a crucial role in pathogenesis of t(8;21) leukemia [120,121]. Though Grimwade et al. [122] showed that t(8;21) was a favorable prognostic factor for AML with a 5-year overall survival rate of 69%, others demonstrated that the median survival time of t(8;21) AML was less than 2 years with a 5-year survival rate of no more than 40% [123–128]. Recently to explore the genetic abnormalities that cooperate with AML1-ETO fusion gene to cause t(8;21) leukemia, SIH screened a number of candidate genes and identified 11 types of mutations in C-KIT gene, including 6 previously undescribed ones among 26 of 54 (48.1%) cases with t(8;21). To address a possible chronological order between AML1-ETO and mutant C-KIT, it has been shown that, among patients with AE and mutant C-KIT, most leukemic cells at disease presentation harbored both genetic alteration, whereas in three such cases investigated during complete remission, only AML1-ETO, but not mutant C-KIT, could be detected by allele-specific PCR. Therefore, mutant C-KIT should be a subsequent event on the basis of t(8;21). Furthermore, induced expression of AML1-ETO in U937 cells significantly up-regulated mRNA and protein levels of C-KIT. This may lead to an alternative way of C-KIT activation and may explain the significantly higher C-KIT expression in 81.3% of patients with t(8;21) than in patients with other leukemias. Additionally, Gleevec suppressed the C-KIT activity and induced proliferation inhibition and apoptosis in cells bearing C-KIT N822K mutation or overexpression, but not in cells with D816 mC-KIT. Gleevec also exerted a synergic effect in apoptosis induction with cytarabine, thus providing a potential therapeutic for t(8;21) leukemia. Since AML1-ETO is crucial for leukemogenesis of AML M2, it is reasonable to develop AML1-ETO targeted therapies for t(8;21) leukemia, as several lines of evidence [120,121,129–133] have proved this hypothesis. Thus AML1-ETO-targeting agents and C-KIT kinase inhibitors together with cytarabine should be an attractive treatment cocktail for AML M2 with t(8;21).

Conclusion and perspectives

The story of successfully developing curative approaches for APL shows that by targeting the molecules critical to the pathogenesis of certain diseases, cells can be induced to return to normal or dead by programmed cell death. The close collaboration between bench and bedside is thus important not
only for unraveling leukemia pathogenesis, designing targeted therapy, elucidating drug mechanism, but also for developing systems biology-based synergistic targeted therapy which may in turn greatly improves clinical outcome. The sequencing of the human genome and ongoing functional genomic research are now accelerating the dissection of disease mechanisms and identification of therapeutic targets. This in turn may facilitate the screening of promising treatments and identification of therapeutic targets. This in turn may facilitate the screening of promising treatments. On the other hand, the history of APL has not come to an end. By extending the model of APL, there is reason to hope that several forms of AML and CML can eventually be cured by specifically tailored cell-modifying treatments.

References