Ham-Wasserman Lecture

Treatment of Acute Leukemia by Inducing Differentiation and Apoptosis

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Conventional treatment of acute leukemia involves the use of cytotoxic agents (chemotherapy), but other strategies have been explored. All-*trans* retinoic acid (ATRA) and arsenic have clearly been effective in the treatment of acute promyelocytic leukemia (APL), which creates the possibility that other types of acute leukemia can be conquered by selectively inducing differentiation and/or apoptosis. A great number of investigations have been performed to elucidate the mechanisms and search for effective agents in the treatment of other types of acute leukemia by these new strategies. Progress at the molecular level has been achieved in explaining the mechanisms of action of ATRA and arsenic compounds, and several new agents have emerged, although their clinical effectiveness remains to be confirmed. Mechanism-/gene-based targeted therapy and a combination of different strategies will improve the treatment of acute leukemia.

Selective induction of differentiation and apoptosis is a new strategy in the treatment of acute leukemia. Successful treatment of acute promyelocytic leukemia (APL) with all-*trans* retinoic acid (ATRA) and arsenic compounds prompts us to further investigate, particularly at the molecular level, the mechanism of differentiation and apoptosis and to seek new differentiation- and apoptosis-inducing agents.[1] The purpose of this article is to review the state-of-the-art in this area.

The Model of Acute Promyelocytic Leukemia

Treatment of APL with ATRA
Clinical effectiveness. ATRA is the drug of first choice in the treatment of newly diagnosed APL. ATRA was first introduced to clinical use for the treatment of APL in 1986.[2] In 1988, we reported that among 24 APL patients treated with ATRA, 23 achieved complete remission (CR).[3] The results were confirmed by Castaigne et al in France,[4] who treated 22 APL patients (4 newly diagnosed and 18 at first to third relapse or refractory to chemotherapy) with ATRA from 1988 to July 1989, and 14 attained CR. Since then, randomized studies in many centers around the world document a rising CR rate, a decrease in severe adverse effects, and a prolongation of remission duration. **Table 1** summarizes the CR rate obtained in most large series of patients (> 50) since 1990.[5-18] ATRA combined with anthracycline-based chemotherapy can achieve CR in 90%–95% of patients with APL and cure the disease in 70%–75% of the cases. Combination therapy with ATRA and chemotherapeutic agents should now be considered as a standard treatment of APL.[19-21] The conventional daily dose of ATRA is 45 mg/m^2, administered orally until remission. A course of the treatment usually requires 28–32 days, and in rare cases up to 42 days. A lower ATRA dose of 30 mg/m^2 can provide a similar response and is indicated in cases of drug intolerance or among elderly patients.[22] An intravenous liposomal ATRA is now available for APL patients who are unable to swal-

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Table 1. Complete remission rate in APL patients treated with ATRA (in series including more than 50 cases).

Year	Researchers	Protocol	n	CR (%)
1991	Chen et al[5]	ATRA	50	94.0
1992	Chinese Coop Study Group[6]	ATRA	400	85.0
		ATRA + chemo	144	76.4
1993	Shanghai Coop Study Group[7]	ATRA	91	81.3
1994	Warrell et al[8]	ATRA	79	84.8
1995	Kanamaru et al[9]	ATRA ± chemo	109	89.0
1997	Tallman et al[10]	ATRA	172	72.1
1997	Soignet et al[11]	ATRA ± chemo	95	83.2
1997	Asou et al[12]	ATRA	62	95.2
		ATRA ± chemo	196	88.3
1997	Mandelli et al[13]	ATRA + chemo	240	95.4
1999	Fenaux et al[14]	ATRA ± chemo	413	92.0
1999	Burnett et al[15]	ATRA (short) + chemo	119	70.0
		ATRA (ext) + chemo	120	87.0
1999	Hu et al[16]	ATRA + chemo	120	88.4
2000	Lengfelder et al[17]	ATRA + HD Ara-C	51	92.0
2001	Asou et al[18]	ATRA ± chemo	369	90.0
		ATRA alone		94.0
		ATRA + initial chemo		89.0
		ATRA + later chemo		88.0
		ATRA + initial, later chemo		86.0

Abbreviations: APL, acute promyelocytic leukemia; Ara-C, cytosine arabinoside; ATRA, all-*trans* retinoic acid; chemo, chemotherapy; CR, complete remission; ext, extended; HD, high dose.

low or absorb the medication. In 56 evaluated patients receiving 4 or more doses of intravenous liposomal ATRA, the CR was 87% (20/23) for the newly diagnosed APL patients, 78% for patients in first relapse (14/18), and 23% for patients in second relapse (3/13).[23] Long-term follow-up confirms the benefit of ATRA incorporation in the treatment regimen of APL, even during maintenance therapy.[21,24]

