PLAINTIFF'S EXHIBIT 1522

Differentiation-Inducing Agents in the Treatment of Myelodysplastic Syndromes

Masahiro Kizaki and H. Phillip Koeffler

THE MYELODYSPLASTIC syndromes (MDS) are a group of hematopoietic stem cell disorders, characterized by bone marrow dysplasia and chronic cytopenias leading to frequent hemorrhage, infections, and need for red cell transfusions; these individuals have an increased risk of transformation to acute myelogenous leukemia (AML).[1-7] The defective maturation, which involve one or more of the hepatopoietic lineages, is the central pathophysiological feature of MDS.[8,9] No specific or effective therapies for MDS exist at this time. Supportive treatment with blood products (red cells and platelets) combined with antibiotics in case of infection has been the basic therapy for MDS. Patients with MDS who receive conventional treatment with combination chemotherapy often have prolonged pancytopenia and the therapy can be fatal in elderly individuals.[10,11] Moreover, MDS patients are often too old for bone marrow transplantation.

Because cells of the MDS clone phenotypically have a block in differentiation, agents that stimulate differentiation of leukemic cells in vitro may have a potential therapeutic role.[12,13] Laboratory studies using leukemic cell lines have provided examples of a variety of agents that are capable of reversing the block in differentiation. These observations have provided the basis for many recent clinical trials aimed at improving marrow function in MDS by stimulating differentiation. This approach is particularly attractive in elderly MDS patients who are unable to tolerate aggressive forms of therapies.[14]

TOOLS TO STUDY DIFFERENTIATION OF LEUKEMIC CELLS

Acute myeloid leukemia arises from neoplastic transformation at the level of the pluripotent hematopoietic stem cells. These stem cells can either replicate or differentiate to committed stem cells that mature into functional blood cells. Induction of differentiation to mature macrophages and granulocytes by physiologic and nonphysiologic agents was demonstrated in vitro in murine[10] and subsequently human leukemic cell lines.[16] The first studies used the murine myeloid leukemic cell line, M-1, established from spontaneous myeloid leukemia in a SL strain of mouse.[17,18] These cells can be induced to differentiate both in vitro and in vivo into macrophages, and granulocytes by treatment with various compounds including differentiation inducing factor (D-factor), colony stimulating factors (CSF), interleukin-1 (IL1), IL6, lipopolysaccharide (LPS), glucocorticoids, 1,25-dihydroxyvitamin D,, dimethyl sulfoxide (DMSO) and lectins.[19] Another intensively studied cell line is the murine erythroleukemia cell lines (MEL), derived from spleens infected with the Friend virus complex.[20] These cells differentiate to red cells after culture with a variety of chemicals, including planar-polar compounds such as hexamethylene bisacetamide (HMBA), purines and their derivatives, hemin, short-chain fatty acids, as well as inhibitors of DNA or RNA synthesis, such as ultraviolet irradiation and X irradiation.[20]

Several human myeloid leukemia cell lines, such as HL-60, K562, KG-1, ML-1, and ML-3 cells, can be induced by various compounds to differentiate into granulocytes, macrophages, or erythroid cells. These leukemic cell lines are blocked at different stages of maturation: KG-1 cells are myeloblasts; HL-60 cells are promyelocytes; ML-1 and ML-3 cells are myelomonoblasts; U937 and THP-1 cells are monocytoid lines; K562 cells have characteristics of very immature cells being able to differentiate down the red cells, megakaryocyte, and macrophage

From the Division of Hematology/Oncology, UCLA School of Medicine, Los Angeles, CA, and Division of Hematology1 Oncology, Department of Medicine, Cedars-Sinai Medical Center, Los Angeles, CA.

Supported in part by grants by US Public Health Service Grants No. DK41936-02, CA26038-11, CA43277-05, CA33936-08, R86LA022, IRT370, the Weisz Family Foundation, 4E Leukemia Fund in memory of Marilyn Levine and the Realtors of Real Estate Industry Division to H.P.K.

Address reprint requests to H. Phillip Koeffler, MD, Cedars-Sinai Medical Center, Division of Hematology/Oncology, 8700 Beverly Blvd, Los Angeles, CA 90048.

Copyright © 1992 by W.B. Saunders Company

0093-7754/92/1901-0007$05.00/0

lineages.[16] A variety of compounds induce the differentiation of HL-60 cells towards granulocytes including DMSO, HMBA, retinoids, interferon alfa (IFN-α), and granulocyte-colony stimulating factor." Phorbor esters, $1,25(OH)_2D_3$ and IFN-γ predominantly induce monocytic differentiation of HL-60, KG-1, ML-1, ML-3, THP-1 and U937 cells.[16,22,23] K562 cells differentiate towards erythroblasts with synthesis of fetal hemoglobin when cultured with either hemin or butyrate."

The use of glucose-6-phosphatasedehydrogenase (G6PD) isoenzymes, karyotypic analysis, and more recently analysis of DNA restriction fragment-length polymorphisms (RFLP) showed that leukemic and preleukemic blast cells can differentiate in vivo to form mature blood cells.[25-27] Theoretically, therefore, agents that foster differentiation in vitro may have therapeutic potential. The remaining chapter reviews the use of specific agents.

COMPOUNDS.USED TO INDUCE DIFFERENTIATION

Retinoids

Vitamin A is an essential micronutrient and deficiencies of this molecule can cause night blindness and xerophthalmia.[28,29] Vitamin A and its analogs (retinoids) have been known since the 1920s to influence growth and differentiation of normal and malignant cells. Retinoids are potent anticarcinogenic agents in many experimental models and they inhibit growth and induce differentiation of transformed neoplastic cells.[30] Retinoids initially were demonstrated to inhibit growth of murine lymphoma and myeloma cell lines.[31] Subsequently, retinoids were found to have profound effects on leukemia cells in vitro.

Retinoic acids (RA) inhibit proliferation and induce differentiation of cells from several human AML cell lines.[32] All-trans retinoic acid (*trans*-RA) induces differentiation and inhibits proliferation of HL-60 cells.[21] More than 90% of the cells differentiate towards granulocytes after 72 to 96 hours of exposure to 10^{-6} M trans-RA. Growth of these cells is also inhibited by RA[33]; *trans* and 13-*cis* forms of RA are equally effective in inhibiting proliferation. U937 cells can differentiate towards mature monocytes by treatment with RA; differentiation of the cells is associated with decreased proliferation without loss of viability." The ML-3 cells show partial monocytic differentiation after exposure to RA[35]; KG-1 cells do not differentiate upon exposure to retinoids, but these cells are very sensitive to the growth-inhibiting effects of retinoids, showing a 50% inhibition of colony formation at 2.4 x 10^{-9} M RA.[33] In contrast, retinoids stimulate the clonal growth of normal human myeloid and erythroid precursors in vitro.[33] The reason for stimulation of proliferation in vitro of normal hematopoietic progenitor cells and for inhibition of clonal proliferation of leukemic cells is unclear.

