MINIREVIEW

Leukemia Cells and the Cytokine Network: Therapeutic Prospects

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The network and balance of cytokines is of major importance in maintaining proper homeostasis of hematopoiesis. Abnormalities in this network may result in a variety of blood disorders; however, the role of this network is not clear in leukemia. The use of antineoplastic agents has improved the survival rate of some types of leukemia, and adjunctive therapy with cytokines may be helpful. Chemotherapeutic approaches are no longer the best choice because cytotoxicity may affect normal and leukemic cells, and leukemic cells may develop resistance to the chemotherapeutic agent. Induction of differentiation to a mature phenotype and the control of apoptotic-gene expression have provided other possible alternative therapies. Combined effects of cytokines and vitamin derivatives such as retinoic acid (RA) and 1,25 dihydroxyvitamin D3 (VD3) were found more beneficial than any of these agents individually. These agents exhibit cooperative effects, potentiate each other's effects, or both. Therefore, understanding the hematopoietic actions of these agents, their interactions with their receptors, and their differentiation signaling pathways may result in the design of new therapies. However, the role of cytokines in apoptosis is controversial because in some cases they were found to increase tumor cell resistance to apoptosis-inducing agents. Recent studies in the molecular biology of gene regulation, transcription factors, and repressors have led to new possible approaches such as differentiation therapy for the treatment of leukemia. In addition, the development of drugs that act on the molecular level such as imatinib is just the beginning of a new era in molecular targeted therapy in which the drug acts specifically on the leukemic cell. There are many possible combinations of cytokines, retinoids, and VD3, and perhaps the best therapeutic combination is yet to be described.

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Cytokines including colony-stimulating factors (CSFs), other growth factors, interferons (IFNs), and interleukins (ILs) (1–10) form a network that regulates proliferation and differentiation in various hematopoietic compartments (1, 10). Cytokines exhibit different effects including pleiotropy, redundancy, synergy, and antagonism. Pleiotropy, in which the same cytokine may act on different cell types to produces different responses, and redundancy, in which several different cytokines may act on a cell individually to induce the same response, renders the cytokine network as one of the most complicated regulatory networks to understand. Although synergy and antagonism may exaggerate the intricate nature of this network, advantages of these two properties were taken to design new therapeutic regimens. The actions of cytokines have been thoroughly reviewed (1, 8, 10–12).

IL-1 induces inflammatory responses and edema and promotes the production of IL-2, prostaglandins, and the growth of leukocytes. IL-2 promotes proliferation and differentiation of additional CD4+ cells and B cells and activates macrophages. IL-3 stimulates the proliferation of precursors in all hematopoietic lineages. IL-4 stimulates production of IgG and IgE by B cells, promotes CD8+ cell growth, and promotes Th2 cell differentiation. IL-4 inhibits production of the proinflammatory cytokines IL-1 and tumor necrosis factor-alpha (TNF-α). IL-8 is a powerful inducer of chemotaxis and IL-10 down-regulates antiviral responses by inhibiting the production of IFN-γ, antigen presentation, and macrophage production of IL-1, IL-6, and TNF-α. It is also

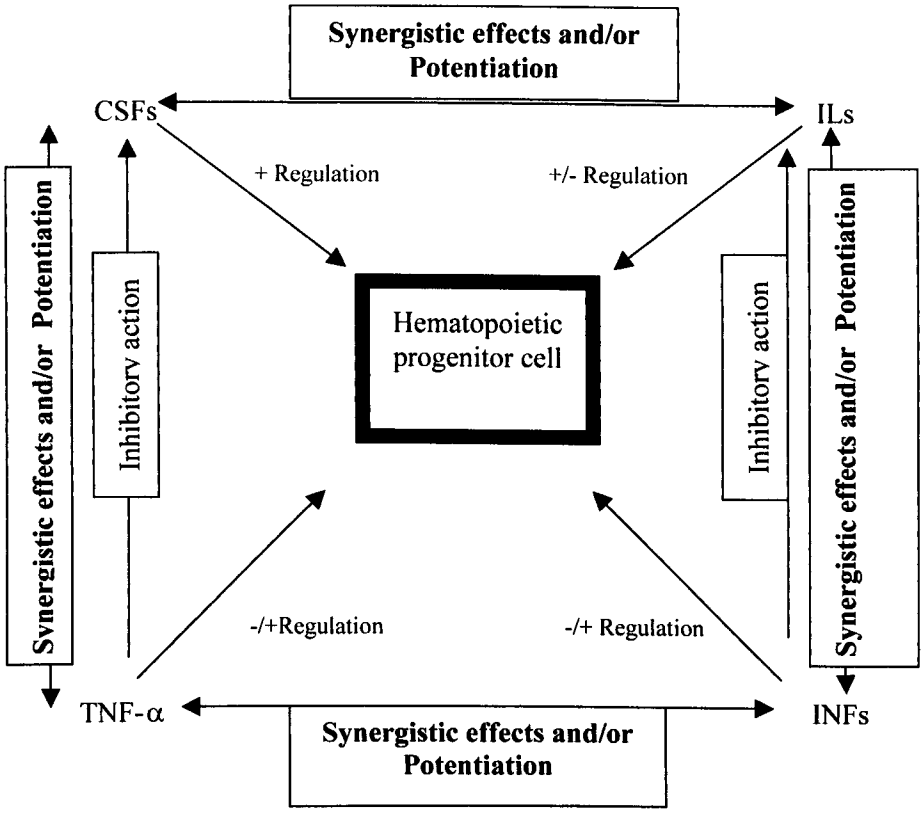

Figure 1. A simplified diagram summary showing the actions and interactions of the main cytokines including positive and negative regulators of hematopoiesis. +/–, more positive than negative regulation; –/+, more negative than positive regulation.

important in B-cell activation. IL-12 promotes Th1 type response in macrophages and NK cells and induces IFN-α production, and IL-13 promotes B-cell differentiation, whereas IL-14 is a B-cell growth factor. IL-17 is a proinflammatory cytokine secreted by activated T cells. The IL-17 family includes IL-17A, IL-17B, IL-17C, IL-17D, IL-17E, and IL-17F, with IL-17A being the prototype member of the family. The cognate receptors for the IL-17 family identified thus far are IL-17R, IL-17RH1, IL-17RL (receptor like), IL-17RD, and IL-17RE (13–15). IL-17 enhances expression of the intracellular adhesion molecule-1 (ICAM-1) in human fibroblasts. It also stimulates epithelial, endothelial, or fibroblastic cells to secrete IL-6, IL-8, and G-CSF. It is also a mediator of neutrophil recruitment (14, 15). IL-18 induces the synthesis of IFN-γ.

In general, there are negative and positive regulators in the cytokine network (Fig. 1; Refs. 1, 8, 10). Although erythropoietin (Epo), stem cell factor (SCF), and IL-3 induce $CD34^+$ cells to differentiate along the erythroid lineage, TNF-α suppresses erythropoiesis by the same cells even in the presence of the three cytokines (9). Recently activin A, bone morphogenetic protein (BMP)2, and BMP4, members of the TGF-β family, have been implicated in the regulation of hematopoiesis (16). BMP2 acts on early erythroid cells, but activin A acts on a more differentiated population. The mechanism of commitment of erythropoiesis by activin A and BMP2 was thought to involve two key events: increase in Epo-R and decrease in GATA2 expression. It was suggested that BMP2 and BMP4 differently affect activin A induction of erythropoiesis (16).

TNF-α inhibits erythropoiesis by inhibiting the generation of glycophorin A^+ generated by $CD34^+$ cells and inhibiting the proliferative capacity of mature erythroid progenitors (9). The action of one cytokine can be amplified by the induction of other cytokines that are required to produce the final cell type, and the network of cytokines couples the events of growth and differentiation. CSFs induce colony formation and stimulate expression of other cytokines to induce differentiation of various cell lineages.

Interleukins can also induce the release of CSFs and/or their synthesis (10, 17–19). For example, IL-1 functions as a positive regulator by inducing the release of CSFs and synergizing with IL-6, G-CSF, and GM-CSF. IL-1 also acts as a negative regulator by inducing the release of TNF-α and prostaglandin E2 (17). G-CSF mobilization of hematopoietic progenitor cells from bone marrow to peripheral blood, in transplant patients, is mediated by IL-6 (20). The recently recognized "IL-10 family" comprises five novel cytokines: IL-19, IL-20, IL-22 (IL-TIF), IL-24 (human MDA-7, mouse FISP, rat C49A/Mob-5), and IL-26 (AK155; Ref. 6). This family of cytokines shares not only homology but also receptor subunits and perhaps activities. They are all involved in regulation of inflammatory and immune responses (6).

In general, ILs and CSFs synergize or mediate each other's actions to stimulate the production of certain hematopoietic lineage (5, 6, 17, 21, 22). Among all hematopoietic growth factors, GM-CSF, G-CSF, and M-CSF are known to regulate primary myelopoietic lineages. However, other cytokines participate in the regulation of myelopoiesis such as SCF, TNF-α, and Flt3 (23–26). GM-CSF and transforming growth factor (TGF)-α are weak inducers of differentiation of the human monoblastic cell line U937 into mature myeloid cells (1, 21, 27). However, synergistic actions were reported when combination of the two agents with each other or with other differentiation inducers, such as RA, VD3, and TNF-α, were used to stimulate differentiation of U937 cells (8, 27–32). These combinations include GM-CSF and TNF-α, GM-CSF and VD3, and TNF-α and TGF-α. These actions indicate the existence of certain differentiation signals generated by the receptors for each cytokine.

IL-6 is a pleiotropic cytokine produced by a variety of cells that respond to viral and bacterial infections (20, 33, 34). Besides its immunological functions such as the induction of immunoglobulin by stimulated B cells, it synergizes with VD3 to induce differentiation of leukemic cells (35). Cytokines exert their multiple biological functions through interactions with their specific receptors. Cytokines mediate their response via cell surface receptors that in turn activate intracellular signaling pathways and lead to gene activation and cell proliferation and differentiation. The binding of cytokines to their receptors induces homo- or heterodimerization of the receptors and triggers activation of intracellular signaling events, which lead to the induction of gene expressions (29, 36). The Janus kinase (JAK) families, which are constitutively associated with the receptors, are the earliest to activate. The signal transducers and activators of transcription (STAT) family is involved in cytokine signal transduction. This JAK-STAT pathway is a simple signaling cascade that directly links the receptor and gene expression. Biological roles of each STAT family protein have been elucidated through studies of gene-targeted mice. For example, STAT1 knockout mice are defective in IFN-mediated functions; STAT4 and STAT6 knockout mice show defective responses to IL-12 and IL-4, respectively; and STAT3 plays a critical roles in cytokine-mediated functions (31). Therefore, cytokines and the cytokine receptor pathways are frequently mutated in disease, thus shedding light on the generation of the inflammatory response, specific immunity, and mechanisms of hematopoiesis. Many approaches are being used to translate this basic research into successful therapies.

Flt3 ligand is a cytokine that affects the growth and proliferation of stem cells and multipotent progenitor cells through stimulation of Flt3 receptor tyrosine kinase by expanding hematopoietic progenitor and dendritic cells (23–25). It supports macrophage differentiation and seems to be involved in lymphoid and myeloid progenitor cell development perhaps by synergizing with IL-6. In this regard, it was demonstrated that early lymphoid and myeloid progenitors were found to express Flt3; however, once these progenitor cells were committed to the B- and T-cell lineage, the receptor was down-regulated (24). Intracellular signaling initiated via Flt3 seems to be important in the leukemogenesis of acute myeloid leukemia (AML) cells. An internal tandem duplication (ITD) of the juxtamembrane domain of Flt3 has been found in 20–30% of patients with AML. ITD-Flt3 has antiapoptotic effects and is associated with leukocytosis and poor prognosis (24, 25). Therefore, Flt3-targeted inhibition has been suggested for the treatment of AML with ITD mutation (23, 25). Moreover, knowledge of the apoptotic pathways may lead to specific and selective induction of the AML cell death. Perhaps a combination of flt3 ligand with differentiation agents such as RA or VD3 may be of therapeutic interest.

Interferons are pleiotropic cytokines that exhibit negative regulatory effects on the growth of normal and leukemic cells (2). Interferons fall in two different classes. Type I includes IFN-α, IFN-β, and IFN-ω, and type II includes IFN-γ only. Interferons signaling pathways in hematopoietic cells involve activation of tyrosine kinases of the STAT and JAK families (2). Type I and type II interferons synergize in blocking the growth of hematopoietic progenitors of all lineages including the progenitors derived from isolated CD34+ CD38– cells (2, 3). CD34+ is a marker of the hematopoietic stem cell (HSC) found in bone marrow, cord blood, and peripheral blood (37). This indicates that interferons may act on early stages of stem cell differentiation. Additionally, interferons are thought to mediate the inhibitory actions of other cytokines (Fig. 1) on the growth of hematopoietic progenitor cells (2). Reported hematopoietic actions of TGF-α varied. It has been shown to inhibit early hematopoietic cell proliferation in the presence of Epo, SCF, GM-CSF, and IL-3. However, others have reported synergistic stimulatory action with GM-CSF on hematopoietic cell proliferation and CD34+ cells from umbilical cord blood (5).

