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 #2
Summary
The review by Yano et al. considers a problem of different sensitivity of cancer cells to various treatments depending on cell cycle phases. The authors published a lot of original articles and are world recognized experts in this area. A new concept of “Decoy, trap, and kill cancer therapy” is formulated and justified. This review will be of interest for a wide auditorium of researchers and clinicians. However, some important issues should be corrected before publication.

Major point 1.
Figure 1 shows wrong colors of FUCCI depending of cell cycle phases. In fact, in the original FUCCI, green fluorescence corresponds to S-G2-M (not G2-M as depicted here), and yellow fluorescence corresponds to a short period at the G1/S transition. See Ref. 23, Fig. 1B. Later in the manuscript, there is a correct description (lines 121-123): “FUCCI-red indicates quiescent G0/G1 phase (23), FUCCI-green indicates proliferating late S/G2/M phase, and FUCCI-yellow means early S phase (Figure 1A).” Also, Fig. 2 should be corrected accordingly as it contains explanatory sketch at the bottom where yellow circle means “Cells in S phase” and green circle means “Cells in G2/M phase”.
Response:
These corrections have been made in the revised manuscript.

Major point 2.
In the section 3 (“Fluorescence ubiquitination cell cycle indicator (FUCCI) …”), it is worth noting the variant Fucci4, which resolves all cell cycle phases by using four orthogonal fluorescent indicators: Bajar BT, Lam AJ, Badiee RK, et al. Fluorescent indicators for simultaneous reporting of all four cell cycle phases. Nat Methods. 2016;13(12):993-996. doi:10.1038/nmeth.4045
Response:
Lines 76-80: FUCCI4 is described in the section 1 in the revised manuscript, and the Bajar et. al. reference is added as #13.

Minor Comment 1.
Line 38: “In normal cells, cell cycle is strictly regulated and organized and comprises in phases G0-G1-S-M.” G2 phase is missed.
Response:
Line 36: This correction has been made in the revised manuscript.

Minor Comment 2.
Line 45-46: “… the confluent cells in culture or plastic or glass.” Perhaps it should be “… the confluent cells in culture on plastic or glass.”
Response:
In the revised focused manuscript, this point is no longer discussed.

Minor Comment 3.
Line 46-47: “The normal cells such as foreskin fibroblasts cease proliferation and are in contrast inhibited. In contrast, the cancer cells continue to proliferate …”.
The first “in contrast” is superfluous, please remove: “The normal cells such as foreskin fibroblasts cease proliferation and are inhibited. In contrast, the cancer cells continue to proliferate …”
Response:
In the revised focused manuscript, this point is no longer discussed.

Minor Comment 4.
Line 55: Incomplete sentence (line 55): “Recently, cancer stem cells (CSCs) are chemo-resistant (17, 18).
Response:
Lines 219-220: This correction has been made in the revised manuscript.

Minor Comment 5.
Line 58: “For examples, especially FUCCI-transgenic mice showed a distinctly regulated and organized cell-cycle distribution”.
Response:
In the revised focused manuscript, this sentence has been eliminated.

**Minor Comment 6.**
Lines 164, 187, 192: “The wording “using with” (lines 164, 187, 192) looks excessive, I would write either “using” or “with”

**Response:**
These corrections have been made in the revised manuscript.

**Minor Comment 7.**
Line 199: “fetal serum bovine”. Please change to the standard “fetal bovine serum”.

**Response:**
Line 229: These corrections have been made in the revised manuscript.

**Minor Comment 8.**
Lines 214-218: “We showed that oncolytic adenovirus decoyed and trapped quiescent cancer stem cells from quiescent G0/G1 phase to in early S phase, where the decoyed cancer cells became sensitive to chemotherapy (110) (Figure 2B). We also demonstrated that oncolytic adenovirus decoyed and trapped quiescent established tumors from quiescent G0/G1 phase to in early S phase, where the decoyed tumors became sensitive to chemotherapy (110) (Figure 2B).”

These two sentences are mostly identical, please combine they into one sentence, e.g. “We showed that oncolytic adenovirus decoyed and trapped both quiescent cancer stem cells and quiescent established tumors from G0/G1 phase to in early S phase, where the decoyed cancer cells became sensitive to chemotherapy (110) (Figure 2B).”

**Response:**
Lines 260-261: In the revised manuscript, these sentences have been combined.

**Minor Comment 9.**
There is no explanation for abbreviations LSC and CML (section 8).

**Response:**
In the revised focused manuscript, this is no longer discussed.