Background and Objective. During the past ten years, the study of retinoids has undergone a total transformation. The Italian Society of Experimental Hematology decided to discuss these advances at a meeting in Florence on April 18, 1996.

Information sources. The material examined in the present review includes articles and abstracts published in journals covered by the Science Citation Index® and Medline®. In addition, all the authors of the present article have been actively working in the field of retinoids and have contributed several papers. Summaries of their oral presentations at the Florence meeting are reported in the Appendix to this review article.

State of Art and Perspectives. One of the most important advances has been the elucidation of new molecular mechanisms of control of gene expression by retinoids. A number of new retinoids have been synthesized by chemists, some of which are being screened for potential clinical use, and a few have already had a tremendous impact on clinical practice. The most important achievements have been obtained in acute promyelocytic leukemia. In 1988 a Chinese group working in Shanghai showed that using all-trans retinoic acid (ATRA) alone 94% of acute promyelocytic leukemic patients obtained complete remission through differentiation of the leukemic clone. This result transformed a dream into reality and allowed researchers to move from laboratory experience to clinical applications of this differentiating therapy. Expanding the spectrum of hematological malignancies that may respond to ATRA remains a challenge; however, several results show some activity of retinoids alone or in combination with other drugs in juvenile chronic myeloid leukemia (CML), myelodysplastic syndrome, cutaneous T-cell lymphoma and CML. Particularly interesting are the studies that explored the potential clinical synergism of ATRA-based combination therapies with growth factors, other differentiating agents such as vitamin D3, immunomodulators like interferons, or chemotherapeutic agents, in particular Ara-C, all of which show promising in vitro effects when used in combination with retinoids. ©1997, Ferrata Storti Foundation

Key words: ATRA, hematological malignancies
There is also considerable evidence that retinoids have a potent antiproliferative effect, and may be effective in the treatment of a variety of human diseases including cancer. Apart from vision, the mechanism by which retinoids are able to elicit these diverse effects ultimately resides in their ability to regulate gene expression at specific targets sites within the cells.

Naturally occurring retinoids are hydrophobic molecules composed of a cyclopentenic ring conjugated to an unsaturated hydrocarbon side chain with a polar terminal group. Figure 1 illustrates the three retinoids of known biological importance: retinol, retinal and retinoic acid.

Retinol (or vitamin A) cannot be synthesized de novo by animals and is derived ultimately from plant carotenoids in the diet. It is stored in and released from the liver and delivered into circulation as a protein complex. In many tissues retinol can be converted by oxidative metabolism to retinal and, irreversibly, to ATRA.

Unlike water soluble peptide hormones and growth factors, which bind to cell-surface receptors, these fat soluble hormones can pass through the lipid bilayer of the cell membrane, after which they are free to interact with intracellular proteins. It is this hormone-receptor complex that initiates a cascade of events that culminates in the cellular response to the hormone. It is postulated that the hormone induces an allosteric change in the receptor such that the complex becomes competent to bind to high-affinity sites in chromatin and modulate transcription of target genes.

Through a series of elegant experimental studies in several laboratories over the past two decades, the retinoid acid (RA) receptor proteins have been biochemically identified and purified, and their sequences have been deduced through molecular cloning of their cDNAs.

Two families of retinoid nuclear receptors have been characterized: retinoic acid receptors, RARs (types α, β, γ and their isoforms α1, α2, β1 to β4, γ1 and γ2), which are activated by all-trans RA and 9-cis RA, a stereoisomer of ATRA; retinoid X receptors, RXRs (types α, β and γ), which are activated by 9-cis RA only.

Alignment of the amino acid sequence of three cloned RARs with each other, as well as with other members of the nuclear receptor superfamilies allowed the identification of six regions (A to F) within the primary sequence which exhibit a differential degree of conservation (Figure 2).

The highly conserved C region (94% to 97 % identity among the three RARs) is also the most conserved throughout the superfamilies of nuclear receptors. This region contains two zinc fingers and corresponds to the core of the DNA binding domain (DBD) of nuclear receptors. Within this region the receptors are targeted to specific DNA sequences known as retinoic acid response elements (RAREs). The RAREs consist of a minimal core hexad consensus sequence, AGGTCA, that can be configured into a variety of repetitive structured motifs. (Figure 3).

Nuclear receptor response elements are composed of DRs (direct repeats), IRs (inverted repeats) or ERs (everted repeats) of the hexad core sequence. Specificity for different hormone responses is given by the number of nucleotides (n) spaced between the two core elements. They directly reflect the mode of receptor binding, which can be as heterodimers, homodimers or monomers. Nuclear magnetic resonance spectroscopy was used to determine the solution structure of both RAR and RXR DBDs in the absence of DNA. The DBD of each of these receptors spans a core of 66 residues that forms a highly conserved domain encompassing the two zinc finger modules followed.
by a carboxyterminal extension (CTE). The core domain is conserved across all members of the receptor family and contains two α helices, one of which (the recognition helix) engages the major groove to make specific contacts with the bases of the half-site.\textsuperscript{12,13}

Region E on the C-terminal half of the receptor, which is 84-90% conserved among the three RARs, corresponds to the ligand binding domain (LBD). This region possesses the essential property of hormone recognition and ensures both the specificity and selectivity of the physiologic response.\textsuperscript{14} In its simplest terms, the LBD can be thought of as a molecular switch that, upon ligand binding, shifts the receptor to a transcriptionally active state. In addition to the LBD, this region contains a dimerization surface and the ligand-dependent transcriptional activation function AF-2 (also termed Tau-4).\textsuperscript{15,16} The core of the AF-2 activating domain has been characterized in the C-terminal part of region E and shown to correspond to an amphipathic α-helix motif whose main features are conserved among all known transcriptionally active members of the nuclear receptor superfamily.\textsuperscript{17}

Region D is also conserved, with the exception of its central D2 sequence. The N-terminal part of this region, D1, is well conserved and contains several basic residues which may correspond to a nuclear localization signal, as is the case of other members of the nuclear receptor superfamily.\textsuperscript{18} No function has as yet been ascribed to the RAR C-terminal F region, which is lacking in some nuclear receptors.