The major life-threatening adverse effect in APL treated with ATRA is the occurrence of retinoic acid syndrome (RAS). Its frequency can be as high as 20%–25%.[1] The addition of chemotherapy at the beginning of the treatment has significantly reduced the incidence to 5%–7%.[25,26] The effect of ATRA on early amelioration of coagulopathy and its possible mode of action are described in Wang and Chen[1] and Degos and Wang.[20]

Mechanism of action of ATRA in differentiation therapy. Over the last decade, tremendous efforts have been made to elucidate the molecular genesis of APL, as well as the mechanism of action of ATRA. The classic chromosome abnormality of APL is a translocation t(15;17)(q22;q21) resulting in the generation of fusion gene and protein PML-RARα, which plays a central role in APL pathogenesis. Experimental evidence has revealed that PML-RARα protein has the following activities: (1) it creates a complex with retinoid x receptor (RXR), nuclear corepressors (N-CoR), Sin3A, and histone deacetylase (HDAC) that represses the transcriptional expression of target genes;[27] (2) it acts in a dominant negative manner on the retinoic acid-signaling pathway,[28] blocking the differentiation of myeloid cells; and (3) it forms a heterodimer with wild-type PML protein and thereby disrupts the PML nuclear body or PML oncogenic domains (PODs).[29] The function of PML as a growth inhibitor and regulator of apoptosis is disturbed when incorporated into PML-RARα complex. The mechanisms of action of ATRA can be summarized as follows: (1) The binding of ATRA to RAR receptors causes degradation of PML-RARα protein through the ubiquitin-protosome and caspase system,[30,31] leading to restoration of terminal differentiation of promyelocytes; (2) Exposure of APL cells to ATRA in vitro or in vivo induces relocalization of PML and restores the normal structure of PODs;[28] and (3) Under the action of ATRA at pharmacological concentration (1 μM), CoR is dissociated from the repressive complex, whereas CoA (coactivator) is recruited to the complex.[27] As a result, the repression of transcriptional activation of target genes is relieved and the differentiation of promyelocytes' process is restored. To further clarify the molecular mechanism of ATRA-induced differentiation, the gene expression profile in the APL cell line NB4 was studied before and after treatment with ATRA using complementary DNA array, suppression-subtractive hybridization, and differential-display polymerase chain reaction (PCR). In a study conducted by the Shanghai group, 169 genes were modulated by ATRA; among them, 100 were upregulated and 69 downregulated. These genes involve transcription factors, DNA synthesis/repair and recombination proteins, cytokines and chemokines, signal transduction modulators and effectors, interferon signaling, cell cycle regulation, apoptosis-related proteins, cell structure/mobility proteins, and cell adhesion proteins, and others. It is interesting to note that the chronology of up- or downregulation of these genes accords with the process of terminal differentiation of the leukemic cell.[32] Recently, using a complementary DNA microarray platform containing 12,630 clones, in conjunction with bioinformatics analysis such as self-organizing map (SOM) and component plane presentation, we profiled gene change in the ANB4 cell line treated with a pharmacological dose of ATRA at 6, 12, 24, and 48 hours. The results showed that ATRA-induced differentiation was a complex and well-organized process. At the early

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stage, it was manifested by cell cycle arrest, cell proliferation suppression, and antagonism of apoptosis. The ubiquitin-proteasome degradation system was activated, chronologically correlated with the degradation of PML-RARα fusion protein and the assembly of PODs. With the process of differentiation and maturation in granulocytes, genes underlying the process of apoptosis were modulated. In addition to genes involved in the protein kinase C (PKC), protein kinase A/cyclic adenosine monophosphate (PKA-cAMP), and Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathways, a large number of genes were found to be involved in other pathways, such as insulin receptor signaling and calcium signaling, that were regulated during ATRA-induced differentiation. In particular, the importance of calcium signaling was detailed in this study. Interestingly, specific members of the histone family were also found to be significantly regulated, suggesting that a chromosome remodeling process occurs during differentiation. Additionally regulated genes included a number of potential hematopoietic regulators involved in various translocations in hematopoietic malignancies. The next steps will be to clarify the relationship between molecular pathways implicated in the differentiation process and the relationship with hematopoietic malignancies (J Zhang et al, unpublished data).