Mechanism of action of RA on hematopoietic cells is unknown, but is more than a nonspecific toxic effects. Cytoplasmic retinoic acid binding protein (CRABP) was hypothesized as the biological mediator of RA.[36] However, we were unable to detect CRABP in HL-60, KG-1, or leukemic blasts from patients.[32] Recently, cDNA clones coding for several retinoic acid receptors (RAR) have been isolated and identified as members of the steroid/thyroid hormone receptor family.[37,38] These receptors are intracellular proteins that mediate complex effects on development, growth, and physiological homeostasis by selective modulation of gene transcription.[39] Cloning of genes coding for human glucocorticoid and estrogen receptors has allowed a detailed biochemical characterization of this family of molecules including identification of discrete DNA- and ligand-binding domains.[39] RAR is represented by at least three species, RAR-α,[37,38] RAR-β,[40] and RAR-γ.[41,42] The RAR-α gene is located on human chromosome 17q22 encoding a 462 amino acid protein with a DNA binding domain (C region) and a ligand-binding domain (E region)." The RAR-β gene is expressed in epithelial cells and expression of RAR-γ may be restricted to skin." More recently, Mangelsdorf et al# discovered a novel receptor (RXR-a), which is a member of the steroid superfamily and which binds and responds specifically to retinoids.[44] These data suggest that different receptors may mediate different functions of retinoic acid. In addition, the temporal and spatial patterns of expression of RAR transcripts suggest that important yet separate roles exist for each of the RAR subtypes during limb morphogenesis.[45] We, as well

as others, have examined the expression of RAR mRNA in hematopoietic cells[46-49] and we have found that all hematopoietic cell lines expressed RAR-γ mRNA including KG-1 (myeloblasts), HL-60 (promyelocytes), ML-3, THP-1, U937 (myelomonoblasts and monoblasts), W62 (erythroblasts), and S-LB1 (T-lymphocytes). Steady-state levels of RAR-α mRNA were not affected by induction of terminal differentiation of HL-60 cells to either granulocytes or macrophages. Furthermore, both actively proliferating and resting lymphocytes from the same individuals expressed equal concentrations of RAR-α mRNA. This data suggest that level of expression of RAR-α mRNA is not related to cellular proliferation or stage of differentiation. Further studies showed that induction of differentiation of HL-60 by RA is mediated through RARα.[50] Taken together, the molecular and cellular biological data suggest that expression of RARα is necessary, but not sufficient to induce differentiation and inhibit proliferation of leukemic cells.

At least 150 reported MDS patients have been treated with 13-*cis* retinoic acid in hopes of improving hematopoiesis and decreasing their incidence of evolution to AML (Table 1). Dose of drug and duration of therapy varied between studies. Response rates were approximately 20%. Gold et al[51] reported the first study in 1983 showing that 5 out of 15 evaluable patients had improvement in hematologic parameters; responses were not generally observed until at least 3 weeks of therapy?' Clark et al[57] randomly assigned 98 MDS patients to either 13-cis retinoic acid or observational control group and found no significant difference in survival between treated and control groups. However, subanalysis of 39 nonsideroblastic refractory anemia patients showed an increase in survival in the group who received the retinoic acid. We reported a multicenter trial in which 68 patients were randomly assigned to either 13-*cis* retinoic acid or placebo; no significant difference was noted between the two groups.'* Another study suggested that long-term administration of the drug was important and responses can require a number of months of therapy.[60]

Hepatotoxicity, as manifested by hyperbilirubinemia and serum glutamic pyruvic transaminase (GOT) elevations, are dose-limiting factors. Other reported side effects include cheliosis, mucositis, erythema of the skin, conjunctivitis, nausea, joint pains, lethargy, and elevation of serum triglyceride levels.[51,58] Alpha tocopherol taken orally and applied to dry skin can reverse some of the skin toxicity of retinoic acid. To avoid these side effects, fenretinide [*N*-(4-hydroxyphenyl) retinamide], a synthetic amide derivative of retionic acid has been studied; however, no responses occurred in 14 evaluable MDS patients:' Moreover, one patient experienced evolution into acute myelomonocytic leukemia.

All-trans and 13-*cis* retinoic acid are naturally occurring isomers of retinoic acid. Hung et al[62] found that all-trans retinoic acid induced 23 complete remissions in 24 patients with acute promyelocytic leukemia (APL, M3).[62] Recently, Castaigne et al[63] reported 14 complete remissions of 22 APL patients using all-trans retinoic acid and Warrell et al[64] showed that 9 of the 11 APL patients entered complete remission while receiving all-trans RA. In APL, the RAR-a is translocated from chromosome 17 to a gene known as *myl* on chromosome 15.6' The t(15;17)

Table 1. Trials of 13-*cis* Retinoic Acid Therapy in Myelodysplastic Syndromes

Investigators	No. of Patients	Dose	Duration of Therapy	GR + PR (%)
Gold et al[51]	15	20-125 mg/m²/d	7-30 W	5 (33)
Greenberg et al[52]	18	1-2 mg/kg/d	>8 W	3 (17)
Swanson et al[53]	10	2.5-4 mg/kg/d	8 W	3 (30)
Picozzi et al[54]	15	2.5-4 mglkgld	8 W	5 (33)
Abraham et al[55]	1	100 mg/m²/d	16 W	1 (100)
Kerndrup et al[56]	6	20-100 mg/m²/d	6 W	0 (0)
Clark et al[57]	33	20 mg/m²/d	8 W	3 (9)
Koeffler et al[58]	35	100 mg/m²/d	6 W	3 (9)
Leoni et al[59]	20	50-100 mg/m²/d	>4 w	12 (60)
Total	153			35 (23)

Abbreviations: GR, good response; PR. partial response; W, weeks.

translocation creates a fusion gene between the amino-termillus of *myl* and C-terminus of RAR-α. Further studies must determine if the responsiveness of APL to all-*trans* RA is either related to unique properties of the fusion protein or reflects the maturity of APL cells allowing them to be more responsive to differentiation agents.

In summary, the role of retinoic acid for treatment of MDS syndromes is still not fully defined. 13-*cis* retinoic acid may have moderate effects on 20% to 30% of patients, but studies have not shown an increased survival in these patients. Most of these studies had small numbers of patients and were not designed to look at survival. If long-term therapy is required for responsiveness to retinoic acid, only MDS patients with relatively indolent disease would be suitable candidates for this therapy. Future studies will determine if all-trans retinoic acid is more effective than 13-*cis* retinoic acid for therapy of MDS.