The N-terminal A/B region contains an autonomous activation function (AF-1), which can activate transcription constitutively in the absence of the ligand-binding domain of region E. Evidence that both AF-1 and AF-2 of RA receptors activate transcription in a promoter and cell-specific fashion has suggested the existence of co-activators or transcriptional intermediary factors (TIFs) that interact specifically with the AF-1 and AF-2 activating domains of the nuclear receptors.\textsuperscript{19,20} In fact, the region containing the AF-2 activating domain has been shown to interact in a ligand-dependent manner with several putative TIFs: ERAP160,\textsuperscript{21} TIF1 (p120),\textsuperscript{22} RIP140,\textsuperscript{23} TRIP-1,\textsuperscript{24} SRC-1.\textsuperscript{25} Not long ago it was demonstrated that the ligand-binding domain interacts strongly in the cell with a conserved domain in the N-terminus of CBP (CREB-binding protein) and p300 in a ligand-dependent manner.\textsuperscript{26} Recent works describe two newly discovered factors, N-CoR (nuclear receptor corepressor)\textsuperscript{27,28} and SMRT (silencing mediator for retinoic and thyroid hormone receptor), that interact with receptors, but rather than activate transcription these factors repress it.\textsuperscript{29}

The complexity of retinoid signaling is further increased by the fact that, at least in vitro, RARs bind to their cognate response elements as heterodimers with RXRs.\textsuperscript{30} Moreover, RXRs, which can also bind in vitro to certain DNA elements as homodimers, are heterodimeric partners for a number of nuclear receptors, such as TRs (thyroid hormone receptors), VDR (vitamin D receptor), PPARs (peroxisome proliferator-activated receptors) and NGFI-B (nerve growth factor receptor-induced orphan receptor).\textsuperscript{31} RAR/RXR heterodimers activate transcription in response to all-trans or 9-cis retinoic acid by binding to direct repeats spaced by five base pairs (DR5 elements) such that RAR occupies the downstream half-site. In the absence of ATRA co-repressor is associated with the complex, thus mediating repression (Figure 4).

Ligand will induce both the dissociation of co-

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Figure 4. Model of differential heterodimeric binding of RAR/RXR to direct rep (DR5 or DR1).
repressor as well as the recruitment of co-activator, and the complex will be active. On DR1 elements, by contrast, the orientation is reversed so that RAR is bound in the 5' site. In this configuration the co-repressor in unable to dissociate from the complex, thereby preventing transcriptional activation.38,39

A RA-associated change at the level of transcription could result from either a direct or an indirect effect of RA. Direct effects of RA on gene transcription are generally assumed to be ones that are mediated by direct binding of RARs or RAR-RXR complexes to a RA-responsive element. Indirect effects are caused by cross-coupling to augment or inhibit signaling pathways mediated by other classes of transcription factors; perhaps the best example is the inhibition of AP-1 (Jun/Fos) action.32,33

In the literature to date, many RA-responsive genes have been identified (growth factors, hormones, cellular enzymes, viral genes, etc.), but often it is still not clear whether they exhibit direct or indirect responses to RA.

Metabolism

ATRA has been proven active against a range of malignancies in isolated tissue culture systems and in human clinical trials, but the duration of its effects has been transient. Recent evidence indicates that the basis for the limited duration of ATRA activity, at least in one form of leukemia, APL, is a pharmacological adaptation that results in reduced serum concentration after prolonged treatment.34

Several pharmacokinetic studies have demonstrated lower plasma ATRA concentrations at the time of relapse and support the hypothesis that acquired resistance to ATRA treatment may have a pharmacokinetic basis.35,37

In fact, decreased intestinal absorption, enhanced enzymatic catabolism and the induction of cellular retinoic acid binding proteins (CRABPs), which leads to drug sequestration, have been suggested as causes of the emergence of clinical drug resistance.

Even though most studies have been carried out in patients affected by APL, no differences have been observed between patients with APL and those with advanced solid tumors with regard to most pharmacokinetic parameters.

ATRA is a natural retinol (vitamin A) metabolite formed by enterocytes from β-carotene and from tissue metabolism of retinol and retinalaldehyde, a process involving nicotinamide-adenine-dinuclotide phosphate (NAD)-linked aldehyde dehydrogenases. The fasting plasma ATRA concentration is in the range of 1.5-3.0 ng/mL (approximately 10⁻⁹ mol/L) in humans. ATRA administered orally is usually formulated as a 10 mg capsule containing an oil suspension of the drug since ATRA is almost insoluble in aqueous solutions. Because no i.v. formulation of free ATRA is available for human trials, the absolute bioavailability of ATRA after oral administration is unknown and a high variability of gastrointestinal absorption cannot be excluded. Drug absorption is dependent upon release from the capsule and is strongly affected by the pH and the fatty acid composition of intralumenal bile. ATRA is mainly absorbed from the intestine into the portal route. At physiologic pH, uncharged ATRA can cross membranes rapidly and spontaneously. In serum, ATRA, presumably in the form of its carboxylated anion, is transported as other free fatty acids bound to serum albumin and not by retinol binding protein (the specific transport protein for plasma retinol). The fact that ATRA does not have a corresponding specific plasma protein may explain why the endogenous plasma concentration of ATRA varies by a factor of two and sometimes more during a single day. ATRA, unlike retinol and retinal, is not stored in any tissue, but is rapidly metabolized to a variety of oxidized and/or conjugated metabolites. ATRA does not accumulate in the liver, thus prolonged assumption of large amounts of ATRA does not result in chronic hepatic damage. Among the tissues examined, the liver, kidney and intestine have relatively high concentrations; serum and adrenal glands intermediate values, testes and fat pads the lowest concentrations.38,39 Recent studies conducted in rabbits have shown that the bone marrow could be an important site of ATRA metabolism and storage.40 This observation may be an important physiologic link between the delivery of retinoids from chylomicrons (that bring dietary lipids and retinoids) to bone marrow and the likely functional role of retinoids in the regulation of blood cell differentiation. Very little ATRA is eliminated in the form of the unchanged compound, with the majority of the material existing in the tissue as various ATRA metabolites.

The principal metabolic reactions responsible for transforming ATRA into its principal metabolites include: isomerization (9-cis-RA and 13-cis-RA), oxidation (4-hydroxy-RA, 4-oxo-RA, 18-hydroxy-RA, 5,6-epoxy-RA, 3,4-didehydro-RA, and retinoic acid), stereoisomerization-oxidation (13-cis-4-oxo-RA), glucuronidation [retinoyl β-glucuronide (RAG), 13-cis-RAG, 4-oxo-RAG, 5,6-epoxy-RAG and 13-cis-4-oxo-RAG], and esterification (retinoic acid esters).

Some of the metabolites of ATRA generated in vivo are active in mediating ATRA function, whereas others are probably catabolic products.41

Following a single oral ATRA dose, plasma concentrations of the drug decrease in a monoexponential fashion, with a terminal half-life of 39-58 minutes. Eight hours after drug ingestion, plasma ATRA concentrations fell below the quantifiable range of a high performance liquid chromatogra-
The mechanisms proposed to explain the progressively decreasing plasma concentration include: 1) decreased intestinal absorption; 2) enhanced enzymatic catabolism (by cytochrome P-450 enzymes and lipid hydroperoxides); 3) induction of CRABPs, which leads to increased drug sequestration.

The most favored explanation for the pharmacological mechanism of ATRA resistance is that continuous ATRA treatment induces catabolic enzymes responsible for conversion of the drug. Administration of ATRA in combination with cytochrome P-450 enzyme inhibitors (ketoconazole or liarozole) showed significant prolonging of the drug’s half-life, supporting the theory of accelerated degradation.

