Point-by-Point Response to the Reviewer’s Comments

Yano et al.: “Real-time fluorescence image guided cell-cycle perturbation for effective cancer treatment” (MS No. Cancers 862286)

Response:
The authors are grateful for the very helpful comments by the reviewer that enabled us to significantly improve the manuscript.

Reviewer #3:
The title of this review, "Real-time fluorescence image guided cell-cycle perturbation for effective cancer treatment" is intriguing, but misleading. Exposing cells to an anti-cancer drug is not "cancer treatment". Even before, and with the advent of cell cycle reporter systems, researchers have been exposing cells to different drugs to study cell cycle specific effects, so this idea is not new. I agree that the approach has seen a renaissance with live imaging approaches, and tumors grown in animal models, but this is not articulated well in the review. The review is very difficult to read, in part due to significant English writing issues, but also because of organization, choice of terms, and oversimplification of the cancer problem. The authors chose to focus in the FUCCI system for tracking cell cycle, however other fluorescent systems exist (e.g. Cdk sensors) that should be included as this is a review article.

Response:
The title of the revised manuscript has been changed. The revised manuscript focuses on how the revolutionary aspect of FUCCI, especially it uniquely images cell-cycle dynamics in real time. Mistakes have been corrected and English has been improved.

Comment 1.
The instances of English writing problems are numerous. Rather than list them, the services of a professional or native English speaker must be used.

Response:
Co-author, Dr. Hoffman, a native English speaker, has improved the English.

Comment 2.
With regard to the discussion of "anti-mitotics", taxanes have very complicated pharmacology, with different cell phenotypes at different, specific concentrations. There is also evidence from Weaver, Mitchison, and others that powerful anti-
tumor effects after taxanes are not due to effects on mitosis itself. The current discussion is superficial and needs to be updated.

Response:
The revised focused manuscript no longer discusses these points.

Comment 3.
Example of writing issue, line 51-52, "However, most cytotoxic agents have limited for for solid cancers."
Response:
Sentences such as this have been corrected in the revised manuscript

Comment 4.
A comment on cancer stem cells being resistant to chemotherapy is made, it states they are resistant because they are quiescent, this is true, but not the only reason, in a review it cannot be this superficial. Even growing cancer stem cells show less cell death. Additional reasons need to be discussed.
Response:
The revised manuscript states that quiescence is just one reason cancer stem cells may be chemoresistant. Further discussion of stem cell biology is not in the scope of the revised focused review.

Comment 5.
The phrase "cell cycle decoyers" is confusing, "decoyers" isn't a word. Perhaps simply "decoys" can be used?
Response:
This correction has been made in the revised manuscript.

Comment 6.
The idea of targeting quiescent cells, or highly-related senescent cells, is an exciting area of drug development. This section needs to be further developed.
Response:
The idea of targeting quiescent stem cells using FUCCI to visualize them in real time is expanded in the revised manuscript and it is emphasized that quiescent cancer stem cells are very dangerous as they commence re-cycling after chemotherapy. Senescence is not discussed as FUCCI cannot yet identify senescence cells.