

# CORRESPONDENCE

## Wilms' Tumor Gene Expression in Human CD34<sup>+</sup> Hematopoietic Progenitors During Fetal Development and Early Clonogenic Growth

To the Editor:

The Wilms' tumor gene (*WT1*) located on chromosome 11p13<sup>1</sup> encodes a transcription factor, which is involved in control of growth and differentiation of various cell types including hematopoietic cells.<sup>2</sup> It activates or suppresses the transcription of target genes depending on their promoter structure and the presence of other transcriptional regulators. The *WT1* gene is expressed in blasts of almost all acute leukemia patients, irrespective of lineage.<sup>3-5</sup> Normal blood cells and CD34<sup>+</sup> hematopoietic progenitors express the *WT1* gene on a far lower level<sup>3</sup> or not at all<sup>4,5</sup> depending on the reverse transcriptase-polymerase chain reaction protocols used. Thus, detection of *WT1* gene transcripts was implied as a diagnostic tool to mark minimal residual disease and imminent relapse in treated acute leukemia patients.<sup>3,4</sup> However, recently increasing evidence suggests that a subset of normal CD34<sup>+</sup> hematopoietic progenitors expresses the *WT1* gene during not yet defined circumstances.<sup>6,7</sup>

Here, we report on *WT1* gene expression in umbilical cord blood cells of human fetuses aged between 19 and 34 weeks of gestation. Further, we found *WT1* gene expression in normal hematopoietic progenitors only during the early exponential growth phase when propagated in clonal growth assays.

Blood of human fetuses (n = 6) was obtained by ultrasound-guided puncture of the umbilical cord vein for intrauterine transfusions. Umbilical cord blood mononuclear cells (MNCs, 1.0 mL) were prepared and subjected to the RT-PCR protocol for *WT1* gene detection as described.<sup>5</sup> *WT1* gene transcripts were found in five of six fetal MNC preparations (Table 1). MNCs of leukapheresis products from solid cancer patients (n = 3) were seeded onto methyl-cellulose agar plates enriched with growth factors at a concentration of 100,000 cells/ $\mu$ L. Colony formation was observed, qualified, and counted. At days 14, 21, and 28 after seeding representative colonies were picked using flame-bent glass-micropipets under light-microscopic control (2.5 $\times$ ). Each colony containing between 100 and 1,000 vital cells (trypan blue uptake in less than 5% of cells) was separately subjected to the *WT1*-RT-PCR protocol. *WT1* gene expression was found in almost all colonies picked at day 14 (100 to 300 cells per colony), irrespective of lineage. Contrary, *WT1* gene transcripts were not detectable in colonies picked at days 21 or 28. MNC preparations (10<sup>6</sup> cells) from leukapheresis products of limited-disease solid cancer patients who were pretreated with stem cell-mobilizing chemotherapy and granulocyte colony-stimulating factor (G-CSF) (breast or esophageal cancer; n = 10; CD34<sup>+</sup> hematopoietic progenitor content: 0.5% to 6.6%),

Table 1. *WT1* Gene Expression at Different Time Spots in Single Hematopoietic Colonies Grown in Soft Agar

|                           | WT1 m-RNA Expression | c-ABL m-RNA Expression |
|---------------------------|----------------------|------------------------|
| Colonies at day 14        | 28/35                | 35/35                  |
| CFU-GEMM                  | 2/3                  | 3/3                    |
| BFU-E                     | 7/7                  | 7/7                    |
| CFU-E                     | 6/6                  | 6/6                    |
| CGU-GM                    | 12/19                | 19/19                  |
| Colonies at day 21        | 1/20                 | 20/20                  |
| CFU-GEMM                  | 0/1                  | 1/1                    |
| BFU-E                     | 0/7                  | 7/7                    |
| CFU-E                     | —                    | —                      |
| CFU-GM                    | 1/12                 | 12/12                  |
| Colonies at day 28        | 0/21                 | 21/21                  |
| CFU-GEMM                  | —                    | —                      |
| BFU-E                     | 0/3                  | 3/3                    |
| CFU-E                     | 0/6                  | 6/6                    |
| CFU-GM                    | 0/12                 | 12/12                  |
| Fetal blood MNC           | 5/6                  | 6/6                    |
| Adult blood MNC           | 0/20                 | 20/20                  |
| Reactive BM MNC           | 0/4                  | 4/4                    |
| Leukapheresis product MNC | 0/10                 | 10/10                  |

