WT1 overexpression: A clinically useful marker in acute and chronic myeloid leukemias

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Monitoring of acute leukemia patients during and after treatment for the presence of remaining leukemic cells minimal residual disease (MRD) have been shown to give major insight into the effectiveness of treatment. However, so far applicability of this strategy has been limited to those leukaemia subsets characterized by genetic markers amenable to sensitive detection by PCR. Although PCR for immunoglobulin and T-cell receptor gene rearrangement represents the gold standard for MRD detection in most cases of acute lymphoblastic leukemias (ALL) lacking the availability of fusion gene transcripts as molecular markers, the situation in AML is more complicated because, at present, more than 50% of them lack any sort of clonality markers suitable for MRD monitoring. Thus, a number of studies have been performed in the attempt to identify cytogenetic and molecular abnormalities associated with leukemic transformation.

The Wilms Tumor Gene (WT1) represents a molecular marker for the detection of the leukemic clone useful for monitoring the presence of leukemic cells in all the patients affected by acute and chronic leukemias as well as myelodysplastic syndromes. The WT1 gene, cloned in 1990 by Call et al. [1] encodes for a protein with the characteristics of a zinc finger transcription factor. WT1 expression is restricted to a small number of tissues [2] including testis, ovaries, myometrium, stromal cells of the uterus, heart, lung, intestine, liver and in the supportive stroma and splenic capsule of the spleen [2]. In contrast, several other tissues and cell lines were negative for WT1 expression. Although the role of the WT1 gene in the development of malignancies in the kidney appears quite well defined, currently its potential function in human hematopoiesis still needs to be clarified. The role of WT1 in the leukemogenesis process appears controversial. The majority of human acute myeloid and lymphoblastic leukemias express high levels of wild type WT1, [3,4] suggesting that this tumor suppressor might have paradoxical oncogenic activity in the hematopoietic cells. Although the potential usefulness of WT1 expression as a panleukemic marker was envisaged by Inoue et al. [5] several years ago, its introduction in the clinical practice was limited principally by the background expression level normally detected in bone marrow (BM) samples from healthy volunteers. More recent data obtained using quantitative RT-PCR methods established that sorted populations of normal progenitors express WT1 at very low levels, sometimes even undetectable by very sensitive methods of nested RT-PCR [6–9].

The introduction of a precise method of quantitative PCR allow to distinguish the levels in normal controls and in leukemic samples, so overcoming the obstacle represented by the low amount of WT1 transcript present in normal hematopoiesis. Using a Real Time quantitative PCR, we were able to demonstrated the presence of high levels of WT1 in the majority of cases of acute myeloid and lymphoblastic leukemias at onset of disease as well as in the different phases of chronic myeloid leukemias (ML) [5]. In addition also the patients affected by myelodysplastic syndromes express increased amount of WT1 transcript with variable level according to the subtypes of MDS [10].

By analyzing a large number of normal BM and peripheral blood (PB) samples we also established that the majority of the PB samples score negative and the median number of WT1 copies detected in the
most cases between 10° and 10² degree of sensitivity that can be estimated to reach in universal marker that can allow a rather sensitive associated with persisting remissions. Therefore, evalua-
relapse appears highly variable. On the opposite, relapse of some months, although the kinetics of the preceded the occurrence of the overt hematological knockers. The increase of the WT1 levels could also predictive of an impending hematological relapse even in AML patients lacking additional molecular markers. WT1 expression can represent a molecular marker extremely useful in the clinical setting of MRD assessment. In particular in cases lacking cytogenetic lesions, it may help to establish the response to therapy and to monitor the behavior of the leukemia clone during follow-up.

To validate the role of WT1 as a marker of MRD, we studied a number of patients bearing a fusion gene transcript suitable for the quantitative assessment of the MRD amount by RQ-PCR and performed a simultaneous analysis of the WT1 amount at sequential time intervals during the follow-up [6]. The WT1 levels were shown to strictly parallel the behavior of the other molecular markers (fusion gene transcripts) used for the MRD monitoring. Furthermore, increased WT1 expression above the range found in normal BM and/or in normal PB samples during follow-up of AML patients was always found to be predictive of an impending hematological relapse even in AML patients lacking additional molecular markers. The increase of the WT1 levels could also precede the occurrence of the overt hematological relapse of some months, although the kinetics of the relapse appears highly variable. On the opposite, normal WT1 values have always been found associated with persisting remissions. Therefore, evaluation of WT1 expression could represent a sort of universal marker that can allow a rather sensitive evaluation of the MRD in all AML patients with a degree of sensitivity that can be estimated to reach in most cases between 10⁻³ and 10⁻⁴ [6]. Furthermore, the finding of extremely low and often undetectable WT1 levels in the PB of normal individuals and in leukaemia patients in CCR, suggests that PB could even more sensitive than BM in revealing impending relapses. Although this point still needs to be demonstrated, replacement of BM with PB sampling could greatly improve patients’ compliance and considerably simplify molecular monitoring.

The clinical application of WT1 gene as a molecular marker for MRD detection was already suggested by Inoue et al. [11] in 1996 and confirmed later on by different studies. Recently published data obtained in a pediatric group of AML patients in which the presence of MRD was assessed by flow cytometric analysis for the blast cell count. They found a strict correlation between the levels of WT1 transcript and the percentage of blast cells detected by flow cytometric analysis. Their results confirm the finding that clinical remission is constantly associated with low levels of WT1 while increasing values are associated with relapse.

Finally, similarly to what demonstrated for AML, [6,9] WT1 levels seem to represent a good marker for MRD detection also in MDS patients treated with intensive therapies aimed at the disease eradication [10]. Moreover, it has been demonstrated that even in the transplant setting, as already demonstrated for acute leukemia patients treated with intensive chemotherapy, the determination of the WT1 amount can represent a useful marker to monitor the persistence or the reappearance of leukemic cells and that the finding of increasing amounts of WT1 transcript during follow-up is predictive of relapse.

References


