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● ● ● CLINICAL TRIALS

Comment on Shanafelt et al, page 4188

The changing definition of CLL

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In this issue of *Blood*, Shanafelt and colleagues provide a clearly written, analytical critique of the ALC in the diagnosis of CLL in daily practice and the role of prognostic factors.

The recent International Workshop on Chronic Lymphocytic Leukemia update of the National Cancer Institute 1996 guidelines for chronic lymphocytic leukemia (CLL) by Hallek et al changed the definition of CLL by requiring an absolute B-cell count (BALC) of 5000 cells/ μ L rather than the previous absolute lymphocyte count (ALC) of 5000 cells/ μ L.¹ This created a considerable amount of controversy for both clinicians and patients. BALCs of 5000 or more cells/ μ L were designated B-cell monoclonal lymphocytosis (MBL), causing many former Rai stage 0 and I patients to be reclassified as MBL or SU (small lymphocytic lymphoma). MBL is well recognized in both the United States and Western Europe in population studies, blood bank donors, in aging individuals, and in unaffected first-degree relatives with familial CLL.² It is now thought to be the precursor in CLL.^{3,4} If the ALC of 5000 was arbitrarily selected, there is the notion that the selection of a BALC of 5000 was likewise arbitrarily selected for “consistency” and was not based on objective clinical outcome data. This situation was further aggravated by the requisite change in diagnosis of CLL to MBL in 40% of patients previously diagnosed with Rai stage 0 CLL⁵ and there is no standardized method to determine the BALC.⁶ Given the seriousness of the diagnosis of

leukemia and an understanding of the evolution of the diagnostic criteria for CLL, Shanafelt et al undertook an evaluation of the ALC, B-cell count and conventional prognostic markers.⁷

The Mayo Clinic CLL Database was used to identify 459 consecutive patients diagnosed with Rai stage 0 CLL over a 7-year period. The database is ongoing, and it allows for a sophisticated statistical analysis of presenting clinical data including ALC and flow cytometry and the prognostic factors of CD38, fluorescence in situ hybridization (FISH), zeta-chain associated protein 70 (ZAP70), and immunoglobulin gene variable reagent heavy chain (IGHV) mutational status. The objective clinical outcome measurements were treatment-free survival (TFS) and overall survival (OS). Both the BALC and the ALC predicted TFS and OS. The B-cell threshold that best predicted both OS and TFS was 11 000 cells/ μ L. The authors noted that the current recommended B-cell count value of 5000 did predict TFS but did not predict OS. Interestingly, the ALC of 5000 did not predict either TFS or OS! What is of even greater interest is that the ALC becomes predictive of OS and TFS at 12 000, which suggests that at this numerical value, the percentage of B cells in such a patient sample will probably be 75% or greater. At that value, the ALC and BALC become interchangeable. The predictability of

the prognostic factors and absolute B-cell counts were then determined. B-cell count, IGHV mutational status, and FISH appeared to be the best predictors of OS while ZAP70 and IGHV mutation status were the stronger predictors of TFS. Interestingly, the predictive value of the B-cell count was similar to or slightly better than FISH and CD38. According to Shanafelt et al, when analyzed together, the combination of B-cell count and ZAP-70 was the best predictor of TFS, while the combination of B-cell count and FISH was the best predictor of OS. The size of the B-cell clone is stressed as it relates to disease outcome. It would now appear that since an absolute B-cell clone size of 11 000 cells/ μ L is an independent prognostic indicator and or a surrogate biomarker, even without a revision in the definition of the diagnosis of CLL (clone size 5000 vs 11 000), the ALC can be conveniently used to determine the second flow cytometric analysis for a more refined B-cell lymphocyte prognostic immunophenotype.

Shanafelt et al have presented the most sophisticated analysis of this single clinical feature to date. The size of the B-cell clone is at the heart of the matter. I would be surprised if these findings are not confirmed on a second cohort. In fact, wherever other CLL databases exist, it should be easy to confirm these findings in subjects who have had multiple B-cell count determinations. From a historical viewpoint, in 1973 Hansen defined CLL as an ALC of 10 000 cells/ μ L with 60% mature lymphocytes.⁸ Regardless of the definition used, the role of prognostic indicators has been enhanced and remain important in the management of CLL. Pre-MBL can now be explored in terms of intraclonal heterogeneity and chronic antigen stimulation for MBL/CLL/SLL.⁹

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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● ● ● HEMATOPOIESIS & STEM CELLS

Comment on Otsu et al, page 4197

Mesenchymally “stemming” angiogenesis

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In this issue of *Blood*, Otsu and colleagues provide evidence that high concentrations of bone marrow–derived MSCs have the capacity to induce endothelial cell apoptosis in culture and inhibit angiogenesis and tumor growth in murine melanomas.

