1. All trans retinoic acid as a targeting drug for differentiation therapy in acute promyelocytic leukemia

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Introduction

Acute promyelocytic leukemia (APL) is a clonal expansion created by a specific translocation t(15;17) (q22;q11.21) [1] of malignant myeloid cells arrested at a specific step of maturation, leading to a characteristic morphological feature—the M3 type in the FAB (French, American, British) cyto logical classification [2].

APL represents 5% to 15% of adult acute nonlymphoblastic leukemias (ANLL) and is clinically associated with a bleeding diathesis, generally increased by conventional chemotherapy. A high percentage of early deaths, around 20%, are due to the hemorrhagic syndrome and to the infections during the chemotherapy-induced aplastic phase. All trans retinoic acid (ATRA), a vitamin A derivative, is able to induce the maturation of malignant cells from APL patients both in vitro and in vivo, leading to a high rate of complete remission (CR). The discovery of the rearrangement of the retinoic acid receptor alpha gene (RARα) by the breakpoint of the translocation t(15;17) on chromosome 17 opens new opportunities for the understanding of leukemogenesis and of mechanisms of action of the drug. This treatment constitutes the first model of differentiation therapy for cancer.

In vitro culture of malignant cells

Malignant cells appear to have been arrested at an early stage of their differentiation. However, the differentiation blockage is sometimes reversible when leukemic cells are incubated with agents inhibiting their proliferation or enhancing their differentiation [3].

In vivo maturation by the inhibition of proliferation is suggested in patients with myeloid leukemia who achieve CR after low-dose ara-C treatment [4,5]. Furthermore, in one third of leukemic patients in CR after conventional chemotherapy, the apparent normal polymorphonuclear cells arise from the leukemic clone [6].

Retinoic acid (RA) is involved in the specific differentiation of malignant
and normal tissues [7] and is one of the most potent differentiating agents known. Leukemic cells are differentiated in vitro culture by various natural and synthetic retinoids. For instance, ATRA and 13-cis RA enhance the growth of normal CFU-GM [8], inhibit the growth of KG1 cells [9], and stimulate the differentiation of HL-60 and U937 [10]. The growth of cells from leukemic patients is either enhanced or inhibited in liquid or methyl cellulose cultures. In vitro specific differentiation of APL cells was observed first by Breitman et al. [11].

The structure–function relationships for different available molecules disclosed an equal and high effectiveness of ATRA and 13-cis RA at $10^{-6}$ M for in vitro differentiation, while ethyl ester was less effective. At $10^{-7}$ M, the ATRA-induced response remains the same, but the 13-cis RA response is reduced [12].

The differentiating effect of ATRA ($10^{-6}$ M) studied in short-term culture on fresh cell suspension from a series of 35 ANLL patients is observed in 2 of 3 AML1 patients, 2 of 8 with AML2, and all 10 with AML3 (APL) cases. In 25 additional cases of APL, all the cell samples differentiated in the presence of ATRA [13]. Furthermore, in vitro leukemia cell differentiation is closely related to the in vivo response [13].

**Clinical results**

ATRA was not originally available in Western countries but was manufactured in China. A collaboration between Shanghai University II (Wang ZY) and Hopital Saint-Louis (Degos L) started in 1985. De novo patients with APL have been treated in China since 1987. Relapsed patients or patients with contraindication to chemotherapy were treated soon after in France, using the Chinese derivative. After the Tienamin Square events, ROCHE FRANCE began to manufacture the drug (June 1989) followed by ROCHE NUTLEY in the U.S. (June 1990) and ROCHE BASEL (December 1990).

*ATRA is an effective drug for inducing complete remission*

Huang et al. [14] first reported the efficacy of various doses of ATRA (30 to 100 mg/m$^2$/day), obtaining 23 CRs among 24 newly diagnosed patients, some of whom had received other cytotoxic drugs at low doses (e.g., haringtonine, cytosine arabinoside). In a first relapse of APL, the French studies documented a 95% CR rate (19 of 20 patients) [15] using ATRA alone at a fixed dose (45 mg/m$^2$/day). Similar results were then reported by the investigators at Memorial Sloan-Kettering Cancer Center on 11 patients [16]. ATRA therapy is now used by several other groups coordinated by the National Cancer Institute (NCI) in the U.S., by Nagoya University in Japan, and by Queensland University in Australia. More than