Treatment of APL with arsenic compounds

Clinical effectiveness. Treatment of APL by arsenic compounds represents a successful example of apoptosis induction therapy of acute leukemia (**Table 2**). Arsenic (As) was used for more than 500 years in traditional Chinese medicine (TCM).[41] In the early 1970s, a group of investigators from Harbin Medical University in the northeastern region of China reported that a crude solution of arsenic trioxide (As_2O_3) Ailin-1 could be used to treat APL, according to the TCM principle of using a toxic agent against something toxic. A paper first appeared in 1992,[42] and a pure solution of As_2O_3 has been used in clinic since 1996.[43] Two groups of clinical investigators reported the results obtained with pure 1% As_2O_3 solution. In one study, 22 of 30 (73.3%) cases of newly diagnosed APL and 22 of 42 (52.4%) cases of relapsed or refractory APL achieved CR.[43] In our study, 15 of 16 APL patients in relapse entered CR, and dual effects (apoptosis and differentiation-inducing effect) on APL cells in vitro and in vivo were demonstrated.[44,45] Use of a TCM compound called *Indigo naturalis,* which consists of realgar (containing mainly tetra-arsenic tetra-

sulfide [As_4S_4]), *Baphicacanthus cusia, Radix salviae miltiorrhizae,* and *Radix pseudostellariae* achieved CR rates as high as 96% in 60 APL patients.[46] In 1999, purified As_4S_4 was reported to be effective in APL treatment.[36] **Table 2** shows recent reports of CR rates in APL patients treated with arsenic compounds; CR rates of 85% to 90% were attained in newly diagnosed APL patients treated with As_2O_3. The CR rate may be higher in relapsed cases.[35,40]

An important question is whether As_2O_3 can achieve molecular remission. When we used a very sensitive nested reverse transcriptase (RT)-PCR system, molecular remission was observed in only 1 of 10 patients who had received a single course of As_2O_3 and were tested immediately after CR.[44] In a recent report by Lazo et al, 7 of 10 evaluated patients achieved a molecular remission.[40] Soignet et al found that after 2 courses of treatment, 8 of 11 patients were converted to PML-RARα negative,[47] and 31 patients (91%) with continued remission after postremission therapy had a negative result for t(15;17).[38] When oral As_4S_4 was used, 14 of 16 patients achieved molecular remission.[48] Therefore, after CR is achieved by arsenic compounds, a molecular remission is obtainable either with arsenic compounds or with ATRA and chemotherapy as consolidation treatment.

Another key question is whether clinical use of arsenic compounds can raise the 5-year survival rate. If yes, how can they be used in postremission treatment of APL? In our study, 33 relapsed APL patients treated with As_2O_3 who achieved CR were followed for 8 to 48 months; the estimated disease-free survival (DFS) rates at 1 and 2 years were 63.6% and 41.6%, respectively.[35] Ma et al reported that overall survival (OS) for 7 years

Table 2. Complete remission rate induced by arsenic compounds (reports from 1998-2003).

Year	Authors	Arsenic Compound	Disease Status	n	CR (%)
1998	Huang et al[33]	As_2O_3	Relapse + refractory	5	57
1998	Ma et al[34]	As_2O_3	De novo	98	87.1
1999	Niu et al[35]	As_2O_3	De novo	11	72.7
			Relapse	47	85.1
1999	Lu et al[36]	As_4S_4	De novo + relapse	100	84.9
2000	Zhang et al[37]	As_2O_3	De novo	124	87.9
			De novo + relapse + refractory	242	74.8
2001	Soignet et al[38]	As_2O_3	Relapse + refractory	40	85
2002	Mathews et al[39]	As_2O_3	De novo	11	91.0
2003	Lazo et al[40]	As_2O_3	Relapse	12	100.0

Abbreviations: As_2O_3, arsenic trioxide; As_4S_4, tetra-arsenic tetra-sulfide; CR, complete remission.

was 58.5% with chemotherapy as consolidation therapy.[34] Zhang et al[37] reported that the 5- and 7-year survival rates were 92.02% and 76.69%, respectively, for patients achieving CR and receiving As_2O_3 and/or chemotherapy as maintenance therapy. In the US multicenter study, As_2O_3 was used in relapsed cases. Among 32 patients achieving CR, 18 received additional As_2O_3 treatment and 11 underwent allogeneic or autologous bone marrow transplantation. The estimated 18-month OS and relapse-free survival rates were 66% and 56%, respectively.[38] Mathews et al reported that 10 newly diagnosed APL patients treated with As_2O_3 and achieving CR remained in CR at a median follow-up of 15 months.[39] Lu et al[48] recently treated 103 APL patients in hematological CR with As_4S_4 orally as maintenance therapy. DFS rates for 1 and 6 years were 96.7% and 87.4%, respectively. Therefore, it seems likely that arsenic compounds appropriately used in postremission therapy could prevent recurrence and achieve a longer survival time. Randomized prospective studies will be necessary to define the arsenic compound regimen that best prevents APL relapse.