1,25 DIHYDROXYVITAMIN D_3 AND ITS ANALOGS

The biologically active metabolite of vitamin D,, 1,25-dihydroxyvitamin D, [$1,25(OH)_2D_3$], is recognized as a major hormonal regulator of calcium metabolism in individuals.[66] Recently, evidence has been obtained for a wider biological role of $1,25(OH)_2D_3$ in tissues not primarily related to mineral metabolism. Studies suggest that this active metabolite may play an important role as an immunohematopoietic regulatory hormone?@'

The $1,25(OH)_2D_3$ is a sew-steroid that is taken up by the target cell and binds to specific vitamin D receptors (VDR) located in the nucleus. The hormone-receptor complex binds to DNA and modulates transcriptional and translational events. The putative DNA-binding regions of VDRs have strong homologies to the structures of other steroid hormone receptor~.@'The receptors for $1,25(OH)_2D_3$ are by no means limited to the classical target tissues of intestine, bone and kidney, but they have a wide distribution in a number of tissues including hematopoietic cells.[66,72-75] Activated, but not quiescent lymphocytes express large numbers of VDR.[76,77] In contrast, nondividing monocytes do express VDR and do respond to $1,25(OH)_2D_3$ and induction of differentiation of HL-60 cells along either the monocytic or granulocytic pathways does not change their levels of steady-state VDR RNA.[77] Furthermore, almost all neoplastic hematopoietic cells express VDR. Further studies should elucidate the physiological role of $1,25(OH)_2D_3$ receptors in hematopoietic cells. The VDR appear to be identical in both normal and neoplastic proliferating hematopoietic cells and their expression, similar to RAR, is probably necessary, but not sufficient for induction of differentiation of normal and leukemic hematopoietic cells.

The $1,25(OH)_2D_3$ induce murine myeloid leukemia cells (M1) to differentiate to monocyte/macrophage-like cells as assessed by morphological and functional criteria.'" Upon treatment with $1,25(OH)_2D_3$, M1 cells become adherent, acquire morphological characteristics of mature macrophages, express Fc and C3 receptors, and develop lysozyme and phagocytic activity. Also, the proliferation of M1 cells is inhibited by $1,25(OH)_2D_3$. Similar findings were made for the human HL-60 and U937 leukemic cells.[73-75,79-82] A large proportion of HL-60 cells (30% to 50%) became multinucleated and gained the ability to bind and degrade bone matrix.[83] This hormone also reduced the clonal growth of freshly harvested leukemic blast cells from patients with myeloid leukemia.[82] In contrast, similar concentrations of $1,25(OH)_2D_3$ slightly stimulated the clonal growth of normal myeloid committed stem cells; this is similar to the paradoxical action of retinoids.[84,85] The reason that $1,25(OH)_2D_3$ stimulates clonal growth of normal and inhibits clonal growth of leukemic hematopoietic cells is unclear.

The ability of $1,25(OH)_2D_3$ to reduce proliferation and induce differentiation of myeloid leukemic cells in vitro formed the basis for several small therapeutic trials of the secosteroid for MDS. Table 2 shows these results.

Table 2. Trials of $1,25(OH)_2D_3$ Therapy in Myelodysplastic Syndromes

Investigators	No. of Patients	Dose	Duration of Therapy	PR + MR (%)
Koeffleretal"	18	2.0 mg/d	4–20 W	8 (44)
Metha et al[87]	6	1.0 mg/d	12 W	0 (0)
Richard et al[88]	7	2.5 mg/d	> 8 W	0 (0)

Abbreviations: PR, partial response; MR, minor response: W, weeks.

We have treated 18 MDS patients with 2 μg/day of $1,25(OH)_2D_3$ for at least 12 weeks.[86] During the course of therapy, most developed an increase in their peripheral blood granulocytes, macrophages, and/or platelets. However, no significant change occurred in median levels of these cells between the initiation and completion of treatment. Only one patient showed a partial response with a 50% increase in platelets, granulocytes, and monocytes for more than 5 weeks. Seven patients revealed a minor response. Six patients had progressive disease with evolution to AML by the end of the study. Serum calcium rose from a median of 8.9 mg/dL at pretreatment to a peak of 10.8 mg/dL during treatment. Five patients developed mildly symptomatic hypercalcemia with anorexia, nausea, polydypsia, polyuria, and/or lethargy. Concentrations of the drug that were required in vitro to induce differentiation produced hypercalcemia in vivo.

Attempts are currently being directed toward the identification of chemically modified vitamin D, analogs that induce hematopoietic cell differentiation without inducing hypercalcemia.[89] We identified over 15 new analogs of $1,25(OH)_2D_3$ that were either equivalent or more potent than $1,25(OH)_2D_3$ as assessed by (1) inhibition of clonal proliferation of HL-60, U937, and patients' myeloid leukemic cells; and (2) induction of differentiation of HL-60 cells.[89-91] Also these analogs stimulated clonal growth of normal human myeloid stem cells. We assessed the effects on calcium metabolism of these novel analogs in vivo by intestinal calcium absorption (ICA) and bone calcium mobilization (BCM). Each of the analogs mediated markedly less (10- to 200-fold) ICA and BCM as compared with $1,25(OH)_2D_3$.[89-91] Among them, $1,25(OH)_2$-16ene-23yne-D_3 was the most potent modulator of growth and differentiation of leukemic cells.[89,92] The therapeutic potential of $1,25(OH)_2$-16ene-23yne-D_3 prompted us to develop in vivo models of AML.[93] We found that WEHI 3BD$^+$, a murine myeloid leukemia line, can be induced by either $1,25(OH)_2D_3$ or $1,25(OH)_2$-16ene-23yne-D_3 to lose clonal proliferative capacity as the cells terminally differentiate. Importantly, $1,25(OH)_2$-16ene-23yne-D_3 was 10 to 25 times less active than $1,25(OH)_2D_3$ in causing hypercalcemia, allowing administration of higher concentrations of the compound than $1,25(OH)_2D_3$ in our murine model.

Other analogs of $1,25(OH)_2D_3$ have been synthesized that also have little calcemic activity but retain the ability to differentiate myeloid leukemia cells. 24-homo-$1,25(OH)_2D_3$ can induce differentiation of HL-60 cells, but do not increase serum calcium when administered to vitamin D-deficient rats.[94] Similar differential activity occurs with MC903 [$1,24(OH)_2$-22ene-24cyclopropyl-D_3], a $1,25(OH)_2D_3$ analog with a cyclopropyl group at the end of the side chain.[95] 22-oxa-$1,25(OH)_2D_3$ (OCT) also has very low bone calcium mobilizing activity in vitro. OCT exhibited an activity 10 times more potent than $1,25(OH)_2D_3$ in suppressing growth of WEHI-3 cells and in inducing differentiation of both HL-60 and WEHI-3 leukemic cells. The binding affinity of OCT for the $1,25(OH)_2D_3$ receptors in HL-60 cells was weaker than that of $1,25(OH)_2D_3$ for its receptors. This suggests that factors other than the binding affinity for the receptor such as stability, cellular uptake, or intracellular metabolism of vitamin D compounds, probably are also important in separating the differentiation-inducing activity from the hypercalcemic action of these new analogs.

