

Historical Review

THE HISTORY OF ACUTE PROMYELOCYTIC LEUKAEMIA

Why should the British Journal of Haematology devote an article to the history of acute promyelocytic leukaemia (APL)? Because APL is probably the best example of a disease where the dialogue between physicians and scientists has provided a chance to make several advances in both clinical practice and basic sciences. Most patients with APL are now cured. Oncogenesis and the reversion of oncogenic events are now better understood. APL was the malignant disease to be treated by cell modulation, using agents which act specifically on oncogenic events. The recent history of APL could, in fact, be subdivided into three periods: before, during and after treatment with all-*trans* retinoic acid (ATRA). During the first period (1957–1988), the disease was defined; during the second (1988–1993), a specific treatment, later recognized as targeting the oncogenic event, dramatically improved the prognosis of the disease and, during the third post-ATRA period (1991–2002), the main achievements were improved knowledge of cellular and molecular biology, including the control of protein expression and degradation, and a new discovery: the beneficial effect of arsenic.

THE PRE-ATRA PERIOD (1957–1988)

The clinical definition of acute promyelocytic leukaemia

Acute promyelocytic leukaemia (APL) was first described in 1957 by the Swedish author Leif Hillestad (Fig 1). He reported three patients characterized by 'a very rapid fatal course of only a few weeks' duration, a white blood cell picture dominated by promyelocytes, and a severe bleeding tendency due to fibrinolysis and thrombocytopenia'. He noted 'a normal ESR (erythrocyte sedimentation rate), probably caused by the reduced fibrinogen concentration in the plasma'. His conclusion was that the disease 'seems to be the most malignant form of acute leukaemia'. One of the three patients had previously been described by Stormorken (1956).

Leif Hillestad also mentioned that previously, Cooperberg and Neiman (1955) had described a patient with acute myelogenous leukaemia with fibrinolytic purpura, which was 'identical to his cases' and that a similar patient had also been described by Pisciotta and Schultz (1955). Leif Hillestad also thought that a patient reported by Risak (1935), with a 'rapid down hill course and the coincident rise of myelocytes in the peripheral blood', might have been APL.

Leif Hillestad concluded the introduction to his 1957 report by the statement that: 'a logical name of this type of leukaemia is acute promyelocytic leukaemia'.

In the first series of 20 patients recorded during the pre-ATRA period, Bernard *et al* (1959) described more detailed features of the disease.

At the sixth European Congress of Haematology in 1957, Jacques Caen (Caen *et al*, 1957) reported the occurrence, in haematological malignancies, of a fibrinolytic syndrome which he defined more precisely 2 years later as acquired fibrinopenia (Caen *et al*, 1959). In fact, the most impressive clinical feature of APL at diagnosis was the occurrence of severe bleeding diathesis. Patients with APL experienced muco-haemorrhages combined with purpura and abundant ecchymotic subcutaneous haemorrhages. A significant proportion of patients (20–30%) died rapidly of cerebral haemorrhage. The disease constituted an individual entity, mainly because of its more hyperacute outcome compared with other forms of acute leukaemia; the two clinical features defining APL were the characteristic morphology of malignant cells and the presence of severe fibrinopenia.

Larger numbers of typically promyelocytic malignant cells were found in the bone marrow than in the blood. The peripheral blood white cell count was low, and blasts had a monocytoid appearance. The predominant malignant cells in bone marrow were described as resembling abnormal promyelocytes, with an immature nucleus and a copious cytoplasm filled with several azurophilic granules. An abundance of large granules sometimes covered and masked the nucleus. Auer rods were found in many cells, grouped into what were called faggots. In 1976, the well-characterized morphology of these malignant cells led the French–American–British (FAB) Nomenclature Committee to assign them the specific classification of M3 cells (Bennett J.M. *et al*, 1976).

Four years later, a rare variant form of APL, the hypogranular variant, was officially recognized by this Committee. It was characterized by cell nuclei that were usually bilobed, with no granules visible on light microscopy and a positive myeloperoxidase reaction (Bennett *et al*, 1980). Patients with this variant form experienced similar coagulation disorders, but often had a high white blood cell (WBC) count.

A third, very rare variant form, basophilic microgranular APL, was described in 1982 (McKenna *et al*, 1982).

Thus, APL and its morphological variants were identified within a 15-year timespan.

Nevertheless, the clinical management of the disease remained a nightmare for physicians as a result of the unpredictable onset of life-threatening bleeding disorders.

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Acute Promyelocytic Leukemia.

By

LEIF K. HILLESTAD.

(Submitted for publication August 13, 1957.)

Summary.

Evidence is presented for the existence of a special type of acute myelogenous leukemia. Three cases are described, characterized by 1) a very rapid fatal course of only a few weeks' duration, 2) a white blood cell picture dominated by promyelocytes, 3) a severe bleeding tendency due to fibrinolysis and thrombocytopenia, 4) a normal ESR, probably caused by the reduced fibrinogen concentration in the plasma.

It is suggested that this type is named acute promyelocytic leukemia. It seems to be the most malignant form of acute leukemia.

Bernard *et al* (1973) reported that the disease was particularly sensitive to treatment by anthracyclines, which resulted in a high rate of complete remission. However, chemotherapy exacerbated the bleeding diathesis, thus increasing the risk of early death. Two major disorders, thrombocytopenia and fibrinogenopenia, were considered responsible for the coagulopathy. Although everybody agreed on the presence of fibrinogenopenia and high levels of serum fibrinogen–fibrin degradation products, the origins of the fibrinogenopenia gave rise to several controversial discussions, which led to the adoption of different therapeutic approaches: was it due to disseminated intravascular coagulopathy (DIC) with secondary fibrinolysis, to primary fibrinolysis, or both? Apart from platelet transfusion, what was the best treatment: low-dose heparin, antifibrinolytic drugs, or no treatment at all? DIC was most frequently incriminated, because of the presence of D-dimers and the decreased levels of factors V and X. Low-dose heparin was usually included in treatment protocols. However, in contrast to the usual features of DIC, normal survival time of platelet and fibrinogen levels had been shown in APL patients (Bennett M. *et al*, 1976). Avvisati *et al* (1988) further reported normal levels of protein C and antithrombin III. The same authors noted an acquired reduction of alpha-2 plasmin inhibitor (α 2PI) levels. They concluded that the fibrinolytic process, rather than DIC, was the main mechanism responsible for the haemorrhagic diathesis.

In 1988, the problems concerning the mechanism of fibrinogenopenia and the best way of managing the bleeding diathesis were still unsolved.

Apart from the description of the two major clinical features, the morphology of malignant cells and the coagulation disorders, APL had been confirmed as a

distinct entity by the presence of an abnormal cytogenetic feature.