Adverse effects of arsenic compounds, which are usually mild and tolerable, include nausea, vomiting, and abdominal pain. Nevertheless, severe hepatic toxicity can occur, particularly in newly diagnosed cases.[35] Cardiac damage with electrocardiographic QT prolongation was observed in 63% of patients in one report.[38] The incidence of RAS-like manifestations accompanied by hyperleukocytosis may be as high as 25%, but it effectively responds to the dexamethasone treatment.[38]

Mechanism of action of As_2O_3. As_2O_3 exerts dual effects on APL cells. Studies in vitro with NB4 cells showed that a higher concentration of As_2O_3 (0.5–1.0 µM) induced apoptosis with typical morphological changes, DNA laddering on agarose gel electrophoresis, appearance of an apoptotic peak on flow cytometric analysis, and increased expression of annexin V on the cell surface membrane. Studies on the mechanism of apoptosis revealed that it was due to the collapse of mitochondrial transmembrane potential, increase in reactive oxygen species generation, release of cytochrome c and apoptosis-inducing factor (AIF) into cytoplasm, activation of caspases, and decreased expression of Bcl-2.[41,49] As_2O_3 acts through activation of Jun N-terminal kinase (JNK) and activator protein-1, inhibition of dual-specificity phosphatases,[50] CD95-independent caspase 8 activation,[51] and inhibition of nuclear factor (NF)-κB.[52] In addition, As_2O_3 could potently enhance phosphoacetylation of serine 10 of histone H3 and phosphoacetylation at the chromatin of caspase-10.[53] The most important finding is that As_2O_3 is able to degrade the PML-RARα oncoprotein at a wide range of concentrations (0.1–1.0 µM).[45,54] As_2O_3 causes PML to localize into the nuclear matrix, where PML proteins become sumoylated and are degraded after recruitment of proteosomes.[55]

At lower concentrations, As_2O_3 can induce APL cells to partially differentiate along the granulocytic pathway, as evidenced by increase in CD11b and CD14 expression and decline in CD33 expression. The mechanism of arsenic-induced differentiation is not clear. Degradation of PML-RARα in the presence of lower concentrations of As_2O_3, although slower in kinetics, favors the release of differentiation arrest. In addition, acetylation of histones 3 and 4 probably contributes to the mechanism of the differentiation process.[41]

Treatment of APL by combining ATRA and As_2O_3

Studies in animal models demonstrate that synergy might exist between ATRA and As_2O_3. The combination of the 2 drugs may also bring clinical advantages or even the possibility of eradicating the leukemia clone in APL patients.[56,57] Three relapsed APL patients achieved CR when treated with ATRA in conjunction with As_2O_3.[58] At our institute, 31 newly diagnosed APL patients were treated with ATRA in combination with As_2O_3, and 29 (93.5%) entered CR. No relapses occurred in a preliminary follow-up study during a median time of 8 months.[59] To further compare the combination therapy with monotherapy, we conducted a prospective study in which remission induction was performed using either ATRA, As_2O_3, or ATRA/As_2O_3 in combination. Fifty-nine newly diagnosed APL patients entered this trial: 20 cases were treated with ATRA, 18 with As_2O_3, and 21 with a combination of ATRA and As_2O_3. The CR rates were 90.5%, 88.9%, and 95.2%, respectively, without significant difference ($P > .05$). Of note, the time to CR was shorter—25.4 ± 5.0 days in the combination group as compared to 40.2 ± 10.5 and 32.6 ± 3.5 days in the ATRA ($P = .0003$) and As_2O_3 ($P = .003$) groups, respectively. Although liver damage was more frequent, it was tolerable and the treatment could continue after dose reduction. Real-time RT-PCR tests revealed that the copy number of PML-RARα transcript was reduced a median of 118.9-fold ($n = 20$) in the combination group, while the median reduction fold was 32.1 and 6.7 in the groups using As_2O_3 ($n = 16$) and ATRA ($n = 19$), respectively. These differences were statistically significant (combination group vs As_2O_3 group, $P = .009$, vs ATRA group, $P = .041$). Most importantly, at a median follow-up duration of 13 months, no relapse was observed in the combination group, as compared to the ATRA (21.0%; $P = .02$) and As_2O_3 (12.5%; $P = .104$) groups (ZZ Shi et al, unpublished data).

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Other Mechanism-Based Anti–Acute Leukemia Agents

Other differentiation-inducing agents

Dozens of differentiation-inducing agents have been studied as potential differentiation therapy of acute leukemia. They can be divided into the following groups[60]:

(1) vitamin analogs: retinoids and vitamin D derivatives

(2) cytokines: granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), interferons, tumor necrosis factor (TNF)

(3) polar-aplanar compounds: hexamethylene bisacetate (HMBA)

(4) histone deacetylase inhibitors: trichostatin (TSA), phenylbutyrate,[61] apicidin,[62] depsipeptide (FR901228)[63]

(5) inhibitors of DNA methylation: 5-aza-2´-deoxycytidine[64]

(6) cyclic AMP analogs: 8-Cl-cAMP, dibutyl cAMP

(7) chemotherapeutic agents: aclarubicin, cytosine arabinoside (Ara-C), hydroxyurea