CRABPs (I and II) are two related high-affinity ATRA binding proteins found in the cytoplasm of many cell types. Adamson et al. observed that the amount of CRABPs measured in skin biopsy specimens was rapidly induced in rhesus monkeys following chronic i.v. administration of ATRA, increasing to approximately 3 times baseline levels by day 3 of ATRA treatment, but that they diminished following a 7-day period without the drug. It is not clear what role CRABP play in the hepatic metabolism of the drug. These authors suggested that the increase in CRABP expression was not related to the increase in the plasma drug clearance (with continuous ATRA administration) but rather to catabolic enzyme induction.

Several strategies aimed at preventing or overcoming induced ATRA resistance have been and are being planned. They include intermittent dosing, administration of pharmacological inhibitors of cytochrome P-450 oxidative enzymes, combination with interferon-α and intravenous administration of liposome-encapsulated ATRA.

An intermittent schedule of ATRA administration has a potential advantage over a continuous one. A period of time without drug administration would allow a return to baseline plasma clearance levels and downregulation of CRABPs, which could result in higher ATRA plasma concentrations and possibly less cytoplasmic binding of the drug.

Clinical studies conducted on patients with APL and on patients with HIV infection and Kaposi’s sarcoma treated with ATRA on an intermittent schedule (7 days on and 7 days off) reported a decrease of plasma AUC values to approximately 4-15% of the baseline level by day 7 of therapy, but this was followed by a rapid recovery of ATRA plasma levels after 7 days off the drug.

We confirmed these results in patients with Ph-positive chronic myelogenous leukemia (CML) by studying the pharmacokinetic profile of ATRA during intermittent therapy in a group of 10 such patients in chronic phase treated with 80 mg/sqm/day in two divided doses for 7 consecutive days every other week (i.e. 1 week on and 1 week off = 1 cycle). Plasma ATRA concentrations decreased significantly during the first week of drug administration, with a decline in plasma AUC values ranging from 26.5% to 92% on day 7 with respect to day 1. Following one week without the drug, ATRA levels on day 1 of cycle 2 were equivalent to those obtained on day 1 of cycle 1 (Figures 5 and 6). All these data confirm the reversibility of the phenomenon of induced metabolism and the possibility of modulating ATRA pharmacokinetics by altering the schedule of drug administration. Studies using an intermittent schedule with shorter intervals without the drug (3 days on/4 days off) are currently being performed.

The coadministration of P-450 inhibitors such as ketoconazole, which can block the rate-limiting step (oxidation) in the catabolism of ATRA, failed to yield a sustained increase in plasma ATRA concentration over time in preliminary clinical studies. To achieve a rise in plasma ATRA concentrations, a single daily dose of ketoconazole between 400 and 1200 mg appears to be necessary.

Cytokines and interferons seem to modulate hepatic cytochrome P-450 enzyme activity and, by limiting the up-regulation of the eliminating process, could ensure adequate plasma ATRA con-

Figure 5. Plasma ATRA concentration-time curves in one patient treated with ATRA on an intermittent schedule (7 days on and 7 days off = 1 cycle).
centrations to target cells.

Lazzarino et al. studied the effect of interferon-α2a (IFN-α) on ATRA pharmacokinetics in two patients with APL in complete remission maintained by alternating 15 days of IFN and 15 days of ATRA. Day 15 ATRA levels obtained during IFN+ATRA treatment were significantly higher than those observed in patients maintained on ATRA alone. The authors suggested a potential role for IFN in modulating ATRA pharmacokinetics; however, more extensive studies are needed to confirm these results.

Intravenous administration of liposomal encapsulated ATRA (L-ATRA) has been proposed as one of several strategies to prevent the induction of accelerated drug catabolism. Lipophilic agents like ATRA that are incompatible with i.v. injection can be given parenterally when encapsulated in liposomes. A recent study indicated that animals treated chronically with L-ATRA metabolized retinoic acid to a lesser extent than those treated with free ATRA. The ATRA lipid formulation seems to bypass the clearance mechanism that evolves in the liver of patients treated with the oral compound, so that the liposomal product should not be subject to the same relapse rate that has been demonstrated in clinical trials of the free formulation. In addition, administration of higher doses of ATRA should be facilitated by liposomal encapsulation of the drug because of its attenuate toxicity. Phase I clinical studies on L-ATRA are currently ongoing.

More extensive and more detailed pharmacokinetic studies are therefore needed to plan adequate strategies aimed at overcoming ATRA resistance and optimizing therapy.

ATRA in hematological malignancies

ATRA in APL

APL is characterized by striking clinical and biological features, of which the t(15;17) (q22;q21) chromosomal translocation is the most important. This unique cytogenetic marker produces two fusion genes, PML/RARα and RARα/PML, that are the result of the PML gene from chromosome 15 and the RARα gene from chromosome 17. It should be noted that additional genes (PLZF and NPM) might be involved in the molecular pathogenesis of APL but their role is presently unclear. While the PML/RARα hybrid is transcribed in virtually all APL patients, RAR/PML is detectable in only about 70% of cases. Therefore detection of PML/RARα fusion protein messenger ribonucleic acid (mRNA) via a reverse transcriptase polymerase chain reaction (RT-PCR) technique has proved to be extremely useful for molecular diagnosis and monitoring of treatment response in patients with APL. Moreover, the PML/RARα fusion protein is believed to play a major role in APL pathogenesis, since it appears to be responsible for the failure of promyelocytes to differentiate.

As for the chromosomal breakpoints, they are consistently located in the second intron of the RARα gene, while three different translocation breakpoints have been detected in the PML gene: intron 6 as bcr1, exon 6 as bcr2 and intron 3 as bcr3. The influence of the different breakpoints on clinical presentation and outcome is still not well defined.

The therapeutic use of ATRA in APL was pioneered in the late eighties by a Chinese group working in Shanghai. Since then their results, 94% complete remissions (CR) using ATRA alone, have generated tremendous interest in the clinical use of ATRA in APL. As a consequence, several groups have utilized this therapeutic agent in relapsing as well as newly diagnosed APL patients, obtaining CR rates between 64% and 100% of treated patients (Table 1).

Compared with standard chemotherapy, ATRA induces a higher CR rate without causing bone marrow hypoplasia or exacerbation of the coagulopathy (Figures 7 and 8). However, in some patients it is responsible for the retinoic acid syndrome. This syndrome is variably characterized by the presence of fever, respiratory distress, pulmonary infiltrates, pleural or pericardial effusion, hypoxemia, episodic hypotension, weight gain, lower-extremity edema and congestive heart failure. This syndrome is frequently associated with hyperleukocytosis but there are also patients with leukocytosis whose clinical course is not complicated by this syndrome. The earliest manifestations of the syndrome are dyspnea, rales, fever, and/or unexplained weight gain.
The retinoic acid syndrome has been described by European and American investigators in about 25% of patients treated with ATRA, while in the Chinese experience it has been observed only in a minority of patients (<2%). So far, this difference in the frequency of the syndrome between Western and Eastern populations remains unexplained. This syndrome, initially confused with pneumonia or congestive heart failure, now appears to be related to leukocytes undergoing differentiation. The phenomenon may be due to the release of vasoactive cytokines by maturing cells or organ infiltration by myeloid cells.