Hepatocyte growth factor (HGF) is a cytokine produced by human bone marrow stromal cells, which also expressed the c-MET/HGF receptor (38). The production of HGF was inhibited by the production of TGF-α. Furthermore, HGF promoted the formation of burst-forming unit–erythroid (BFU-E) and colony-forming unit–granulocyte erythroid macrophage by bone marrow mononuclear cells in the presence of Epo and GM-CSF (38). IL-9 provides another example on the complicated interactions of cytokines. IL-9 has been demonstrated to support early erythroid progenitor cells, BFU-E, in the presence of Epo (39), to enhance the development of eosinophils from $CD34^+$ cells and most recently to potentiate megakaryocytopoiesis in the presence of Epo, SCF, or both (39, 40).

Abnormalities. Abnormalities in the cytokine network have been involved in many types of leukemia (1, 3, 21, 22, 41–46). Autocrine and paracrine activities by leukemic and normal cells represent largely the mechanisms of

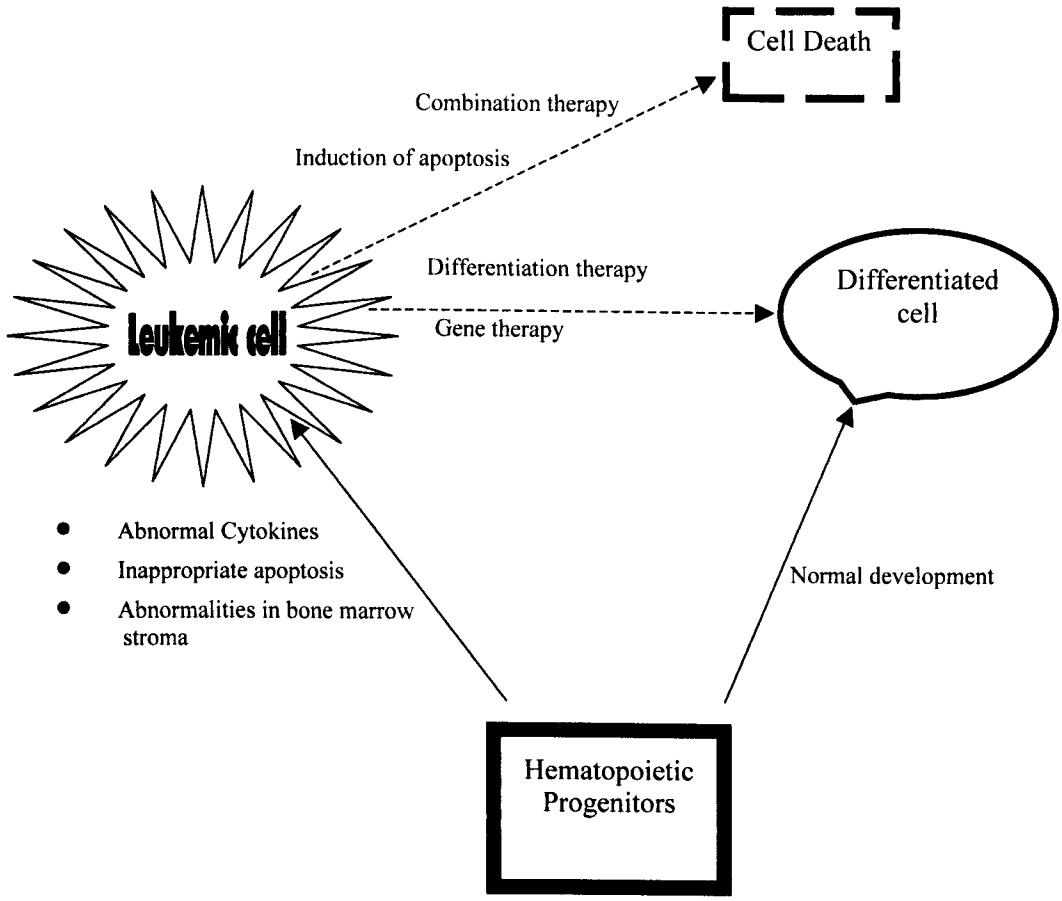

Figure 2. A simplified diagrammatic representation showing factors that can lead to the development of leukemic condition and the possible experimental therapeutic approaches. Dotted arrows indicate proposed therapies.

survival for these cells (1, 43–45). Therefore, a successful therapy for this type of leukemia should deprive leukemic cells of the cytokine(s) necessary for their survival with minimal effect on normal cells. Such treatment may be difficult *in vivo* because (i) many cell types in the body may produce any type of cytokine(s), (ii) the cytokine(s) produced is/are likely to be necessary for growth and function of normal hematopoietic cells, and (iii) malignant cells survival may not be strictly depending on one specific cytokine. In chronic myelomonocytic leukemia (CMML), the increase in the number of monocytes in blood and bone marrow is thought to be caused by GM-CSF produced either in autocrine or paracrine mechanisms.

It has been demonstrated recently that cultured stromal cells and macrophages, from patients with myelodysplastic syndrome, produced excessive amounts of TNF-α and IL-6, compared with their counterparts in healthy donors (46). This supports the view that production of cytokines by stromal microenvironmental cells is impaired in patients with various forms of myelodysplastic syndrome (Fig. 2). Other cytokines such as IL-4, IL-6, IL-10 and TNF-α were also reported to be involved in chronic myelocytic leukemia (CML; Ref. 44). Bone marrow cells from patients with CML formed spontaneous colonies in methylcellulose cultures and showed an *in vitro* and *in vivo* dependence on GM-CSF (44). CMML colony growth from patients' bone marrow cells was reduced by 92% when E12R, a specific GM-CSF antagonist, was added to CMML methylcellulose cultures (44). Elevated levels of endogenous serum and surface receptor expression of GM-CSF and IL-3 were reported in patients with myelodysplastic syndromes (45). Moreover, progenitor cells in juvenile myeloid leukemia were shown to be hypersensitive to GM-CSF (47). Nonetheless, GM-CSF is also used clinically to repair irradiation and chemotherapy associated suppression and to stimulate normal granulocyte development of normal hematopoiesis in leukemic patients.

A successful therapeutic approach should determine the cytokines that are necessary for the initiation or progression of a given disease. Consequently, the therapeutic combination, which results in maximum cytotoxicity to leukemic cells and minimal effect on normal cells, may be determined. Dysregulation of cell surface structures may contribute to neoplastic transformation or leukemic cell proliferation. Furthermore, proliferation of leukemic cells is stimulated by autocrine and paracrine processes. These processes are mediated by the cytokine network or interactions with other cells and molecules. The expression of molecules such as CD30, CD40 by AML blasts, and their interaction with their

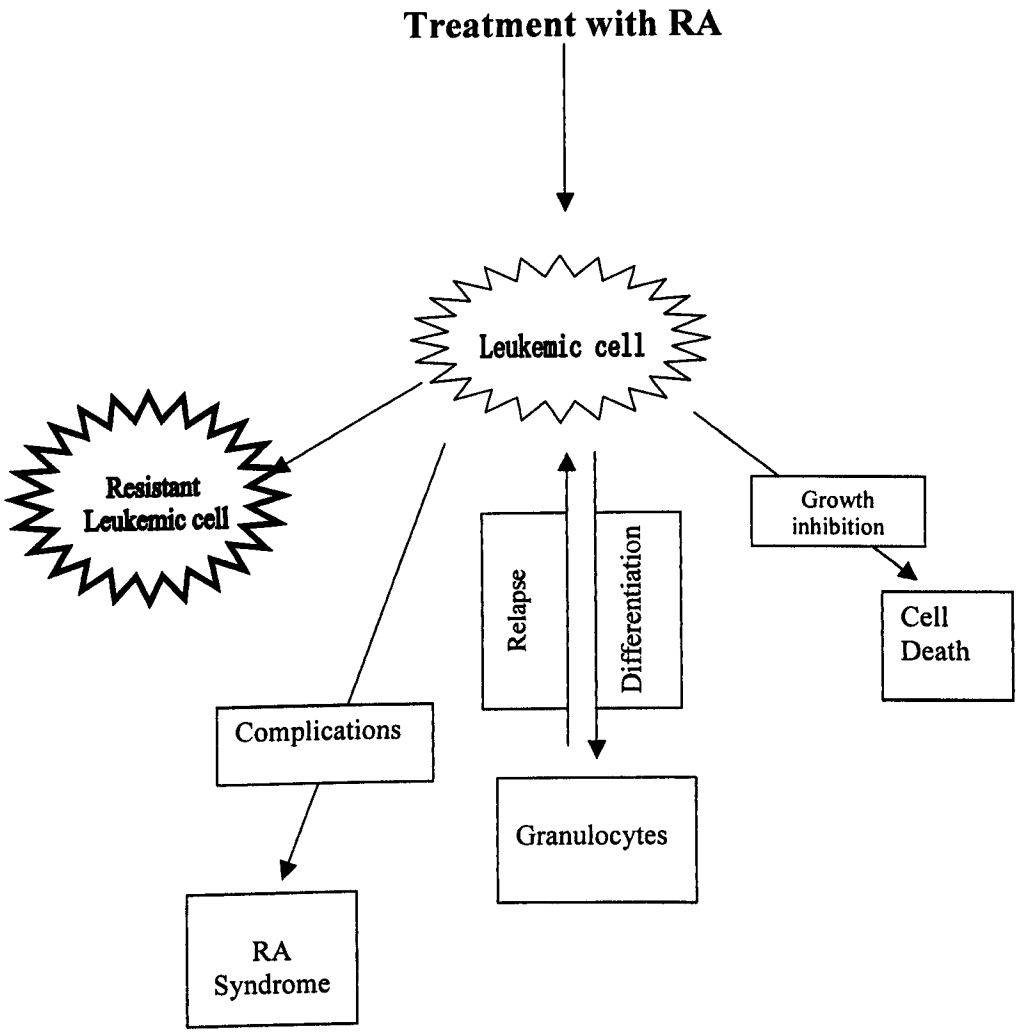

Figure 3. A simplified representation of the effects of RA treatment on leukemic cells.

ligands CD30L and CD40L have been associated with production of cytokines such as IL-4 and IL6 and triggers pleiotropic activities such as proliferation, self-renewal, and rescue from apoptosis (48, 49). Results of such studies should be taken in consideration when designing immunotherapies.

Retinoic Acid. Retinoic acid is a natural derivative of vitamin A and plays an important role in the growth, differentiation, and development of normal and transformed hematopoietic cells (22, 30, 50–55). It has been implicated as a chemopreventive and differentiating agent in a variety of leukemias (Fig. 3). These leukemias have been shown to induce differentiation of transformed cells and inhibit their proliferation (27, 28, 30, 56, 57). Retinoids promote granulocytic differentiation of HL-60 cells, U937 cells, and fresh acute promyelocytic leukemia (APL) cells. All trans RA induce complete remission in patients with APL (1, 22, 28, 30, 50–55). Retinoids play a critical role in embryonic morphogenesis, epidermal cellular growth, and hematopoiesis (1, 28). Topical and systemic retinoids are emerging as a therapy of choice (58) for the treatment of AIDS-related Kaposi's sarcoma. Topical retinoid treatment appears to be associated with greater efficacy and less toxicity than systemic retinoids. Retinoids inhibit the clonal growth of fresh leukemic cells and cell lines from patients with AML *in vitro* and *in vivo* (1, 22, 50–52). However, RA syndrome is a major complication in patients with APL treated with RA. This syndrome is represented by leukocytosis during RA therapy (59). In such cases combination therapy might be the therapy of choice (discussed in "Combination Therapies" section).

Recent studies on cytokines, RA, and VD3 receptors have made significant progress on the road to understanding the process of growth and differentiation. The RA-induced cell cycle arrest and differentiation of myeloblastic leukemia cells is mediated by retinoic acid receptors (RARs) and retinoid receptor (RXRs; Fig. 1). However, recent studies have shown the involvement of the Fc gammaRII immunoglobulin receptor in mediating RA-induced growth arrest and differentiation of HL-60 myeloid leukemia cells (60). Similar results were obtained with VD3 (60). More understanding of the RA-and VD3-induced myeloid differentiation has recently been possible (61–63). In the case of

RA-induced myeloid differentiation, HL-60 cells transfected with polyomavirus small t antigen (PST) underwent apoptosis instead of differentiating into a mature phenotype. This apoptotic effect by PST was associated with the expression of an early cell surface differentiation marker, CD11b, whereas the more mature differentiation marker was inhibited. On the other hand, treatment of the PST transfectants with VD3 resulted in slowing down the differentiation process but did not block it (63).