This abnormality was at first considered by Golomb *et al* (1976) as a partial deletion of chromosome 17. However, a year later, the same group identified it as a balanced reciprocal translocation between the long arms of chromosomes 15 and 17 (Rowley *et al*, 1977), and APL was consistently associated with this 15;17 translocation by Larson *et al* (1984). However some APL patients did not have the t(5;17) because of an insertion or complex chromosomal exchanges.

In the 1980s, APL was, therefore, defined by three features: the presence of the M3 cell (a morphological subtype of the FAB nomenclature), the occurrence of fibrinogenopenia and the presence of the specific 15;17 translocation.

Between 1980 and 1988, studies focused on the management of APL treatment and on prognostic factors, the obsession being to avoid a fatal haemorrhage of the central nervous system during the first days of treatment (Rodighiero *et al*, 1990).

The beneficial effect of the anthracycline treatment initiated by Bernard *et al* (1973) was confirmed in Europe and the USA.

Some prognostic factors of APL were rapidly identified by Bernard *et al* (1973), such as the intensity of the fibrinogenopenia and the high WBC count. The latter parameter was also assessed by others, using various criteria: high blast counts or an increased level of lactate dehydrogenase. Other prognostic factors investigated included age, fever, and serum creatinine or albumin levels.

Failure to obtain complete remission was not only due to resistance to chemotherapy, which was reduced by

Fig 1. The first description of APL by Leif K. Hillestad. From: Hillestad, L.K. (1957) Acute promyelocytic leukaemia. *Acta Medica Scandinavica*, 159, 189–194; copyright Blackwell Publishing Ltd.

aggressive daunorubicin treatment (Marty *et al.*, 1984), a reduction later confirmed by the South-west Oncology group (Head *et al.*, 1995), but also to fatal haemorrhages. The dispute as to whether the fibrinogenopenia and haemorrhages were due to DIC or primary fibrinolysis continued, as well as the differences between recommended treatments. The only positive confirmed therapeutic requirement was the intensive need for platelet transfusions during chemotherapy.

To increase patient survival, two studies suggested that maintenance therapy using 6-mercaptopurine and methotrexate could result in longer remissions than short consolidation regimens (Marty *et al.*, 1984; Kantarjian *et al.*, 1986).

By 1988, the end of this initial period, APL was well characterized, both clinically and cytogenetically, and was being treated with anthracyclines and frequent platelet transfusions. The complete remission rate was around 75%, the early mortality rate 15% and the resistance rate 10%. At 2 years, the relapse rate after complete remission was 35%. Thus, about 25% of patients survived for more than 2 years and were considered cured because late relapses are rare in this type of acute leukaemia.

THE SECOND PERIOD (1988–1993): TREATMENT WITH ATRA

The dialogue between clinicians and biologists (1980–1989)

In 1978, Leo Sachs introduced the new concept that certain agents can trigger a differentiation process in leukaemic cells, thus contradicting the dogma of the irreversible status of malignant cells. To understand the disorders affecting cell regulation of blood cell development in leukaemia, he had developed the first culture system in which normal blood cells from mice could be cloned and expanded (Ginsburg & Sachs, 1963). This procedure enabled the identification of the molecules that regulate the differentiation and proliferation of haematopoietic cells. Dr Sachs' group also demonstrated that certain leukaemic cells obtained from mouse cell lines after cloning and culture could be reprogrammed to resume normal differentiation and to become non-dividing mature granulocytes or macrophages as a result of stimulation by various cytokines (Paran *et al.*, 1970). Different clones of myeloid leukaemic cells formed different blocks in this cytokine-induced differentiation (Fibach *et al.*, 1973). Leo Sachs' team also demonstrated that when leukaemic cells from leukaemic mice were injected into mouse embryos they participated in normal haemopoiesis after birth (Gootwine *et al.*, 1982).

In addition, the establishment of the human myeloid leukaemic HL-60 cell line by Dalton *et al.* (1988) enabled the identification of agents that were capable of inducing terminal differentiation. More than 100 agents were listed, some of which, e.g. retinoic acid, induced differentiation into mature granulocytes, others, e.g. dimethyl sulphoxide, into monocytes–macrophages, and others again, e.g. anthracyclines, into erythroid cells.

One of the best candidates for the treatment of several types of human leukaemia was low-dose cytosine

arabinoside (ARA-C), according to the *in vitro* maturation of two myeloid cell lines, HL-60 and U937, using a low concentration of ARA-C (Chomienne *et al.*, 1986a; Poirier *et al.*, 1986).

Accordingly, patients treated with low-dose ARA-C (Houset *et al.*, 1982) achieved a complete remission. In a cohort of elderly patients, 35% achieved a complete remission after 3 weeks of treatment with low-dose ARA-C (Tilly *et al.*, 1985). However, various cytotoxic effects hampered the demonstration of the pure induction of differentiation.

In 1980, the HL-60 myeloid cell was considered to be derived from promyelocytic leukaemic cells. However, we now know that this cell is not a promyelocytic leukaemic cell because it does not carry the specific 15;17 translocation and it only possesses one chromosome 17. Breitman *et al.* (1981) showed terminal differentiation in primary cultures of HL-60 cells and of cells from APL patients in the presence of retinoic acid (RA), a derivative of vitamin A that plays a major role in embryonic development. Using the list of potential RA derivatives that can induce differentiation in the myeloid HL-60 and U937 cell lines, Chomienne *et al.* (1986a, 1990a) assayed primary cultures of fresh malignant cells from the bone marrow of leukaemic patients (instead of cell lines). By testing over 60 bone marrow samples from patients with acute myeloid leukaemia (AML), they demonstrated that the differentiating effect of these derivatives was specific to APL and that different retinoid derivatives exhibited different abilities to induce differentiation. Etretinate was not effective (Chomienne *et al.*, 1986b) and ATRA was potentially 10 times more effective than 13-*cis* (or 4 OXO) retinoids. However, during the 1980s, etretinate was the only derivative available in Europe, and only 13-*cis* RA was available in the USA. ATRA was not manufactured in western countries. Two patients were treated with 13-*cis* RA, but this induced no *in vivo* maturation, despite some *in vitro* differentiation (Chomienne *et al.*, 1989).

In 1985, thanks to travel facilities offered to Chinese medical personalities by Air France, the first informal meeting took place in Paris between Wang Zhen Yi and Laurent Degos. Their initial discussion concerned treatments designed to induce differentiation, using low-dose ARA-C in Paris and low-dose homoharringtonine in Shanghai. They also discussed the specific activity of ATRA in APL. These discussions resulted in a close collaboration between the Shanghai Institute of Haematology (Medical University number 2) and the Institute of Haematology of the Saint Louis Hospital in Paris (University of Paris 7) using ATRA manufactured by the Pharmaceutical Unit number 6 in Shanghai (Fig 2).