Another serious adverse effect of ATRA treatment, especially in pediatric patients, is the so-called pseudotumor cerebri syndrome, characterized by variable association of the following symptoms and signs: severe headache, nausea and vomiting, papilledema, retinal hemorrhages, visual changes such as intermittent loss of vision and ophthalmoplegia. It may or may not be associated with the retinoic acid syndrome and leukocytosis. However, so far it does not seem that there is a close relationship among these toxicities.

Moreover, since continued use of ATRA alone, once CR has been achieved, is responsible for the development of resistance to this drug, some studies have evaluated the combination of ATRA with chemotherapeutic regimens in newly diagnosed, relapsed or refractory APL patients, and have obtained better results than historical controls (Table 2).

These results have also been confirmed in a randomized study comparing ATRA + chemotherapy versus chemotherapy alone. The conclusion of this study was that ATRA + chemotherapy increased

### Table 1. ATRA alone as induction in APL patients.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Year</th>
<th>ATRA Dosage Mg/m²</th>
<th>Number of patients treated</th>
<th>N° Cr</th>
<th>% Cr</th>
<th>Relapses number</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Huang et al. (58)</td>
<td>1988</td>
<td>45-100</td>
<td>TOTAL: 23</td>
<td>23</td>
<td>100</td>
<td>7</td>
<td>CR Duration: from 1+ to 11+ months</td>
</tr>
<tr>
<td></td>
<td></td>
<td>45-50</td>
<td>15 Untreated</td>
<td>15</td>
<td>100</td>
<td>3</td>
<td>from 1+ to 11+ months</td>
</tr>
<tr>
<td></td>
<td></td>
<td>45-80</td>
<td>3 Relapsed</td>
<td>3</td>
<td>100</td>
<td>2</td>
<td>from 4 to 5+ months</td>
</tr>
<tr>
<td></td>
<td></td>
<td>45-100</td>
<td>5 Refractory</td>
<td>5</td>
<td>100</td>
<td>2</td>
<td>from 1+ to 3+ months</td>
</tr>
<tr>
<td>Castaigne et al. (59)</td>
<td>1990</td>
<td>45</td>
<td>TOTAL: 22</td>
<td>22</td>
<td>64</td>
<td>9</td>
<td>CR Duration: from 1+ to 11+ months</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 Untreated</td>
<td>4</td>
<td>50</td>
<td>1</td>
<td>from 2 to 13+ months</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>16 Relapsed</td>
<td>16</td>
<td>69</td>
<td>8</td>
<td>from 2 to 13+ months</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 Refractory</td>
<td>2</td>
<td>50</td>
<td>0</td>
<td>8+ months</td>
<td></td>
</tr>
<tr>
<td>Warrel et al. (60)</td>
<td>1991</td>
<td>45</td>
<td>TOTAL: 11</td>
<td>11</td>
<td>82</td>
<td>1</td>
<td>CR Duration: from 1.5+ to 6+ months</td>
</tr>
<tr>
<td></td>
<td></td>
<td>45-50</td>
<td>6 Untreated</td>
<td>6</td>
<td>83</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>45-80</td>
<td>5 Relapsed</td>
<td>5</td>
<td>80</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Chen et al. (61)</td>
<td>1991</td>
<td>60-80</td>
<td>TOTAL: 50</td>
<td>50</td>
<td>94</td>
<td>19</td>
<td>CR Duration: from 2+ to 38+ months</td>
</tr>
<tr>
<td></td>
<td></td>
<td>47 Untreated</td>
<td>47</td>
<td>94</td>
<td>16</td>
<td>from 2 to 38+ months</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 Relapsed</td>
<td>3</td>
<td>100</td>
<td>3</td>
<td>from 7 to 18+ months</td>
<td></td>
</tr>
<tr>
<td>Frankel et al. (62)</td>
<td>1994</td>
<td>45</td>
<td>TOTAL: 51</td>
<td>51</td>
<td>86</td>
<td>19</td>
<td>CR Duration: from 1+ to 34+ months</td>
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<tr>
<td></td>
<td></td>
<td>30 Untreated</td>
<td>30</td>
<td>87</td>
<td>7</td>
<td>All 13 patients treated and maintained</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>21 Relapsed</td>
<td>21</td>
<td>86</td>
<td>12</td>
<td>only with ATRA, relapsed within 10 months after starting treatment.</td>
<td></td>
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</table>
EFS in newly diagnosed APL, suggesting that ATRA should be incorporated in the front line therapy of newly diagnosed APL. As a consequence, several international studies are now in progress to evaluate varying combinations of ATRA and chemotherapy for treating APL. Among these studies is that of the Italian cooperative groups GIMEMA and AIEOP, called AIDA-LAP 0493, that has also been adopted by the EORTC group. Patients are considered eligible for this study only if a diagnosis of APL is confirmed with molecular or cytogenetic studies for the PML-RARα hybrid gene or t(15;17). Patients enrolled in the study will receive a combination of ATRA + idarubicin during the induction phase, followed by three courses of consolidation. Thereafter, patients RT-PCR negative for the PML-RARα hybrid gene will receive maintenance therapy for two years according to 4 randomization arms, while those RT-PCR positive will undergo a transplantation procedure. Therefore the AIDA protocol is the first example of molecularly adapted therapy in acute leukemias, and the preliminary findings presented at the last ASH meeting in Seattle are very impressive as regards both clinical and biological results. In particular, of the first 170 patients who completed the induction phase, 159 (94%) achieved CR; moreover, 110/113 patients (97%) who completed the consolidation phase are in molecular CR. ATRA but show no morphologic, histochemical, or functional differentiation. U937 cells undergo monocytoid differentiation when treated with 10⁻⁶ M concentrations of ATRA. An interaction between G-CSF and ATRA was observed in the AML-193 cell line derived from M4 leukemia with an +i(17q),-17 abnormality. This suggested that differentiation of leukemic cells may require overriding the blockade, which allows the normal stimulus of the growth factor to act. ATRA has also exhibited variable effects on the growth and differentiation of fresh human leukemia cells in short-term culture. Eight out of 9 patients with non M3 leukemia, showed Bcl-2 downregulation when exposed to ATRA in vitro. While differentiation and growth inhibition have been reported in some AML cells, stimulatory effects were seen in others. The capacity for blast self-renewal may also be decreased. Two thirds of the cases in a small series treated in vitro with RA showed inhibited cluster formation. A similar proportion of leukemic patients had clonal growth inhibited by ATRA treatment and demonstrated enhanced normal myeloid colony formation. Combinations of retinoids with other agents have been studied in fresh leukemic cells. The addition of IFN-α to ATRA resulted in additive inhibition of the growth of day 7 colonies cultured in methylcellulose in five of eight patients who showed a response to either agent used alone. Further myeloid differentiation or inhibition of clonal cell growth was induced in two of five cases treated in vitro with ATRA following in vivo IFN treatment. Although the most profound effect was observed in two cases of APL, individual M1 and M4 cases also appeared to