The CXC chemokine receptor is a product of the Burkitt's lymphoma receptor 1 blr1 gene. It has been found to enhance and accelerate the RA- and VD3-induced myelomonocytic cell differentiation (61, 62). Experiments on the U937 human leukemia cell line showed that treatment of these cells with RA induced the blr1 mRNA within 12 hrs. The U937 cells transfected with blr1 differentiated faster and in higher percentages when treated with either RA or VD3 (61). Other studies have shown that RA-induced myeloid differentiation of HL-60 human myeloblastic leukemia cells is accompanied with an increase in RAF phosphorylation that requires mitogen-activated protein kinase kinase-dependent ERK2 activation. The induced RAF phosphorylation, as well as anteceding extracellularly regulated kinase (ERK)2 activation, was shown to depend on ligand-induced activation of both an RAR-α and an RXR receptor (64, 65). In fact, RA-induced myeloid differentiation and G1/G0 growth arrest of HL-60 cells require the activation of the RAR-α and RXR retinoid receptors, as well as activation of the mitogen-activated protein kinase, ERK2 (65). In this regard, Battle *et al.* (61) showed that the signals known to be required for HL-60 differentiation, activated RAR-α, RXR, and ERK2, are necessary for blr1 mRNA expression. These findings may contribute to the understanding of the signals controlling cell differentiation and ultimately may help in the design of new therapies. In this regard, understanding of the mechanism by which myeloid leukemia cells become resistance to RA-induced differentiation may be of great therapeutic value.

1,25 dihydroxyvitamin D3. 1,25 dihydroxyvitamin D3 has been proven to be a differentiating agent for several leukemic cell lines in supraphysiological levels. It regulates myeloid cell line differentiation along the monocyte/macrophage pathway (66–70). In a recent study by Grande *et al.* (71), it was demonstrated that physiological levels of this vitamin play a role in the regulation of normal hematopoiesis. Physiological concentrations of VD3 promoted differentiation of CD34+ hematopoietic progenitors in the presence of different combinations of cytokines. This differentiation was characterized by the induction of all the monocyte/macrophage immunophenotypic and morphological markers. This effect was observed at both the terminal maturation and the commitment levels. These differentiation effects are thought to be mediated by the VD3 genomic signaling pathway (71).

NB4 cells represent an *in vitro* model of differentiation for APL. Although these cells respond to all-*trans*-retinoic acid to form neutrophils, NB4 cells are also capable of terminal monocytic differentiation in response to combined treatment with VD3 and 12-O-tetradecanoylphorbol-13-acetate (TPA) (1, 66). Experiments utilizing tyrosine kinase and phosphatase inhibitors supported the hypothesis that VD3 signaling was mediated by both serine/threonine and tyrosine phosphorylation cascades. This provided evidence to support the hypothesis that VD3 signaling occurs via nongenomic mechanisms, which, when combined with the signaling effects of TPA, allows for the terminal differentiation of APL cells (72). On the other hand, as the acute promyelocytic leukemia cell line NB4 undergoes monocyte/macrophage differentiation, it strongly expresses alkaline phosphatase activity after exposure to the combination of VD3 and phorbol 12-myristate 13-acetate treatment (68, 69).

VD3 is a potent stimulator for M-CSF production by human monocytic cells. This effect of VD3 on human monocytes is attributed, at least in part, to the stimulated secretion of M-CSF (69). However, VD3 inhibits proliferation and cytokine production by activated T lymphocytes (41, 69, 72). VD3 directs monocytic differentiation of normal and leukemic cells by interacting with its nuclear receptors (VDRs), which have been demonstrated in many hematopoietic cells (41, 66). VD3 may be involved in the regulation of the immune system because it stimulates differentiation of human and murine leukemia cells into macrophages and granulocytes. VD3 receptors are not present in normal resting human T and B lymphocytes. However, the receptors were demonstrated in human T lymphocytes 24 hrs after activation by phytohemagglutinin P or concanavalin A or in human B lymphocytes after infection with Epstein-Barr virus (41). Other studies have demonstrated the absolute requirement of VDRs for VD3-induced monocyte/macrophage differentiation, and the monocyte/macrophage differentiation can occur in the absence of this receptor (41, 66, 67). Other hematopoietic actions of VD3 include the up-regulation of the proliferative response of erythroid progenitors to Epo by increasing the Epo receptors on the mRNA and protein levels. VD3 can also enhance proliferation of erythroid progenitors in the absence of Epo (73).

Molecular Aspects

In the past few years, there has been much literature on molecular and cellular characteristics of leukemic stem cells (LSCs); however, it is not within the scope of this article to describe in detail the latest advances in this area. In brief, it is known that identification and characterization of specific chromosomal translocations associated with leukemia are important for management of leukemias such as CML and AML. Genetic alterations in AML involve chromosomal translocations, deletions, and inversions. Characterization of

these genetic lesions including alterations in the structure and function of transcription factors and other nuclear proteins has provided some insight into the mechanism of leukemogenesis (74, 75). Different AML subtypes have been categorized in spite of the fact that no morphologic or immunophenotypic distinction exists between cells that contain any one of four different chromosomal translocations. The (11; 17) translocation, which encodes the PLZFRARα fusion protein, predicts resistance to RA, whereas the other translocations do not (74, 76).

Recent studies (76) suggest that the population of malignant cells found in human AML arises from a rare population of LSCs that have been phenotypically described as CD34+/CD38– or CD34+/HLA-DR–. In these LSCs the IL-3 receptor alpha chain (IL-3Rα or CD123) was strongly expressed. However, normal bone marrow–derived CD34+/CD38– cells did not express the CD123 antigen (76). It was suggested that IL-3Rα represents the marker for primitive LSCs because it is strongly expressed. Therefore, it was proposed that targeting of IL-3Rα might be a promising therapeutic approach.

Translocations and inversions commonly generate fusion or chimeric proteins. Characterization of these proteins, mainly transcriptional regulators, has allowed for the identification of key pathways that lead to leukemogenesis. The involvement of the *AML1/CBF*β, *RAR*α, *MLL*, *NUP98*, and *TEL* genes suggests that their target genes play critical roles in hematopoiesis. The mechanisms involved in gene dysregulation suggest that attacking pathways commonly utilized by the leukemia cell will be effective in patients with these diseases. The biologic functions of fusion transcription factors, generated by specific chromosomal translocations in AML or acute lymphoblastic leukemia (ALL), are not clearly understood but may be important to regulating the disease. The leukemic differentiation block is attributed to deregulated transcription caused by chimeric fusion proteins, which aberrantly recruit histone-deacetylase (HDAC) activity. In many cases these proteins function as transcriptional repressors, and the mechanism of transcriptional repression has recently been identified (61, 64, 65, 74, 77–82). The PML-RARα, PLZF-RARα, AML1-ETO, and TEL-AML1 fusion proteins have all been shown to exert transcriptional inhibitory activity on gene expression, which is attributable to their ability to bind or "recruit" corepressor molecules such as mSin3, N-CoR, or SMRT, which bind to one or both portions of these fusion proteins (77, 78, 80, 81, 83). For example, a study by Lutterbach *et al.* (81) demonstrated the interaction of ETO, a target of t(8:21), with the corepressors mSin3 and N-CoR, and provided a model for how AML1/ETO can repress transcription of AML-1 targets.

Other studies (84) described the importance of the PML-RAR-α and PLZF-RARα/HDAC complex in the development of APL. Inhibitors of HDAC dramatically potentiate retinoid-induced differentiation. On the other hand, the leukemic fusion proteins and inhibitors of HDAC block the VD3 signaling differentiation pathway. The AML-associated translocation products block differentiation not only by interfering with chromatin modeling but also by sequestering factors involved in the differentiation signaling pathways, such as VDR in the VD3-induced differentiation (83). As a result, new classes of drugs, which have the potential to restore gene expression, are considered for potential clinical trials.

The Philadelphia chromosome (Ph) is one of the well-characterized cytogenetic abnormalities in leukemia. It is detected in many types of leukemias such as CML, ALL, and monocytic leukemia (85–87). CML is characterized by the presence of the Ph, and 20% of ALL patients also show this genetic abnormality. In CML the Ph chromosome is formed by a reciprocal translocation between chromosomes 9 and 22 that fuses BCR-encoded sequences upstream of exon 2 of c-ABL. The BCR-ABL fusion creates a gene whose protein product, p210BCR-ABL, has been implicated as the cause of the disease (85). The Ph has been shown to be an independent risk factor in some leukemias such as ALL (86, 87). Furthermore, survivin, an inhibitor of the apoptosis, is overexpressed in many cancers but not in normal differentiated adult tissues. A study done on a group of 12 patients with CML, representing both chronic and blastic phases of the disease, showed that patients in chronic phase CML were uniformly negative for the survivin transcript (88). In contrast, Ph-positive CML patients in blastic crisis were positive for survivin. This study indicated that the up-regulation of survivin expression might be associated with the evolution from the chronic into the blastic phase of CML.

Stromal Cells and Leukemia

The role of bone marrow stroma cells in leukemogenesis is important but not clearly understood. Stromal cells are major components of bone marrow, liver, and spleen microenvironments (1, 89, 90). Their importance in normal hematopoiesis and the initiation of leukemia is well documented. The essential role of stromal cells in normal and abnormal hematopoiesis is a consequence of their role in adhesion, migration, and cytokine production (Fig. 4). Recent studies suggested that different microenvironments (bone marrow, fetal liver, and spleen) might use different mediators for hematopoietic support. For example, liver cell lines express higher levels of extracellular matrix proteins such as osteopontin, fibronectin, and laminin, whereas bone marrow cell lines express higher levels of the matrix proteins Sca-1 and lower levels of vascular cell adhesion molecule-1 (VACAM-1; Ref. 89). Therefore, homing of HSCs may depend on expression of various surface and matrix proteins. In addition to that, cytokines, as part of the bone marrow microenvironment, play a major role in the support provided by stromal cells to the differentiating hematopoietic progenitors cells. In this regard, experiments on blast colony–forming cells (CFU-BL) derived from bone

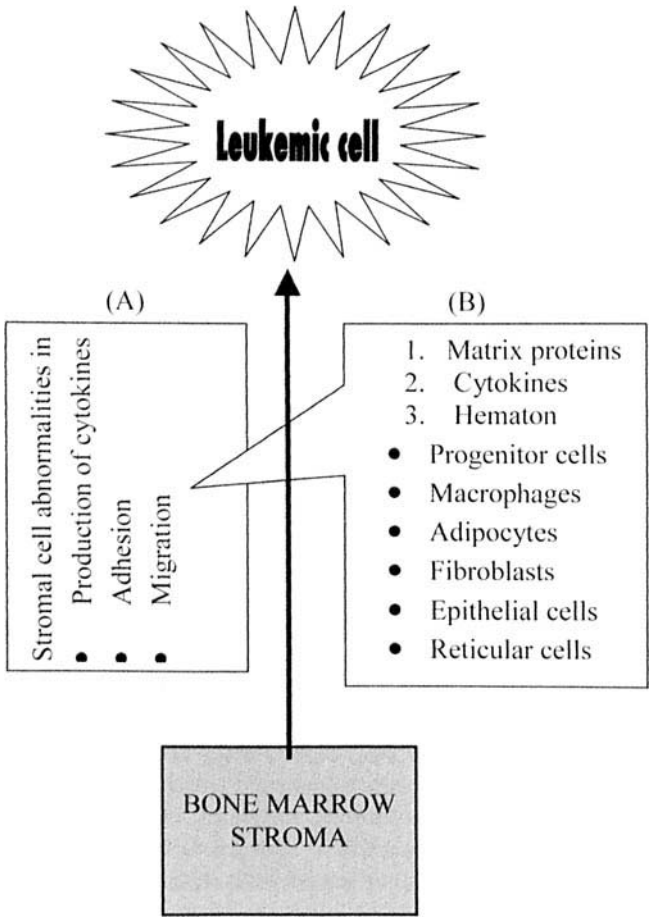

Figure 4. Box (A) shows the abnormal changes in the components of bone marrow stroma, which either may lead to leukemic condition or may be the result of a leukemic condition. Cellular components of bone marrow stroma are shown in box (B).

marrow of CML patients have been shown to adhere poorly to bone marrow–derived stromal layers (91). CFU-BL is a specific subpopulation of primitive progenitors that can form colonies only when in close contact with a stromal layer. Consequently, the appearance of progenitors and precursors in the circulation is due, therefore, to a defective adhesion of these cells to the bone marrow microenvironment. An *in vitro* study (91) demonstrated that local concentration of hemopoietic growth factors such as G-CSF and SCF did influence the attachment of CML progenitors to the stroma.