Wang Zhen Yi was educated in Shanghai at Aurore University, which was managed by French Jesuits prior to the Chinese communist revolution. This is why he spoke perfect French, which facilitated communication with his French colleagues. After 1985, he came to Paris several times to visit his young student Chen Zhu (currently Vice-President of the Chinese Academy of Sciences and a prominent figure in the development of arsenic treatment) who spent 5 years of his scientific training in Paris.

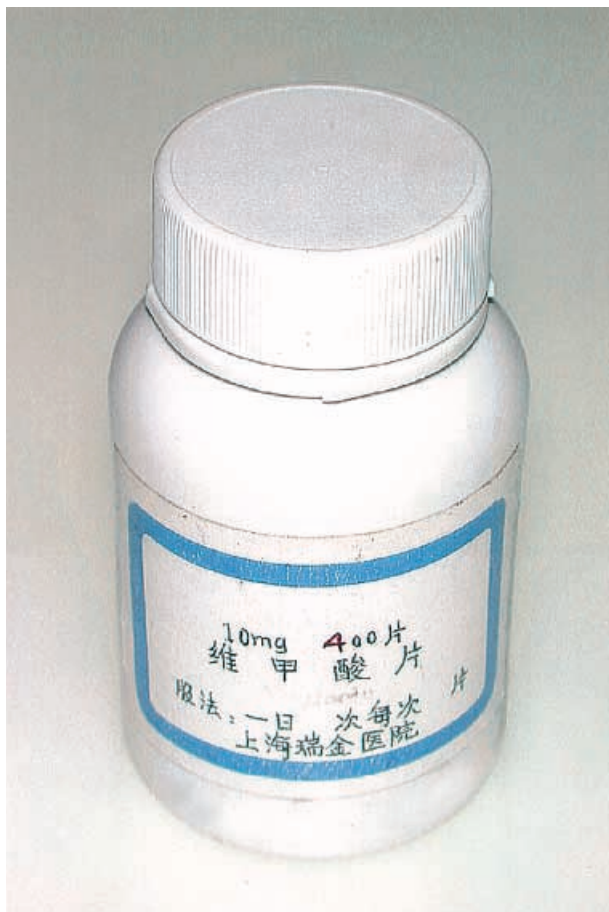


Fig 2. The first production of all-*trans* retinoic acid for clinical use, as 10 mg tablets, made by the Shanghai Pharmaceutical Unit. Courtesy of L. Degos.

ATRA was first used to treat APL patients in 1987 at the Rui-Jin Hospital in Shanghai (Fig 3). During a second collaborative meeting in Shanghai in 1987 between the Saint-Louis and Shanghai Institutes of Haematology, the remarkable results of this treatment were reported: they showed that ATRA could induce differentiation of malignant cells in APL patients until they reached the stage of complete clinical remission (Huang *et al*, 1988). A joint presentation of differentiation therapies using low-dose ARA-C in AML and ATRA in APL was presented at the second conference of Differentiation Therapy of Cancer, in September 1987 (Degos *et al*, 1988). However, despite the demonstration of these positive results, western pharmaceutical companies refused to manufacture ATRA and the Chinese kindly provided the drug for the treatment of French APL patients. It was transported by Chinese students when they travelled to Paris for their training, the first of whom was Huang Meng Er.

In Shanghai, treatment with 45 mg/m²/d ATRA was proposed for newly diagnosed patients (Huang *et al*, 1988) and in Paris for patients experiencing a first or subsequent relapse (Castaigne *et al*, 1990; Degos *et al*, 1990). The complete remission rates reached 95%. The clinical features observed were unusual for a treatment that induced complete remission. Thus, instead of the initial worsening of coagulopathy and bleeding diathesis usually observed during chemotherapy, patients experienced a rapid improvement. No aplasia, no primary resistance to the drug, no alopecia and few infectious episodes were observed. The most striking feature was the gradual terminal differentiation of malignant cells in the bone marrow, sometimes combined with the presence of Auer rods in mature granulocytes (Castaigne *et al*, 1990).

In June 1989, governmental rules required all French Research Institutions to stop their cooperation with Chinese



Fig 3. Huang Meng Er (first author of the first report on ATRA in APL), Wang Zhen Yi, Degos Laurent and Dr Chang in Shanghai in 1987 when the first APL patients were treated with all-*trans* retinoic acid. Courtesy of L. Degos.

EDITORIAL**Acute Promyelocytic Leukemia: Another Pseudoleukemia?**

IN 1875, William Pepper described the bone marrow of a fatal case of pernicious anemia as pseudoleukemia.¹ In the first part of this century, Minot and Murphy² abolished anemia in a series of 45 pernicious anemia patients with daily ingestions of beef liver for months. Ultimately, vitamin B₁₂ was demonstrated to be the missing maturation and differentiation inducer,³ and continuous treatment with that agent uniformly cures the manifestations of the disease, but not the disease itself.

Another pseudoleukemia could be on the way out.

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Fig 4. Opinions expressed after the striking results obtained with all-*trans* retinoic acid. Is acute promyelocytic leukaemia a true leukaemia or a pseudo-leukaemia? Malignancy was not reversible in 1990. From: Wiernik, P.H. (1990) Acute promyelocytic leukaemia: another pseudo-leukaemia? *Blood*, 76, 1675–1677. Copyright American Society of Hematology, used with permission.

groups because of the Tien An Men Square events. ATRA from China was, therefore, no longer available, but several French patients were undergoing treatment. Faced with this situation, Laurent Degos asked representatives of Roche France, a subsidiary of Roche Switzerland, to make the drug. They agreed, but restricted its indication to French patients, to avoid going against the policy of the Roche headquarters in Basel.

At that time, Roche was developing interferon for the treatment of hairy cell leukaemia. Loretta Itri, the vice-president of Roche Nutley, in the USA, chaired a controversial meeting in Paris on the neutral or neutralizing effect of antibodies against interferon. Laurent Degos took the opportunity to contact Loretta Itri, to obtain a source of ATRA production. She consulted her husband, Raymond Warrell, who was in Paris with her. Raymond Warrell was surprised by the effects of ATRA, as he thought that the induction of a complete remission by *in vivo* terminal differentiation of malignant cells was innovative, and suggested to Laurent Degos that his results of ATRA treatment be presented at the Memorial Sloan Kettering Cancer Center in August 1989. After the presentation, which showed series of bone marrow samples from several APL patients at various times after treatment, the audience was convinced by this new treatment, and Raymond Warrell immediately asked Loretta Itri to make the drug. As a result of her great efforts at persuasion, Roche Nutley manufactured ATRA tablets, not only for the treatment of APL but also for an extensive clinical trial involving various types of malignancies conducted under the auspices of the National Cancer Institute.