(8) medicinal plant-derived products and plant growth regulators: meisoindigo (derivative of indirubin, ingredient of *Indigofera suffruticosa Mill*),[65] flavonoids from *Morus alba* leaves,[66] cotylenin A (a plant growth regulator),[67] tanshinone IIA (a component of *Salvia miltiorrhiza*),[68] pyranocoumarins (isolated from *Peucedanum praeruptorum Dunn*),[69] intermedeol (isolated from the leaves of *Ligularia fischeri var. spiciformis*),[70] and magnolialide (a 1beta-hydroxyeudesmanolide isolated from *Cichorium intybus*).[71]

Even though many new differentiation-inducing agents have been explored during the past few years, the majority of them have only been studied in vitro on only leukemia cell lines, such as NB4, H-60, U937, K562, or Kasumi-2, or in a primary leukemia cells culture assay. Therefore, the results of clinical trials of their effectiveness are limited. The following reports show some positive results in differentiation therapy for acute leukemia other than APL with ATRA. First, among 41 cases of acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) treated with HMBA, 3 patients achieved CR and 6 partial remission (PR), with a median duration of CR of 6.8 months (1.3–16 months).[72] Second, an APL patient who had had multiple relapses and was resistant to ATRA treatment achieved clinical and cytogenetic CR after being treated with sodium phenylbutyrate. Immunofluorescence and Western blot analysis revealed that phenylbutyrate caused a time-dependent increase in histone acetyla-tion in blood and marrow mononuclear cells.[73] Third, CR was achieved in a case of M2a subtype of AML treated with ATRA alone.[74] Fourth, low-dose cytarabine was used for the treatment of AML in elderly patients; 24/48 (50%) achieved CR with a median remission duration of 8 months.[75] However, this schedule is not advised in patients with monocytic leukemia or in those with hypercellular marrow.[76] Fifth, a combination of different inducers of differentiation could yield a higher remission rate, as illustrated in a report that concurrent administration of G-CSF, low-dose cytarabine (10 mg/m^2/d, for 14 days), and aclarubicin (10–14 mg/m^2/d, for 4 days) in 18 patients with relapsed AML, achieved a CR rate of 83% (15/18).[77] A protocol combining low-dose cytarabine and harringtonine was tried in 10 AML patients, and 8 out of the 10 achieved CR.[78] When ATRA was used in conjunction with low-dose Ara-C in the treatment of "poor prognosis" AML, 48% (16/33) of the patients entered CR, and the CR rate was 88% in 17 patients with < 50% blasts in bone marrow.[79] It is noteworthy that a cytotoxic adverse effect cannot be excluded when low-dose chemotherapeutic drugs are used as differentiation-inducing agents.

Other apoptosis-inducing agents based on modulation of distinct pathways

A great number of new apoptosis-inducing agents are being studied. **Table 3** displays some new compounds (other than the the arsenic compounds previously described) reported recently, and **Table 4** shows medicinal plant–derived or other natural products. The mechanisms of apoptosis induction implicated for these agents can be grouped into the following well-clarified or not-yet-well-clarified categories:

(1) activation of death pathway receptors (FAS, TNF) and signaling, as with DT(338) GM-CSF,[99] tryptanthrin[103]

(2) calcium-dependent apoptosis signal pathway, as with calphostin C[81]

(3) activation of caspases, as with se-methyl selenocysteine,[88] novel retinoid CD437 and its non-retinoidal analog MM002,[96] curcumin,[107] diallyl disulfide,[97] and resveratrol[87]

(4) increase of reactive oxygen species (ROS) production or ROS-dependent apoptosis, as with falconensone A dioxime,[86] β2 microglobulin,[98] and baicalin[111]

(5) disruption or modification of mitochondrial transmembrane potential (Δψμ) or cytochrome c, AIF release, as with potassium antimonyl tartrate,[92] homoharringtonine,[106] theasinensin A,[101] artepillin C,[102] carnosol,[104] curcumin,[107] baicalin,[111] sophoranone,[109] dolichyl monophos-

Table 3. Apoptosis-inducing compounds for acute leukemia cells.

