TARTE ET AL REFER to three studies, two letters to the editor and one recent publication from their group, that have failed to show HHV-8 in multiple myeloma (MM) bone marrow stroma cells (BMSCs). Importantly, the types of BMSCs produced by long-term marrow culture are highly heterogeneous, and the cell populations that were evaluated for the presence of HHV-8 were not fully characterized in these studies. In addition, no positive controls (Kaposi’s sarcoma [KS] patients) were included in the analysis; thus, the sensitivity of the polymerase chain reaction (PCR) assay could not be assessed. Anderson’s group has recently confirmed the presence of HHV-8 in MM BMSCs with a similar immunophenotype to our own. Although the Tisdale group was able to amplify ORF26 but not ORF72 products in most MM BM samples, we and Anderson’s group have easily amplified product in these patients using primers from both of these ORFs in addition to other HHV-8 ORFs. The inability to find HHV-8 in BM aspirates from myeloma patients is consistent with our own experience. In contrast, we and others have detected HHV-8 in the BM biopsies from most MM patients. We have used primer pairs from many HHV-8 ORFs and have found consistent detection in fresh BM biopsies from MM patients but not in normal subjects or in patients with other malignancies. Importantly, the conditions necessary to amplify product are optimized for each of our primer pairs before use. In addition, the integrity of the sample DNA is assured by serially diluting it until only one copy of it is present and then performing PCR amplification with actin primers. The lack of viral interleukin-6 (vIL-6) expression in the BM biopsies is consistent with our recent report showing the infrequent expression of this viral homologue in fresh BM biopsies from these patients.

Tarte et al also showed lack of HHV-8 in 30 BM aspirates from MM patients collected after a second high-dose therapy procedure. This is consistent with our previous results on BM aspirates posttransplant. Unfortunately, studies using fresh BM biopsies have not been reported by this group, except in a single case in which the clinical status of the patient is not recorded. In addition, the lack of HHV-8 in BM aspirates obtained from the 4 patients who relapsed after high-dose therapy is consistent with our ability to detect HHV-8 in aspirates from less than 10% of similarly treated patients.

It is intriguing that MM patients have weak or no detectable antibodies against HHV-8. This finding may reflect the relative general immunodeficiency that is the hallmark of MM or a specific immune defect that does not allow generation of antibodies against HHV-8. A more interesting possibility is that the type of HHV-8 present in MM patients is different than that present in KS and body cavity lymphoma (BCL) patients. In support of this, we have identified in MM patients consistent changes in the HHV-8 sequence present in regions of the virus that are largely responsible for the immune response. These differences may help explain the inability to identify antibodies that were developed using KS tissues. Recently, specific viral strains of another gammaherpesvirus, Epstein-Barr, have been associated with the development of lymphoid malignancies.

Similarly, HHV-8 derived from KS tissues show distinct genetic differences from BCL-derived viruses, and these different viral isolates show different biological effects. Moreover, recent data from KS patients show that certain subtypes of HHV-8 may even predict more aggressive clinical characteristics of the tumor.

The investigators suggest that the HHV-8–infected BMSCs in myeloma patients are not actually dendritic cells (DCs), because they have not been demonstrated to provide antigen-presenting cell function. Quite to the contrary, Anderson’s group has shown that these virally infected DCs are, in fact, functional. There is also marked heterogeneity in the type of DCs generated in these different culture systems. To assume that the DCs analyzed for viral presence by other groups were identical to those shown to contain virus in our and Anderson’s long-term marrow cultures is a leap of faith. The studies from Tarte et al and Yi et al characterized their DCs as CD1a and CD4-expressing cells, in contrast to the HHV-8–containing DCs in our and Anderson’s studies that lacked expression of both of these markers. In addition, the DCs in Yi et al’s study also lacked CD83, which was found on the HHV-8–containing DCs. Consistent with Tarte et al and others and the absence of CD34 on the BMSCs infected with HHV-8 from our and Anderson’s groups, we also have rarely found HHV-8 in CD34-enriched autograft material. We certainly believe that it may be possible to generate functional DCs from CD34-selected and other autograft material for clinical use based on our results and these other studies as well as the recent report from Raju et al.

Thus, our studies support the presence of HHV-8 in the vast majority of bone marrow and peripheral blood samples from MM patients. It also suggests that the viral strain may be unique in these patients and may help explain the weak or lack of a

**Rebuttal to Tarte, Chang, and Klein**
serological response in these individuals. It remains to be determined the part that this virus plays in the development of this B-cell malignancy, but its uniqueness among these patients suggests that it has an important role in the disease pathogenesis.

REFERENCES


6. Tisdale JF, Stewart AK, Dickstein B, Little RF, Dube I, Cappe D, Dunbar CE, Brown KE: Molecular and serological examination of the relationship of human herpes virus 8 to multiple myeloma: orf 26 sequences in bone marrow stroma are not restricted to myeloma patients and other regions of the genome are not detected. Blood 92:2681, 1998


**Rebuttal to Berenson and Vescio**

**R**eproducibility is a requirement in establishing whether a scientific finding is valid. Not only must results be reproducible, but they must be routinely reproducible in multiple laboratories. The field of KSHV research in its short history has a number of examples of startling reports of disease associations, including sarcoidosis, posttransplant skin tumors, angiosarcoma, and T-cell lymphomas that have not been reproducible. The dispute over whether KSHV plays a role in multiple myeloma involves the reproducibility of the initial findings based on Rettig et al.

Assistant from Berenson’s group, three other groups have reported results that could be consistent with the hypothesis that KSHV is causally associated with multiple myeloma. Brousset et al. have been cited as a group that was able to confirm the association in their French series. Although Berenson has asserted that KSHV/HHV8 cannot be detected by PCR on fresh bone marrow biopsies, nevertheless, Brousset et al were able to detect KSHV/HHV8 from 18 of 20 acetone-fixed and paraffin-embedded bone marrow biopsies. Agbaliki et al. were also able to detect KSHV from 5 of 10 paraffin-embedded bone
Rebuttal to Tarte, Chang, and Klein