French clinical findings for ATRA produced in China and by Roche France, as well as the cellular investigations, were published together in *Blood* (Castaing *et al*, 1990; Chomienne *et al*, 1990a), in conjunction with an editorial in the

same issue (Wiernik, 1990), which asked the questions: is APL treated by vitamin A derivative similar to pernicious anaemia treated by vitamin B₁₂? Does a malignant cell still remain malignant if its status is reversible (Fig 4)? The concept that Leo Sachs had formulated in 1978 had still not been really accepted in 1990.

*From the therapeutic product to gene abnormality
(1990–1991)*

The specificity of ATRA treatment for APL, and the location of the retinoic acid receptor alpha (RARA) gene by Mattei *et al* (1988) on the long arm of chromosome 17, at a site close to the one at which cytogeneticists had located the breakpoints, prompted Laurent Degos, Christine Chomienne and Hugues de Thé to undertake further investigations. Using the RARA and probes kindly provided by Martin Petkovich (Petkovich *et al*, 1987) of the Pierre Chambon Laboratory in Strasbourg (France), and by Anne Dejean and Hugues de Thé of the Pierre Tiollais Laboratory at the Pasteur Institute in Paris, they observed an unusual pattern of RARA mRNA in blast cells from APL patients, a pattern not found in normal individuals or in patients with other types of leukaemia. They also noted multiple abnormalities in genomic DNA, as shown by Southern blotting, and concluded that, in APL, the RARA receptor gene undergoes rearrangement. Early in 1989, an article describing these observations was submitted to a prominent medical journal but was rejected, on the grounds that the results were due to artefacts and polymorphisms at restriction sites. The reviewers quoted a previous study conducted in the USA showing no particular RARA mRNA pattern in APL. The French paper was later published in another journal (Chomienne *et al*, 1990b). Meanwhile, Christine Chomienne, together with Laurent Degos (St Louis Hospital in Paris), and Hugues de Thé and Anne Dejean at the Pasteur

Institute, continued their research, and cloned and sequenced the breakpoint on the *RARA* gene (de Thé *et al*, 1990) using the NB4 cell line, established by Michel Lanotte from a patient with APL (Lanotte *et al*, 1991) and fresh APL cells. The partner gene was at first named *myl*. Two other teams conducted concomitant investigations: one, headed by Ellen Solomon (Borrow *et al*, 1990), worked on chromosome 17 and, the other, headed by Pier Giuseppe Pelicci (Longo *et al*, 1990), focused on several candidate partner genes located on chromosome 17, found the same breakpoint on the *RARA* gene. The three studies were published simultaneously at the end of 1990.

One year later, the partner gene of *RARA* in the 15;17 translocation was completely sequenced simultaneously by de Thé *et al* (1991) and Kakizuka *et al* (1991), and both teams published its sequence in the same issue of *Cell*. The gene was renamed *PML* for promyelocytic leukaemia, instead of *myl*, which might have led to confusion with a myosin light chain gene.

ATRA trials (1990–1993)

An extremely beneficial effect (1990–1993). The first two reports of ATRA treatment showed a truly beneficial effect with regard to the number of complete remissions obtained: 22 out of 23 *de novo* APL patients (Huang *et al*, 1988) and 19 out of 20 patients in first relapse (Degos *et al*, 1990).

The differentiation process and the rapid improvement of coagulopathy were considered to constitute a breakthrough in the treatment of APL. These data were described in greater detail by Castaigne *et al* (1990) and Chen *et al* (1991), and confirmed by Warrell *et al* (1991), who supplied evidence of the differentiation process by serial fluorescence *in situ* hybridization (FISH) and of the clonality, using X chromosome-linked polymorphism.

However, the treatment had one major drawback: patients who achieved complete remission with ATRA, either alone or combined with low-dose maintenance therapy, usually relapsed within a few months (median 5 months during continuous ATRA treatment) (Castaigne *et al*, 1990; Chen *et al*, 1991; Warrell *et al*, 1991). These results led French clinicians to initiate a combination therapy, consisting first of ATRA until complete remission, followed by intensive chemotherapy in order to combine the positive effect of the two treatments, i.e. the high rate of complete remission with rapid improvement of bleeding diathesis obtained by ATRA and the long-term remission obtained by the intensive chemotherapy.

In the first non-randomized study (Fenaux *et al*, 1992), the results for 26 newly diagnosed patients with APL treated with ATRA followed by three courses of daunorubicin and ARA-C were compared with the results of a historic control group treated by chemotherapy alone. ATRA followed by chemotherapy greatly reduced the number of early relapses that occurred within 18 months of complete remission (Fenaux *et al*, 1992), although a later follow-up showed that the low number of late relapses was similar to that seen after chemotherapy alone (Fenaux *et al*, 1994). Such dramatic progress prompted the immediate

launch, early in 1991, of the first randomized European ATRA trial (APL 91).

In this trial, the results of chemotherapy alone, comprising three courses of daunorubicin and ARA-C, were compared with those for ATRA until complete remission followed by the same three courses of chemotherapy. The trial was stopped prematurely after 18 months, at the end of 1992, because event-free survival was significantly better in the ATRA group (Fenaux *et al*, 1993). The intergroup difference was confirmed in the subsequent 4-year follow-up. By the end of 1992, the European Cooperative Group considered any regimen for the treatment of APL that did not include ATRA to be unethical, and this attitude was supported by the Memorial Sloan Kettering Cancer Center in New York.

On the other hand, the American–Australian Cooperative Intergroup elaborated a protocol for a randomized trial in which the results of ATRA plus two courses of chemotherapy were compared with those of three courses of chemotherapy without ATRA.

In 1993, the Europeans launched a second trial (APL 93), in which patients were randomly assigned to receive either ATRA followed by chemotherapy (the reference treatment) or ATRA and chemotherapy simultaneously. The resulting relapse rate was far better for the group receiving ATRA and concomitant chemotherapy (Degos *et al*, 1995; Fenaux *et al*, 1999). At the same time, the results of the USA Intergroup Study confirmed those of the first European randomized trial (fewer relapses when ATRA is administered during induction treatment) but also included an unexplained lower complete remission rate (68%) in the cohort of patients given ATRA and chemotherapy concomitantly (Tallman *et al*, 1997).