Authors (Year)	Apoptosis-Inducing Agents	In Vitro Study Cell Lines	Mechanism
Fujimura et al[80] (1998)	ATRA 9-*cis* RA 13-*cis* RA	ATL	p21Waf1/Cipl protein↑pRb hypophosphorylation
Zhu et al[81] (1998)	Calphostin C	ALL-1 (pre-pre-B) RS4;11 (pro-B) NALM-6 (pre-B) Daudi (B-ALL) Molt-3 (T-ALL) Jurkat (T-ALL)	Calcium-dependent apoptotic signal pathway
Uckun et al[82] (1999)	B43-genistein	CD19$^+$ B-ALL CLL	CD19-receptor-directed tyrosine kinase inhibitor
Dimitroulakos et al[83] (1999)	Lovastatin	AML cell lines fresh cells	Δψμ↓, glutathione↓
Gong et al[84] (2000)	Arginine deiminase	ALL	Cell cycle arrest in G_1
El-Sabban et al[85] (2000)	As + IFN-α	HTLV-1 transformed cells	Tax downregulation, inactivation of NF-κB pathway
Takahashi et al[86] (2000)	Falconensone A, dioxime	HL-60	Intracellular ROS↑
Dorrie et al[87] (2001)	Resveratrol	HL-60	Independent of CD95 signaling, mitochondria/caspase 9 pathway
Kim et al[88] (2001)	Se-methyl-selenocysteine	HL-60	Activation of caspase 3
Nimmanapalli et al[89] (2001)	STI-571 + TRAIL	bcr-abl$^+$/HL-60	Enhance Apo-2L/ TRAIL induced-apoptosis, increase of caspases 9,3,XIAP processing
Ogata et al[90] (2001)	Picolinic acid-related comp	HL-60	NC
Narla et al[91] (2001)	VO(SO$_4$)(Me$_2$-Phen)$_2$	NALM-6 leukemia cells	Δψμ↓ ROS
Lecureur et al[92] (2002)	K antimonyl tartrate	HL-60	ROS↑, Dym↓
Yasugi et al[93] (2002)	Dolichyl monophosphate	U937	Δψμ↓, AIF translocation, activation caspase 3-like protease
Park et al[94] (2002)	Monensin Na$^+$ ionophore	10 AML cells including HL-60	CDK6 Cyclin D1↓ Cyclin A↓, p27↑, changes in Δψμ, caspase 3, 8, Bax
Hamada et al[95] (2002)	Farnesylpyridinium	HL-60	cytochrome c↑, cytochalasin-like effect
Zhang et al[96] (2002)	Novel retinoid CD437, MM002	WSU-B-CLL primary B-CLL ALL	Caspase 2, 3↑, cleavage of Bcl-XL, activation of caspase 2, 3
Kwon[97] (2002)	Diallyl disulfide	HL-60	Caspase 3 hydrogen peroxide generation increased
Gordon[98] (2003)	β2-microglobulin	CCRF-HSB-2 ALL cell line	Caspase, ROS dependent, release of cytochrome c, AIF
Thorburn[99] (2003)	DT(388)GM-CSF	U937	Activation of FAS-associated DD protein

Abbreviations: AIF, apoptosis-inducing factor; ALL, acute lymphoblastic leukemia; ATRA, all-*trans* retinoic acid; mRNA, messenger RNA; RA, retinoic acid; NC, not clear; AML,acute myeloid leukemia; CLL, chronic lymphoid leukemia; ROS, reactive oxygen species; Dym, mitochondrial transmembrane potential; DD, death domain;TRAIL, tumor necrosis factor alpha-related apoptosis-inducing factor; IFN, interferon; NF, nuclear factor; XIAP, x chromosome-linked inhibitor of apoptosis protein

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Table 4. Medicinal plant-derived apoptosis-inducing agents and other products.

Authors (Year)	Products	Origin	In Vitro Study Cell Line	Mechanism
Tan et al[100] (2000)	Extract	*Sophora flavescens*	HL-60	Apoptosis 5-20 mg/L Differentiation 1.5 mg/L
Pan et al[101] (2000)	Polyphenol theasinensin A	Oolong tea	U937	Caspase 9↑, release of cytochrome c, ROS↑$\Delta\psi\mu\downarrow$
Kimoto et al[102] (2001)	Artepillin C	*Brazilian propolis*	7 T-cell 5 B-cell myeloid, monocytic non-L, M leukemia cell lines	FAS antigen expression $\Delta\psi\mu\downarrow$
Kimoto et al[103] (2001)	Tryptanthrin	*Polygonum tinctorium Lour*	U937 HL-60	Enhances FAS-induced apoptosis, caspase 3↑
Dorrie et al[104] (2001)	Carnosol	Herb rosemary	pro-B ALL pre-B ALL	Bcl-2↓ $\Delta\psi\mu\downarrow$
Li et al[105] (2001)	Erianin	Dendrobium	HL-60	Bcl-2 gene↓, Bax gene↑
Cai et al[106] (2001)	HHT	*Cephatotaxus hainanensis Li*	Molt3	Cytochrome c release, translocation of Bax, activation of caspase
Anto et al[107] (2002)	Curcumin	*Curcuma longa*	HL-60	Caspase 8↑, cleavage of BID, cytochrome c release
Kim et al[108] (2002)	Momordin I	*Ampelopsis japonica*	HL-60	Bcl-2/Bax↓, activation of caspase 3
Kajimoto et al[109] (2002)	Sophoranone	*Sophora tonkinensis gagnep*	U937	Opening of mitochondrial permeability transition pores, ROS↑
Candra et al[110] (2002)	Saponins 7,8	Liliacae	L1210	Not clear
Ueda et al[111] (2002)	Baicalin	*Scrutellaris baicalensis georgi*	Jurkat cells	Caspase 3↑, ROS↑ cytochrome c release, $\Delta\psi\mu\downarrow$
Zhu et al[112] (2002)	Squamocin	Annonaceae (*Annonaceous acetogenins*)	HL-60	Activation of caspase 3 related to SAPK/JNK
Zhou et al (2003)*	SIH-10	Medicinal herb	Kasumi-1	$\Delta\psi\mu\downarrow$, caspase 3↑

