Madam/Sir,

Thank you very much for returning the reviews of our manuscript, “[How virus persistence can initiate the tumorigenesis process](http://www.wjgnet.com/esps/ManuscriptDetail.aspx?id=6h3tKkN2h8yuOZrg0pzp4w%3d%3d)” by *Simone Avanzi et al*.

We appreciated the reviewers' favorable comments, and we also appreciated their critical comments, suggestions, and questions. We hope our answers will satisfy the reviewers comments. We are very grateful to their feedback, because it allowed us to rethink carefully to our hypothesis and challenge its standalone character. We have included in the revised text the possible contribution that viral products can exert on destabilizing genomic regulation and integrity through epigenetic control.

Attached electronically, please find a revised version of our manuscript. Below, we describe our responses to each of the reviewer's comments.

We thank you and the reviewers for your help with this manuscript. We hope you will now find it acceptable for publication in *the World Journal of Virology*.

Sincerely,

Alessandro Ripalti

Reviewer n. 1

**The authors focus the review only on the human viruses; I think that this restriction is consistent with the major idea which they want to develop in this paper: the virus is not per se the initiator of the oncogenesis but would be a “catalyst” or an agent favoring the development of the tumors (Viruses have been shown to influence tumor sustainment and progression and induce escape pathways from apoptosis and immunesurveillance [1, 4], however in no case has it been proven that a virus can be the initiator, the primum movens, and not merely an “influential passenger” of a tumor). But, this deliberated choice, without any discussion of potential contradictory hypotheses, limits the interest of this paper which is only written in one way.**

*The major idea we want to develop in this paper is that viruses CAN be initiators of tumorigenesis, contrary to the actually commonly accepted concept that viruses are more “catalysts” or “influential passengers” of a tumor. It is therefore this accepted hypothesis that we attempt to contradict.*

*We avoid discussion on animal viruses because the best known animal oncogenic viruses either infect avian or rodents species, that is animals that have a shorter life span than humans and therefore a lesser efficient DNA damage repair system. In fact animal tumor viruses are much more efficient in causing tumors in their natural host than human tumor viruses in man. It is for that reason that animal viruses are known and accepted since 1908 (chicken leukemia), while the first human oncogenic virus was recognized only in 1972. This can be explained only by a profoundly different mechanism by which animal and human tumor viruses induce tumor formation. While it is well established that viral oncogenes and genome insertional mutagenesis are the driving engines for virus driven tumor formation in animals, these mechanisms do not explain why a human tissue expressing viral oncogenes (e.g.: HPV E6 and E7 in cervical lesions) takes years to develop cancer cells. This is true for all six human accepted oncogenic viruses: cancer develops in a very limited percentage of the infected population and over periods of time ranging over decades, while Avian leukosis takes 3 to 4 month to develop after a bird is infected by Avian sarcoma leukosis virus.*

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*This in a nutshell our reasoning for discussing in this paper an hypothesis referred exclusively to human viruses.*

**Furthermore, I am not completely in agreement with the definition of DNA damage as the principal criterion of latency establishment and oncogenesis.**

*One of the original points of our hypothesis is indeed that the process of establishing a latency state is at high risk of producing genomic damage in the host cell. However this is an hypothesis, therefore we certainly do not pretend to offer a definition of DNA damage as one of the principal criterions of latency establishment.*

*DNA damages are important in the late phases of the oncogenesis, but nothing is clear on the role of the DNA damages on the implementation of the genesis of the tumors and the viral latency, in vivo.*

*There is a vast scientific literature investigating and reporting on the debate if DNA damage comes before or after cell transformation during tumor development (regardless of viral involvement in the process), however in recent years compelling evidence supports the notion that chromosomal damage, aneuploidy and chromosomal instability can drive tumorigenesis (Cancer Res. 2007; 67(21):10103-10105; BioEssays 2012; 34(11):963–972). We relay on this assumption to build on our hypothesis. We could be wrong, but then this is how one formulates an hypothesis: one makes an assumption (that can be matter of debate) and takes it to its reasonable, hypothetical, consequences.*

