Author's response to reviews

Title: Gene expression profile of AIDS-related Kaposi's sarcoma

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We have addressed the reviewer's comments as follows:
Reviewer 1, Dr. Patrick Moore:
Major comments:
1. We have now included a new table (Table 4) where our KS SAGE libraries have been compared with relevant publicly available SAGE libraries, including the HMVEC libraries suggested by Dr. Moore.
2. Semi-quantitative limited-dilution RT-PCR might not be the most optimal technique to quantify mRNA differences, but it is widely used, so we feel that the experiments described in this paper are justified. At present, we are determining expression levels of some of the mRNA's in the paper by real-time quantitative RT-PCR (TaqMan).
3. We agree that PBMC might also provide a useful means of comparison to find a blood marker of KS. However, at the time of analysis, no PBMC library was available. We did compare our KS libraries to a library we ourselves generated from CD4 T-cells (see also table 4). A drawback of using a PBMC library from a healthy individual with one from AIDS-KS tissue might be that not so much gene expression, as well the composition of the PBMC’s (e.g. different levels of different cells) could have been changed.
4. I do not quite understand the remark of the reviewer that one of the properties of SAGE, the identification of unknown transcripts, is not taken advantage of, with respect to three transcripts in (now) Table 5, namely no. 20, 21 and 30. Identifying unknown transcripts after SAGE is trying to determine to which mRNA tags belong that have yet no ID in the SAGEmap database. This involves amplifying a (longer) cDNA fragment using the SAGE tag as a primer. However, we did not detect any of such tags expressed at high levels in the KS libraries. Otherwise, I imagine the reviewer wants more information on the function of the mRNA/protein these tags belong to, as the mentioned transcripts all have ID's. However, SAGE itself is not able to give any clues about this. Two of the mentioned transcripts (calgranulin B and keratin 16) are discussed in the manuscript.
5. All SAGE libraries have now been deposited with the GEO database at NCBI (www.ncbi.nlm.nih.gov/geo/) with accession numbers: GSM3240 (KSa), GSM3241 (KSb) and GSM3242 (NS).

Reviewer 2, Dr. Barbara Ensoli:
Discretionary revisions
a). 1. We have removed all data on blood expression of CD169 from the present manuscript, and will publish these together with new data of risk patients in our cohort in another paper.
   a.) 2. The two KS biopsies were from nodular tissue, this has been added to M&M and Fig. 1.
b.) The paragraph mentioned has been moved to the results section.
c.) The nucleotide sequences of the primers are now presented in Table 1.
d.) The reference from Blasig et al. has been added accordingly.
Compulsory revisions
b) We have removed all sections dealing with blood marker analysis and prognostic value from the revised manuscript, to be presented later in a next paper, and we have rephrased the text accordingly.
c) We have chosen the alternative option, and deleted all reference to the three clusters mentioned in the first draft of the manuscript.
d) "Endothelial cells" has been replaced by "endothelial macrophages".
e) Legend has been corrected.
f) Asterisks has been added (it accidentally disappeared from the figure).
g) Legend explaining asterisks has been added (it accidentally disappeared from the table).