Reviewer #1:
Scientific Quality: Grade D (Fair)
Language Quality: Grade B (Minor language polishing)
Conclusion: Rejection
Specific Comments to Authors: It's too complex to distinguish HIV specific miRNAs, HPV specific miRNAs, and HIV and HPV co-infection specific miRNAs, which all should be related to anal squamous cell cancers. There are some limits for cancers to use miRNAs as biomarkers, anal cancer is much easier than other digestive tract tumors which need more invasive tests, however, pathology is the "gold standard". I don't see the benefits using miRNAs as biomarkers.

Response:

Language quality (Minor language polishing): The manuscript was reviewed and amended accordingly.

Scientific quality:

This manuscript summarizes the pathogenesis of HIV and HPV infections in relation to anal cancer. It provides a comprehensive review of the existing data on miRNA deregulation during HIV and HPV infections and the possible implementation of miRNAs as biomarkers for screening for and early detection of anal cancer. It is important to note that some miRNAs that are deregulated during HPV and HIV infections have reached clinical trials. For example, and as per reference 122] in the manuscript, miR-34a mimics already reached clinical phase 2 trials. Also, anti-miRs targeted at miR-122 has reached clinical phase II trials and were investigated for treating hepatitis C infection [123]. Importantly, miR-122 has been shown to be upregulated in HIV-1 infected jurkat cells as per reference [185] cited in the manuscript.

We agree with the reviewer about the limitations of using miRNA and as such a section on the limitations of the use of miRNAs for anal cancer screening has been added at the end of the manuscript:

Limitations and considerations for the use of miRNAs as biomarkers for anal cancer screening

In the case of anal cancer, where HIV and HPV pathogenesis play a role in the development of the disease in PLWH, a major challenge is to distinguish HIV-specific miRNAs, HPV-specific miRNAs, and HIV and HPV co-infection specific miRNAs. Major limitations include the absence of studies implementing computational models to identify these miRNAs, technical issues associated with conventional miRNA extraction and detection tools, and scarcity of anal cancer in vitro and in vivo models. Ongoing studies are still being conducted to study miRNA profiles during HIV [124-126] and HPV [127-129] infections. With the appropriate application of advanced bioinformatic analysis tools and computational models, the identification of the most predictive miRNAs, even from complex datasets would be possible. These tools are becoming widespread and have already been used to identify potential miRNA biomarkers for Ebola[130] and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)[131], in addition, these tools have been used to decipher potential miRNA biomarkers in a wide variety of cancers, including melanoma[132, 133], breast[134], colon[135], and lung cancer[136].

In addition to the conventional miRNA detection platforms which include Northern blotting, in situ hybridization, next generation sequencing (NGS), reverse transcription qPCR, microarrays[137], new miRNA extraction and detection platforms have emerged to compensate for the limitations of conventional assays[138] . These technologies are referred to as point-of-care (PoC) technologies and include isothermal amplification-based assays[139], lateral flow assay (LFA)-based systems[140], nanobead-based[141], electro-chemical-based[142], and microfluidic chip-based[143] strategies. The

latter, which is also known as Lab-on-a chip or microchip, is highly specific, cost-effective, and a quick approach for the multiplexed detection of miRNAs[138]. It has been used to test miRNAs in several biological samples including blood of breast cancer patients[144]. Importantly, this system has been also used to quantify miRNAs in plasma extracellular vesicles (EVs), including exosomes. EVs are secreted by body cells and are found in body fluids including plasma, urine, and synovial fluid[145]. They have been shown to carry and stabilize miRNAs in the blood[146]. A unique feature of exosomes is the presence of cell-specific proteins[147], which enables identification of exosomes released from cancer cells. Examining specific miRNAs released from tumors and tumor niche, instead of whole blood miRNA profiling would provide a more accurate way of distinguishing HIV-specific and HPV-specific miRNAs, given the unique viral tropism of each. Exosomal miRNAs would enable the identification of the cell origin and might be better source when compared to non-exosomal, cell-free miRNAs. Recently, studies that profiled and analyzed miRNAs from different sources were reviewed[148]. Authors concluded that 71% of the studies stated that exosomes are the best source of miRNAs as biomarkers. Detecting EVs miRNA signature has already been proven to be a good prognostic tool in several cancers including colorectal[149] and pancreatic cancer[150].

Interestingly, organ-on-chip and organoids are being used to study infectious diseases and cancer. These models can be used to assess HPV virus-Langerhans cells interactions[151] and HPV-oral mucosa epithelia interactions[152]. Cell-to-cell communication can be also studied by co-culturing cancer cells with immune cells, and thus allows the study of cancer-immune interaction. Organoids can be used to model tumor-derived EVs, also known as oncosomes, in addition to EVs released by stromal cells in tumor microenvironment[153]. Very recently, researchers established organoid cultures from human ecto- and endocervix. Cells collected using Pap brush method were used to derive organoids from cervical tissue. The established patient-derived model system resembled causative HPV infection[154], and thus could be used for modeling HPV-related pathogenesis, in addition to exploring the role of HPV and HIV in deregulating miRNAs. The same derivation method can be used to derive organoids from healthy or tumor anal tissue to assess miRNA deregulation by HIV and/or HPV. These model systems could be used to test the efficacy of engineered miRNA loaded EVs in targeting anal cancer cells to deliver potential miRNA therapeutic molecules.

It is important to note that although extensive research has been conducted to identify candidate miRNA biomarkers for cancer screening, the development of new techniques, such as POC for miRNA detection is still at the very early stage and a work on progress. Further progress is required to achieve the desired goal of using POC testing for detecting and distinguishing miRNAs deregulated by oncogenic viral infections, including HPV. Therefore, the identification of miRNAs deregulated by HIV, HPV, and HIV-HPV co-infection warrants further research. More accurate and standardized methods are required for implementation of miRNAs as biomarkers for anal cancer diagnosis. Importantly, the widespread use of high-throughput sequencing, POC technologies, and advanced computational analysis tools may facilitate discovering and distinguishing these miRNAs.

Reviewer #2:
Scientific Quality: Grade A (Excellent)
Language Quality: Grade A (Priority publishing)
Conclusion: Minor revision
Specific Comments to Authors: Congratulations for our work, it is a comprehensive review on a very interesting topic. As observation, though, I would recommend to shorten a little the introduction and the sections regarding HIV infection and pathogenesis, so the reader could follow more easily the content.

Response:

As requested, the introduction and sections pertaining to HIV infection and pathogenesis were shortened.