The structural integrity of the bone marrow microenvironment is usually evaluated by analyzing the morphogenetic unit, the hematon. The hematon is a multicellular, tightly packed, stromal web that includes fibroblasts, adipocytes, endothelial cells, resident macrophages, hematopoietic cobblestone area–forming cells, high-proliferative potential colony-forming cells, granulocyte-macrophage colony-forming unit, BFU-E, and terminally differentiated cells in normal bone marrow (92). Primary myelodysplasia (MDP), AML, and CML are considered disorders of clonal stem cell division. Blazsek *et al.* (93) demonstrated that although normal amounts of hematon complexes were present in bone marrow aspirates from healthy donors, hematon complexes were absent in bone marrow aspirates from patients with MDP and AML in the first perceptible phase and from those with CML throughout the disease.

Recent *in vitro* studies indicate that both cell composition and functional abnormalities of the hemopoietic microenvironment are present in a proportion of patients with myeloid leukemia, both chronic and acute. In addition, VD3 was also absent from the plasma of some of the patients, thus indicating a severe impairment in the metabolism of low-density lipids and lipophilic hormones in the bone marrow microenvironment of those patients. Therefore, it was concluded that disorganized stromal network and hormonal disturbances might contribute to the onset and progression of human myeloid leukemias. Other studies have reported cell composition abnormalities in patients with AML; these abnormalities include reduced numbers of fibroblast progenitors, macrophages, and adipocytes and deficient hematopoietic supportive capacity of stromal cells *in vitro* (93, 94). Macrophages and fibroblasts from myelodysplastic syndrome (MDS) marrow produced significantly higher levels of TNF-α and IL-6 than their normal counterparts (95). These abnormalities in MDS bone marrow were accompanied with an increased apoptotic index.

Apoptosis

Apoptosis-inducing therapy is one of the most recent strategies for leukemia therapy because leukemia is the result of an inappropriate apoptosis and excessive proliferation (1, 96, 97). IL-6 is a B-cell growth and differentiation factor; it is thought to promote the growth of B-cell neoplasms. In chronic lymphocytic leukemia (CLL), the increase in the level of IL-6 is correlated with the advancement of the disease and the patient's survival time. Higher IL-6 level correlated with shorter survival, suggesting that IL-6 may be a useful prognostic marker (42). M1-t-p53 myeloid leukemic cells have a temperature-sensitive p53 protein that changes its conformation to wild-type p53 after transfer from 37°C to 32°C (97). An early lysosomal rupture, mitochondrial damage, and the appearance of apoptotic cells occurred in these cells after transfer to 32°C. Lysosomal rupture, mitochondrial damage, and apoptosis were all inhibited by the cytokine IL-6. This clearly indicates that cytokines can decrease apoptosis in leukemic cells, and, therefore, inhibition of cytokine activity may improve therapy by enhancing apoptosis (96–98). Both leukemic cells and normal hematopoietic cells are similarly stimulated by cytokines. G-CSF was found to initiate apoptosis of the radiation-induced murine leukemia cell line, C2M-A5. Therefore, cytokines seem to be a key substance of apoptosis of leukemic cells (96).

Knowledge of apoptosis and apoptotic genes has contributed to new therapeutic approaches to leukemia. That is accomplished by manipulating apoptosis-inducing or apoptosis-suppressing gene products (10, 98). IL-3, IL-6, IL-7, G-CSF, and GM-CSF are mainly the cytokines that regulate the apoptotic machinery. Cytokines suppress apoptosis induced by wild-type p53 and by irradiation and cytotoxic cancer chemotherapeutic compounds (10, 98). Therefore, cytotoxic chemotherapy can improve by increasing the expression of apoptosis-inducing genes in cancer cells. The *c-myb* gene is highly expressed in immature hematopoietic cells, and its expression declines as the cells mature (99, 100). It has been demonstrated that *c-myb* gene binds to the *bcl-2* gene, a promoter of the antiapoptotic oncoproteins and regulates its transcription (101). Overexpression of the *c-myb* gene is thought to be involved in leukemogenesis, and, therefore, disruption of it function would inhibit the *bcl-2* function (102). Yi *et al.* (102) were able to induce apoptosis in K562 leukemic cells by disruption of the c-myb and therefore suggested that dominant negative gene therapy could be an effective alternative in providing treatment for leukemic patients.

Retinoic acid was found to inhibit G-CSF-induced proliferation of CD34+ cells but not GM-CSF-induced proliferation. However, RA opposed antiapoptotic effects of G-CSF and GM-CSF on bone marrow CD34+ cells. Moreover, RA-induced apoptosis of CD34+ cells and CD34+CD71+ cells were stimulated with Epo. Study of the receptor-signaling pathways involved in RA-induced apoptosis showed that only RARs were involved in RA-mediated apoptosis of myeloid progenitor cells, whereas both RARs as well as RXRs were involved in RA-mediated apoptosis of erythroid progenitor cells (103). This variation in apoptotic mediation of the RARs is worth further investigation. Perhaps one can selectively induce apoptosis in either the erythroid or myeloid compartments of bone marrow using RA in combination with other agents such as cytokines or VD3. Studies have shown that, using differentiating agents, induction of differentiation is associated with increased resistance to apoptosis-inducing agents, such as chemotherapy and gamma-irradiation (104). Among the differentiation agents used in the treatment of AML and other types of leukemia are RA, VD3, and GM-CSF. It has been demonstrated that incubation of human myeloid leukemic cells with RA or VD3 induced resistance to idarubicin-induced apoptosis, which was modulated by coincubation with GM-CSF (104). Therefore, understanding of the processes by which the cells escape cytotoxic drug-mediated apoptosis is essential.

It is known that apoptosis can be initiated through the TNF family of death receptors (7). Unlike other members of the TNF ligand family, TNF-related apoptosis-inducing ligand (TRAIL) preferentially induces apoptosis in tumor cell lines but not in normal cells, suggesting that TRAIL could potentially represent a powerful cancer therapy. The TRAIL receptors that have been identified are TRAIL-R1/DR4 and TRAIL-R2/DR5 (7). These receptors contain cytoplasmic death domains and signal apoptosis. Needless to say, knowledge of the TRAIL signaling pathway and understanding their receptors' regulation and physiological role can help in the design of a successful therapy.

Combination Therapies

In normal hematopoiesis, there is a balance between cell proliferation and differentiation on the one hand, and programmed cell death (apoptosis) and cell senescence on the other hand. Leukemia may result from disturbances or irregularities in this balance (Fig. 2). Cytokines are among the tools that regulate some of the molecular pathways leading to normal hematopoiesis. As a result, cytokines might contribute to leukemogenesis or be used to treat leukemia. In addition, hematopoietic growth factors are being used in combination therapies not only to stimulate proliferation of hematopoietic cells affected by the chemotherapy but also to increase the percentage of leukemic cells in the S phase and render them susceptible to the cytotoxicity of the chemotherapeutic agent. Furthermore, combinations of cytokines have been used to enhance body immunity. It has been demonstrated that AML cells can be sensitized to lymphokine-activated-killer (LAK) cells by preincubation of AML bone marrow cells with combination of the cytokines IL-1α, IL-3, IL-6, SCF, Epo, and GM-CSF (105, 106). This *in vitro* study demonstrated a synergistic action between cytokines and LAK cells to kill AML clonal cells.

In a study by Meloni *et al.* (107), 55% of the patients affected by AML in advanced phase of the disease achieved complete remission with a high dose of intravenous recombinant IL-2. However, only less than 50% of those achieved long-term survival (more than 9 years) with subcutaneous prolonged IL2 therapy. This study showed that subcutaneous low doses of IL-2 could be given for a prolonged period of time without serious organ-specific late follow-up. On the other hand, combinations of cytokines, such as IL-2 and rexinoids, were recommended for lymphoid malignancies because rexinoids were found capable of up-regulating high-affinity IL-2 receptor expression. Rexinoids binding to both the RAR and RXR families of retinoid receptors have been shown to mediate genes associated with both growth and differentiation. RXR rexinoids have demonstrated efficacy in the treatment of cutaneous T-cell lymphomas and human T- and B-cell leukemia cells (36). Furthermore, synergistic effects were reported between RA and TNF-α (108) and IL-6 (109) on glioblastoma and HL-60 cells, respectively.

This synergism was the result of the up-regulation of TNF-α and IL-6 receptors by RA. Treatment of human myeloblastic leukemia ML-1 with all-trans RA- and GM-CSF–induced differentiation of these cells to granulocytes, whereas all-trans RA alone was unable to induce differentiation of the ML-1 cells (110). The induction of RAR alpha by GM-CSF was suggested as a mechanism for

stimulation of RA by GM-CSF. Similar effects were suggested in other myeloid cell lines such as THP-1 and KG-1 (110). Other studies (53) have revealed that RA induces morphological changes and growth inhibition in cultures of human medulloblastomas. This effect correlated with down-regulation of the leukemia inhibitory factor that is expressed in human medulloblastomas *in vitro* and *in vivo* (53).

Retinoids, VD3, and cytokines exert similar effects on hematopoietic cells; nevertheless, their actions vary according to the target cell and agent in concern. Experiments on these agents have demonstrated that combinations of these compounds were superior to their individual effects (41, 55). The reported effects of combinations of the three classes of compounds varied enormously, depending on the combination and the target cell. Cytokines as well as retinoids combined with VD3 and analogues synergistically enhanced differentiation induction in human transformed hemopoietic cell lines (27, 28, 56). Retinoids may act through the up-regulation of some cytokines. Am-80, a synthetic retinoid, exerts its inhibitory effect by modulating the production of IL-6 (56). Cytokines such as IFN-α, IFN-γ, G-CSF, TNF-α, IL-1, and IL-4 markedly potentiate differentiation-inducing effects of retinoids (10, 26, 29, 111). Other studies (19) demonstrated that IL-3 and GM-CSF regulate the transcriptional activity of retinoic acid receptors during myeloid cell differentiation. Human APL, which is associated with a block to granulocytic differentiation, is characterized by disruption of RARs. Retinoids were also reported to synergize with cytokines such as IFN-α, IFN-γ, TNF-α, TGF-β, and with VD3 and analogues on inhibition of proliferation of transformed cells (29–31, 57).

Transforming growth factor-α has been shown to be an effective tumor suppressor in the early stages of treatment. It is thought to mediate the actions of retinoids during the early stage. However, at later stages of the treatment, a resistant generation of the tumor develops and dominates the tumor population (32). Therefore, TGF-α may be best used in combination with other cytokines or differentiation agents.

Although RA alone has been found to induce granulocytic differentiation of myeloid leukemia cells *in vitro* and *in vivo* (1, 112), RA and VD3 were found to be cooperative in their effects on differentiation of some leukemia cell lines such as HL-60, U937, and NB4 (27, 67, 113, 114). In this regard, VD3 can potentiate apoptosis induced by RA (66). Their combined actions were described as additive, synergistic, or both.

Evidence indicates that VD3 induces leukemic cell differentiation by increasing TNF-α, which in turn may influence nitric oxide production. Nitric oxide is thought to be a differentiation agent (66). In earlier studies, (29) it was shown that both RA and VD3 reduced the clonogenicity and inhibited proliferation of L1210 lymphocytic leukemia cells, *in vitro* and *in vivo*, individually and combined. However, combination of the two agents was more effective than either one alone. VD3 analogues interact additively with RA in reducing *c-myc* mRNA expression (50). Combination of RA and VD analogues, which had little effect on calcium metabolism or mobilization, was seen as a possible interesting chemotherapeutic regimen (114–118). Induction of differentiation of myeloid leukemia cells by RA and VD3 separately and in combination were reported (115–117); however, one of the main obstacles in the treatment with RA was the development of resistance by leukemic cells (Fig. 3; Ref. 114). Synergistic effects between RA and VD analogues were reported on differentiation of HL60 cells. In this regard VD3 has been shown to be capable of inhibiting expression of multiple antiapoptotic proteins leading to activation of the mitochondrial pathway for apoptosis (115–118).

Although RA induced differentiation and complete remission in patients with APL, one of the main adverse effects of the treatment of APL with RA is the RA syndrome (Fig. 3). In a study by Fenaux and De Botton (119), 25% of the APL patients treated with RA suffered from RA syndrome. RA syndrome is associated with increase in leukocyte count. Leukocytosis may be due to the release of cytokines such as IL-1α, SCF, IL-3, IL-6, and TNF-α (112). The RA syndrome clinical picture includes respiratory distress, serous effusion, cardiac and renal failure, and an increase in body weight (119). More recent studies demonstrated a release of the inflammatory cytokine IL-8, IL-1-α, TNF-α, and the adhesion molecule ICAM-1 by APL cells treated with RA (120). The release of inflammatory cytokines is amplified by G-CSF and reduced by IL-1α (103). The RA control of the IL-8 gene expression and the early release of the inflammatory cytokines are thought to play an important role in the manifestation of RA syndrome (120).