* Unpublished data
Abbreviations: HHT, homoharringtonine; L, lymphoid; M, myeloid; ALL, acute lymphoblastic leukemia; ROS, reactive oxygen species; SAPK/JNK, stress-activated protein kinase/JUN IV-terminal kinase;

phate,[93] farnesylpyridinium,[95] lovastatin[83] and bis(4,7-dimethyl-1,10 phenanthroline) sulfato-oxovanadium (IV)$[VO(SO_4)(Me_2\text{-Phen})_2]$[91]

(6) modulation of Bcl-2/Bax, or Bcl-2, as with carnosol,[104] erianin,[105] and momordin I[108]

(7) modulation of cell cycle, inhibition of cyclin D kinase (CDK), as with arginine deiminase[84] and monensin[94]

(8) decrease or inactivation of antiapoptosis activation gene or protein, such as XIAP processing with STI571 + TRAIL[89] and NF-κB with arsenic + interferon (IFN)-α[85]

(9) caspase-3 activation and related to SAPK/JNK, as with squamocin[112]

For the majority of apoptosis-inducing agents, the pathways implicated in the mechanism of apoptosis are usually not unique as shown in **Table 3** and **Table 4** but may involve much more complex networks of modulation.

In general, arsenic compounds are not effective in the treatment of acute leukemia other than APL. The majority of apoptosis-inducing agents or products listed in **Tables 3** and **4** are based on in vitro studies. However, the apoptosis-inducing CD19-directed tyrosine kinase inhibitor B43-genistein has been studied in a clinical trial.[82] Seven children and 8 adults with CD19+ B-lineage acute lymphoblastic leukemia (ALL), and 1 adult with chronic lymphocytic leukemia were treated

with this drug. All patients had failed to respond to previous chemotherapy, and 6 had relapsed after bone marrow transplantation; there were 2 transient responses and only 1 durable CR. In spite of few positive results from clinical trials, the treatment of acute leukemia by triggering apoptosis is still a promising new area deserving further exploration.

Apoptosis-inducing activity in chemotherapeutic agents

A number of known cytotoxic drugs can induce an apoptotic pathway. Treatment of human acute leukemia cells HL-60, U937, and Jurkat cells with etoposide, Ara-C, or doxorubicin can upregulate DR5 levels in a p53-independent manner and sensitize them to TRAIL (Apo-2L)-induced apoptosis.[113] Another mechanism in etoposide-induced apoptosis is the engagement of the mitochondrial pathway by releasing cytochrome c, in which caspase 2 is considered to play an important role.[114] There is evidence that doxorubicin-induced apoptosis in HL-60 cells is ROS- and proteinase-3-dependent, which is downregulated in its doxorubicin-resistant variant.[115] DNA damage caused by doxorubicin can lead to activation of the p53-survivin signaling pathway, inducing cell cycle arrest and apoptosis in childhood ALL cells. Survivin is a novel member of the inhibitors of apoptosis protein.[116] Mitoxantrone and anisomycin are able to induce apoptosis in HL-60 cells by stimulating JNK/SAPK activity in a time- and dose-dependent manner.[117] Adriamycin is capable of inducing apoptosis in the T-cell leukemia line, H9, probably through activating JNK.[118] Cladribine, cytarabine, cisplastin, and 5-fluorouracil have apoptosis- and necrosis-inducing potential in human leukemia cell lines HSB2 and Jurkat.[119] Homoharringtonine (HHT), extracted from a medicinal plant (*Cephalotaxus hainanensis Li*) in China, has long been used as a cytotoxic agent in the treatment of acute leukemia.[120] An in vitro study on HL-60 and K562 cells revealed that HHT exerts its action by inducing apoptosis with downregulation of BCR/ABL gene expression. Laser scanning confocal microscopy examination disclosed that there is an alteration of intracellular calcium distribution in apoptosis of HL-60 cells induced by harringtonine (another active component isolated from *C hainanensis Li*).[121] The apoptotic response to HHT in the human wild-type p53 leukemic cell line MOLT-3 is independent of ROS generation, while Bax translocation, mitochondrial cytochrome c release, and caspases activation have been implicated.[106]