In addition to the non-randomized trials conducted by Wang Zhen Yi in Shanghai, Raymond Warrell in New York, and Akihisa Kanamaru in Japan (Kanamaru *et al*, 1995), the Italians and Japanese later launched randomized trials. In 1989, the Italian group (Gruppo Italiano Malattie Ematologiche Maligne dell'Adulto; GIMEMA) tested the role of ARA-C in the treatment of APL. This trial delayed the following one (Mandelli *et al*, 1997), which included ATRA. The Japanese tried to enlarge their cooperative group (Japan Adult Leukaemia Study Group; JALSG) in order to have enough patients for a randomized trial.

Two of the randomized trials (APL 93) in Europe and the USA Intergroup trial also tested the effect of maintenance therapy. The results strongly indicated that ATRA maintenance treatment was beneficial in newly diagnosed APL. The European trial showed the additive effects of 2 years of intermittent ATRA treatment (for 2 weeks every 3 months) combined with low-dose chemotherapy (6-Mercaptopurine daily plus methotrexate weekly) for 2 years in reducing the risk of relapse, even (and mainly) in the poor-prognosis group with a high WBC count at diagnosis. In the USA, Intergroup Trial, patients who had not received ATRA during induction therapy also benefited from ATRA maintenance therapy, i.e. continuous ATRA treatment for 1 year (Tallman *et al*, 1997).

At the end of this period (1991–1993), the high rate of complete remission with ATRA (reduced risk of early

mortality and absence of primary resistance) and the low relapse rate generated the hope of curing the disease, at least in 75% of patients (Degos, 1994). In view of the results obtained with a combination of ATRA and chemotherapy, neither autologous nor allogeneic haemopoietic progenitor transplantation in first remission were recommended. Moreover, they seemed to have a detrimental effect. In 1993, Franco Mandelli organized a meeting in Rome on the theme 'APL: a curable disease'.

Reactions of biologists and clinicians to adverse effects (1992). Treatment with ATRA led to three major adverse effects: leucocyte activation during treatment, secondary resistance to ATRA generated by continuous ATRA treatment and some unpredictable relapses. On the other hand, ATRA treatment rapidly eliminated the bleeding disorders. Biologists used their talents to elaborate guidelines and procedures designed to counteract these adverse effects and manage the coagulopathy.

Castaigne *et al* (1990) described patients whose WBC counts increased during treatment, and who also developed fever, dyspnoea, and even renal failure and coma. These severe disorders were reversible in some patients who achieved complete remission, but others died. The disorders were well defined as the 'ATRA syndrome' (Frankel *et al*, 1992). It included fever, respiratory distress, weight gain, and pleural and pericardial effusion, and sometimes renal failure. The syndrome affected about one-third of patients in western countries and Japan, but was less common in China (Wang *et al*, 1999). It was often preceded by an increase in the number of white cells in peripheral blood. Biologists found that the syndrome was due to leucocyte activation and appeared to be related to cytokine release by the differentiating cells (Dubois *et al*, 1994). Two approaches were attempted to counteract this syndrome: (1) the European approach, which was to prevent the syndrome by chemotherapy when the WBC count increased (Fenaux *et al*, 1993), and (2) the approach in New York, which was to treat the syndrome with high doses of intravenous corticosteroids (Frankel *et al*, 1992; Warrell *et al*, 1994).

Secondary resistance, which was recognized in the first series of patients, occurred in all patients treated with ATRA for long periods (Warrell *et al*, 1994). On account of this resistance, patients receiving ATRA alone only achieved complete remission for a short time and then became refractory to ATRA for periods of 6–12 months. After these periods, the acquired resistance was reversible. Some biologists tried to explain this by establishing artificial ATRA-resistant cell lines with additional genetic defects in the *RARA* gene. In fact, however, it appeared that secondary genetic change was not usually involved in this process because all patients acquired resistance and because it was usually reversible.

Consequently, the resistance observed was probably due in almost all patients to a catabolic process or to feedback mechanisms that reduced the ATRA concentration in the organism. Accordingly, Muindi *et al* (1992) showed a significant drop in plasma ATRA levels after only a few days of treatment. ATRA remained at low and sometimes undetectable levels, even if the dose was doubled, because of

an increase in cytochrome P450 activity induced by ATRA itself. This enzyme complex was shown to be involved in the catabolism of ATRA. Cornic *et al* (1992) further noted that ATRA upregulated the expression of a cytoplasmic protein binding ATRA (CRABP II) in the myeloid cells of patients with AML M3, thereby suggesting a reduction in the nuclear concentration of the drug, probably leading to a decrease in activation of the nuclear receptors of ATRA. Optimal intracellular ATRA concentrations correlated with the differentiation of APL cells (Agadir *et al*, 1995). Differentiation induction was considered as a prognostic factor (Cassinat *et al*, 2001). Biologists tried to find new derivatives independent of the catabolic processes, while clinicians adapted the treatments by adding chemotherapy (e.g. in the APL 91 trial) and proposed that maintenance treatments should not be continuous, but intermittent, as in the APL 93 trial.

As soon as the fusion gene *PML-RARA* was cloned in 1990, biologists and clinicians established a reverse transcription polymerase chain reaction (RT-PCR) method designed to ascertain molecular remission and to monitor patients for minimal residual disease (Biondi *et al*, 1992; Castaigne *et al*, 1992). As the sensitivity of the technique was dependent on various parameters, Christine Chomienne initiated a series of technical workshops on RT-PCR in APL with the European Biomed Program.

RT-PCR not only contributed to the diagnostic assessment but was also a sensitive tool for patient follow-up. GIMENA studies (Lo Coco *et al*, 1992) first demonstrated that RT-PCR status was negative in about 95% of patients after complete consolidation. However, the interlaboratory sensitivity of the assay test varied and this proved to be a major limitation. The GIMENA group (Lo Coco *et al*, 1992) clearly demonstrated that conversion from PCR negativity to PCR positivity after consolidation therapy was always associated with subsequent haematological relapse. This introduced a new concept, which led the Italians to anticipate salvage therapy in the event of molecular relapse, thus improving outcome (Diverio *et al*, 1998). This strategy was rapidly included in the design of trials implemented in several other countries.

Lastly, Dombret *et al* (1993, 1995) found that during treatment with ATRA, the bleeding diathesis rapidly disappeared, with a concomitant decrease in primary fibrinolysis, although DIC was not affected. These results confirmed the previously suggested main role of primary fibrinolysis in the fibrinogenopenia and bleeding diathesis. Dombret *et al* (1995) also described a third possible mechanism causing the coagulation disorders, i.e. extended proteolysis due to the release of the proteolytic endocellular enzymes cathepsin G, elastase and myeloblastin, which cleaved several coagulation factors including von Willebrand factor. Primary fibrinolysis was stopped by ATRA treatment whereas DIC persisted, which explained the severe thrombosis occurring in some patients. Clinicians proposed giving low-dose heparin during ATRA treatment in order to avoid such complications.

