Clonal nature of hematopoietic stem cell disorders

In a paper published in the present issue of Haematologica,[1] Guidetti *et al.* investigate the clonal origin of hematopoietic cells in patients with myelodysplastic syndromes (MDS). Several cell populations were analyzed, including peripheral blood granulocytes and T lymphocytes, bone marrow hematopoietic progenitors (CFU-GM and BFU-E) and long-term culture-initiating cells (LTC-IC), which represent the most immature human hematopoietic cells that can be isolated and cultured *in vitro*. It was was established that these cell populations were clonal through analysis of the X-chromosome inactivation pattern in female patients. This assay is based on the random inactivation of one X-chromosome in female cells. Such inactivation occurs early during embryogenesis, probably at a stage when fewer than 20 precursors of hematopoietic stem cells are present,[2] and the progeny of a single cell shows inactivation of the same parental chromosome X as the common progenitor cell. The consequence is that if tumors derive from the neoplastic transformation of a single cell, the same chromosome X would be inactivated in each tumor cell.

X-chromosome inactivation studies have been used to investigate the clonal nature of several disease entities,[3-5] the multistep pathogenesis of MDS,[6] the involvement of different cell lineages in hematologic malignancies,[7,8] and to show the polyclonal nature of complete remissions following aggressive chemotherapy in acute (AML) and chronic (CML) myeloid leukemias.[9,10]

The results reported by Guidetti *et al.* show that whereas granulocytes, CFU-GM and BFU-E are clonal in MDS patients, more immature LTC-IC are polyclonal. Although these data were obtained in a small group of MDS patients, they not only confirm results of previous works indicating that polyclonal hemopoietic stem cells are still present in some MDS patients who obtain hematologic remissions following chemotherapy,[11] but further suggest that some, perhaps most, residual stem cells in MDS are polyclonal in origin and do not belong to the dysplastic clone. *In vivo*, however, such polyclonal stem cells do not give origin to an adequate progeny of differentiated cells because they are suppressed by the clonal, dysplastic cell population. Similar observations had already been made in patients with CML by Frassoni *et al.*[12] who found that during the early phases of CML most LTC-IC are Philadelphia negative. Since it is generally accepted that Philadelphia negative cells in CML are polyclonal, it now appears that a relatively large population of normal stem cells persists in several hemopoietic malignancies, such as CML, MDS, and possibly AML, al least in the early phases of the disease.

One potential criticism of this kind of study is that the LTCT-IC assay, as it is actually performed, may provide more favorable conditions for the growth of normal hemopoietic cells than for leukemic and MDS cells. However, this might not be the case, since the induction of polyclonal remissions and/or polyclonal stem cell mobilization with chemotherapy and growth factors indicate that normal stem cells are still present in some leukemia/MDS patients and have different kinetic and drug sensitivity properties from those of their leukemic/MDS counterparts. Therefore, the prevalence of normal/polyclonal LTC-IC in MDS[1] and CML[12] patients may reliably represent the situation at the stem cell level.

Attempts to exploit residual polyclonal stem cells for therapy are still based on traditional approaches, such as chemotherapy and autologous stem cell transplantation, which have only limited success in the long-term control of hematologic malignancies. In addition, clonality analysis through X-chromosome inactivation studies has intrinsic limitations in detecting neoplastic cells in a given population. In fact, the assay has a good sensitivity for detecting residual polyclonal cells, but it is not quantitative and, in the presence of a balanced pattern of X-chromosome inactivation, typical of a polyclonal population, it provides no information about residual clonal (neoplastic) cells which can be present in the sample. This means that in the absence of other markers of clonality, such as cytogenetic and/or molecular abnormalities, X-chromosome inactivation studies do not allow the presence of a significant level of neoplastic contamination to be ruled out.

The therapy of hematologic stem cell malignancies through the use of autologous polyclonal stem cells[13-15] needs further improvements in the procedures of normal stem cell identification and selection and malignant stem cell suppression by chemotherapy, immunotherapy or agents directed against specific molecular targets. However, the presence of a polyclonal population of stem cells in several, perhaps most patients with MDS at diagnosis suggests that this is a research field that deserves to be further pursued in the future.

Gaetano Bergamaschi, MD
Internal Medicine and Medical Oncology
IRCCS Policlinico San Matteo
27100 Pavia, Italy
E-mail: n.bergamaschi@smatteo.pv.it

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Conventional and novel tools for defining the risk of the individual patient with chronic myeloid leukemia and for monitoring treatment

Since chronic myelogenous leukemia (CML) was shown to be associated with a specific chromosomal translocation, t(9;22)(q34;q11), which generates the Philadelphia (Ph) chromosome and the hybrid BCR-ABL gene,[1,2] these specific cytogenetic and molecular alterations have constituted the basis for therapy surveillance of the disease and, to this purpose, novel and more sophisticated tools have been progressively introduced into clinical practice. Though some notions that emerged from the huge number of studies performed on this subject are universally accepted, there are still aspects which appear controversial and that will require further investigation.

It is already clear, particularly for patients treated with interferon-α (IFN-α), the first biological agent capable of inducing cytogenetic remission in patients with CML, that the degree of tumor load reduction during therapy is an important prognostic factor for CML patients.[3] However, the hematologic response, which is achieved with the normalization of peripheral blood counts and absence of signs and symptoms of disease and which corresponds to a 1 log reduction in the leukemia burden, does not represent *per se* a sufficient therapeutic goal in CML, as patients in hematologic remission but who are still 100% Ph-positive invariably progress to a blastic phase and die from its complications. In contrast, the degree of cytogenetic remission, which, if complete, indicates an approximately 2 log reduction of leukemia cell load, has been shown to represent a strong prognostic indicator and it has often been suggested in clinical trials as a possible surrogate marker for overall survival.[3] The cytogenetic response is established on the basis of the proportion of residual Ph-positive metaphases and is defined as complete (0% of Ph-positive metaphases), partial (1-33%), minor (34-66%), or minimal (67-99%), whereas a major response represents the sum of the complete and partial cytogenetic responses. Only major (complete and partial) cytogenetic remissions have been shown to be associated with an increased survival, whereas the impact of minor or minimal cytogenetic responses on prognosis remains negligible.[3]

Finally, molecular remission was traditionally defined on the basis of the detection of residual BCR-ABL transcripts by conventional qualitative nested reverse transcripatse polymerase chain reaction (RT PCR). Indeed, data on the prognostic significance of achieving a molecular remission as defined above, have been obtained mainly in cohorts of patients subjected to allogeneic bone marrow transplantation, the only category of patients able to achieve this condition in a consistent percentage of cases.[4] For all the other patients treated in a different way and in particular for those treated with IFN-α, in whom the number of absolute molecular remissions in terms of persistent polymerase chain reaction (PCR) negativity was very