In summary, these novel vitamin D analogs are more potent than $1,25(OH)_2D_3$ in inducing differentiation and inhibiting proliferation of leukemic cells. Moreover, these compounds appear to have the potential to be markedly less toxic as measured by the development of hypercalcemia. For example, about 10-fold more $1,25(OH)_2$-16ene-23yne-D_3 is required to produce hypercalcemia as compared to $1,25(OH)_2D_3$. These novel vitamin D, analogs may prove superior to $1,25(OH)_2D_3$ in a number of clinical situations including MDS and acute leukemia. In addition, they will provide a tool to dissect the mechanism of action of vitamin D, compounds in promoting cellular differentiation.

INTERFERONS

Interferons are a family of proteins sharing the capacity to exert distinctive effects on cell functions. IFNs are secreted by many cell types in response to various stimuli. Interferon gamma is produced by immunocompetent T lymphocytes, in response to a sensitizing antigen.[97] Fibroblasts usually produce only IFN-β.[98] Leu-

kocytes predominantly produce IFN-a and a small percentage of IFN-β.[99] Interferons render cells resistant to viral growth.[100] They also play a physiological role in the regulation of immune responses. Depending on the target cell, IFNs can function as differentiation factors, as cellular growth inhibitors, and as modulators of gene expression in differentiated cells. They have also been reported to contribute to control of autocrine growth. Human IFN-a, -β, and -γ enhance the differentiation of the myeloblastic leukemic HL-60 cells into macrophages.[101-104] In vitro IFN-γ also induces monocytic differentiation of blasts from AML and MDS patients.[105] Interferon alfa has not been found to be clinically effective in AML[106]; but it is effective for treatment of patients with chronic myeloid leukemia.[107] Partial remissions with IFN-α were reported in MDS[108,109]; however, confirmatory data from clinical trials are not available. In one patient the complete disappearance of the cytogenetic anomaly of trisomy 8 was observed after 34 days of IFN-α treatment, indicative of eradication of the malignant hematopoietic clone.[110] Recently, a study examined the effects of long-term application of IFN-α at higher dosages in patients with "low-risk" MDS.[111] Ten patients were included in the study; 8 were treated for 6 to 36 months. IFN-α was administered at a median dosage of 9, 6, and 4 million units per week during the first, second, and third years, respectively. Responses occurred in 3 of 10 (30%) of the patients; disease progression was thought to have been retarded during the third year of therapy in an additional case. Only a few case studies have been published on treatment of MDS patients with IFN-γ in vivo.[112-114] Only minimal benefit was observed. Flu-like symptoms, which can be debilitating at times, are examples of the side-effects of therapy with interferons.

HEXAMETHYLENE BISACETAMIDE AND 5-AZACYTIDINE

A number of nonphysicological agents induce in vitro differentiation of leukemic cells and some of them have been tried in patients with AML and MDS. Hexamethylene bisacetamide, a low-molecular weight polar-planar compound, was introduced into clinical trials because of its potent tumor-differentiating capabilities in vitro.[115-117] Hexamethylene bisacetamide induces differentiation of Friend MEL and human myeloblastic HL-60 cells.[118,119] Hexamethylene bisacetamide concentrations that induced differentiation in vitro (2-5 mM) inhibited the clonal growth of myeloid and erythroid committed hematopoietic stem cells in 15 patients with MDS and these concentrations of HMBA are achievable in clinical trials (1-2 mM).[120] However, these same concentrations also inhibited in vitro clonal growth of committed hematopoietic stem cells of normal individuals suggesting lack of a selective cytotoxicity to neoplastic cells. Phase I trials have demonstrated that HMBA produces some hematologic toxicity.[121,122] Therapeutic efficacy of HMBA in MDS is currently being evaluated.[123,124] These trials are examining the effects of maximally tolerated HMBA concentrations infused for 5 and 10 consecutive days on peripheral blood cell counts and blast cell fractions.

5-azacytidine is a ring analog of cytidine that is capable of inducing remissions in approximately 20% of patients with relapsed AML.[125] 5-azacytidine is incorporated into newly synthesized DNA.[125] Incorporation leads to a decrease in the activity of DNA methyl transferase thus reducing methylation of newly synthesized DNA; this hypomethylation can modify gene expression.[126] Differentiation of HL-60 cells along the myeloid lineage occurs after exposure to 5-azacytidine.[127] Therefore, a study was conducted of low-dose 5-azacytidine (75 mg/m^2 per day) administered as a continuous infusion for 7 days in 11 patients with AML; no patient had a response as defined by bone marrow remission.[128] However, in 44 evaluable patients with MDS, administration of 5-azacytidine in the same dose and schedule resulted in normalization of peripheral blood and bone marrow in 5 patients (11%) and improvement in an additional 16, for an overall response rate of 48%.[129] Further clinical trials are necessary, however, before this approach can be recommended for therapy of MDS.

COMBINATIONS OF DIFFERENTIATION INDUCING AGENTS

Numerous reports on low-dose cytarabine treatment in MDS and AML have been pub-

lished since the initial successful case report in 1979.[130] Recently, combination of differentiation-inducing agents with DNA synthesis inhibitors, such as cytarabine, resulted in vitro in a 10- to 1,000-fold greater differentiation of leukemic cells in vitro than either agent alone.[131] A phase II clinical trial with low-dose cytarabine (5 mg/M^2 per 12 h subcutaneously [SC]) and 13-*cis* retinoic acid (60 mg/M^2 per day orally) in 14 patients with MDS resulted in one partial and one minor response.[132] Another innovative study combined IFN-α (3 M U/day, SC), 13-cis retinoic acid (1 mg/kg per day) and 1,25(OH)$_2$D$_3$ (1 μg/d) for treatment of MDS.[133] Four complete and 9 partial remissions were obtained out of 47 patients. This result was comparable to the response rate of treatment with low-dose cytarabine; in addition, this treatment had a high rate of side effects, perhaps due to IFN-γ. This therapy did not appear to prolong survivals of the patients. In conclusion, combinations of cytarabine and inducers of differentiation does not seem to offer a therapeutic benefit to patients with MDS as compared to single agents alone.

CONCLUSIONS

Myelodysplastic syndromes are clonal hematologic malignancies with an increased risk of transformation to AML. Natural history of MDS ranges from a relatively benign slowly progressive to a very aggressive disease resulting in either life-threatening cytopenias or evolution to AML. Therapies for MDS have included hormones, chemotherapy, bone marrow transplantation, and differentiating agents. Recently, hematopoietic growth factors have been tested in clinical trials of MDS because they increase the number of neutrophils, platelets, and erythrocytes of these patients.[134-139] However, hematopoietic growth factors, especially granulocyte-macrophage colony stimulating factors, have a risk for increasing the number of blast cells in patients with refractory anemia with excess blasts in transition and also chronic myelomonocytic leukemia.[138,139] Inducers of differentiation are often well tolerated by elderly patients as compared to cytotoxic agents. However, results with these agents have not been satisfactory. Further studies should continue to try to identify new compounds that can induce differentiation of the most immature hematopoietic stem cells of the MDS clone. In addition, major effort should be directed towards understanding the molecular biology of MDS; such an understanding may permit us to taylor our therapy to correct chemically the molecular defect.

