Reviewer’s report

Title: Integrated microarray and multiplex cytokine analyses of Kaposi’s Sarcoma Associated Herpesvirus viral FLICE Inhibitory Protein K13 affected genes and cytokines in human blood vascular endothelial cells

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Reviewer: Subhash Verma

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Manuscript entitled “Integrated microarray and multiplex cytokine analysis of Kaposi’s sarcoma Associated Herpesvirus viral FLICE Inhibitory Protein K13 affected genes and cytokines in human blood vascular endothelial cells” by Punj et al has looked at the global gene expression profile induced by the expression of K13 in human vascular endothelial cells (HUVECs). By using previously published HUVEC cell line, which expresses K13 in a 4-OHT dependent manner, authors show that 174 genes were differentially regulated in K13 expressing HUVECs. Out of these genes the numbers of up-regulated genes (123) were higher that the down regulated ones (51). Using pathways analysis tools, Ingenuity Pathways Analysis (IPA) and Gene Ontology (GO) analysis software, authors generated a biologically relevant network of the K13 modulated genes. This network identified NF-kB as the key pathway linked to modulation by the K13 expression. Authors have also confirmed the expression of cytokines using multiplex cytokine assay which corroborated with the microarray data. Authors also show data that up regulation of one chemokine, CXCL10 is due to the upregulation of its promoter through NF-kB pathway. CXCL10 promoter was highly up regulated by K13 but not with either NF-kB defective mutant or phosphorylation resistant mutants of IkB# suggesting specificity of NF-kB pathway. However, K13 was not able to up regulate PROX-1, a major regulator of lymphatic development.

This manuscript provides list of genes which were modulated by the expression of K13, an important gene expressed during KSHV infection. However, data presented here does not tell much other than the list of genes and NF-kB being the important pathways in K13 mediated gene modulation. Here are specific comments which will strengthen the manuscript.

1- Fig 2. Authors should show western blot for some more up and down regulated proteins in HUVEC cells with K13 with and without 4OHT.

2- Fig 3. Western blot showing expressions of K13, K13 58AAA, MC159, IkB# and IkB# SS32/36AA in these reporter assays are needed.

3- It would be good to compare the expression profiles of some of the up- and down-regulated genes in KSHV infected cells to comment that these changes are specific to KSHV infection.

In conclusion, this manuscript provides good insight how K13 may be regulating
cellular genes in KSHV infected cells but need additional experiment to support their conclusions.

**Level of interest**: An article whose findings are important to those with closely related research interests

**Quality of written English**: Acceptable

**Statistical review**: No, the manuscript does not need to be seen by a statistician.