An understanding of the mechanism of leukocytosis is necessary to identify candidates that may develop RA syndrome. In such cases, a combination therapy or an alternative therapy might be more beneficial. Carnosic acid, a strong dietary antioxidant, is a polyphenolic diterpene derivative from rosemary. It exhibits anticarcinogenic activity and inhibits proliferation of HL-60 and U937 human myeloid leukemia cells (113). It was reported to enhance differentiation and antiproliferative effects of both RA and VD3. Perhaps such combination may speed the RA treatment and decrease the RA dose required so that RA may have a minimal effect. Obviously, it would be ideal to combine RA with an agent that inhibits the release of inflammatory cytokines to avoid at least some of the complications during RA treatment. G-CSF was reported to inhibit inflammatory immune response by modulating the release of inflammatory cytokines such as TNF-α, GM-CSF, INF-γ, and IL-2 (4).

One of the objectives of treatment for patients with APL is to induce tumor cell differentiation and block cell proliferation. NB4 cells respond to the combination treatment of VD3 plus phorbol 12-myristate 13-acetate (PMA) and differentiate into monocyte/macrophage-like cells (41, 66–69). This differentiation is accompanied by

a strong alkaline phosphatase ALP activity. Because PMA has limited clinical application because of its tumor-promoting effect, another protein kinase C activator, bryostatin-1, was tested for its interaction with VD3. Bryostatin-1 synergistically interacts with VD3 to stimulate ALP expression and further promote appearance of monocyte/macrophage-like cells. This combination treatment was suggested to be potentially an alternative therapy regimen for APL patients because ALP may be an easier and more sensitive way to monitor the possible remission of patients with APL (70).

Normal cells may become leukemic cells because of genetic abnormalities. Recent differentiation studies on myeloid leukemia cells indicate that induction of differentiation is accomplished by using an alternative differentiation pathway without correcting the genetic abnormalities (66). The effects of combinations of cytokines and retinoids on differentiation of myeloid leukemia cells were recently under focus. The expression of the matrix metalloproteonase-9 (MMP-9) produced by neutrophils might be linked with myeloid cell differentiation. Shibakura *et al.* (121) reported that RA induced MMP-9 and IL-8 in myeloid leukemia cell lines; however, IL-8 was not involved in MMP-9 expression induced by RA. Phospholipase C–beta-2 expression was found to be up-regulated in NB4 cell line and CD34+ cells treated with RA and cytokines, respectively (122). The RA-induced cell cycle arrest of U-937 cells was associated with down-regulation of c-myc expression (50), and RARs are thought to be important regulators of normal myeloid differentiation (19, 22). The cytokines IL-3 and GM-CSF, which normally regulate myeloid commitment and differentiation, were demonstrated to mediate ligand-independent activation of transcriptional activity of RARs.

These experiments may participate in the enlightening of the molecular basis for the sensitivity of myelogenous leukemia cells to retinoids. In addition, it demonstrates the interrelation and close association in the actions of retinoids and cytokines in normal and malignant cells (22). Retinoids, on the other hand, may potentiate a cytokine's action. An *in vivo* study showed that a combination of all-trans RA and interferon was more superior in achieving cytogenetic remission in the first chronic phase of CML (51). Reduced or absent neutrophil alkaline phosphatase (NAP) activity is a common feature of neutrophilic granulocytes from patients with CML. Stagno *et al.* (52) demonstrated that the *in vitro* activity of NAP was restored when CML cells were stimulated with all-trans RA, granulocyte-macrophage colony-stimulating factor (GM-CSF), or granulocyte colony-stimulating factor (G-CSF), individually and in combination.

Complete remission in hypoplastic AML was induced by G-CSF without chemotherapy (123). In a report on three cases, it was demonstrated that G-CSF stimulatory action is not limited to granulopoiesis (1, 17). G-CSF induces proliferation of multipotential HSCs and mobilizes them from bone marrow into peripheral circulation. G-CSF increases the number of all types of hematopoietic precursors, including CD34+ cells, in peripheral blood. This represents an alternative to bone marrow cells in autologous bone marrow transplantation (17, 18). On the other hand, G-CSF was reported to inhibit inflammatory immune response by modulating the release of inflammatory cytokines such as TNF-α, GM-CSF, INF-γ and IL-2. One of the mechanisms in which this is accomplished is by the inhibition of lipopolysaccharide-induced cytokine production by monocytes (4). Other cytokines also have been reported to stimulate the release of hematopoietic stem cells from bone marrow into peripheral blood in response to chemotherapy or other cytokines. These include IL-3, IL-7, IL-8, IL-12, SCF, and flt-3 ligand (26).

Combinations of cytokines with each other and with other agents such as RA have proven to be more effective in the treatment of various malignancies than any individual cytokine or retinoid. Combination of RA and INF were superior in achieving cytogenetic remission in the first chronic phase (124) and growth inhibition (125) of CML. Leukocytosis induced by RA correlated with increase in the G-CSF levels in the sera of APL (54). In fact, it is suggested that RA induces maturation of APL cells by reducing the serum inhibitory activity on hematopoiesis and increasing the level of CSFs such as G-CSF (43). Combinations of cytokines are also being used indirectly or as an adjunct therapy in the treatment of leukemia.

The use of mononuclear cells from umbilical cord blood (UCB) in bone marrow transplantation for the treatment of hematopoietic disorders has recently been under focus (126). Cells from UCB provide lower incidence of severe acute graft-versus-host disease (GVHD) following transplantation, compared with the transplantation of similar unrelated bone marrow cells (127). However, neonatal and UCB T cells were found either to be functionally immature or have limited capacity to produce cytokines (126). Therefore, post-UCB transplantation of unrelated UCB mononuclear cells is associated with several limitations. These limitations include the delayed T-cell reconstitution, which may account for the high rate of infectious morbidity, and the lack of available donor immunoeffector cells for adoptive cellular immunotherapy (127, 128). Robinson *et al.* (126) were able to activate and induce maturity of T lymphocytes using *ex vivo* combination of IL-2, IL-7, IL-12, and anti-CD3. Combination of GM-CSF and Epo were also used in the treatment of anemic, neutropenic patients with myelodysplastic syndrome (129). Treated patients showed an increase in neutrophil count and hemoglobin and required less blood transfusions, compared with the placebo group. However, some patients suffered from GM-CSF toxicity.

Most recently the drug imatinib was found to produce complete cytogenetic remission in Ph-positive, but to a lesser extent in the Ph-negative (accelerated or blastic phase), patients with CML (87). This drug specifically inhibits

tyrosine kinase in leukemic cells. High response rates were obtained after administration of imatinib to newly diagnosed chronic-phase patients with CML who have had no response to INF-α (130). This study has also shown that treatment with imatinib was less toxic and more effective than treatment with INF-α and cytarabine combination chemotherapy. Some of the difficulties encountering the treatment of patients with Ph-positive CML in the accelerated and blastic phases are the acquired chromosomal aberrations associated with the progression of the disease (131). Studying the morphological effects of imatinib on bone marrow showed that after 3 and 6 months of treatment, there was a complete hematological response with reduced bone marrow cellularity and significant clearance of leukemic cells in patients who had no abnormal Ph-positive cells (132). In some cases, allogeneic bone marrow transplantation has been suggested as a post-imatinib treatment for the patients with Ph-positive chronic-phase CML (133).

Monoclonal antibodies are considered for some types of therapy combinations. For example, antibody to CD33 provides some antileukemic activity against relapsed AML. Furthermore, and very promising, is rituximab, which is a humanized monoclonal antibody against the tumor antigen CD20. Rituximab is particularly effective and specific for therapy of lymphoid tumors and lymphomas. Rituximab produces response rates of up to 73% in patients with previously untreated indolent non-Hodgkin's lymphoma (134). It has been demonstrated that combining rituximab with cyclophosphamide, mitoxantrone, vincristine, and prednisone achieves high remission rates without significant additional toxicity in patients with previously untreated indolent non-Hodgkin's lymphoma (134). Supportive therapy with cytokines remains possible in some cases.

Gene Therapy

Gene therapy has a great potential, providing all the safety features are followed. In this regard, uncontrolled transfer of viral or nonviral genetic information and prevention of the formation of replicating retroviruses are of great concern (135). Approximately 60% of patients with AML treated with a chemotherapeutic or differentiation agent achieve remission. Of those, only 30% may achieve the long-term event-free survival. In an attempt to increase the survival of patients, Koya *et al.* (136) suggested an immunotherapeutic approach by genetic modification of AML cells for possible use in a vaccine. Transduced AML expressing CD80 and/or GM-CSF demonstrated that expression of either transgene enhanced T-cell activation. Adenovirus-mediated gene expression has been used to transfect cell lines that are derived from lymphoid tumors. Many other studies have examined the use of recombinant adenovirus vectors expressing cytotoxic genes for gene therapy in lymphomas, CLL, and multiple myeloma (137). However, vectors and specificity of action are of major concern for this type of therapy in humans, and more promising research is needed.

For a long time, the only curative treatment for the X-linked severe combined immune deficiency (X-SCID) was allogeneic bone marrow transplantation (BMT). However, in addition to incomplete restoration of B-cell function, BMT is often associated with other complications such as GVHD. Recent advances in gene transfer and better understanding of the genetic aspects of some immunodeficiencies have offered new opportunities in the treatment of children with X-SCID. Genetic defects of expression and function of the common gamma chain (γc) of cytokine receptors have been shown to be responsible for this disease (138). The involvement of γc in multiple cytokine receptors, including those for IL-2, IL-4, IL-7, IL-9, and IL-15, explains the severe impairment of lymphoid development and function in patients with X-SCID.

Patients with X-SCID who received autologous progenitor cells transduced with the therapeutic gene achieved remarkable levels of immune reconstitution without being exposed to the risk of GVHD (138). A major setback in this therapy was reported when two patients, of the 11 involved in this trial, developed therapy-induced T-cell leukemia (138, 139). Both patients with the adverse events were less than 3 months old, indicating that age may have been a crucial factor in this trial. The therapeutic gene is thought to play a crucial role in this therapy-induced leukemia. It is postulated that the retrovirus may have inserted itself near or into the *LMO2* gene. Cooperation between the *LMO2* and the γc may have resulted in the abnormal cell growth. Modification of the gene therapy is seen necessary to reduce the risk.

Both IL-12 and CD154 share several pathways in mediating immune responses. IL-12 gene has been shown to be a potent candidate for gene therapy. IL-12 is an essential cytokine for the generation of T-helper 1 response, natural killer cells, and cytotoxic T-lymphocyte stimulation (140). Gene therapy is entering a new stage of treating hematological malignancies. This seems to be possible by activating the immune response against leukemia-associated antigens. For example, it was possible to induce a cellular antileukemia immunity by transfer of genes encoding recombinant membrane-stabilized proteins of the TNF family, such as the one encoding CD154, the ligand for CD40, using autologous CD154-transduced leukemia cells as a cellular vaccine (141). CD154 induces antigen presentation by triggering CD40 on antigen-presenting cells and induces production of IL-12. Live leukemic cells transduced by IL-12, CD154, and both IL-12 and CD154 showed reduced leukemogenicity. In addition, natural killer cell cytotoxicity was enhanced because transduced cells induced IFN-γ mRNA in CD4+ and CD8+ T cells isolated from the spleen of vaccinated animal (140). Additionally, leukemogenicity was reduced in a murine leukemia model when transduced by the L-12 gene (140).

Apoptotic cell death has recently been induced using double-stranded RNA (ds-RNA) molecules (142). This mechanism is called RNA interference. It is extremely

potent and requires only a few ds-RNA molecules per cell to silence homologous gene mRNA expression. Recently Wilda *et al.* (142) were able to induce apoptosis in K562 cells using this method. Transfection of ds-RNA specific for the M-BCR/ABL fusion mRNA into K562 cells depleted the corresponding mRNA and the M-BCR/ABL oncoprotein. Gene therapeutic approaches using RNA interference to kill tumor cells seem to be promising.

Mutations in genes regulating development of hematopoietic progenitor cells can lead to various types of leukemia. Products of these genes have been under focus in recent years. Mutations in the ras genes are the most common molecular abnormalities in myeloid leukemia and preleukemia. In a study by Darley and Burnett (143), ras inhibited neutrophilic differentiation under the influence of GM-CSF and G-CSF. Cells expressing mutant ras continued to proliferate in the metamyelocyte stage indefinitely.