REFERENCES

1. Block M, Jacobson LO, Bethard WF: Preleukemic acute leukemia. JAMA 152:1018-1028, 1953

2. Linman JW, Bagby GC Jr: The preleukemic syndrome (hematopoietic dysplasia). Cancer 42:854-864, 1978

3. Greenberg PL The smoldering myeloid leukemic states: Clinical and biologic features. Blood 61:1035-1044, 1983

4. Mufti GJ, Stevens JR, Oscier DG, et al: Myelodysplastic syndromes: A scoring system with prognostic significance. Br J Haematol 59:425-433, 1985

5. Tricot G, Vlietinck R, Boogaerts MA, et al: Prognostic factors in the myelodysplastic syndromes: Importance of initial data on peripheral blood counts, bone marrow cytology, trephine biopsy and chromosomal analysis. Br J Haematol 60:19-32, 1985

6. Koeffler H P Myelodysplastic syndromes (preleukemia). Semin Hematol 23:284-299, 1986

7. Foucar K, Langdon RM, Armitage JO, et al: Myelodysplastic syndromes: A clinical and pathologic analysis of 109 cases. Cancer 56553-561, 1985

8. Tricot G, Boogaerts MA, DeWolf-Peeters C, et al: The myelodysplastic syndromes: Different evolution patterns based on sequential morphological and cytogenetic investigations. Br J Haematol 59:659-670, 1985

9. Verwilghen RL, Boogaerts MA: The myelodysplastic syndromes. Blood Rev 1:34-43, 1987

10. Koeffler HP: Preleukemia. Clinics Hematol 15:829-850, 1986

11. Vallespi T, Torrabadella M, Julia A, et al: Myelodysplastic syndromes: A study of 101 cases according to the FAB classification. Br J Haematol 61:83-92, 1985

12. Lotan R Effects of vitamin A and its analogues (retinoids) on normal and neoplastic cells. Biochem Biophys Acta 605:33-91, 1980

13. Fohlmeister I, Fischer R, Schaefer HE: Preleukemic myelodysplastic syndromes (MDS): Pathogenetical considerations based on retrospective clinico-morphological sequential studies. Anticancer Res 5:179-188, 1985

14. Layton DM, Mufti GJ: Myelodysplastic syndromes: Their history, evolution and relation to acute myeloid leukemia. Blut 53:423-436, 1986

15. Sachs L The differentiation of myeloid leukemia cells: New possibilities for therapy. Br J Haematol 40:509-517, 1978

16. Koeffler HP: Induction of differentiation of human

acute myelogeneous leukemia cells: Therapeutic implications. Blood 62:709-721, 1983

17. Ichikawa Y: Differentiation of a cell line of myeloid leukemia. J Cell Physiol 74:223-234, 1%9

18. Sachs L Control of normal cell differentiation and phenotypic reversion of malignancy in myeloid leukemia. Nature 274535-539,1978

19. Hozumi M: Fundamentals of chemotherapy of myeloid leukemia by induction of leukemia cell differentiation. Adv Cancer Res 38:121-169, 1983

20. Marks PA, Rifkind RA: Erythroleukemic differentiation. Ann Rev Biochem 47419-448,1978

21. Breitman TR, Selonic SE, Collins SJ: Induction of differentiation of the human promyelocytic leukemia cell line (HL-60) by retinoic acid. Proc Natl Acad Sci USA 772936-2945,1980

22. Koeffler HP: Human acute myeloid leukemia lines: Models of leukemogenesis. Semin Hematol 23:223-236, 1986

23. Koeffler HP, Golde DW: Acute myelogeneous leukemia: A human cell line responsive to colony-stimulating activity. Science 200:1153-1154, 1978

24. Miller CW, Young K, Dumenil D, et al: Specific globin mRNAs in human erythroleukemia (K562) cells. Blood 63:195-200, 1984

25. Fialkow PJ, Jacobson RJ, Papayannopoulou T: Chronic myelocytic leukemia: Clonal origin in a stem cell common to the granulocyte, erythrocyte, platelet and monocyte/macrophage. Am J Med 63:125-130, 1977

26. Fearon ER, Burke PJ, Schiffer CA, et al: Differentiation of leukemia cells to polymorphonuclear leukocytes in patients with acute nonlymphocytic leukemia. N Engl J Med 315:15-24, 1986

27. Kere J, Ruutu T, Chapelle A Monosomy 7 in granulocytes and monocytes in myelodysplastic syndrome. N Engl J Med 316:499-503, 1987

28. Goodman D: Vitamin A and retinoids in health and disease. N Engl J Med 310:1023-1031, 1984

29. Sporn MB, Roberts AB: Role of retinoids in differentiation and carcinogenesis. Cancer Res 43:3034-3040, 1983

30. Lotan R: Effects of vitamin A and its analogs (retinoids) on normal and neoplastic cells. Biochem Biophys Acta 605:33-91, 1980

31. Lotan R, Nicolson GL: Inhibitory effects of RA or retinyl acetate on the growth of untransformed, transformed and tumor cells in vitro. J Natl Cancer Inst 59:1717-1722,1977

32. Douer D, Koeffler H P Retinoic acid inhibition of the clonal growth of human myeloid leukemic cells. J Clin Invest 69:277-283, 1982

33. Douer D, Koeffler H P Retinoic acid enhances colony-stimulation factor-induced clonal growth of normal human myeloid progenitor cells in vitro. Exp Cell Res 138:193-198, 1982

34. Olsson IL, Breitman TR: Induction of differentiation of human histiocytic lymphoma cell line U937 by RA and cyclic adenosine 3':5'-monophosphate-inducing agents. Cancer Res 42:3924-3927, 1982

35. Ferrero D, Pessano S, Giovanni LP, et al: Induction of differentiation of human myeloid leukemias: Surface changes probed with monoclonal antibodies. Blood 61:171-179,1983

36. Chytil F, Ong D E Cellular retinol- and retinoic acid binding proteins in vitamin A action. Fred Proc 38:2510-2514,1979

37. Giguere V, Ong ES, Sequi P, et al: Identification of a receptor for the morphogen retinoic acid. Nature 330:624-629,1987

38. Petkovich M, Brand NJ, Krust A, et al: A human retinoic acid receptor which belongs to the family of nuclear receptors. Nature 330444-450,1987

39. Evans RM: The steroid and thyroid receptor superfamily. Science 240:889-895, 1988

40. Brand N, Petkovich M, Krust A, et al: Identification of a second human retinoic acid receptor. Nature 332:850-853,1988