However, the controversy about the respective roles of primary fibrinolysis and DIC in the pathogenesis of the

bleeding diathesis still persists. On one hand, the high levels of annexin II, a plasminogen receptor, and of one of its activators, the tissue plasminogen activator (tPA) detected on leukaemic cells from APL patients, favoured the notion of plasminogen activation into plasmin, the most important fibrinolytic enzyme (Menell *et al.*, 1999). On the other hand, the DIC theory was supported by the release of tissue factors by APL cells (Zhu *et al.*, 1999).

THE POST-ATRA PERIOD (1991–2002): THE MOLECULAR BIOLOGY OF APL

In October 2001, a series of reviews were published in a special issue of *Oncogene* (Chelbi-Alix & de Thé, 2001). These reviews reflected the vast amount of data collected during the previous decade. An exhaustive report of the original data and discoveries concerning oncogenesis and the restoration of normal malignant cell behaviour cannot be condensed into a few lines. In addition, a major event occurred in the middle of this period: the description of the therapeutic role of arsenic, presented at the meeting of the Chinese Society of Haematology in October 1995 (Da Lian, Manchuria).

1991–1995: the pre-arsenic period

The topic most intensively explored was the relationship between the fusion gene (*PML-RARA*), the *PML-RARA* protein and APL. When the gene *PML-RARA* was transfected into a Cos cell, it acted as a dominant negative gene and product, and impaired the functioning of the normal receptor (de Thé *et al.*, 1991). A high concentration of ATRA partially suppressed this negative effect. Further, the myeloid cell line HL 60, known to be differentiated by retinoic acid, became insensitive to ATRA after transfection of *PML-RARA* but not to other differentiating agents (Rousselot *et al.*, 1994). Thus, the fusion protein *PML-RARA* interfered in the myeloid differentiation cell programme by acting as a dominant negative molecule on the transcription of genes targeted by RARA.

These results raised two important questions: the role of RARA in myeloid differentiation and the identification of the RARA-controlled target genes whose function is impaired by the *PML-RARA* protein.

The first attempts to answer these questions were initiated by de Thé *et al.* (1989), who noted that among the retinoic acid receptors, the *RARA* gene is the one most involved in myelopoiesis. Collins *et al.* (1990) rendered HL-60 cells resistant to the differentiating activity of RA by inhibiting expression using a dominant negative mutation of *RARA*. These experiments were similar to those subsequently conducted by Rousselot *et al.* (1994) with *PML-RARA*. Further, Tsai *et al.* (1993) demonstrated that a multipotential haemopoietic cell with a *RARA* mutation located on the retinoic acid binding site switched the lineage commitment from the granulocyte–monocyte to the mast cell lineage.

However, Lufkin *et al.* (1993) found normal haemopoiesis in *RARA*-deficient mice. The paradox between the dramatic effect of the *PML-RARA* protein, which impaired normal

RARA function and induced a leukaemia, and the normal granulopoiesis observed in *RARA*-deficient mice was intriguing.

As regards the identification of the *RARA*-controlled target gene, Chen Zhu, in collaboration with Michel Lanotte, used the differential display technique on the NB4 cell line before and after RA treatment, and found several retinoic-acid-induced genes (RIG) (Mao *et al.*, 1996). This search is still continuing, and more than 150 genes modulated by RA in NB4 cells have already been identified.

The novel *PML* gene, the partner gene of the *PML-RARA* fusion gene, was also one of the main preoccupations of molecular biologists. The first descriptions were of several protein isoforms (Fagioli *et al.*, 1992) and the ubiquitous expression of *PML* (de Thé *et al.*, 1991).

Thanks to antibodies established by Hugues de Thé, the location of *PML* was noted on the outer shell of the nuclear bodies (Koken *et al.*, 1994). These nuclear bodies had previously been described by Guy de Thé (de Thé *et al.*, 1960), the father of Hugues de Thé. They were not co-located with any other known nuclear substructures, and were not related to any known nuclear function (replication, transcription or translation). A new function had to be discovered for 'a new and rediscovered nuclear organelle' (Brasch & Ochs, 1992)

PML was also present in numerous isoforms which were divided into seven groups (see review by Jensen *et al.*, 2001) as already suggested by Fagioli *et al.* (1992). Several other molecules were found to be partners of the outer shell of the nuclear bodies, with close or weaker links to *PML*. A very intriguing molecule.

Marie Thérèse Daniel, who studied the location of *PML* and *PML-RARA* in APL cells from patients, was surprised to find not only that the nuclear bodies were totally disrupted into multiple spots, spread out in the nucleus and the cytoplasm, but also that after patients had been treated with RA for 5 d, the nuclear bodies began to be reconstructed, and that the reconstruction was complete in 12 d (Daniel *et al.*, 1993). This restoration of nuclear structure was a new effect of RA.

At the end of 1995, the theory proposed was that: (1) APL is due to the fusion gene (*PML-RARA*) and protein *PML-RARA*; (2) the impaired *RARA* moiety acts as a dominant negative molecule by repressing the normal programme of *RARA* for myeloid differentiation, leading to a blockage at the promyelocyte stage; and (3) the impaired *PML* moiety disrupts the nuclear bodies. By an unknown process, retinoic acid restored both the myeloid differentiation and the disrupted nuclear structures.

1995–1996: arsenic treatment of APL: a Chinese story

In 1971, a Chinese group from Harbin Medical University in Manchuria began to use arsenic to treat various malignancies. In 1992, in a relatively confidential Chinese Journal (Sun *et al.*, 1992; quoted by Zang *et al.*, 2001), they reported the effect on APL of an anticancer solution called Ailing-1 (anticancer-1 or Al-1), containing 1% of arsenic trioxide and traces of mercury chloride: 21 of the 32 patients treated (65%) achieved a complete remission. The 5-year survival



Fig 5. Production of the first arsenic preparation by the Harbin Medical University (1995). Courtesy of L. Degos.

rate was 50% and the 10-year rate was 18.8%. In 1995, during the meeting of the Chinese Society of Haematology at Da Lian in Manchuria, to which Laurent Degos and Ruizo Ohno were invited, two groups from Harbin reported that 10 mg/d of purified arsenic trioxide had been used with similar positive results in each of their series. The first trial, which had started in 1971, included 60 patients: 30 *de novo* patients and 30 in relapse, of whom, respectively, 23 and 16 (73% and 53%) experienced complete remissions. The second trial included 72 patients: 30 *de novo* patients of whom 22 (73%) achieved complete remission, five (17%) partial remission and three were resistant to treatment, as well as 42 refractory and relapsed patients, 22 of whom (52%) achieved complete remission, five (12%) partial remission and 15 (36%) failed to respond to treatment.