### Table 2. ATRA ± chemotherapy as induction in APL patients.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Year</th>
<th>ATRA Dosage Mg/m²</th>
<th>Number of patients treated</th>
<th>N° Cr</th>
<th>% Cr</th>
<th>Relapses number</th>
<th>Comments</th>
</tr>
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<tbody>
<tr>
<td>Fenaux et al. (67)</td>
<td>1992</td>
<td>45</td>
<td>TOTAL: 26 untreated 12 ATRA+Chemotherapy</td>
<td>25</td>
<td>96</td>
<td>3</td>
<td>DFS= 87% after 18 months</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>12 ATRA+Chemotherapy</td>
<td>11</td>
<td>92</td>
<td>3</td>
<td>EFS= 77% after 18 months</td>
</tr>
<tr>
<td>Ohno et al. (68)</td>
<td>1993</td>
<td>45</td>
<td>TOTAL: 64 7' Untreated 26 Relapsed 21 Refractory 10 Refractory relapsed</td>
<td>54</td>
<td>84</td>
<td>?</td>
<td>DFS for 1st Cr= 76% at 20 months</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>7' Untreated</td>
<td>7</td>
<td>100</td>
<td>?</td>
<td>DFS for &gt;2nd Cr= 28% at 17 months</td>
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<td></td>
<td></td>
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<td>26 Relapsed</td>
<td>22</td>
<td>85</td>
<td>?</td>
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<td></td>
<td></td>
<td></td>
<td>21 Refractory</td>
<td>20</td>
<td>95</td>
<td>?</td>
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<td></td>
<td></td>
<td></td>
<td>10 Refractory relapsed</td>
<td>5</td>
<td>50</td>
<td>?</td>
<td></td>
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<tr>
<td>Castaigne et al. (69)</td>
<td>1993</td>
<td>25</td>
<td>TOTAL: 30 12 Untreated 14 Relapsed 4 Refractory</td>
<td>24</td>
<td>80</td>
<td>2</td>
<td>CR Duration: from 3 to 26+ months</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>12 Untreated</td>
<td>10</td>
<td>83</td>
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<td></td>
<td></td>
<td></td>
<td>14 Relapsed</td>
<td>10</td>
<td>71</td>
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<td>4</td>
<td>100</td>
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<td>Avvisati et al. (70)</td>
<td>1993</td>
<td>45</td>
<td>TOTAL: 86 55 ATRA alone 31 ATRA+Chemotherapy</td>
<td>67</td>
<td>78</td>
<td>?</td>
<td>CR Duration: not specified</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>55 ATRA alone</td>
<td>46</td>
<td>83</td>
<td>5</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>31 ATRA+Chemotherapy</td>
<td>21</td>
<td>68</td>
<td>?</td>
<td></td>
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<tr>
<td>Kanamaru et al (71)</td>
<td>1995</td>
<td>45</td>
<td>TOTAL: 109 Untreated 28 ATRA alone 30 ATRA+Late Chemotherapy 5 ATRA+Early Chemotherapy</td>
<td>97</td>
<td>89</td>
<td>?</td>
<td>DFS= 81% after 20 months of median follow up</td>
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<tr>
<td></td>
<td></td>
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<td>28 ATRA alone</td>
<td>25</td>
<td>89</td>
<td>?</td>
<td>DFS= 75% after 23 months</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>30 ATRA+Late Chemotherapy</td>
<td>26</td>
<td>87</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5 ATRA+Early Chemotherapy</td>
<td>46</td>
<td>90</td>
<td>?</td>
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respond.  

In vitro studies have indicated that the effectiveness of ATRA in promoting differentiation of leukemic cells is enhanced by associating cytosine arabinoside (ARA-C). Moreover, Lishner found that brief exposure of two established cell lines to ATRA increased the ARA-C sensitivity of blasts.

On the basis of these observations, Venditti et al. treated 33 patients with poor prognosis AML no longer suitable for aggressive chemotherapy with ATRA 45 mg/sqm/daily and ARA C 20 mg, twice a day for 10 days, every 4 weeks.

A total of 16 patients (48%) obtained CR. Median duration of CR was 34.4 weeks. The authors concluded that this treatment seems to be an effective regimen for inducing CR in poor prognosis AML and that patients with <50% bone marrow infiltration are likely to represent the ideal target for this combination therapy.

**ATRA in myelodysplastic syndromes**

The therapeutic management of myelodysplastic syndromes (MDS) still remains an unsolved problem. Despite the use of steroid hormones or chemotherapy, overall effects on the disease and survival have not been encouraging. Until now, there has been no effective standard therapy capable of improving the defective hematopoietic maturation in MDS, or of correcting the leukemic transformation. In fact, none of the therapeutic approaches used in MDS have been able to achieve complete remissions in more than 10-15% of the cases at most, and then only for a relatively short period.

Thus most patients, especially the elderly, are given only supportive treatment. Since bone marrow failure is the main feature of these disorders and since it has been shown that differentiation can be induced in leukemic cells by a variety of agents, clinical trials have focused on treating MDS with colony-stimulating factors (CSFs) or inducers of differentiation.

Recombinant growth factors such as G-CSF have been shown to be useful in a significant percentage of patients; however, negative aspects, such as brevity of response, financial cost and enhancement of disease progression, have limited their use to selected groups of patients. The results obtained with differentiation promoting agents are controversial.

The first retinoic derivative used in MDS, 13 cis-RA, produced longer survival in patients with refractory anemia (77% vs 36% in controls; p=0.004), and this was attributed by the authors to restoration of the neutrophil count and, consequently, lower mortality from sepsis and severe infections.

Gold treated 17 patients with 13 cis-RA acid and found improvement in hematological parameters in 5 of them; however, not without a high degree of hepatic and epithelial side effects. By contrast, prospective randomized trials, including one double-blind placebo-controlled study, demonstrated no significant differences in response rates or survival. The response rate defined as hematological improvement varies from 3 to 60%. ATRA has also been applied in MDS. Twenty-three patients with MDS (2 refractory anemia, 11 RAEB, 10 RAEBt) were treated with daily doses of oral ATRA 45 mg/sqm in a multi institutional prospective study. Only two with RAEB and one with RAEBt experienced a transient increase in peripheral neutrophil counts, with a reduction in the percentage of blasts in the bone marrow.

These data are consistent with those recently reported by Kurzrock et al. in 1993. In their study, only one of 29 MDS patients (3%) responded to ATRA therapy, which ranged from 10 to 250 mg/sqm/day, and this response was unrelated to the dose used. Visani treated 2 MDS patients with ATRA and noted a transient improvement in erythropoiesis. The same author then tested ATRA in a series of 10 MDS patients and the results were slightly different. An improvement in erythropoiesis was observed in 3/10 patients but there was also a rise in platelet count in 1 case.

All these effects were transient; the values started to decline once again by the 4th week, despite continuation of treatment.