41. Krust A, Kastner P, Petkovich M, et al: A third human retinoic acid receptor, hRAR-γ. Proc Natl Acad Sci USA 865310-5314,1989

42. Zelent A, Krust A, Petkovich M, et al: Cloning of murine a and β retinoic acid receptors and a novel receptor γ predominantly expressed in skin. Nature 339:714-717, 1989

43. Mattei MG, Petkovich M, Mattei JF, et al: Mapping of the human retinoic acid receptor to the 921 band of chromosome 17. Hum Genet 80:186-188, 1988

44. Mangelsdorf DJ, Ong ES, Dyck JA, et al: Nuclear receptor that identifies a novel retinoic acid response pathway. Nature 345224229,1990

45. Doll P, Ruberte E, Kastner P, et al: Differential expression of genes encoding a, β and γ retinoic acid receptors and CRABP in the developing limbs of the mouse. Nature 342:702-705, 1989

46. Kizaki M, Koeffler HP, Lin CW, et al: Expression of retinoic acid receptor mRNA in hematopoietic cells. Leuk Res 14:645-655, 1990

47. Largman C, Detmer K, Corral JC, et al: Expression of retinoic acid receptor alpha mRNA in human leukemia cells. Blood 7499-102,1989

48. Gallagher RE, Said F, Pua I, et al: Expression of retinoic acid receptor alpha mRNA in human leukemia cells with variable responsiveness to retinoic acid. Leukemia 3:789-795, 1989

49. Wang C, Curtis JE, Minden MA, et al: Expression of retinoic acid receptor gene in myeloid leukemic cells. Leukemia 3:264-269, 1989

50. Collins SJ, Robertson KA, Mueller L: Retinoic acid-induced granulocytic differentiation of HL-60 myeloid leukemia cells is mediated directly through the retinoic acid receptor (RAR-alpha). Mol Cell Biol 10:2154-2163, 1990

51. Gold EJ, Mertelsmann RH, Itri LM, et al: Phase I clinical trial of 13-cis-retinoic acid in myelodysplastic syndromes. Cancer Treat Rep 67:981-986, 1983

52. Greenberg BR, Durie BG, Barnett TC, et al: Phase 1-11 study of 13-cis-retinoic acid in myelodysplastic syndrome. Cancer Treat Rep 691369-1374,1985

53. Swanson G, Picozzi V, Morgan R, et al: Response of hematopoieti precursors to 13-cis-retinoic acid and 1,25 dihydroxyvitamin D_3 in the myelodysplastic syndromes. Blood 67:1154-1161, 1986

54. Picozzi VJ Jr, Swanson GF, Morgan R, et al: 13-cis-retinoic acid treatment for myelodysplastic syndrome. J Clin Oncol 4:589-595, 1986

55. Abrahm J, Besa EC, Hyzinski M, et al: Disappearance of cytogenetic abnormalities and clinical remission during therapy with 13-cis-retinoic acid in a patient with myelodysplastic syndrome: Inhibition of growth of the patient's malignant monocytoid clone. Blood 67:1323-1327, 1986

56. Kernadrup G, Bendix-Hansen K, Pedersen B, et al: 13-cis-retinoic acid treatment of myelodysplastic syndromes. Leuk Res 11:7-16, 1987

57. Clark RE, Ismail SA, Jacobs A, et al: A randomized trial of 13-cis-retinoic acid with or without cytosine arabinoside in patients with the myelodysplastic syndrome. Br J Haematol 6677-83, 1987

58. Koeffeler HP, Heitjan D, Mertelsmann R, et al: Randomized study of 13-cis-retinoic acid V placebo in the myelodysplastic disorders. Blood 71:703-708, 1988

59. Leoni F, Ciolli S, Longo G, et al: 13-cis-retinoic acid treatment in patients with myelodysplastic syndrome. Acta Haematol 80:8-12, 1988

60. Besa EC, Abrahm JL, Bartholomew MJ, et al: Treatment with 13-*cis* retinoic acid in transfusion-dependent patients with myelodysplastic syndrome and decreased toxicity with addition of alpha-tocopherol. *Am* J Med 89:739-747, 1990

61. Garewal HS, List A, Meyskens F, et al: Phase II trial of fenretinide [*N*-(4-hydroxyphenyl) retinamide] in myelodysplasia: Possible retinoid-induced disease acceleration. Leuk Res 13:339-343, 1989

62. Huang M, Ye HC, Chen SR, et al: Use of all-trans retinoic acid in the treatment of acute promyelocytic leukemia. Blood 72567-572, 1988

63. Castaigne S, Chromienne C, Daniel MT, et al: All-trans retinoic acid as a differentiation therapy for acute promyelocytic leukemia. I. Clinical results. Blood 76:1704-1709, 1990

64. Warrell PR, Frankel SR, Miller WH, et al: Differentiation therapy of acute promyelocytic leukemia with tretinoin (all-*trans*-retinoic acid). N Engl J Med 324:1385-1393, 1991

65. De The H, Chomienne C, Lanotte M, et al: The t(15;17) translocation of acute promyelocytic leukemia fuses the retinoic acid receptor alpha gene to a novel transcribed locus. Nature 347:558-561, 1990

66. Norman AW, Roth J, Orchi L The vitamin D endocrine system: Steroid metabolism, hormone receptors, and biological response (calcium binding proteins). Endocr Rev 3:331-366, 1982

67. Bell NH: Vitamin D-Endocrine system. J Clin Invest 761-6, 1985

68. Monolagas SC, Deftos LJ: The vitamin D endocrine system and hematolymphopoietic tissue. Ann Intern Med 100:144-146, 1986

69. McDonnell DP, Mangelsdorf DJ, Pike JW, et al: Molecular cloning of complementary DNA encoding the avian receptor for vitamin D. Science 235:1214-1217, 1987

70. Baker *AR*, McDonnell DP, Hughes M, et al: Cloning and expression of full-length cDNA encoding human vitamin D receptor. Proc Natl Acad Sci USA 85:3294-3298, 1988

71. Burmester JK, Wiese RJ, Maeda N, et al: Structure and regulation of the rat 1, 25-dihydroxyvitamin D, receptor. Proc Natl Acad Sci USA 85:9499-9502, 1988

72. Henry HL, Norman AW: Vitamin D Metabolism and biological actions. Ann Rev Nutr 4493-520, 1984

73. Tanaka H, Abe E, Miyaura C, et al: 1, 25-dihydroxycholecalciferol and a human myeloid leukemia cell line (HL-60) The presence of a cytosol receptor and induction of differentiation. Biochem J 204:713-719, 1982

74. Amento EP, Bhalla AK, Kurnick JT, et al: 1a, 25-dihydroxyvitamin D, induces maturation of the human monocyte cell line U937, and, in association with a factor from human T lymphocytes, augments production of the monokine mononuclear cell factor. J Clin Invest 73:731-739, 1984