The adherent bone marrow stromal cell fractions contain pluripotent mesenchymal stem cells (PMSCs). The PMSCs are capable of self-renewal by proliferation to give rise to the differentiated stromal cells of various lineages. A more primitive adherent stem cell was also recently identified, the multipotent adult progenitor cell (MAPC; Ref. 144). These MAPCs differentiate into PMSCs, endothelial and epithelial, and even hematopoietic cells. Bone marrow stromal cells, including the PMSCs and MAPCs, are candidates for cell and gene therapy. They can be engineered to secrete proteins that are deficient in bone marrow stroma or to give rise to cells that are deficient in leukemic bone marrow stroma. This study and other similar studies offer the potential to treat bone marrow stromal disorders that may lead to leukemic conditions.

Concluding Remarks

In the last decade, our understanding of some types of leukemia has advanced tremendously. Retinoic acid, VD3, and cytokines have been recognized as powerful therapeutic agents. Although cytokines were found to be useful as therapeutic or adjunct therapeutic agents, much still remains to determine with regard to the choice of combination and the schedule of treatment. Cytokines are being successfully used as adjunct therapeutics to stimulate certain hematopoietic compartments; however, their use as long-term therapeutic agents is a difficult task because of the complexity of their actions on hematopoietic and leukemic cells. As a result, therapeutic challenges in achieving long-term survival and minimal hematological side effects are still there.

Among the future challenges in the treatment of leukemia is improving the current techniques in treating and predicting complete remission and possible cure. Understanding the signal pathways that mediate the actions of cytokines, RA and VD3, and the interactions of these agents with their receptors is essential in tailoring a proper combination therapy for a certain type of leukemia. Moreover, selective induction of apoptosis in leukemic cells seems to be a promising therapy. Furthermore, gene transduction can be used to produce the proper cytokine to induce differentiation, arrest proliferation, and induce apoptosis of leukemic cells.

Translocations and inversions commonly generate fusion or chimeric proteins. Characterization of these proteins, mainly transcriptional regulators, has allowed us to identify key pathways that lead to leukemogenesis. The involvement of the *AML1/CBFβ*, *RARα*, *MLL*, *NUP98*, (and *TEL*) genes suggests that their target genes play critical roles in hematopoiesis. The mechanisms involved in gene dysregulation suggest that attacking pathways commonly utilized by the leukemia cell will be effective in patients with these diseases.

Imatinib is a promising molecularly targeted drug that proved to be useful in the treatment of patients with Ph-positive CML and superior to INF-α. However, treatment of patients with Ph-negative CML in the accelerated phase and the blastic phase is less successful and may require to be followed with bone marrow transplant. Further investigation is required to explore the effects of Imatinib in combination with cytokines.

Finally, the use of cytokines during periods of anemia or cytopenia following therapy is for the most part safe if monitored carefully. However, cytokine therapy as an adjunctive treatment for some specific diseases like AML remains controversial, even though the agent may render leukemic cells more sensitive to chemotherapy. In addition, differentiation agents need to be employed with other chemotherapeutic agents to get the best results. Many human leukemias do not require known cytokines for growth *in vivo*; however, some animal models do. Thus, a common cytokine network or theme for the role in leukemia is not clear at this time.

1. Moqattash S, Lutton JD. Leukemia cells and the cytokine network: a review of the literature. Proc Soc Exp Biol Med 219:8–27, 1998.

2. Platanias LC, Fish EN. Signaling pathways activated by interferons. Exp Hematol 27:1583–1592, 1999.

3. Weekx SF, Van Bockstaele DR, Plum J, Moulijin A, Rodrigus I, Lardon F, De Smedt M, Nijs G, Lenjou M, Loquet P, Berneman ZN, Snoeck HW. CD34+ CD38– and CD34+ CD38+ human hematopoietic progenitors from fetal liver, cord blood, and adult bone marrow respond differently to hematopoietic cytokines depending on the ontogenic source. Exp Hematol 26:1034–1042, 1999.

4. Hareng L, Hartung T. Induction and regulation of endogenous granulocyte colony-stimulating factor formation. Biol Chem 383: 1501–1517, 2002.

5. Suzuki M, Harashima A, Okochi A, Yamamoto M, Matsuo Y, Motoda R, Yoshioka T, Orita K. Transforming growth factor–β1 augments granulocyte-macrophage colony-stimulating factor-induced proliferation of umbilical cord blood CD34+ cells with an associated tyrosine phosphorylation of STAT5. Exp Hematol 30:1132–1138, 2002.

6. Kotenko SV. The family of IL-10-related cytokines and their receptors: related, but to what extent? Cytokine Growth Factor Rev 13:223–240, 2002.

7. Baetu TM, Hiscott J. On the TRAIL to apoptosis. Cytokine Growth Factor Rev 13:199–207, 2002.
8. Sachs L. The control of hematopoiesis and leukemia: from basic biology to the clinic. Proc Natl Acad Sci U S A 93:4742–4749, 1996.
9. Xiao W, Koizumi K, Nishio M, Endo T, Osawa M, Fujimoto K, Sato I, Sakai T, Koike T, Sawada K. Tumor necrosis factor-α inhibits generation of glycoprotein A^+ cells by $CD34^+$ cells. Exp Hematol 30: 1238–1247, 2002.
10. Lotem J, Sachs L. Cytokine control of developmental programs in normal hematopoiesis and leukemia. Oncogene 13:3284–3294, 2002.
11. Borish LC, Steinke JW. Cytokines and chemokines. J Allergy Clin Immunol 111:S460–S475, 2003.
12. Shen JT, Falanga V. Growth factors, signal transduction, and cellular responses. J Dermatol 30:5–16, 2003.
13. Moseley TA, Haudenschild DR, Rose L, Reddi AH. Interleukin-17 family and IL-17 receptors. Cytokine Growth Factor Rev 14:155–174, 2003.
14. Miyamoto M, Prause O, Sjostrand M, Laan M, Lotvall J, Linden A. Endogenous IL-17 as a mediator of neutrophil recruitment caused by endotoxin exposure in mouse airways. Immunol 170:4665–4672, 2003.
15. Aggarwal S, Gurney AL. IL-17: prototype member of an emerging cytokine family. J Leukoc Biol 7:1–8, 2002.
16. Maguer-Satta V, Bartholin L, Jeanpierre S, Ffrench M, Martel S, Magaud JP, Rimokh R. Regulation of human erythropoiesis by activin A, BMP2, and BMP4, members of the TGF-beta family. Exp Cell Res 282:110–120, 2003.
17. Saito M, Kiyokawa N, Tagachi T, Suzuki K, SekinoT, Mimori K, Nakajima H, Katagiri Y, Fujimura J, Fujita H, Ishimato K, Yamashiro Y, Junichiro F. Granulocyte colony-stimulating factor directly affects human monocytes and modulates cytokines secretion. Exp Hematol 30:1115–1123, 2002.
18. Bolwell BJ, Fishleder A, Anderson SW, Lichtin AE, Koo A, Yanssens T, Burwell R, Baucco P, Green R. G-CSF primed peripheral blood progenitor cells in autologous bone marrow transplantation: parameters affecting bone marrow engraftment. Bone Marrow Transplant 12:609–614, 1993.
19. Johnson BS, Mueller L, Si J, Collins SJ. The cytokines IL-3 and GM-CSF regulate the transcriptional activity of retinoic acid in different in vitro models of myeloid differentiation. Blood 99:746–753, 2002.
20. Carstanjen D, Regenfus M, Muller C, Salama A. Interleukin-6 is a major effector molecule of short-term G-CSF treatment inducing bone metabolism and an acute phase response. Exp Hematol 29:812–82[illegible]2001.
21. Okuma E, Inazawa Y, Saeki K, You A. Potential role of extracellular signal-regulated kinase but not p38 during myeloid differentiation of U937 cells stimulated by cytokines: augmentation of differentiation via prolonged activation of extracellular signal-regulated kinase. Exp Hematol 30:571–581, 2002.
22. Shimizu T, Takeda K. Induction of retinoic acid receptor-alpha by granulocyte macrophage colony-stimulating factor in human myeloid leukemia cell lines. Cancer Res 60:4544–4549, 2000.
23. Reilly JT. FLT3 and its role in the pathogenesis of acute myeloid leukemia. Leuk Lymphoma 44:1–7, 2003.
24. Karsunky H, Merad M, Cozzio A, Weissman IL, Manz MG. Flt3 ligand regulates dendritic cell development from Flt3+ lymphoid and myeloid-committed progenitors to Flt3+ dendritic cells in vivo. J Exp Med 198:305–313, 2003.
25. Bruserud O, Hovland R, Wergeland L, Huang TS, Gjertsen BT. Flt3-mediated signaling in human acute myelogenous leukemia (AML) blasts: a functional characterization of Flt3-ligand effects in AML cell populations with and without genetic Flt3 abnormalities. Haematologica 88:416–428, 2003.
26. Lapidot T, Petit I. Current understanding of stem cell mobilization: the roles of chemokines, proteolytic enzymes, adhesion molecules, cytokines and stromal cells. Exp Hematol 30:973–981, 2002.
27. Reiss M. Transforming growth factor and cancer. Microbes and Infection 15:1327–1347, 1999.
28. Bollag W. Experimental basis of cancer combination chemotherapy with retinoids, cytokines, 1,25-dihydroxyvitamin D3, and analogues. J Cell Biochem 56:427–435, 1994.
29. Moqattash S, Abraham NG, Lutton JD. Modulation of growth supporting and oncogenic properties of murine leukemia cells. Leuk Res 18:531–539, 1994.
30. Motomura K, Sakai H, Isobe H, Nawata H. Effects of retinoids on the production of tumour necrosis factor-alpha and nitric oxide by lipopolysaccharide-stimulated rat Kupffer cells in vitro: evidence for participation of retinoid X receptor signaling pathway. Cell Biochem Funct 15:95–101, 1997.
31. Takeda K, Akira S. STAT family of transcription factors in cytokine-mediated biological responses. Cytokine Growth Factor Rev 11:199–207, 2000.
32. Bollag W, Retinoids and interferon: a new promising combination. Br J Haematol 79:87–91, 1991.
33. Naka T, Nishimoto N, Kishimoto T. The paradigm of IL-6: from basic science to medicine. Arthritis Res 4(Suppl 3):S233–S242, 2002.
34. Nakashima K, Raga T. gp130 and the IL-6 family of cytokines: signaling mechanisms and thrombopoietic activities. Semin Hematol 35:210–221, 1999.
35. Duits AJ, Dimjati W, Van de Winkel JGJ, Capel PJA. Synergism of interleukin 6 and 1,25-dihydroxyvitamin D_3 in induction of myeloid differentiation of human leukemic cell lines. J Leukoc Biol 51:237–243, 1992.
36. Gorgun G, Foss F. Immunomodulatory effects of RXR rexinoids: modulation of high-affinity IL-2R expression enhances susceptibility to denileukin diftitox. Blood 100:1399–1403, 2002.
37. Drew E, Merkens H, Chelliah S, Doyonnas R, McNagny KM. CD34 is a specific marker of mature murine mast cells. Exp Hematol 30:1211–1218, 2002.
38. Takai K, Hara J, Matsumoto K, Hosoi G, Osugi Y, Tawa A, Okada S, Nakamura T. Hepatocyte growth factor is constitutively produced by human bone marrow stromal cells and indirectly promotes hematopoiesis. Blood 89:1560–1565, 1997.
39. Fujiki H, Kimura T, Minamiguchi H, Harada S, Wang J, Nakao M, Yokota S, Urata Y, Ueda Y, Yamagishi H, Sonoda Y. Role of human Interleukin-9 as a megakaryocyte potentiator in culture. Exp Hematol 30:1373–1380, 2002.
40. Gounni AS, Gregory B, Nutku E, Aris F, Latifa K, Minshall E, North J, Tavernier J, Levit R, Nicolaides N, Robinson D, Hamid Q. Interleukin-9 enhances interleukin-5 receptor expression, differentiation, and survival of human eosinophils. Blood 96:2163–2171, 2000.
41. Sachs L. The cellular and molecular environment in leukemia. Blood Cells 19:709–730, 1993.
42. Lai R, O'Brien S, Maushouri T, Rogers A, Kantarjian H, Keating M, Albitar M. Prognostic value of plasma interleukin-6 levels in patients with chronic lymphocytic leukemia. Cancer 95:1071–1075, 2002.
43. Zhou R, Sun G, Wu W. The effect of sera of acute promyelocytic leukemia patients and culture supernatants of NB4 cells on normal granulo-macrophage hematopoiesis. Zhonghua Xue Ye Xue Za Zhi 20:343–346, 1999.
44. Ramshaw HS, Bardy PG, Lee MA, Lopez AF. Chronic myelomonocytic leukemia requires granulocyte-macrophage colony-stimulating factor for growth in vitro and in vivo. Exp Hematol 30:1124–1131, 2002.
45. Zweirzina H, Schollenberger S, Herold M, Schmalzl F, Bezemer J. Endogenous serum levels and surface receptors of GM-CSF and IL-3 in patients with myelodysplastic syndromes. Leuk Res 17:1181–1186, 1992.