This phenomenon could be attributed to exhaustion of an ATRA-responding cell pool or, alternatively, to cellular resistance to ATRA as in APL, or to a reduction in plasma ATRA levels after prolonged treatment. Nevertheless, given these results, it seems that ATRA may have some activity in MDS. Two major goals have to be achieved in the future: increasing the percentage of responding patients and prolonging the duration of response. These could possibly be attained by exploring the potential clinical synergism of ATRA-based combination therapies, using growth factors and other differentiation agents such as vitamin D3 or immunomodulators (like interferons), all of which show promising in vitro effects when combined with retinoids.

**ATRA in cutaneous T-cell lymphoma**

Retinoids, employed as a single agent, have proven useful in the treatment of cutaneous T-cell lymphoma. In one trial conducted in Taiwan with 13-cis RA, 6 of 12 patients with peripheral T-cell lymphoma achieved a response, while none of 6 with B-cell lymphoma did. Only a few studies on ATRA in lymphomas have been reported so far. The drug has been utilized both in its oral formulation and, more recently, in the liposomally encapsulated form. In one study on 13 patients with refractory cutaneous T-cell lymphomas treated with oral
ATRA at a dose of 45 mg/sqm/day, 4 achieved a partial remission. L-ATRA, which allows intravenous administration of the drug, was recently used at various dosages in a phase I study on patients with different hematologic malignancies. Three patients with cutaneous T-cell lymphomas resistant to a prior retinoid displayed a minor transient response, and one of 4 disseminated T-cell lymphoma patients achieved a partial remission.  

Retinoids seem to have a preferential effect on patients with mature T-cell lymphomas. However, in vitro studies show that L-ATRA renders B-cell lymphoma lines more susceptible to apoptosis by down-regulating bcl-2 gene expression, suggesting that L-ATRA might also be useful for treating B-cell non-Hodgkin's lymphoma.

**ATRA in multiple myeloma**

Sidell and Siegel showed that ATRA alone or in combination with IFN-α can induce growth inhibition of myeloma cell lines via downregulation of the interleukin-6 receptor (IL-6R) and via a mechanism distinct from IL-6 modulation or induction of apoptosis. More recently, Ogata reported interesting results from the study of fresh human myelomatous plasma cells. He showed that in vitro ATRA can inhibit proliferation of myeloma cells by the downregulation of IL-6 receptors and/or its signal transducer glycoprotein 130 (gp130) surface expression on neoplastic cells, and by inhibition of IL-6 production by myelomatous and stromal cells. Despite these convincing in vitro results, none of the 27 patients reported so far has shown a significant level of response to treatment with ATRA alone. Although we have to consider that all these patients were affected by advanced myeloma and were resistant or relapsed after at least three lines of chemotherapy, these results nonetheless do not encourage the use of ATRA alone in patients with advanced myeloma. The role of ATRA alone in patients with less advanced myeloma and the possible therapeutic impact of ATRA in combination with IFN-α or dexamethasone remain to be elucidated.

**ATRA in chronic myeloid leukemia**

The therapeutic role of ATRA in the treatment of CML is undefined, and many questions about the feasibility and efficacy of treatment with it and its mechanism of action are still open.

The rationale for using ATRA in CML is essentially based on preclinical data suggesting that it could be active on Ph1+ cells and act synergistically with IFN-α. In vitro studies have shown that ATRA: i) can exert dose-dependent inhibitory and granulo-monocytic differentiating effects on different leukemic cell lines and on CML progenitor cells; ii) can act synergistically with IFN-α to induce both growth inhibition and differentiation; iii) can induce apoptosis both in normal hematopoietic progenitors and in leukemic ones.

One or more of the above mentioned mechanisms could play a role in controlling the proliferation of Ph1+ leukemic clone; however, it cannot be excluded that ATRA may lead to leukemic suppression by stimulating normal hematopoiesis. This hypothetical mechanism seems to be suggested by in vitro studies showing that RA stimulates normal progenitor cells rather than leukemic cells, probably by increasing responsiveness to growth factors.

Based on these experimental observations, the clinical use of ATRA in combination with IFN-α appears to be an encouraging attempt to improve the therapeutic results of IFN-α alone.

So far a few responses have been described in patients with CML in blast crisis who were treated with ATRA, but no data have been reported on the activity of ATRA in CML patients in chronic phase.

Quite recently Russo et al. reported the preliminary results of a phase II study to evaluate: i) the feasibility of intermittent treatment with ATRA in Ph1+ CML patients in chronic phase; ii) the pharmacokinetic profile of ATRA and the correlation between ATRA plasma levels and response. In this study 10 CML patients in chronic phase were treated with ATRA 80 mg/sqm/day, divided into two doses, for 7 consecutive days every other week (i.e. 1 week on and 1 week off = 1 course). Two out of 10 patients completed 6 courses of therapy and obtained a partial hematologic response; 8/10 patients went off the study due to hyperleukocytosis (7 cases) and thrombocytosis (1 case). Treatment with ATRA was well tolerated; xerostomia, dry skin and headache were frequent but mild, and in only one case was the ATRA dosage reduced by 50% because of headache (WHO grade II).

Plasma concentrations of ATRA, determined by a sensitive HPLC method, decreased significantly during the first week of drug administration, but after one week without the drug, ATRA AUC values on day 1 of course 2 were equivalent to those achieved on day 1 of course 1 (see also the section on Metabolism and Figures 5 and 6). These preliminary results suggest that ATRA given intermittently at a dose of 80 mg/sqm/day for 7 consecutive days every other week is feasible, but that as a single agent it seems to be incapable of producing good hematologic disease control, considering that ATRA did not induce relevant cytoreductive effects, it could be tested for its differential activities either with cytotoxic agents or with IFN-α.

In such a case an intermittent schedule of ATRA administration appears to offer a pharmacokinetic advantage over daily administration because it could induce less resistance. Although a larger number of cases are needed to evaluate any rela-
A Spanish group working in Madrid has shown that 94% of APL patients obtained CR through differentiation of the leukemic clone; this result was obtained with ATRA alone, and without any sign of bone marrow hypoplasia or exacerbation of coagulopathy. The role of ATRA has been limited by its inability to maintain patients in CR; however, several studies evaluated the combination of ATRA with chemotherapeutic regimens and obtained better results than historical controls, as well as increased EFS in newly diagnosed APL patients. These results suggest that ATRA should be incorporated into the front line therapy of newly diagnosed APL. Expanding the spectrum of hematological malignancies that may respond to ATRA remains a challenge, but several results show some activity of retinoids alone or in combination with other drugs in juvenile CML, MDS, cutaneous T-cell lymphoma and CML. Studies exploring the potential clinical synergism of ATRA-based combination therapies (e.g., with growth factors, other differentiating agents such as vitamin D3, immunomodulators like interferons or chemotherapeutic agents, in particular Ara-C, all of which show promising in vitro effects when used together with retinoids) appear to be especially interesting. Whether the clinical benefits of ATRA therapy can be extended to other hematological malignancies awaits further clinical studies.

Conclusions
Studies conducted over the last two decades demonstrated the capacity of some substances to induce in vitro differentiation of human leukemia cell lines; however, only the in vivo use of ATRA in the treatment of APL allowed researchers to move from the laboratory to clinical application of this differentiating therapy, transforming a dream into reality.