Initial interest in bone marrow–derived mesenchymal stem cells (MSCs) focused on their potential for restoring organ function by engraftment and differentiation.^{1,2} More recent work has focused on the immunomodulatory properties of MSCs on T cells, B cells, natural killer cells, and neutrophils, all of which may have value in treating inflammatory disorders including acute lung injury, acute kidney injury, and inflammatory bowel disease.^{3,4} There is also clinical data that MSCs may have activity in treating acute graft-versus-host disease.⁵ In addition to their effects on immune responses, MSCs produce endothelial and epithelial growth factors that might promote tissue repair. However, because of their capacity to release angiogenic growth factors,

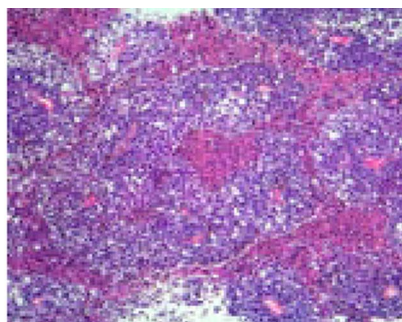
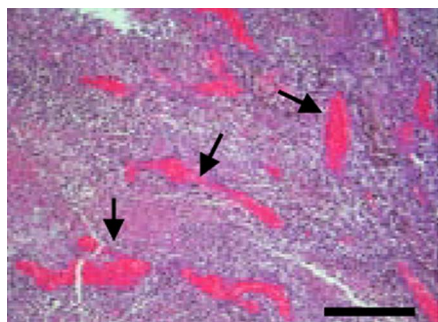
such as vascular endothelial growth factor and basic fibroblast growth factor, there has been concern that MSCs might favor the development or growth of tumors in patients by stimulating angiogenesis.⁶ There has also been concern that MSCs have the potential to become neoplastic.^{3,6} Thus, the observation in the current article that bone marrow–derived MSCs can inhibit growth of melanoma tumors in mice is interesting and potentially important.

Using an in vitro Matrigel angiogenesis assay, Otsu et al found that MSCs migrated to capillaries, established gap junction intercellular communications, and induced a marked increase in reactive oxygen species (ROS) in the cultured endothelial cells, resulting in endothelial cell apoptosis and cap-

illary degeneration.⁷ Direct inoculation of MSCs into subcutaneous melanomas in C57BL/6 mice induced apoptosis in the microcirculation of the tumors, findings that were associated with marked inhibition of tumor growth. Control experiments with mouse lung fibroblasts had no effect. There was a remarkable decrease in vascular density in the MSC-inoculated melanoma tumors. Molecular markers of endothelium and tissue levels of hemoglobin in the tumors were also reduced. Based on the in vitro studies, the mechanism for inducing endothelial cell apoptosis may be explained by the generation of ROS, since ROS inhibitors blocked MSC-induced endothelial cytotoxicity in the Matrigel capillaries. The investigators demonstrated transfer of mitochondria from the MSCs to the endothelial cells, raising the possibility that MSC mitochondria may have been the source for ROS in the endothelial cell. Work from another group indicated that intravenously injected MSCs can home to highly vascular Kaposi sarcoma tumors in athymic nude mice and markedly reduce tumor growth, an effect that depends on cell to cell contact and Akt inhibition.⁸

Although the current study is interesting and well done, there are some issues that will require further study. The authors needed to use a high concentration of MSCs to endothelial cells (1:1) in order to induce apoptosis in capillaries in the Matrigel assay. It was not clear what the ratio of MSCs to tumor cells was in the mouse melanoma experiments, but the effect was only reported with direct injection into the tumors, not with intravenous delivery. Thus, we do not know if the MSCs would home to the melanoma tumors if delivered in the systemic circulation, and if the effect would be transient or sustained with repeated delivery of the MSCs. Further experiments will be needed to assess how MSCs would perform in other mouse tumor models, especially on highly vascular, rapidly growing tumors that are highly dependent on active angiogenesis for ongoing growth and metastatic potential. Finally, the in vivo effects of MSCs could involve immune responses that were not evaluated in this study.

Despite these limitations, there are several important implications from these studies. First, more preclinical studies on the capacity of MSCs to control tumor growth



The antiangiogenic effects of MSCs injected into melanoma are illustrated by the histologic sections that show blood vessels in an untreated (left panel) and MSC-inoculated (right panel) tumor. There is markedly reduced vascular density in the MSC-treated tumors. Bar equals 300 μm . See the complete figure in the article beginning on page 4197.