Members of the Shanghai Institute of Haematology used arsenic trioxide produced in Harbin (Fig 5) to treat 10 patients in relapse after ATRA and chemotherapy, and reported that nine of them achieved complete remission. The only non-responder had lost the 15:17 translocation in malignant cells at relapse (Chen *et al*, 1996). In fact, arsenic is an old drug that was used in the 19th century to treat leukaemia (Fig 6). It is conceivable that the few patients with a good outcome at that time were indeed APL patients.

Biologists from the Shanghai Institute of Haematology performed pharmacokinetic studies and cellular investigations demonstrating mild differentiation of malignant cells at low concentrations and apoptosis at higher concentrations (Chen *et al*, 1996). They have acquired great experience with this drug and found that it was highly toxic for the liver in *de novo* patients (Niu *et al*, 1999).

The clinical effect of arsenic trioxide was confirmed, first by Soignet *et al* (1998) at the Memorial Sloan Kettering Cancer Center, and then by several groups in Europe and Japan.

Like ATRA, arsenic improved the bleeding diathesis in APL and eliminated not only the primary fibrinolysis but also the DIC, thus reducing the membrane procoagulant activity and tissue factor content of APL cells (Zhu *et al*, 1999).

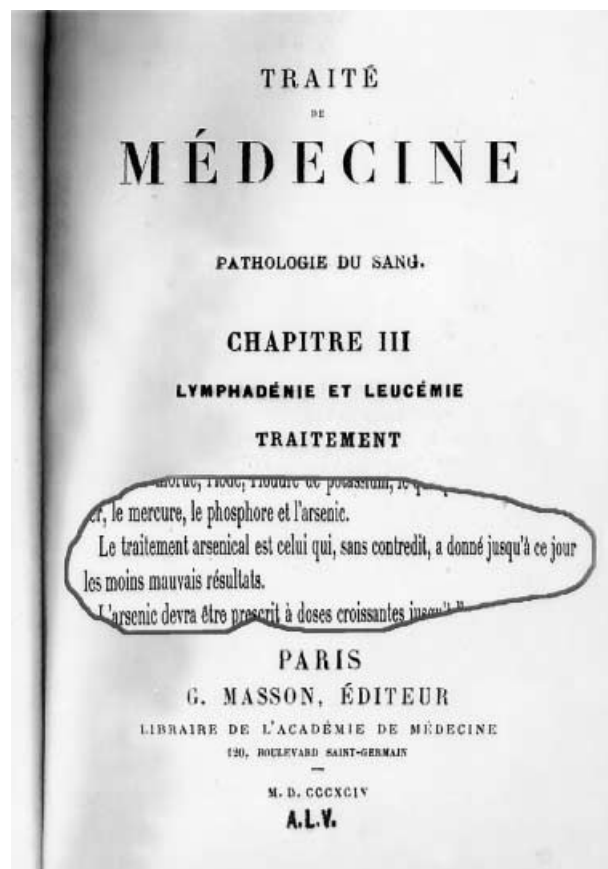


Fig 6. Textbook of medicine for students in 1894, stating that 'the best treatment for leukaemia is arsenic...'. With the permission of Masson, Paris, France.

Also like ATRA, arsenic induced the ATRA syndrome, now known as the leucocyte activation syndrome.

1996–2002: how ATRA and arsenic restore normal function in APL

RARA is a nuclear receptor for ATRA and acts as a dimer with RXR (retinoic X receptor). The RAR–RXR complex has two main functions: the repression and activation of transcription. In the absence of ligand, the heterodimer binds to co-repressors called SMRT (silencing mediator of retinoic acid and thyroid hormone receptor) and N.COR (nuclear receptor co-repressor), which are attached to histone deacetylase–Sin 3A complexes, leading to repression of the target genes transcription (Nagy *et al*, 1997). Histone deacetylase compacts the DNA on histones, thus inhibiting the transcription mechanism. In addition, RA binding induces an exchange between co-repressors and co-activators linked to histone acetylase, thus opening the histone attachments. The co-activator–co-repressor complex also includes the cytoplasmic retinoic acid binding protein II (CRABP II), which is the first ligand binding co-activator of nuclear receptors (Delva *et al*, 1999).

PML-RAR was known to be a strong repressor of RARA signalling (de Thé *et al*, 1991). An Italian group (Grignani

et al., 1998), headed by Pier Giuseppe Pelicci, as well as the group headed by Ron Evans (Lin *et al.*, 1998), made a valuable contribution to the understanding of the molecular disorders in APL. Grignani *et al.* (1998) showed that: (1) the fusion protein PML-RARA recruited histone deacetylase; (2) PML-RARA formed a stable complex with N-COR-histone deacetylase; and (3) PML-RARA functioned as a constitutive transcriptional repressor. Treatment with a pharmacological dose of ATRA released the repressor complex from the PML-RARA molecule. Histone deacetylase inhibitors were able, *in vitro*, to reverse the leukaemic phenotype (He *et al.*, 1998). This explanation opened a new phase in the treatment of AML, as other types of leukaemia such as the M2 subtype with t(8;21) AML-1 ETO had a similar constitutive transcriptional repression through a complex that also included histone deacetylase. APL is a model for the study of the link between gene expression and chromatin reshaping. The repression of gene expression other than the methylation of promoters is mainly due to deacetylation (and methylation) of histones. New avenues of cancer treatment could be envisaged using drugs such as inhibitors of histone deacetylation or demethylating agents. Thus, the theory was that a pharmacological dose of ATRA counteracted the repressor complex binding on RARA, which facilitated the exchange between co-repressors and co-activators of PML-RARA.

To improve understanding of the mechanism of action, clinicians and biologists listed the forms of acute myeloid leukaemias which were not true APL but harboured a translocation in which one of the partners of the fusion gene was RARA (Table I). Morphological features of RARA-associated translocations were not exactly similar to standard APL.

The first of these translocations (11;17) was identified in APL by Chen *et al.* (1993), and the RARA partner was called *PLZF*. The disease was resistant to ATRA, as a result of a second binding to histone deacetylase through *PLZF*, with a link insensitive to ATRA (Hong *et al.*, 1997; He *et al.*, 1998). However, in 1999, the group headed by Bob Lowenberg noted that, for unknown reasons, the disease was sensitive to the combination of ATRA and granulocyte colony-stimulating factor (Jansen *et al.*, 1999).