46. Manakova TE, Tsvetaeva NV, Levina AA, Momotyuk KS, Sarkisyan GP, Khoroshko ND, Gerasimova LP. In vivo production of cytokines by bone marrow stromal cells and macrophages from patients with myelodysplastic syndrome. Bull Exp Biol Med 132:633–636, 2001.
47. Emanuel PD, Bates LJ, Castleberry RP, Gualtieri RJ, Zuckerman KS. Selective hypersensitivity to granulocyte-macrophage colony-stimulating factor by juvenile chronic myeloid leukemia hematopoietic progenitors. Blood 77:925–929, 1991.
48. Rossi FM, Degan M, Tassan Mazzocco L, Di Francia R, Aldinucci D, Poletto D, Vellenga E, Pinto A, Gattei V. Co-expression of CD30L and interleukin-4 (IL-4) receptors by acute myeloid leukemia blasts is associated with the expression of IL-4-producing $CD30^+$ normal T cells. Br J Hematol 117:59–69, 2002.
49. Aldinucci D, Poletto D, Nanni P, Degan M, Rupolo M, Pinto A, Gattei V. CD40L induces proliferation, self-renewal, rescue from apoptosis and production of cytokines by CD40-expressing AML blasts. Exp Hematol 30:1283–1292, 2002.
50. Dimberg A, Bahram F, Karlberg I, Larsson LG, Nilsson K, Oberg F. Retinoic acid-induced cell cycle arrest of human myeloid cell lines is associated with sequential down-regulation of c-Myc and cyclin E and posttranscriptional up-regulation of p27 (Kip1). Blood 99:2199–2206, 2002.
51. Egyed M, Mihalyfalvi Z, Kollar B, Rumi G, Keller E, Vass J, Fekete S. Effect of retinoic acid on the cytogenetic remission in the first chronic phase of chronic myeloid leukemia treated with interferon. Orv Hetil 142:2421–2425, 2001.
52. Stagno F, Guglielmo P, Consoli U, Fiumara P, Longo GS, Giustolisi R. All-trans-retinoic-acid- and growth-factor-mediated induction of alkaline phosphatase activity in freshly isolated chronic myeloid leukemia cells. Acta Haematol 102:61–65, 1999.
53. Liu J, Guo L, Luo Y, Li JW, Li H. All trans-retinoic acid suppresses in vitro growth and down-regulates LIF gene expression as well as telomerase activity of human medulloblastoma cells. Anticancer Res 20:2659–2664, 2000.
54. Wu W, Sun G, Wu W, Zhou R, Li X, Shen Z, Wang Z. The relationship between the levels of granulocyte colony-stimulating factor and leukocytosis induced by all-trans retinoic acid in acute promyelocytic leukemia. Chin Med J (Engl) 12:1085–1087, 1999.
55. Ganser A, Seipelt G, Verbeek W, Ottmann OG, Maurer A, Kolbe K, Hess U, Elsner S, Reutzel R, Wormann B, et al. Effect of combination therapy with all trans retinoic acid and recombinant human granulocyte colony-stimulating factor in patients with myelodysplastic syndromes. Leukemia 1994 8:369–375, 1994.
56. Hiroichi N, Satoko M, Keiko B, Yuko T, Ting W, Satoru N, Koichi S. Effect of Am-80, a synthetic derivative of retinoid, on experimental arthritis in mice. Pharmacology 58:101–112, 1999.
57. Peck R, Bolla W. Potentiation of retinoid-induced differentiation of HL-60 and U937 cell lines by cytokines. Eur J Cancer 27:153–157, 1991.
58. Jie C, Tulpule A, Zheng T, Masood R, Espina B, Gill PS. Treatment of epidemic (AIDS-related) Kaposi's sarcoma. Curr Opin Oncol 9:433–439, 1997.
59. Federici AB, Stabile F, Castaman G. Treatment of acquired von Willebrand syndrome in patients with monoclonal gammopathy of uncertain significance: comparison of three different therapeutic approaches. Blood 92:2707–2711, 1998.
60. Wightman J, Roberson MS, Lamkin TJ, Varvayanis S, Yen A. Retinoic acid-induced growth arrest and differentiation: retinoic acid up-regulates CD32 (Fc gammaRII) expression, the ectopic expression of which retards the cell cycle. Mol Cancer Ther 1:493–506, 2002.
61. Battle TE, Yen A. Ectopic expression of CXCR5/BLR1 accelerates retinoic acid- and vitamin D(3)-induced monocytic differentiation of U937 cells. Exp Biol Med (Maywood) 227:753–762, 2002.
62. Battle TE, Levine RA, Yen A. Retinoic acid-induced blr1 expression promotes ERK2 activation and cell differentiation in HL-60 cells. Exp Cell Res 254:287–298, 2000.
63. Yen A, Placanica L, Bloom S, Varvayanis S. Polyomavirus small t antigen prevents retinoic acid-induced retinoblastoma protein hypophosphorylation and redirects retinoic acid-induced G0 arrest and differentiation to apoptosis. J Virol 75:5302–5314, 2001.
64. Mannervik M, Nibu Y, Zhang H, Levine M. Transcriptional coregulators in development. Science 284:606–609, 1999.
65. Battle TE, Roberson MS, Zhang T, Varvayanis S, Yen A. Retinoic acid-induced blr1 expression requires RAR-α, RXR, and MAPK activation and uses ERK2 but not JNK/SAPK to accelerate cell differentiation. Eur J Cell Biol 80:59–67, 2001.
66. Bhatia M, Kirkland JB, Meckling-Gill KA. 1,25-Dihydroxyvitamin D3 primes acute promyelocytic cells for TPA-induced monocytic differentiation through both PKC and tyrosine phosphorylation cascades. Exp Cell Res 222:61–69, 1996.
67. Sharon SY, Williams MA, Newland AC, Colston KW. Leukemia cell differentiation, cellular and molecular interactions of retinoids and vitamin D. Gen Pharmacol 32:143–154, 1999.
68. Song X, Norman AW. 1,Alpha,25-dihydroxyvitamin D3 and phorbol ester mediate the expression of alkaline phosphatase in NB4 acute promyelocytic leukemia cells. Leuk Res 22:69–76, 1998.
69. Kaneki M, Inoue S, Hosoi T, Mizuno Y, Akedo Y, Ikegami A, Nakamura T, Shiraki M, Ito H, Suzu S, et al. Effects of 1 alpha, 25-dihydroxyvitamin D3 on macrophage colony-stimulating factor production and proliferation of human monocytic cells. Blood 83: 2285–2293, 1994.
70. Song XD, Norman AW. Bryostatin-1 and 1,alpha,25-dihydroxyvitamin D3 synergistically stimulate the differentiation of NB4 acute promyelocytic leukemia cells. Leukemia 13:275–281, 1999.
71. Grande A, Montanari M, Tagliafico E, Manfredini R, Marani TZ, Siena M, Tenedini E, Gallinelli A, Ferrari S. Physiological levels of 1,alpha, 25 dihydroxyvitamin D3 induce the monocytic commitment of CD34+ hematopoietic progenitors. Leukoc Biol 71:641–651, 2002.
72. Lotem J, Sachs L. Epigenetics wins over genetics: induction of differentiation in tumor cells. Semin Cancer Biol 12:339–346, 2002.
73. Alon DB, Chaimovitz C, Dvilanski A, Lugassy G, Douvdevani A, Shany S, Nathan I. Novel role of $1,25(OH)_2$ vitamin D3 in induction of erythroid progenitor cell proliferation. Exp Hematol 30:403–409, 2002.
74. Jakubowiak A, Cammenga J, Sokolic R, Alvarez S, Jackson G, Hedvat C, Nimer S. Molecular biology of leukemia. Curr Oncol Rep 2:123–131, 2000.
75. Jordan CT. Unique molecular and cellular features of acute myelogenous leukemia stem cells. Leukemia 16:559–562, 2002.
76. Jordan CT, Upchurch D, Szilvassy SJ, Guzman ML, Howard DS, Pettigrew AL, Meyerrose T, Rossi R, Grimes B, Rizzieri DA, Luger SM, Phillips GL. The interleukin-3 receptor alpha chain is a unique marker for human acute myelogenous leukemia stem cells. Leukemia 14:1777–1784, 2000.
77. Grignani F, De Matteis S, Nervi C. Fusion proteins of the retinoic acid receptor-alpha recruit histone deacetylase in promyelocytic leukaemia. Nature 391:815–818, 1998.
78. Wong CW, Privalsky ML. Components of the SMRT corepressor complex exhibit distinctive interactions with the POZ domain oncoproteins PLZF, PLZF-RAR-alpha, and BCL-6. J Biol Chem 273:27695–27702, 1998.
79. Gelmetti V, Zhang J, Fanelli M. Aberrant recruitment of the nuclear receptor corepressor-histone deacetylase complex by the acute myeloid leukemia fusion partner ETO. Mol Cell Biol 18:7185–7191, 1998.
80. Fenrick R, Amann JM, Lutterbach B. Both TEL and AML-1 contribute repression domains to the t(12;21) fusion protein. Mol Cell Biol 19:6566–6574, 1999.

