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**IDENTIFICATION OF BCR/ABL-NEGATIVE HEMATOPOIETIC
PROGENITOR CELLS WITHIN
CHRONIC MYELOID LEUKEMIA MARROW**

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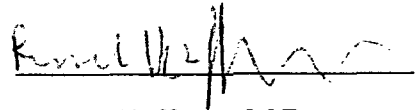
Submitted to the faculty of the University Graduate School
in partial fulfillment of the requirements
for the degree
Doctor of Philosophy
in the Department of Pathology
Indiana University

October, 1993

Accepted by the Graduate Faculty, Indiana University, in partial fulfillment
of the requirements for the degree of Doctor of Philosophy

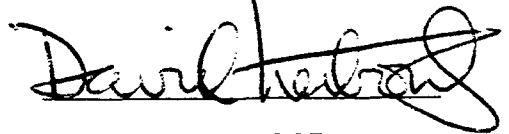


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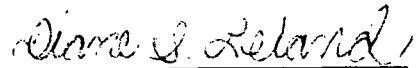


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July 19, 1993

ABSTRACT

Chronic myeloid leukemia (CML) is a clonal neoplastic disorder of the hematopoietic stem cell. Despite the stem cell origin, it is believed that normal stem cells co-exist with the malignant stem cells in the bone marrow of patients with chronic phase CML. Immunofluorescence cell sorting and differential stromal adhesion were investigated as possible ways that normal primitive progenitor cells and stem cells could be isolated from within CML marrow for use in autologous transplantation. Initially, CD34⁺DR⁻ and CD34⁺DR⁺ cells were isolated using centrifugal elutriation, monoclonal antibody labeling, and flow cytometric cell sorting. Polymerase chain reaction (PCR) analysis of these CD34⁺ subpopulations was used to detect the presence of the BCR/ABL translocation characteristic of CML. The CD34⁺DR⁺ subpopulation contained BCR/ABL(+) cells in 13 of 14 marrow specimens, while the CD34⁺DR⁻ subpopulation contained BCR/ABL(+) cells in 7 of 12 CML marrows. Progenitor cell assay results from 8 patients revealed that the CD34⁺DR⁻ subpopulation contained significantly fewer BCR/ABL(+) progenitor cells than either low density bone marrow (LDBM) or the CD34⁺DR⁺ fraction. After 28 days of stromal cell-free long-term culture, 5 of 9 cultures initiated with LDBM, and 4 of 6 cultures initiated with CD34⁺DR⁻ cells produced BCR/ABL(-) cells. Taken together, these results confirm that normal and leukemic progenitor cells co-exist within CML bone marrow. Adherence to selected components of bone marrow stroma was also examined as a potential means of removing BCR/ABL(+) stem cells from CML marrow. There was a marginally significant ($0.05 < p < 0.1$) decrease in the capacity of CML progenitors to adhere to thrombospondin (TSP), but there was no difference in their capacity to attach to *c-kit*-ligand (KL) or to the combination of TSP and KL. PCR analysis of the myeloid progenitors within the adherent and non-adherent fractions revealed no significant difference in the percentage of BCR/ABL(+) colonies between any of the fractions and the input population.

Although the cytoadhesion assay did not appear to select for normal progenitors, flow cytometric selection for CD34⁺DR⁻ cells may be an appropriate primary enrichment step for the isolation of normal stem cells for use in autologous transplantation.

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