Sometimes, a patient's cells also showed other genetic defects such as tandem duplication of the gene coding for

the kinase *FTL3* or several karyotypic abnormalities (Schoch *et al.*, 1996). In both cases, treatment by ATRA was effective and long-term follow-up confirmed a prognosis similar to APL. However, in cases of *FTL3* duplication, the patients usually had a high WBC count.

In addition, joint research by Chen Zhu and Hugues de Thé demonstrated that arsenic induced the targeting of PML molecules and the PML moiety of PML-RARA onto nuclear bodies, and that this was followed by the degradation of these proteins (Zhu *et al.*, 1997), through enhanced sumo-lation of the PML molecule (Lallemant *et al.*, 2001).

The two drugs, ATRA and arsenic, are able to restore normal function to the malignant cell, through two mechanisms. Each drug acts on one partner of the fusion protein. ATRA first induces an exchange of transcription modulators on RARA and then degrades RARA and PML-RARA proteins, and arsenic degrades the PML and the PML-RARA oncogenic molecule in nuclear bodies. In the end, both drugs are able to clear the oncoprotein PML-RARA.

The identification of this oncoprotein prompted biologists to experiment with animal models. The first results were disappointing, because RARA knock-outs were viable, and had no obvious defect in myelopoiesis. This was due to the natural repression of myelopoiesis by RARA when no ligand is present. The absence of RA receptors leads not only to permissive but also to accelerated myelopoiesis.

The second phase of animal model experimentation was to reproduce the APL disease. *PML-RARA* transgenic mice were generated using regulatory elements of gene expressed in promyelocytes. Two approaches gave positive results, one using human cathepsin G (Grisolano *et al.*, 1997) and the other using hMRP8 expression vectors (Brown *et al.*, 1997).

Both teams obtained mice that developed leukaemia after a long preleukaemic state leading to a state that resembled human APL and was sensitive to ATRA and arsenic.

Pier Paolo Pandolfi generated several transgenic mice using not only the *PML-RARA* fusion gene but also the *PLZF-RARA* and other fusion genes of which RARA is a partner. He concluded that X-RAR is oncogenic (see review in Piazza *et al.*, 2001). Mice are serially produced in his laboratory with all kinds of combinations (*PML-RARA*, *RARA-PML*, *PLZF-RARA* and others), using transgenic, double transgenic and knock-out mice.

Table I. APL and APL-like acute leukaemia with a translocation involving RARA (17q21).

Gene	Location	Name	Function	Sensitive to ATRA	Frequency	References
<i>PML</i>	15q22	Promyelocytic leukaemia	Apoptosis	Yes	99%	
<i>PLZF</i>	11q23	Promyelocytic leukaemia zinc finger	Morphogenesis (limbs, skeleton)	No	0.8%	Chen <i>et al.</i> (1993)
<i>NPM</i>	5q35	Nucleophosmin	Ribosome biogenesis	Yes	<0.01%	Redner <i>et al.</i> (1996)
<i>NuMA</i>	11q13	Nuclear mitotic apparatus	Chromatin compaction	Yes	<0.01%	Wells <i>et al.</i> (1997)
<i>Stat 5b</i>	17q11	Signal transducer activ. transcript.	Transcription factor for EPO, G-CSF, IL-2, IL-3, IL-7	No	<0.01%	Arnould <i>et al.</i> (1999)

Epo, erythropoietin; G-CSF, granulocyte colony stimulating factor; IL, interleukin.

Further, Lallemand *et al* (1999), using an APL model obtained by transplanting spleen cells from APL transgenic mice (Brown *et al*, 1997), demonstrated not only that ATRA or arsenic had a beneficial effect on APL but, more important, that the combination of ATRA and arsenic eradicated the disease. The mice died without any clinical, haematological or molecular signs of APL. The effect of the ATRA and arsenic combination on *de novo* patients is not known. In practice, nobody is tempted to give arsenic to *de novo* patients as a front-line treatment, because two out of 10 *de novo* patients treated in China experienced severe liver failure (one died) (Chen *et al*, 2001). Arsenic is presently proposed as a consolidation in induction treatment or for patients in relapse, and is sequentially or simultaneously combined with ATRA in several countries, including the USA, China and Europe, but it is not used as a front-line induction treatment.

Finally, several groups are presently working on the immune response, in order to elaborate new vaccines against the fusion protein, in the hope of obtaining a permanent cure for APL, by clearing or controlling the minimal residual disease (Padua *et al*, 2002).

CONCLUSION

APL has been defined as 'the most malignant form of acute leukaemia' among all the forms of acute myeloid leukaemia (Hillestad, 1957) and is now curable in at least 75% of patients.

APL is the first model of a malignant disease to be treated by drugs targeted on an oncogenic event that alters the biological process of the diseased cells. Other cell modifiers, such as antikinase in chronic myeloid leukaemia, are currently being investigated. APL is the main form of leukaemia in which malignancies are treated by differentiating agents, with two drugs, ATRA and arsenic, respectively, acting on the RARA and PML moieties in the fusion protein PML-RARA. The knowledge of the mechanism of action of these drugs gives reason to hope that differentiation treatment can be extended to forms of leukaemia other than APL, mainly by inhibiting the activity of transcription suppressors, such as histone deacetylase inhibitors, demethylation and enhancers of exchanges between co-repressors and co-activators. Studies of APL suggest that acute myeloid leukaemias are due to transcriptional factor activity impairment, which induces maturation arrest. APL is also a valuable model for the rationale of molecular screening of AML. Minimal residual disease assessment is now recognized as a key independent prognostic factor after the induction treatment of AML.

APL is a pioneer model for diseases treated by drugs that target oncogenic pathways. As such, it provides lessons and a source of new concepts. For instance, targeted treatment was equally effective in patients with a pure genetic defect in malignant cells (*i.e.* PML-RAR fusion gene) and in patients with additional karyotypic abnormalities. Similar data were obtained in chronic myeloid leukaemia patients treated with GLIVEC® (O'Dwyer *et al*, 2002).

A second lesson concerns resistance due to the presence of minor subclones harbouring a mutation of the ligand binding pocket domain of the gene involved in oncogenesis (*i.e.* ATRA binding pocket on RARA molecules). These subclones are selected during treatment and lead to a drug-resistant relapse. Similar clonal selection is also found in patients with chronic myeloid leukaemia during treatment with GLIVEC®. Treatments combining several drugs are now proposed to eradicate eventual subclones, in the same way that several antibiotics are used in the treatment of infectious diseases.

The history of APL has not come to an end. Several intriguing questions remain, and several doors have been opened in basic science and clinical research. By extending the model of APL, there is reason to hope that several forms of acute leukaemia can eventually be cured by specifically tailored cell-modifying treatments.

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