our RT-PCR protocol enabled us to detect WT1 gene expression in the same percentage of acute leukemia MNC preparations as compared to others. 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. 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. 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.

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To the Editor:

Lately, arsenic trioxide (As$_2$O$_3$) has been described in the treatment of acute myeloid leukemia. Experiments in vitro showed that As$_2$O$_3$ induced the acute promyelocytic leukemia (APL) cell line NB4 to downregulate bcl-2 expression, as well as to undergo apoptosis. Clinically efficacy has been shown in 14 of 15 patients with relapsed APL, where the use of intravenous As$_2$O$_3$ at a dose of 10 mg/d for 4 to 9 weeks resulted in complete morphologic remission without associated bone marrow suppression. In these cases, partial differentiation of the APL cells and downregulation of the fusion protein PML/RARA could also be shown, which might account for the pharmacologic action of the drug.

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$_2$O$_3$ in potassium bicarbonate (1% wt/vol), known generally as Fowler’s solution, 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 ‘‘leucocytoma’’ studied at Boston City Hospital, MA was first described in 1878. This lead to the use of As$_2$O$_3$ 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, also at Boston City Hospital, described nine of 10 patients with chronic myeloid leukemia (CML) who responded to As$_2$O$_3$ treatment. These results were subsequently confirmed by other reports, so that As$_2$O$_3$ was considered next to irradiation as the most effective treatment of CML before the development of modern chemotherapy. 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$_2$O$_3$, including skin pigmentation and keratosis, cirrhosis, polyneuritis, and gastrointestinal problems. In this department, As$_2$O$_3$ 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$_2$O$_3$ for CML in chronic phase. As As$_2$O$_3$ 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$_2$O$_3$ induced apoptosis and differentiation of APL cells is a novel observation, its clinical use represents but a
The present study was undertaken to determine if pleckstrin was filtered by staining with Ponceau (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-
Delicious Poison: Arsenic Trioxide for the Treatment of Leukemia

Y.L. Kwong and D. Todd

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