our RT-PCR protocol enabled us to detect *WT1* gene expression in the same percentage of acute leukemia MNC preparations as compared to others.[3,4] Since more sensitive RT-PCR protocols detect low *WT1* gene expression levels in normal blood and bone marrow (BM) MNCs, quantitative RT-PCR had to be implemented to discriminate between a physiologic and a malignant, leukemia-associated expression level of this gene.[3] Contrary to acute leukemia, we never detected the WT1 nuclear protein in MNC preparations from normal blood and BM, or from leukapheresis products of solid cancer patients, using a single cell indirect immunofluorescence assay with anti-WT1 monoclonal antibodies.[8] Thus, it remains unclear, whether the detection of low-level *WT1* gene expression in normal blood cells and hematopoietic progenitors by highly-sensitive RT-PCR protocols reflects ''illegitimate or ectopic transcripts'' or may have a physiologic significance. To our surprise, we found *WT1* gene transcripts in almost all hematopoietic soft agar colonies at day 14 but not thereafter, although single colonies at day 14 contain only 100 to 300 as compared to 800 to 1,000 cells at day 28, indicating transient *WT1* gene expression in hematopoietic progenitor cells during their early exponential growth.

Finally, we hypothesize that expression of the *WT1* gene is relevant to the fetal development and physiologic expansion of immature $CD34^+$ hematopoietic progenitors, and that the WT1 gene is functionally switched off on their determination and differentiation. This hypothesis explains acute leukemia as a proliferative disorder, which is at least partly arrested in a state of *WT1* gene-expressing stem cell expansion. It further explains, why the *WT1* gene is downregulated in differentiation-induced leukemia cell lines, why antisense-*WT1* oligonucleotides reduce growth of acute leukemia cell lines, and why subsets of normal regenerating BM $CD34^+$ hematopoietic progenitors express the *WT1* gene on levels comparable to leukemia blasts.[6]

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Delicious Poison: Arsenic Trioxide for the Treatment of Leukemia

To the Editor:

Lately, arsenic trioxide (As_2O_3) has been described in the treatment of acute myeloid leukemia. Experiments in vitro showed that As_2O_3 induced the acute promyelocytic leukemia (APL) cell line NB4 to downregulate bcl-2 expression, as well as to undergo apoptosis.[1] Clinically efficacy has been shown in 14 of 15 patients with relapsed APL, where the use of intravenous As_2O_3 at a dose of 10 mg/d for 4 to 9 weeks resulted in complete morphologic remission without associated bone marrow suppression.[2] In these cases, partial differentiation of the APL cells and downregulation of the fusion protein PML/RARα could also be shown, which might account for the pharmacologic action of the drug.[3]

Arsenic has been known to be poisonous for centuries. Medicinal use of arsenic began in the 15th century. In the 18th century, Dr Thomas Fowler developed a solution preparation of As_2O_3 in potassium bicarbonate (1% wt/vol), known generally as Fowler's solution,[4] which was used empirically for the treatment of a variety of infectious and malignant diseases. The effect of Fowler's solution on the reduction of white cells in two normal people and one patient with ''leucocythemia'' studied at Boston City Hospital, MA was first described in 1878.[5] This lead to the use of As_2O_3 for the treatment of leukemia, until the advent of radiotherapy caused a decline in its clinical application. Its popularity waxed again when Forkner and Scott,[6] also at Boston City Hospital, described nine of 10 patients with chronic myeloid leukemia (CML) who responded to As_2O_3 treatment. These results were subsequently confirmed by other reports,[7] so that As_2O_3 was considered next to irradiation as the most effective treatment of CML before the development of modern chemotherapy.[8] Clinical improvement of the leukemia, including the control of fever, reduction of white cell count, amelioration of anemia and decrease in the size of spleen, could often be achieved. Sometimes, a remission might be maintained for a long period. As expected, toxic side effects were observed in the majority of patients given long-term As_2O_3, including skin pigmentation and keratosis, cirrhosis, polyneuritis, and gastrointestinal problems.[9] In this department, As_2O_3 was used by hematologists in the 1950's for the treatment of a variety of leukemias. Figure 1 shows the typical course of a patient treated with As_2O_3 for CML in chronic phase. As As_2O_3 appeared to be effective for leukemias of different morphologic types, the action was probably related to an intrinsic toxicity of arsenic to marrow cells.

