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Cindy Jacobs, PhD, M.D.
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Dear Dr. Jacobs:

This letter is written regarding our recent discovery of the Kaposi's sarcoma herpes associated virus (KSHV) in multiple myeloma and its possible applicability to the Ceprate device. We believe that this virus is likely to play a critical role in the development of multiple myeloma. We also believe that the removal of virally infected dendritic cells using the Ceprate device may provide a new rationale to use this technique clinically.

In our studies published in Science last week, we were able to show that 100% of myeloma patient's bone marrow dendritic cells are infected with the KSHV. In addition, we were also able to show that the cells infected with the virus did not express the CD34 antigen. Further studies characterized the cell infected in the bone marrow as belonging to the dendritic cell lineage. This was established by showing that the cells infected with the virus contained CD68, CD83 and fascin (a new dendritic cell marker) on their cell surface. By contrast, the cells do not stain with CD31 or CD34. Electron microscopy studies further characterized the cells as demonstrating the morphologic appearance of macrophages.

We also have studied patients with monoclonal gammopathy of undetermined significance (MGUS). The virus was present in the dendritic cells of approximately one-fourth of these patients as well. It is well known that approximately one-fourth of MGUS patients will eventually develop multiple myeloma. Therefore, the possibility exists that the infection is required for the development of fullblown myeloma. As a result, preventing the infection may prevent the development of myeloma.

Besides the above mentioned data, we believe the virus is important because the virus itself makes its own set of cytokines which have been shown to be critical in multiple myeloma development. These include interleukin 6 (IL-6) as well as an interferon regulatory factor which has been shown to upregulate IL-6 in other studies. The virus also makes a host of factors that put it at a survival advantage over uninfected dendritic cells. We have also shown that many of the cells in sections from the fresh bone marrow of myeloma patients stain with fascin and that these same cells are infected with virus as shown by *in situ* hybridization. In addition, these bone marrow dendritic cells contain long foot processes that contact nearly all cells in the bone marrow compartment.

Because of the lack of CD34 expression on the dendritic cells infected with the virus, it became logical to initiate studies looking at whether the Ceprate device could purge the stem cell products of KSHV-containing cells. First, we have now been able to demonstrate the virus in the peripheral blood cells of all myeloma patients analyzed. In addition, studies of unselected leukapheresis products have shown the virus present in approximately half the cases. Furthermore, recent studies by other groups have shown that dendritic cell members greatly increase during mobilization chemotherapy. Thus, it becomes likely that the dendritic cells infected with virus are a significant part of the peripheral stem cell harvest. It becomes potentially important to rid the stem cell product of virally infected product, if this plays a role in development and maintenance of the myeloma clone. As a result, we performed preliminary studies in order to determine whether virus was present before and after CD34 selection in stem cell products from patients on the Phase III CellPro trial. In these studies, using PCR technology with DNA from 100,000 cells, we were able to demonstrate amplified product in half of the leukapheresis products prior to CD34 selection, whereas following CD34 selection none of the products contained virus. I believe this is potentially important since not only do the virally infected cells continue to provide a mechanism for spreading the virus in the patient, but the virally infected dendritic cells are likely to be poor antigen-presenting cells (APCs). Similar infection of dendritic cells by the related EBV leads to their poor function as APCs. Thus, the immune deficiency in myeloma patients may be partially explained by the infection of dendritic cells by KSHV. As a result, giving back a virally free product may give rise to a set of cells which are lacking virus and able to function normally as antigen presenting cells.

Thus, I believe that removal of virally infected dendritic cells from the stem cell product may benefit patients in several important ways. We are continuing to accumulate more data on the mobilization of virally infected cells during mobilization chemotherapy as well as during the leukapheresis procedure (before and after CD34 selection).

I feel that this represents an exciting new use for the Ceprate device.

Sincerely,

James R. Berenson MD

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