75. Mangelsdorf DJ, Koeffler HP, Donaldson CA, et al: 1,25-dihydroxyvitamin D_3-induced differentiation in a human promyelocytic leukemia cell line (HL-60). Receptor-mediated maturation to macrophage-like cells. J Cell Biol 98391-398, 1984

76. Prowedini DM, Tsoukas KD, Deftos LJ, et al: 1, 25-dihydroxyvitamin D, receptors in human leukocytes. Science 221:1181-1183, 1983

77. Kizaki M, Norman AW, Bishop JE, et al: 1, 25-dihydroxyvitamin D_3 receptor RNA Expression in hematopoietic cells. Blood 7731238-1247, 1991

78. Abe E, Miyaura C, Sakagami H, et al: Differentiation of mouse myeloid leukemia cells induced by 1, 25-dihydroxyvitamin D_3. Proc Natl Acad Sci USA 78:4990-4994, 1981

79. Murao S, Gemmell MA, Callaham MF, et al: The control of macrophage cell differentiation in human promyelocytic HL-60 leukemia cells by 1, 25-dihydroxyvitamin D_3 and phorbol-12-myristate-13-acetate. Cancer Res 43:4989-4996, 1983

80. Olsson I, Gullberg U, Ivhed I, et al: Induction of differentiation of the human histiocytic lymphoma cell line U937 by 1a, 25-dihydroxycholecalciferol. Cancer Res 43: 5862-5867, 1983

81. Rigby WFC, Shen L, Bal ED, et al: Differentiation of a human monocytic cell line by 1,25-dihydroxyvitamin D, (calcitriol): A morphologic, phenotypic, and functional analysis. Blood 64:1110-1115, 1984

82. Munker R, Norman AW, Koeffler HP: Vitamin D compounds. Effects on clonal proliferation and differentiation of human myeloid cells. J Clin Invest 78:424-430, 1986

83. Bar-Shavit Z, Teitelbaum SL, Reitsma P, et al: Induction of monocytic differentiation and bone resorption by 1,25-dihydroxyvitamin D_3. Proc Natl Acad Sci USA 80:5907-5911, 1983

84. McCarthy DM, SanMiguel JF, Freake HC, et al: 1, 25-dihydroxyvitamin D, inhibits proliferation of human promyelocytic leukaemia (HL-60) cells and induces monocyte macrophage differentiation in HL-60 and normal human bone marrow cells. Leuk Res 7:51-55, 1983

85. Koeffler HP, Amatruda T, Ikekawa N, et al: Induction of macrophage differentiation of human normal and leukemic myeloid stem cells by 1,25-dihydroxyvitamin D_3

and its fluorinated analogues. Cancer Res 44:5624-5628, 1984

86. Koeffler HP, Hirji K, Itri L, et al: 1,25-dihydroxyvitamin D,: In vivo and in vitro effects on human preleukemic and leukemic cells. Cancer Treat Rep 691399-1407,1985

87. Mehta AB, Kumaran TO, Marsh G W Treatment of advanced myelodysplastic syndrome with alfacalcidol [letter]. Lancet 2:761, 1984

88. Richard C, Mazo E, Cuadrado MA, et al: Treatment of myelodysplastic syndrome with 1,25-dihydroxyvitamin D3. Am J Hematol 73:175-178, 1986

89. Zhou JY, Norman AW, Lubbert M, et al: Novel vitamin D analogs that modulate leukemic cell growth and differentiation with little effect on either intestinal calcium absorption or bone calcium mobilization. Blood 74:82-93, 1989

90. Norman AW, Zhou JY, Henry HL, et al: Structure-function studies on analogs of 1α,25-dihydroxyvitamin D,: Differential effects on leukemic cell growth, differentiation, and intestinal calcium absorption. Cancer Res 50:6857-6864, 1990

91. Zhou JY, Norman AW, Akashi M, et al: Development of a novel 1,25(OH)$_2$-Vitamin D, analogwith potentiability to induce HL-60 cell differentiation without modulating calcium metabolism. Blood 7875-82,1991

92. Norman AW, Zhou JY, Henry HL, et al: Structure-function studies on analogues of 1a,25-dihydroxyvitamin D,: Differential effects on leukemic cell growth, differentiation and intestinal calcium absorption. Cancer Res 50:6857-6864,1990

93. Zhou JY, Norman AW, Chen DL, et al: 1,25(OH)$_2$-16ene-23yne vitamin D, prolongs survival time of leukemic mice. Proc Natl Acad Sci USA 87:3929-3932, 1990

94. Ostrem VK, Tanaka Y, Prahl J, et al: 24- and 26-homo-1,25-dihydroxyvitamin D,: Preferential activity in inducing differentiation of human leukemia cells HL-60 in vitro. Proc Natl Acad Sci USA 84:2610-2614, 1987

95. Binderup L, Bramm E: Effects of a novel vitamin D analogue MC903 on cell proliferation and differentiation in vitro and on calcium metabolism in vivo. Biochem Pharmacol 37:889-895, 1988

96. Abe J, Morikawa M, Miyamoto K, et al: Synthetic analogues of vitamin D, with an oxygen atom in the side chain. FEBS Lett 226:58-62, 1987

97. Epstein LB: The special significance of interferon-gamma, in Vilcek J, De Mayer E (eds): Interferon. Interferons and Immune System (vol 2). Amsterdam, Elsevier, 1984, pp 185-219

98. Havell EA, Hayes TG, Vilcek J Synthesis of two distinct interferons by human fibroblasts. Virology 89:330-334,1978

99. Brehm G, Kirchner H: Analysis of the interferons induced in mice in vivo and in macrophages in vitro by Newcastle Disease Virus and by polyionosinic-polycytidylic acid. J Interferon Res 6:21-28, 1986

100. Isaacs A, Lindenmann J: The interferon. Proc R Soc Lond B 147:258-267, 1957

101. Tomida M, Yamamoto Y, Hozumi M: Stimulation by interferon of induction of differentiation of human promyelocytic leukemia cells. Biochem Biophys Res Commun 104:30-37, 1982

102. Ball ED, Guyre PM, Shen L, et al: Gamma interferon induces monocytoid differentiation in the HL-60 cell line. J Clin Invest 73:1072-1077, 1984

103. Dayton ET, Matsumoto-Kobayashi M, Perussia B, et al: Role of immune interferon in the monocytic differentiation of human promyelocytic cell lines induced by leukocyte conditioned medium. Blood 66:583-594, 1985

104. Harris PE, Ralph P, Litcofsky P, et al: Distinct activities of interferon gamma, lymphokine and cytokine differentiation-inducingfactors acting on the human monoblastoid leukemia cell line U937. Cancer Res 45:9-13, 1985

105. Stella CC, Cazzola M, Ganser A, et al: Recombinant gamma-interferon induces in vitro monocytic differentiation of blast cells from patients with acute nonlymphocytic leukemia and myelodysplastic syndromes. Leukemia 12:55-59,1988