Therefore, while As_2O_3 induced apoptosis and differentiation of APL cells is a novel observation, its clinical use represents but a

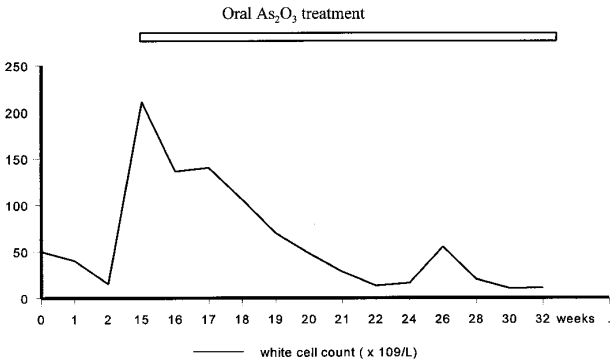

Fig 1. A 30-year-old man presented in March 1954 with splenomegaly and CML in chronic phase was diagnosed. No specific treatment was given until October 1954 when his splenomegaly increased to 5 cm and his white cell count increased to 50 × 10^9/L. Fowler's solution 5 minims (1 minim = 0.06 mL, equivalent to 0.6 mg As_2O_3) three times daily was administered, resulting in a satisfactory control of his white cell count to about 10 × 10^9/L. Treatment was stopped. Six months later, he was readmitted with progressive splenomegaly (10 cm) and leucocytosis (211 × 10^9/L). Fowler's solution was recommenced at 5 minims three times daily, and increased to 10 minims three times daily. This resulted in gradual control of his white cell count. The dose of As_2O_3 was decreased to a maintenance dose of 5 minims three times daily. However, 8 months later, signs and symptoms of chronic arsenic poisoning developed, including skin pigmentation, diarrhea, and chronic gastrointestinal hemorrhage. As_2O_3 was stopped and he was put on melphalan. Splenomegaly and leucocytosis progressed despite treatment, and he died 11 months later of pneumonia. The maximum daily dose (10 minims × 3) of As_2O_3 given orally was 18 mg, which is comparable to 10 mg/d when used intravenously for the treatment of relapsed APL.

resurgence of the use of arsenicals in the treatment of leukemia. Because of the considerable toxicities and the possible and still undefined long-term sequelae, the usefulness of As_2O_3 in the modern treatment of leukemia is still unclear. For this reason, Fig 1 is only of historical interest. However, the use of heavy metals in the treatment of malignancies is not unprecedented, with platinum being a prominent example. Therefore, the rekindling of the interest in arsenicals is of potential importance, but the biological and pharmacological actions of arsenic must be further investigated to define its role in the treatment of leukemia and other types of malignancies.

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Evidence That the Expression and Phosphorylation Status of Pleckstrin Is Modulated by Epstein-Barr Virus in Human B Lymphocytes

To the Editor:

Pleckstrin is exclusively expressed in human hemopoietic cells and is induced during differentiation. Pleckstrin protein contains two copies of the prototypic pleckstrin homology (PH) domain and has been shown to be hyperphosphorylated and appears to have a role in signal transduction.[1] Recently we reported that the coordinate expression of the Epstein-Barr virus (EBV) encoded EBNA3, 4 and 6 proteins (EBNA 3 family) lead to the upregulation of pleckstrin protein in the transfected Burkitt's lymphoma (BL) cell line dG75.[2] The present study was undertaken to determine if pleckstrin was upregulated in EBV^+ cells and to evaluate its phosphorylation status.

Isogenic cell pairs of BL cells or the corresponding lymphoblastoid cell line expressing either the EBNA1 protein alone (Mutu-I, BL29) or the full set of EBV latency proteins (Mutu-III, IARC167) were used. The cell phenotype and the expression of EBV genes were analyzed by fluorescence-activated cell sorter and immunoblot analysis. Equal numbers of exponentially growing cells were radiolabeled with ^{32}P and the cell lysates subjected to immunoprecipitation using rabbit antipleckstrin serum[1] followed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Fig 1A). To determine the amount of total pleckstrin protein in the radiolabeled cells, aliquots of the cell lysates were analyzed in parallel by immunoblot using the rabbit antipleckstrin serum (Fig 1B). Analysis of the polyacrylamide gels by silverstaining and of the nitrocellulose filters by staining with Ponceaus (Sigma, Castle Hill, NSW, Australia) and with an anti-βII microglobulin antibody confirmed that each sample contained similar amounts of protein.

Pleckstrin protein was highly expressed in each of the cell lines expressing the EBV-latency antigens (Fig 1B, lanes 2 and 4). How-