81. Lutterbach B, Westendorf JJ, Linggi B. ETO, a target of t(8;21) in acute leukemia, interacts with the N-CoR and mSin3 corepressors. Mol Cell Biol 18:7176–7184, 1998.
82. Fenrick R, Amann JM, Lutterbach B. Both TEL and AML-1 contribute repression domains to the t(12;21) fusion protein. Mol Cell Biol 19:6566–6574, 1999.
83. Puccetti E, Obradovic D, Beissert T, Bianchini A, Washburn B, Chiaradonna F, Boehrer S, Hoelzer D, Ottmann OG, Pelicci PG, Nervi C, Ruthardt M. AML-associated translocation products block vitamin D(3)-induced differentiation by sequestering the vitamin D(3) receptor. Cancer Res 62:7050–7058, 2002.
84. Lin RJ, Nagy L, Inoue S. Role of the histone deacetylase complex in acute promyelocytic leukaemia. Nature 391:811–814, 1998.
85. Bhat A, Kolibaba K, Oda T, Ohno-Jones S, Heaney C, Druker BJ. Interaction of CBL with BCR-ABL and CRKL in BCR-ABL transformed myeloid cells. J Biol Chem 272:16170–16175, 1997.
86. Beyermann B, Adams HP, Henze G. Philadelphia chromosome in relapsed childhood acute lymphoblastic leukemia: a matched-pair analysis. J Clin Oncol 15:2231–2237, 1997.
87. Schiffer CA, Hehlmann R, Larson R. Perspectives on the treatment of chronic phase and advanced phase CML and Philadelphia chromosome positive ALL. Leukemia 17:691–699, 2003.
88. Badran A, Yoshida A, Wano Y, Imamura S, Kawai Y, Tsutani H, Inuzuka M, Ueda T. Expression of the antiapoptotic gene survivin in chronic myeloid leukemia. Anticancer Res 23:589–592, 2003.
89. Charbord P, Oostendorp R, Pang W, Hérault O, Noel F, Tsuji T, Dzierzak E, Péault B. Comparative study of stromal cell lines derived from embryonic, fetal, and postnatal mouse blood-forming tissue. Exp Hematol 30:1202–1210, 2002.
90. Deryugina EI, Muller-Sieburg CE. Stromal cells in long-term cultures: keys to the elucidation of hematopoietic development. Crit Rev Immunol 13:115–150, 1993.
91. Gidali J, Laszlo E, Halm G, Feher I. Blast colony-forming cell binding from CML bone marrow, or blood, on stromal layers pretreated with G-CSF or SCF. Cell Prolif 35:1–7, 2002.
92. Blazsek I, Delmas Marsalet B, Legras S, Marion S, Machover D, Misset JL. Large scale recovery and characterization of stromal cell-associated primitive haemopoietic progenitor cells from filter-retained human bone marrow. Bone Marrow Transplant 23:647–657, 1999.
93. Blazsek I, Farabos C, Quittet P, Labat ML, Bringuier AF, Triana BK, Machover D, Reynes M, Misset JL. Bone marrow stromal cell defects and 1 alpha,25-dihydroxyvitamin D3 deficiency underlying human myeloid leukemias. Cancer Detect Prev 20:31–34, 1996.
94. Mayani H. Composition and function of the hemopoietic microenvironment in human myeloid leukemia. Leukemia 10:1041–1047, 1996.
95. Flores-Figueroa E, Gutierrez-Espindola G, Montesinos JJ, Arana-Trejo RM, Mayani H. In vitro characterization of hematopoietic microenvironment cells from patients with myelodysplastic syndrome. Leuk Res 26:677–686, 2002.
96. Afford S, Randhawas S. Apoptosis. Mol Path 53:55–63, 2002.
97. Yuan XM, Li W, Dalen H, Lotem J, Kama R, Sachs L, Brunk UT. Lysosomal destabilization in p53-induced apoptosis. Proc Natl Acad Sci U S A 99:6286–6291, 2002.
98. Lotem J, Sachs L. Cytokines as suppressors of apoptosis. Apoptosis 4:187–196, 1999.
99. Duprey SP, Boettinger D. Developmental regulation of c-myb in normal myeloid progenitor cells. Proc Natl Acad Sci U S A 82:6937–6941, 1985.
100. Fu SL, Lipsick JS. Constitutive expression of full-length c-Myb transforms avian cells characteristic of both the monocytic and granulocytic lineages. Cell Growth Differ 8:35–45, 1997.
101. Wolff L, Schmidt M, Koller R, Haviernik P, Watson R, Bies J, Maciag K. Three genes with different function in transformation are regulated by c-myb in myeloid cells. Blood Cells Mol Dis 27:483–488, 2001.
102. Yi HK, Nam SY, Kim JC, Kim JS, Lee DY, Hwang PH. Induction of apoptosis in K562 cells by dominant negative c-myb. Exp Hematol 30:1139–1146, 2002.
103. Josefsen D, Blomhoff HK, Lomo J, Blystad AK, Smeland EB. Retinoic acid induces apoptosis of human CD34+ hematopoietic progenitor cells: involvement of retinoic acid receptors and retinoid X receptors depends on lineage commitment of the hematopoietic progenitor cells. Exp Hematol 27:642–653, 1999.
104. Ketley NJ, Allen PD, Kelsey SM, Newland AC. Modulation of idarubicin-induced apoptosis in human acute myeloid leukemia blasts by all-trans retinoic acid, 1,25(OH)2 vitamin D3, and granulocyte-macrophage colony-stimulating factor. Blood 90:4578–4587, 1997.
105. Braun S, Gerhartz HH, Schmetzer HM. Lymphokine-activated killer (LAK) cells and cytokines synergize to kill clonal cells in acute myeloid leukemia (AML) in vitro. Haematologia (Budap) 30:271–288, 2000.
106. Braun SW, Gerhartz HH, Schmetzer HM. Influence of cytokines and autologous lymphokine-activated killer cells on leukemic bone marrow cells and colonies in AML. Acta Haematol 105:209–221, 2001.
107. Meloni G, Trisolini SM, Capria S, Torelli GF, Baldacci E, Torromeo C, Valesini G, Mandelli F. How long can we give interleukin-2? Clinical and immunological evaluation of AML patients after 10 or more years of IL2 administration. Leukemia 16:2016–2018, 2002.
108. Chambaut-Guerin AM, Costa SL, Lefrancois T, Fages C, Gauthereau X, Tardy M. Effects of retinoic acid and tumor necrosis factor alpha on GL-15 glioblastoma cells. Neuroreport 11:389–393, 2000.
109. Xie P, Chan FS, Ip NY, Leung MF. IL-6 enhanced the retinoic acid-induced differentiation of human acute promyelocytic leukemia cells. Cancer Lett 148:207–213, 2000.
110. Shimizu T, Takeda K. Induction of retinoic acid receptor-alpha by granulocyte macrophage colony-stimulating factor in human myeloid leukemia cell lines. Cancer 60:4544–4549, 2000.
111. Visani G, Ottaviani E, Zauli G, Tosi P, Pellacani A, Isidori A, Pierpaoli S, Tura S. All-trans retinoic acid at low concentrations directly stimulates adult megakaryocytopoiesis in the presence of thrombopoietin or combined cytokines. Eur J Haematol 63:149–153, 1999.
112. Grande A, Manfredini R, Tagliafico E, Balestri R, Pizzanelli M, Papa S, Zucchini P, Bonsi L, Bagnara G, Torelli U. All-trans-retinoic acid induces simultaneously granulocytic differentiation and expression of inflammatory cytokines in HL-60 cells. Exp Hematol 23:117–125, 1995.
113. Steiner M, Priel I, Giat J, Levy J, Sharoni Y, Danilenko M. Carnosic acid inhibits proliferation and augments differentiation of human leukemic cells induced by 1,25-dihydroxyvitamin D3 and retinoic acid. Nutr Cancer 41:135–144, 2001.
114. Dore BT, Uskokovic MR, Momparler RL. Mutation in the ligand-binding domain of the retinoic acid receptor alpha in HL-60 leukemic cells resistant to retinoic acid and with increased sensitivity to vitamin D3 analogues. Leuk Res 20:761–769, 1996.
115. Taimi M, Breitman TR. Growth, differentiation, and death of retinoic acid-treated human acute promyelocytic leukemia NB4 cells. Exp Cell Res 10:230:69–75, 1997.
116. Guzey M, Kitada S, Reed JC. Apoptosis induction by 1α,25 dihydroxyvitamin D3 in prostate cancer. Mol Cancer Ther 1:667–677, 2002.
117. Wightman J, Roberson MS, Lamkin TJ, Varvayanis S, Yen A. Retinoic acid-induced growth arrest and differentiation: retinoic acid up-regulates CD32 (Fc γRII) expression, the ectopic expression of which retards the cell cycle. Mol Cancer Ther 1:493–506, 2002.
118. Coyne DW, Grieff M, Ahya SN, Giles K, Norwood K, Slatopolsky E. Differential effects of acute administration of 19-Nor-1,25-dihydroxy-vitamin D2 and 1,25-dihydroxy-vitamin D3 on serum calcium and phosphorus in hemodialysis patients. Am J Kidney Dis 40:1283–1288, 2002.

119. Fenaux P, De Botton S. Retinoic acid syndrome. Recognition, prevention and management. Drug Saf 18:272–279, 1998.
120. Hsu HC, Tsai WH, Chen PG, Hsu ML, Ho CK, Wang SY. In vitro effect of granulocyte-colony stimulating factor and all-trans retinoic acid on the expression of inflammatory cytokines and adhesion molecules in acute promyelocytic leukemia cells. Eur J Hematol 63:11–18, 1999.
121. Shibakura M, Niiya K, Kiguchi T, Shinagawa K, Ishimaru F, Ikeda K, Namba M, Nakata Y, Harada M, Tanimoto M. Simultaneous induction of matrix metalloproteinase-9 and interleukin 8 by all-trans retinoic acid in human PL-21 and NB4 myeloid leukaemia cells. Br J Haematol 118:419–425, 2002.
122. Bertagnolo V, Marchisio M, Pierpaoli S, Colamussi ML, Brugnoli F, Visani G, Zauli G, Capitani S. Selective up-regulation of phospholipase C-β2 during granulocytic differentiation of normal and leukemic hematopoietic progenitors. J Leukoc Biol 71:957–965, 2002.
123. Nimubona S, Grulois I, Bernard M, Drenou B, Godard M, Fauchet R, Lamy T. Complete remission in hypoplastic acute myeloid leukemia induced by G-CSF without chemotherapy: report on three cases. Leukemia 16:1871–1873, 2002.
124. Egyed M, Mihalyfalvi Z, Kollar B, Rumi G, Keller E, Vass J, Fekete S. Effect of retinoic acid on the cytogenetic remission in the first chronic phase of chronic myeloid leukemia treated with interferon. Orv Hetil 142:2421–2425, 2001.
125. Benthin M, Dallmann I, Atzpodien J. 13-Cis- and all trans-retinoic acid have antiproliferative effects on CML cells and render IFN-α antiproliferative potency after combined treatment in vitro. Cancer Biother Radiopharm 16:323–331, 2001.
126. Robinson KL, Ayello J, Hughes R, Ven van de C, Issitt L, Kurtzberg J, Cairo MS. Ex vivo expansion maturation and activation of umbilical cord blood-derived T lymphocytes with IL-2, IL-12, anti CD3, and IL-7: potential for adoptive immunotherapy post-umbilical cord blood transplantation. Exp Hematol 30:245–251, 2002.
127. Gluckman E. Current status of umbilical cord blood hematopoietic stem cell transplantation. Exp Hematol 28:1197–1205, 2000.
128. Abu-Ghosh A, Goldman S, Slone V, van de Ven C, Suen Y, Murphy L, Sender L, Cairo M. Immunological reconstitution and correlation of circulating serum inflammatory mediators/cytokines with the incidence of acute graft-vs-host disease during the first 100 days following unrelated umbilical cord blood transplantation. Bone Marrow Transplant 24:535–544, 1999.
129. Thompson JA, Gilliland DG, Prchal JT, Bennett JM, Larholt K, Nelson RA, Rose EH, Dugan MH. Effect of recombinant human erythropoietin combined with granulocyte/macrophage colony-stimulating factor in the treatment of patients with myelodysplastic syndrome. Blood 95:1175–1179, 2000.
130. O'Brien SG, Guilhot F, Larson RA, Gathmann I, Baccarani M, Cervantes F, Cornelissen JJ, Fischer T, Hochhaus A, Hughes T, Lechner K, Nielsen JL, Rousselot P, Reiffers J, Saglio G, Shepherd J, Simonsson B, Gratwohl A, Goldman JM, Kantarjian H, Taylor K, Verhoef G, Bolton AE, Capdeville R, Druker BJ. IRIS Investigators. Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. N Engl J Med 348:994–1004, 2003.
131. Mohamed AN, Pemberton P, Zonder J, Schiffer CA. The effect of imatinib mesylate on patients with Philadelphia chromosome-positive chronic myeloid leukemia with secondary chromosomal aberrations. Clin Cancer Res 9:1333–1337, 2003.
132. McNamara C, Grigg A, Szer J, Roberts A, Campbell L, Hoyt R, Lynch K, Juneja S. Morphological effects of imatinib mesylate (STI571) on the bone marrow and blood of patients with Philadelphia chromosome (Ph) positive chronic myeloid leukaemia. Clin Lab Haematol 25:119–125, 2003.
133. Kantarjian H, Talpaz M, O'Brien S, Giles F, Beth Rios M, White K, Garcia-Manero G, Ferrajoli A, Verstovsek S, Wierda W, Kornblau S, Cortes J. Prediction of initial cytogenetic response for subsequent major and complete cytogenetic response to imatinib mesylate therapy in patients with Philadelphia chromosome-positive chronic myelogenous leukemia. Cancer 97:2225–2228, 2003.
134. Economopoulos T, Fountzilas G, Pavlidis N, Kalantzis D, Papageorgiou E, Christodoulou C, Hamilos G, Nicolaides C, Dimopoulos M. Rituximab in combination with CNOP chemotherapy in patients with previously untreated indolent non-Hodgkin's lymphoma. Hematol J 4:110–115, 2003.
135. Hansen AC, Pedersen FS. Safety features of retroviral vectors. Curr Opin Mol Ther 4:324–333, 2002.
136. Koya RC, Kasahara N, Pullarkat V, Levine AM, Stripecke R. Transduction of acute myeloid leukemia cells with third generation self-inactivating lentiviral vectors expressing CD80 and GM-CSF: effects on proliferation, differentiation, and stimulation of allogeneic and autologous anti-leukemia immune responses. Leukemia 16:1645–1654, 2002.
137. Turturro F. Recombinant adenovirus-mediated cytotoxic gene therapy and lymphoproliferative disorders: analysis based on pharmacodynamics. Curr Drug Targets Immune Endocr Metabol Disord 2:109–118, 2002.
138. Otsu M, Sugamura K, Candotti F. Lack of dominant-negative effects of a truncated γc on retroviral-mediated gene correction of immunodeficient mice. Blood 97:1618–1624, 2001.
139. Khon DB, Sadelain M, Glorioso JC. Occurrence of leukemia following gene therapy of X-linked SCID. Nat Rev Cancer 3:477–488, 2003.
140. Saudemont A, Buffenoir G, Denys A, Desreumaux P, Jouy N, Hetuin D, Bauters F, Fenaux P, Quesnel B. Gene transfer of CD154 and IL-12 cDNA induces an anti-leukemic immunity in a murine model of acute leukemia. Leukemia 16:1637–1644, 2002.
141. Kipps TJ. Immune and cell therapy of hematologic malignancies. Int J Hematol Suppl 1:269–273, 2002.
142. Wilda M, Fuchs U, Wossmann W, Borkhardt A. Killing of leukemic cells with a BCR/ABL fusion gene by RNA interference (RNAi). Oncogene 21:5716–5724, 2002.
143. Darley RL, Burnett AK. Mutant RAS inhibits neutrophil but not macrophage differentiation and allows continued growth of neutrophil precursors. Exp Hematol 27:1599–1608, 1999.
144. Van Damme A, Vanden Driessche T, Collen D, Chuah MK. Bone marrow stromal cells as targets for gene therapy. Curr Gene Ther 2:195–209, 2002.