Perspectives and Conclusion

Oncogene- or mechanism-based targeted treatment

ATRA and arsenic compounds are very effective in the treatment of APL, providing a model of success in the treatment of APL through induction of differentiation and apoptosis. However, there is an interesting question: Why do they have limited or even no effect at all in the treatment of other types of acute leukemia? The aforementioned findings may help us to understand the reasons. APL has a unique and specific chromosome aberration t(15;17) resulting in the fusion gene and protein PML-RARα, which plays a crucial role in APL genesis with arrest of differentiation and impairment of apoptosis. Although the mechanism of action of ATRA and arsenic compounds has not yet completely been explained, the data mentioned above suggest a common pharmacological activity of these 2 agents: to target the fusion protein and to cause its degradation. The success in the treatment of APL with ATRA or arsenic compounds furnishes a model of mechanism- or oncogene-based targeted therapy. A second example of considerable success through molecularly based targeted therapy is offered by chronic myelogenous leukemia treated with Gleevec, a specific inhibitor of tyrosine kinase located on ABL of the BCR-ABL fusion protein, resulting from a specific aberration of chromosome translocation t(9;22). A third candidate for study of oncogene-based targeted therapy is M2b AML. This subtype of AML accounts for about 20% to 25% of acute myeloid leukemia and is characterized by a chromosome translocation t(8;21)(q22;q22). The result of this chromosomal aberration is to generate a chimeric fusion product AML1-ETO that plays a central role in genesis of this leukemia. Similar to PML-RARa in APL, the fusion protein recruits a transcription repression complex containing histone deacetylase. A potent histone deacetylase inhibitor, TSA, and a less specific inhibitor, phenylbutyrate, partially reversed ETO-mediated repression,[122] but the clinical result was not as promising. A new candidate is the FLT3 gene. In up to 41% of patients with AML, there are constitutively activating internal tandem duplication (ITD) and point mutations of the receptor tyrosine kinase FLT3.[123] An inhibitor of FLT3-targeted tyrosine kinase CEP-70 has been developed, and the compound is able to inhibit FLT3 in primary leukemia blasts from AML patients harboring the FLT3/ITD mutation. This drug can prolong survival in a mouse leukemia model bearing FLT3/ITD.[123] Recently, SIH-10, isolated from a Chinese medicinal herb, has been shown to induce apoptosis of Kasumi cell line and primary M2 AML (GP Zhou et al, unpublished data). A preliminary in vivo study demonstrated that

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intraperitoneal administration of this compound into Nod/Scid mice bearing M2 AML leukemia model can induce apoptosis of leukemia cells and prolong the life span of the animal by 44%. It seems likely that this new compound could bring benefit in gene/mechanism target-based treatment of M2 AML. Further studies are in progress.

Rational combination of differentiation treatment, apoptosis-inducing treatment, and chemotherapy

The superiority of combining differentiation and/or apoptosis inducers and chemotherapy has already been proved in the treatment of APL. With this strategy, the CR rates are higher, the survival time is longer, and the risk of adverse effects is lower; even drug resistance can be overcome. In vitro studies show that combination of a potent D3 analogue (KH 1060) with 9-cis retinoic acid can irreversibly inhibit clonal growth, decrease Bcl-2 expression, and induce apoptosis in HL-60 cells.[124] IFN-γ is able to sensitize human myeloid leukemia cells to death receptor–induced and mitochondria-mediated apoptosis.[125] Susceptibility to TRAIL-induced apoptosis is augmented by doxorubicin in some primary leukemia cells.[126] A synergic effect is observed in APL cell differentiation through combining arsenic trioxide with cAMP, subtending a new signaling pathway.[127] Arsenic is capable of inducing apoptosis in multidrug-resistant human myeloid leukemia cells that express BCR-ABL or overexpress MDR, MRP, Bcl-2, or Bcl-x(L).[128] Treatment of BCR-ABL-positive leukemia cells with As_2O_3 combined with STI-571 can potentially induce apoptosis and differentiation.[129] Studies on the treatment of acute leukemia by combining different strategies should yield promising results. As indicated above, the treatment of APL with ATRA in conjunction with As_2O_3 could achieve not only a high CR rate with shorter time for remission induction but also a better reduction of leukemic burden, as shown by a much higher reduction of copy number of PML-RARα mRNA transcripts.

In conclusion, the success in the treatment of APL by inducing differentiation and apoptosis with ATRA and arsenic compounds offers convincing evidence to support further investigation of new medications in the treatment of acute leukemia by these 2 new approaches. The key problem is how to find the oncogenes or genes and related proteins involved in the genesis of the leukemia and to elucidate the mechanism by which the leukemia develops. If this were accomplished, it will be possible to design and create new compounds based on oncogene or mechanism-targeted principles. The rational combination of different strategies based on regimens defined by prospective randomized studies will bring benefits in future therapies that might help us conquer the disease.

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