MNC preparations of leukapheresis products obtained from three solid cancer patients were seeded onto soft agar plates. Before leukapheresis, patients were treated with stem cell-mobilizing chemotherapy and G-CSF. At days 14, 21, and 28 colonies of different lineages were separately subjected to the *WT1*-RT-PCR protocol to detect *WT1* mRNA transcripts.

Abbreviations: BFU-E, burst-forming unit erythrocytes; CFU, colony-forming unit; GEMM, colonies containing granulocytes, erythrocytes, monocytes, and megakaryocytes; E, erythrocyte; GM, granulocyte and monocytes.

including the preparations already used for the clonal growth assays, did consistently not express the *WT1* gene (Table 1, Fig 1).

Due to a large first amplification product (1,745 base pairs [bp]), the nested-primer RT-PCR protocol we are using is not as sensitive as other protocols employed for *WT1* mRNA detection. However,

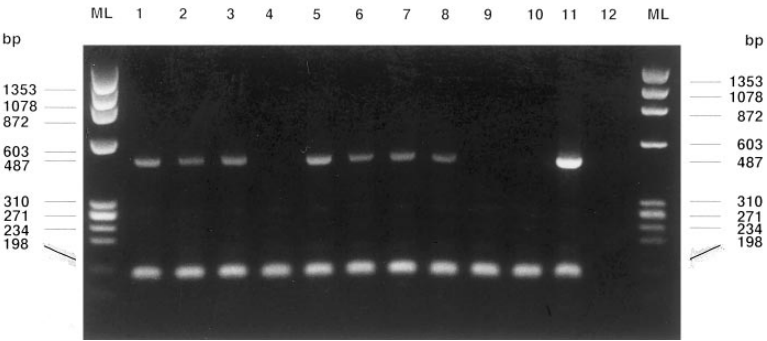


Fig 1. *WT1* gene (487 bp) and *cABL* gene (198 bp, control of RNA-integrity) RT-PCR amplification products of fetal blood MNCs and different single soft agar colonies are shown on an electrophoresis gel stained with ethidium bromide. Lanes from left: Fetal blood MNCs from patients S.M. (1), K.B. (2), and K.Z. (3), MNCs from the leukapheresis product of a solid cancer patient treated with stem cell-mobilizing chemotherapy and G-CSF<sup>4</sup>, colonies at day 14: CFU-GEMM (5), BFU-E (6), CFU-E (7), CFU-GEMM (8), colonies at day 21: BFU-E (9), CFU-GM (10), HL60 cells (11, positive control), water control (12), and marker lanes (ML).

our RT-PCR protocol enabled us to detect *WT1* gene expression in the same percentage of acute leukemia MNC preparations as compared to others.<sup>3,4</sup> 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.<sup>3</sup> 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.<sup>8</sup> 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<sup>+</sup> 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<sup>+</sup> hematopoietic progenitors express the *WT1* gene on levels comparable to leukemia blasts.<sup>6</sup>

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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.<sup>1</sup> 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.<sup>2</sup> In these cases, partial differentiation of the APL cells and downregulation of the fusion protein *PML/RAR $\alpha$*  could also be shown, which might account for the pharmacologic action of the drug.<sup>3</sup>

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,<sup>4</sup> 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.<sup>5</sup> This led 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,<sup>6</sup> 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,<sup>7</sup> so that  $As_2O_3$  was considered next to irradiation as the most effective treatment of CML before the development of modern chemotherapy.<sup>8</sup> 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.<sup>9</sup> 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