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References
14:651-6.


Acute promyelocytic leukemia (APL) is characterized by the accumulation of blasts blocked at the promyelocytic stage of differentiation. The differentiation block is a major determinant in the maintenance of the leukemic phenotype, since induction of terminal differentiation with retinoic acid (RA) therapy leads to clinical remission. Cytogenetically, APL is characterized by chromosome translocations that involve 17 and 15 [t(15;17)] or, less frequently, 11 [t(11;17)]. The chromosome breakpoints are located with the locus that encodes for the RAR (RAR) receptor induced growth of normal human myeloid progenitor cells in vitro. Blood 1994; 84: 3040-6.

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All-trans retinoic acid (ATRA) induces complete remission in almost all patients with acute promyelocytic leukemia (APL). Remission durations are brief, and continuous oral treatment is characterized by a progressive decline in plasma ATRA concentrations. Our experience, similar to that of others, demonstrated a rapid (7 days) and sustained (40-90%) decrease in plasma drug concentrations in APL, Ph chronic myelogenous leukemia (CML) and solid tumors. The most favored explanation for the increased disappearance of ATRA from plasma is that continuous ATRA treatment induces pharmacological inhibitors of CYP 450 oxidative enzymes; intravenous administration of liposome-encapsulated aimed at preventing or overcoming induced ATRA resistance has been planned. Pharmacokinetic modulation of ATRA by an intermittent schedule of drug administration (i.e. 1 week on and 1 week off = 1 cycle) was studied in a group of 10 patients with CML in chronic phase treated every...
other week with 80 mg/sqm/day in two divided doses. Plasma ATRA concentrations decreased significantly during the first week of drug administration (mean reduction 70%), but AUC values on day 1 of cycle 2 were equivalent to those obtained on day 1 cycle 1. Besides ketoconazole and liarozole, interferon administration appeared to attenuate the reduction in plasma ATRA levels presumably modulating hepatic CYP-450 enzyme activity: in two patients with APL in molecular remission maintained by alternating 15 days of IFN and 15 days of ATRA, day 15 ATRA levels during IFN+ATRA treatment were significantly higher than those observed in patients maintained on ATRA alone. However, a considerable inter- and intra-individual variability in ATRA pharmacokinetic profile was observed in all studied patients during administration of ATRA by continuous or intermittent or combination treatment. In conclusion, our experience confirms the reversibility of the phenomenon of induced ATRA metabolism and the possibility of modulating ATRA pharmacokinetics. Larger studies are needed to determine the efficacy and toxicity of these new schedules of ATRA administration.

All-trans retinoic acid and in vitro cytokine production by acute promyelocytic leukemia cells

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All-trans retinoic acid (ATRA) is able to produce morphological and functional differentiation of acute promyelocytic leukemia cells (APL) both in vitro and in vivo. Leukemic cells spontaneously secrete cytokines involved in the proliferation of the clone; in this study we thus evaluated the effects of ATRA on the in vitro autocrine production of cytokines by acute myeloid leukemia cells.

Thirty acute non-lymphoid leukemia cases (ANLL: 10 APL and 20 ANLL of cytotypes other than APL) were studied; the in vitro secretions of IL-1α, IL-3, IL-4, IL-6, IL-10, G-CSF, GM-CSF, TNF-α was tested with and without ATRA addition. After 5 days' exposure to ATRA 10^7 M APL-treated samples showed a significant reduction of IL-6 (p<0.01) and GM-CSF (p<0.03), and a significant increase of IL-1α (p<0.03) production, if compared to untreated APL samples. No difference was seen in IL-3, IL-10, IL-4 production; G-CSF production was absent in all but three APL cases, in which addition of ATRA determined an increase in production. Interestingly, the three G-CSF-producing cases did not obtain clinical remission with ATRA; confirming previously published results; in contrast, GM-CSF and IL-6 production did not seem related to an unfavorable clinical outcome since they were spontaneously produced by all cases, and 7 out of 10 APL patients subsequently obtained complete remission after induction. TNF-α was produced only in one case; no correlation was possible in this case. No statistical difference was seen in any of the production obtained from cells other than promyelocytic acute leukemia cells, with or without ATRA addition. However, it is noteworthy that the production of IL-6 was more than three times greater in ANLL non APL than in APL cases. It is well known that GM-CSF, IL-6 and IL-1α, at different levels, are involved in the regulation of leukemic cell proliferation. These data could thus suggest possible complementary mechanisms for exhaustion of the leukemic clone upon treatment with ATRA.

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ATRA induces autocrine hematopoietic growth factor production in primary acute myeloid leukemia cells in vitro

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All-trans retinoic acid (ATRA) is widely employed in the treatment of acute promyelocytic leukemia. ATRA has also been shown to promote partial maturation and, in some instances, increase the proliferation rate of acute myeloid leukemia (AML) blasts. We wished to investigate whether these effects could be attributed to ATRA induction of HGF autocrine production in AML blasts. To this end, we isolated leukemic blasts from 27 cases of de novo AML, and evaluated G-CSF, GM-CSF, IL-6, TNF-α, IL-3 production after 18 hours of culture in the presence of ATRA 1 and 10 µM. After this period, cells were harvested, RNA was extracted to perform RT-PCR with oligomers specific for the above mentioned HGFs. At the same time, supernatant from cell cultures was tested for the presence of HGFs, using both the ELISA and biological assay, the latter as stimulation of in vitro growth of HGF-dependent cell lines TF-1 and 32d cl-4. Fifteen out of 27 AML cases were able to produce GM-CSF protein and, of these, 13 were induced by ATRA. IL-6 production was induced in 10 cases, whereas TNFα expression was increased by ATRA in only 3 cases. Although in some AML cases HGF autocrine production was spontaneous, in a conspicuous fraction it was induced by ATRA. We therefore argue that ATRA can act as a regulatory molecule in HGF expression in AML, albeit with some heterogeneity among cases. Whether this activity of ATRA is related to molecular characteristics typical of AML subtypes or to mere susceptibility of AML blasts to mature (and thus also produce HGFs) is a matter that deserves further investigation.

Differentiation of promyelocytic leukemia cells in vitro and in vivo with ATRA and CSFs

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APL is a distinct type of AML characterized by specific morphological, phenotypical and genetic markers. In vitro, differentiation of promyelocytic cells and derivative cell lines has been demonstrated after exposure to phorbol esters, retinoic acid and cytokines such as G-CSF, TNF-α interferon and tumor necrosis factor. In addition, ATRA therapy characteristically induces differentiation of APLs in vivo. We therefore investigated the surface antigen expression and morphocytochemical characteristics of bone marrow cells in a cohort of 13 APL patients, 10 classified APL classic and 3 APL variant, following an uninterrupted treatment with ATRA and in vitro exposure to ATRA alone or in combination with G-CSF or GM-CSF. The promylocytes of patients with M3 variant expressed CD2 but this adhesion molecule was lacking in patients with classic M3. Samples from all patients were cultured in the presence of ATRA, G-CSF and GM-CSF, with and without ATRA. The phenotype of the blast cells was studied at the start (day 0) of the investigation and after 4, 8 and 12 days. In vitro a downregulation of CD2 in blast cells treated with ATRA was observed, whereas CD2 seems to upregulate in blast cells incubated with G-CSF, and GM-CSF without or with ATRA. CD2 expression seems to be higher in the cells treated with G-CSF than with GM-CSF. Similarly, CD2 expression was clearly downregulated in vivo in a patient with APLv treated with ATRA and idarubicin. Morphological and functional (NBT) in vitro differentiation of APL was induced after 8 and 12 days by ATRA and ATRA plus G-CSF. Delayed APL maturation features (more than 12 days) were observed in vitro with G-CSF and GM-CSF. The downregulating mechanism of CD2 exerted by ATRA in APL is unknown.