106. Bonnem EM, Speigel RJ: Interferon-a: Current status and future promise. J Biol Response Modif 3:580-598,1984

107. Talpaz M, McCredie KB, Mavligit GM, et al: Leukocyte interferon-induced myeloid cytoreduction in chronic myelogeneous leukemia. Blood 62:689-692, 1983

108. Elias L, Hoffman R, Boswell S, et al: A trial of recombinant a2 interferon in the myelodysplastic syndromes: I. Clinical results. Leukemia 1:105-110, 1987

109. Galvani DW, Cawley JC, Nethersell A, et al: a-interferon in myelodysplasia. Br J Haematol 66:145-146, 1987

110. Block WL, Lowenberg B, Sizoo W, et al: Disappearance of trisomy 8 after alpha-2 interferon in a patient with myelodysplastic syndrome [letter]. N Engl J Med 318:787-788,1988

111. Gisslinger H, Chott A, Linkesch W, et al: Long-term alpha-interferontherapy in myelodysplasticsyndromes.Leukemia 491-94,1990

112. Beran M, Andersson B, Kantarjian H, et al: Hematologic response of four patients with smoldering acute myelogeneous leukemia to partially pure gamma interferon. Leukemia 1:52-57, 1987

113. SchwarzingerI, Stain C, Bettelheim P, et al: Gamma-interferon in myelodysplastic syndromes: A pilot study. Oncology 47:322-326, 1990

114. Maiolo AT, Cortelezzi A, Calori R, et al: Recombinant gamma-interferon as first line therapy for high risk myelodysplastic syndromes. Leukemia 4:480-485, 1990

115. Fibach E, Reuben RC, Rifkind RA, et al: Effect of hexamethylene bisacetamide on the commitment to differentiation of murine erythroleukemia cells. Cancer Res 37440-444,1977

116. Rabson AS, Stem R, Tralka TS, et al: Hexamethylene bisacetamide induces morphologic changes and increased synthesis of procollagen in cell line from glioblastoma multiforme. Proc Natl Acad Sci USA 74:5060-5064, 1977

117. Chun HG, Leyland-Jones B, Hoth D, et al: Hexamethylene bisacetamide: A polar-planar compound entering clinical trials as a differentiating agent. Cancer Treat Rep 70991-996,1986

118. Friend C, Freedman HA: Effects and possible mechanism of action of dimethylsulfoxide on Friend cell differentiation. Biochem Pharmacol 27:1309-1313, 1978

119. Spremulli EN, Dexter DL: Polar solvents: A novel class of antineoplastic agents. J Clin Oncol 2:227-241, 1984

120. Rowinsky EK, Donehower RC, Spivak JL, et al: Effects of the differentiating agent hexamethylenebisacetamide on normal and myelodysplastic hematopoietic progenitors. J Natl Cancer Inst 82:1926-1931, 1990

121. Rowinsky EK, Ettinger DS, Grochow LB, et al: Phase I and pharmacologic study of hexamethylene bisacetamide in patients with advanced cancer. J Clin Oncol 4:1835-1844, 1986

122. Egorin MJ, Sigman LM, Van Echo DA, et al: Phase I clinical and pharmacokinetic study of hexamethylene bisacetamide (NSC 95580) administered as a five-day continuous infusion. Cancer Res 47:617-623, 1987

123. Rowinsky EK, Conley BA, Spivak J, et al: The efficacy of hexamethylene bisacetamide (HMBA) in myelodysplastic syndromes (MDS): 5-day exposure to maximal levels. Proc Am Assoc Clin Res 31:1132, 1990

124. Andreeff M, Young C, Clarkson B, et al: Treatment of myelodysplastic syndromes (mds) with hexamethylene bisacetamide (HMBA). Blood 72:656a, 1988 (suppl 1)

125. Glover AB, Leyland-Jones BR, Chun HG, et al: Azacytidine: 10 years later. Cancer Treat Reports 71:737-746, 1987

126. Jones PA, Taylor SM: Cellular differentiation, cytidine analogs and DNA methylation. Cell 20:85-93, 1980

127. Christman JK, Mendelsohn N, Herzog D, et al: Effect of 5-azacytidine on differentiation and DNA methylation in human promyelocytic leukemic cells (HL-60). Cancer Res 43:763-769, 1983

128. Lee EJ, Hogge DE, Gallagher R, et al: Low dose 5-azacytidine is in effective for remission induction in patients with acute myeloid leukemia. Leukemia 4835-838, 1990

129. Silverman LR, Davis RB, Holland JF, et al: 5-azacytidine (AZA) as a low dose continuous infusion is an effective therapy of patients with myelodysplastic syndromes (MDS). Blood 73:263-270, 1989

130. Baccarani M, Tura S: Differentiation of myeloid leukemic cells: New possibilities for therapy. Br J Haematol 40:509-517, 1979

131. Francis GE, Guimaraes JET, Berney JJ, et al: Synergistic interactions between differentiation inducer and DNA synthesis inhibitors: A new approach to differentiation induction in myelodysplasia and acute myeloid leukemia. Leuk Res 9:573-581, 1985

132. Ho AD, Martin H, Knauf W, et al: Combination of low-dose cytarabine and 13-cis-retinoic acid in the treatment of myelodysplastic syndromes. Leuk Res 11:1041-1044, 1987

133. Hellström E, Robert KH, Gahrton G, et al: Therapeutic effects of low-dose cytosine arabinoside, alpha-interferon, la-dihydroxyvitamin D, and retinoic acid in acute leukemia and myelodysplastic syndromes. Eur J Haematol 40:449-459, 1988

134. Vadhan-Raj S, Keating M, LeMaistre A, et al: Effect of recombinant human granulocyte-macrophage colony-stimulating factor in patients with myelodysplastic syndromes. N Engl J Med 317:1545-1552, 1987

135. Thompson JA, Lee DJ, Kidd P, et al: Subcutaneous granulocyte-macrophage colony-stimulating factor in patient with myelodysplastic syndrome: Toxicity, pharmacokinetics, and hematological effects. J Clin Oncol 7:629-637, 1989

136. Antin JH, Smith BR, Holmes W, et al: Phase 1/11 study of recombinant human granulocyte-macrophage colony-stimulating factor in aplastic anemia and myelodysplastic syndrome. Blood 72705-713, 1988

137. Negrin K, Kurzrock M, Talpaz M, et al: Treatment of myelodysplastic syndromes with recombinant human granulocyte colony stimulating factor. A phase 1-11 trial. Ann Int Med 110:976-984, 1989

138. Ganser A, Volkers B, Greher J, et al: Recombinant human granulocyte-macrophage colony-stimulating factor in patients with myelodysplastic syndromes—A phase 1/11 trial. Blood 73:31-37, 1989

139. Herrmann F, Lindemann A, Klein H, et al: Effect of recombinant human granulocyte-macrophage colony-stimulating factor in patients with myelodysplastic syndrome with excess blasts. Leukemia 3:335-338, 1989