**I think that part on the paper should be rewrite by integrating the data known on the tumor induced by viruses in the animal models. Notably, a lot of data in the chicken model (oncogenic retrovirus-RSV, ALV- and herpevirus-MDV-) are not entirely in agreement with the hypothesis developed in this paper.  It seems important to present the data of the different animal models which can give a beneficial perspective to the human model. Precisely, oppositely to the human model, where the descriptive approach have been essentially carried out, the animal model have allowed more interventionists approaches (deletion or over expression of oncogenes, miRNA… in recombinant viral genomes, following by direct evaluation of the impact of the targeted gene by in vivo infection and not only in cells as for the human model). So, if the authors showed in one paragraph the data corresponding to the animal models, the last paragraph (test of the hypothesis) could be modify by including the potential interest of the human versus animal compared oncogenesis which could enhance the significance of the retrospective and prospective studies which consist essentially in descriptive approaches between infected or non infected humans.**

*Again, we strongly emphasize the profound difference between virus-induced tumors in animals and humans. Human adenoviruses can induce tumors in laboratory animals and transform rodent cells in vitro, however they are considered non-tumor viruses in humans. We have avoided any discussion of the animal model on purpose, because we suggest an oncogenic mechanism working in the human model, and we believe that in the context of our hypothesis the animal model would only generate confusion.*

**Finally, I do not disagree with the main hypothesis of the article, giving an essential role of the DNA damages in the tumorigenesis. However, its exclusive presentation is too restrictive. It seems indispensable to discuss this hypothesis without excluding other hypotheses (for example: deregulation of the miR expression, epigenetic modifications…), in order to increase the real interest of this paper.**

*We thank the reviewer for this observation. Although our hypothesis is focusing on a very limited period of the life cycle of human viruses, that is the time interval between virus entry in human cells and the establishment of latency, we agree that early virus induced epigenetic modifications might have consequences on genome regulation and integrity. We therefore discussed this aspect of virus induced damage and added a third table to better illustrate the concept.*

**Reviewer n. 2**

**The manuscript by Avanzi et al. hypothesizes that virus persistence could represent the first step in viral induced oncogenesis. After its entry, the virus encoded products could damage the DNA of the host cell and predispose the cell to further genetic damage following viral reactivation or re-infection. Additional DNA damage may lead to genetic instability, immortalization and tumor development.**

**The hypothesis is interesting and stimulating for the scientists working in this field. However, the sole viral persistence cannot explain the occurrence of tumors in immunocompromised individuals such as solid organ transplant patients.**

**Several other factors are likely involved and act concomitantly or before viral infection or reactivation. It is known that immunosuppressive drugs favor the appearance of tumors in transplant patients. For instance cyclosporine increases the risk of developing skin cancer or lymphomas. Moreover, cyclosporine reduces the ability of the cells to repair DNA damage. So, other factors have to be considered in immunocompromised beside viral persistence.**

**So, the authors should rewrite the paper keeping into consideration the other possible mechanisms involved in human cancerogenesis: cellular and immunosuppressive therapies.**

*We mention the post-transplant setting because it is a situation where virus induced tumors occur at an accelerated pace with respect to virus induced tumors in immunocompetent subjects. This helps us in supporting the mechanistic process of virus induced DNA damage suggested by our hypothesis. Viral reactivation is an extremely common event in the post-transplant setting, and life-threatening infections are a primary concern in post-transplant patient management. Considering post-transplant lymphoproliferative disorders, the vast majority of cases are associated with EBV infection/reactivation.*

*Our hypothesis suggests viruses are DNA damaging agents, possibly acting in primary genetic damage. Immunesuppressant drugs are not additional factors provoking DNA damage; they, especially calcineurin inhibitors, increase UV-induced skin cancer risk, because they reduce the ability of cells to repair DNA damage, and by hampering the function of the immune system they reduce its ability to recognize and kill cancer cells. With the same two mechanisms they facilitate the insurgence of parasites, and limit the cell’s ability to repair virus induced DNA damage, actually supporting our hypothesis. However post-transplant tumors are a rare occurrence (0,7%) and therefore are not a major subject of virus induced cancer. This is why we feel that a comprehensive discussion of all possible contributors involved in cancerogenesis in the post-transplant setting would not add any interest to an hypothesis that attempts to theorize a general mechanism for virus-driven DNA damage as one possible “ primum movens” in virus induced tumorigenesis.*

*In other words we are not attempting here to give a comprehensive picture of all possible stages and actors that can drive, or contribute to drive, primary DNA damage in human cells. We are trying to draw the attention on the fact that, hypothetically, viruses may generate on their own, without any co-factor required, primary DNA damage, an idea that is strongly opposed by current thinking. This does not mean that we exclude other factors contributing to primary damage.*