Differential hemostatic events during all-trans retinoic acid (ATRA) therapy in acute promyelocytic leukemia

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Acute promyelocytic leukemia (APL) is frequently associated with life-threatening hemorrhagic diathesis, responsible for 10-20% of early deaths. Remission induction therapy of APL with ATRA induces CR (up to 90% of cases) and a rapid resolution of the coagulation/bleeding syndrome, even when ATRA is given with chemotherapy. Factors related to the leukemic cells are the major pathogenetic determinants of the coagulopathy, i.e.: 1. expression of procoagulant (including tissue factor [TF] and cancer procoagulant), fibrinolytic and proteolytic activities; 2. release of cytokines, which in turn regulate the expression of endothelial cell (EC) products active on the hemostatic system.
Koyama et al., 1994; Dubois et al./H11003 maintenance of leukemic clonogenic cells (CFU-L) in some of these cases, increasing cytokine production, upregulating TM expression, regulating the surface adhesion receptors, with an increase of blast attachment) may be associated with ATRA administration. When ATRA therapy was stopped, we observed a progressive normalization of the MDR phenotype in leukemic blasts treated in vitro with all-trans retinoic acid (ATRA): preliminary results.

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In order to evaluate both the biological effects of ATRA and its potential activity to modify the MDR phenotype of human blasts, leukemic cells from 8 patients affected by acute myeloid leukemia were cultured with or without 5 x 10^-5 M ATRA in RPMI 1640 medium for 6 days. Next the MDR phenotype was tested by using 6 unconjugated monoclonal antibodies (MoAbs). Four MoAbs were directed against an inner surface cytoplasmic epitope (C-219, JSB-1 from dilute ascites, JSB-1 purified, C494) and two MoAbs recognized an external epitope (MRK16 and 4E3). Blast cells, washed with PBS solution, were fixed and permeabilized in two steps: i) 3.5% paraformaldehyde/PBS; ii) 50% cold acetone/PBS. Moreover, the samples were processed in parallel either with isotype matched negative controls or with primary pure MoAbs at 4°C for 30’. Cells were then incubated with a FITC-conjugated F(ab)2 fragment of goat antinouse Ig (dilution 1:20). The analysis was carried out by an Epics Profile flow cytometer (Coulter). A marked decrease was observed in the mean fluorescence intensity (MFI) ratio, calculated by dividing the MFI of samples stained with anti-P-170 MoAbs and that of negative controls, was found in 3 samples treated with ATRA. In the remaining cases a slight decrease of this ratio was observed. No significant differences were noted among these MoAbs when comparing the MFI ratio. These preliminary data would suggest that the differentiative activity of ATRA on human blasts may be associated with a decrease of MDR expression.

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All-trans retinoic acid and low-dose cytosine arabinoside for the treatment of ‘poor prognosis’ acute myeloid leukemia

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Thirty-five acute myeloid leukemia (AML) patients, no longer suitable for aggressive chemotherapy because of advanced age, poor clinical condition (WHO performance status >2) and other severe diseases associated with organ failure, were treated with daily oral all-trans retinoic acid (45 mg/m²) and subcutaneous cytosine arabinoside (20 mg twice a day, days 1 to 10, every 4 weeks). Median age was 67 (range 39-83) years, 17 patients were females and 18 were males. Eleven patients were at onset of disease, 15 were refractory to previous therapies, 3 were in first relapse and 3 were in second relapse. Median bone marrow blast infiltration was 45% (range 20-90). A total of 16 (46%) patients achieved complete remission (CR); a median of 3 cycles (range 1-5) was required. Median duration of CR was 41 weeks (range 6-138). The rate of CR increased to 83% in those patients with <50% blast infiltration at the time of entering the study (n=18); conversely for those with >50% blast infiltration (n=17) CR rate was 6%. This difference was statistically significant (p<0.001). Eleven patients experienced a relapse and died in leukemic progression, 3 patients died in CR of cerebral hemorrhage (1) and infections (2); 2 patients are still alive and being followed up (+138 and +98 weeks, respectively). Mild to moderate hematologic toxicity was the most common side effect: 5 patients suffered serious infectious episodes (WHO >2) and 14 experienced grade 3-4 thrombocytopenia. Neither oral mucositis nor gastrointestinal complications were observed. In conclusion, ATRA and LDARac appear to be an effective regimen for inducing CR in ‘poor prognosis’ AML. Patients with <50% bone marrow infiltration are likely to represent the ideal candidates to receive this combination therapy.

All-trans retinoic acid, alone and in combination with α-interferon and dexamethasone, for the treatment of advanced multiple myeloma

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It was recently demonstrated that ATRA has an inhibitory effect on myeloma cell growth in culture by lowering the expression of IL-6 receptors (IL-6R) and/or its signal transducer gp130, and by inhibition of IL-6 production by myelomatous and stromal cells. Both IFN and DEX, two drugs effective in MM patients, have been reported to act synergistically in vitro when used in combination with ATRA. On this basis, we conducted a first pilot study in 10 relapsed or refractory multiple myeloma (MM) patients, using ATRA alone at a dose of 100 mg/d p.o., for at least two months. Three patients interrupted the trial early because of relevant side effects. None of the 7 patients who completed the study showed significant reduction of M-component, marrow plasmacytosis or clinical symptoms. Disease accelerated in 4 patients under ATRA treatment and was associated with hypercalcemia and increased serum levels of IL-6 and IL-6R. More recently, we treated 10 additional patients with advanced MM, using lower doses of ATRA (50 mg/d p.o.), IFN (3 MU tiw s.c.), and DEX (40 mg i.v. 4 days every month). All patients had experienced progressive disease after chemotherapy, including DEX. One patient did not tolerate the treatment. To date, of the 5 evaluable subjects who have received at least 3 months of therapy, one has shown a reduction of M-component > 25%, two have maintained a plateau phase with improvement of performance status and reduction of bone pain, and two have undergone further progression of MM. No patient developed hypercalcemia and serum levels of IL-6 and IL-6R increased only in one of the subjects with progressive disease. We conclude that ATRA alone is ineffective and potentially dangerous in patients with advanced MM, while its combination with IFN and DEX merits further investigation.