Reviewers' comments:  
  
  
  
Reviewer's Responses to Questions  
  
~~Note: In order to effectively convey your recommendations for improvement to the author(s), and help editors make well-informed and efficient decisions, we ask you to answer the following specific questions about the manuscript and provide additional suggestions where appropriate.<br><br>1. Are the objectives and the rationale of the study clearly stated?<br><br>Please provide suggestions to the author(s) on how to improve the clarity of the objectives and rationale of the study. Please number each suggestion so that author(s) can more easily respond.  
  
Reviewer #1: Yes  
  
Reviewer #2: Yes, the objectives and rationale are clearly stated.~~  
  
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**2.** If applicable, is the application/theory/method/study reported in sufficient detail to allow for its replicability and/or reproducibility?<br><br>Please provide suggestions to the author(s) on how to improve the replicability/reproducibility of their study. Please number each suggestion so that the author(s) can more easily respond.  
  
~~Reviewer #1: Mark as appropriate with an X:  
Yes [X] No [] N/A []  
Provide further comments here:~~  
  
Reviewer #2: Mark as appropriate with an X:  
Yes [] No [X] N/A []  
Provide further comments here:  
~~1. Section 3.1: In the LHS, what were taken as the lower and upper values of the range for each parameter?  The authors mention “wider parameter sets” but this is insufficient.~~

*Addressed in section 3.1, parameter ranges given for LHS*

~~2. How were the PRCC values calculated?  Did you use a program? Did you write your own code?  If you wrote your own code based upon the method, please cite the paper.~~

*Addressed in Section 3.1*

~~3. Section 3.5: The LHS method is just a sampling method; it is not a method to detect sensitivity to parameters.  Sensitivity is measured using PRCC analysis.  The clarity of the writing in this section needs to be improved, because as is, it veers on being inaccurate.~~

*Section 3.5 has been extensively rewritten.*  
  
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**3.** If applicable, are statistical analyses, controls, sampling mechanism, and statistical reporting (e.g., P-values, CIs, effect sizes) appropriate and well described?<br><br>Please clearly indicate if the manuscript requires additional peer review by a statistician. Kindly provide suggestions to the author(s) on how to improve the statistical analyses, controls, sampling mechanism, or statistical reporting. Please number each suggestion so that the author(s) can more easily respond.  
  
~~Reviewer #1: Mark as appropriate with an X:  
Yes [] No [] N/A [X]  
Provide further comments here:~~  
  
Reviewer #2: Mark as appropriate with an X:  
Yes [] No [X] N/A []  
Provide further comments here:  
~~1. “Significant” is a word with a strict scientific meaning (p.10 and p.15).  Use of this word necessitates a remark on the statistics that back the claim.  Another word would perhaps be more appropriate here.~~

*Wording has been changed on p. 10 and p. 15*  
  
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**4.** Could the manuscript benefit from additional tables or figures, or from improving or removing (some of the) existing ones?<br><br>Please provide specific suggestions for improvements, removals, or additions of figures or tables. Please number each suggestion so that author(s) can more easily respond.  
  
~~Reviewer #1: No~~  
Reviewer #2: Yes, the manuscript would benefit from improving and removing existing figures.

1. Figure 2:~~Label the units of the Morphine concentration (ug/l),~~ ~~on the x-axis on and in the figure caption's second sentence (i.e., M\_thresh=54 ug/l).  On the right, label the colorbar so the reader knows what changes as the color changes. Is this also M\_thresh?  The red line is misleading, pointing down at a 45 degree angle:  B barely has an effect on the color past B=7 or so. It looks like it is almost just as easy to have a yellow region when B=10 as when B=50.  Contrary to the figure caption, in this figure, M\_thresh increases moving along nearly a vertical line, rather than the arrow from the upper left to lower right. Please correct.~~

*We’ve replaced the heatmap with a figure showing F vs Mthresh for several values of B*

~~2. Fig 3a is difficult to interpret.  Please explain.~~

*New writing added in 3.3.3 and the caption for figure 3a rewritten*

~~3. Enlarge the font size in Fig 5cd as in Fig 5ab.~~

*Done.*

4. Fig 6: This figure is very interesting but the results need to be normalized.  If the VL starts out higher, then clearly it would take longer to reach 50, but does the % drop relative to the ss VL also lag?  Please clarify.

~~5. Fig 7 is hard to follow.  Is M=0 on the left and M=200 on the right (just a guess)? If so, this should be indicated. However,    PRCC values below 0.5 are not high enough to be indicative, so this figure is not meaningful and can be removed without any loss.  Alternatively, include a discussion of what changes between one subfigure and the next (not simply listing the parameter names that are different, but discuss in terms of the mechanisms that the parameters represent and what this means for the results), why this is interesting, and relate it to morphine levels.~~

*We’ve opted to rewrite section 3.5 to better explain this figure and the meaning behind changes in global sensitivities for morphine vs no morphine and the responsible mechanisms. We’ve also added text to each subfigure to clarify what each set of PRCCs are for.*  
  
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**5.** If applicable, are the interpretation of results and study conclusions supported by the data?<br><br>Please provide suggestions (if needed) to the author(s) on how to improve, tone down, or expand the study interpretations/conclusions. Please number each suggestion so that the author(s) can more easily respond.  
  
~~Reviewer #1: Mark as appropriate with an X:  
Yes [X] No [] N/A []  
Provide further comments here:~~  
  
Reviewer #2: Mark as appropriate with an X:  
Yes [] No [X] N/A []  
Provide further comments here:  
This is a very interesting idea and model on an extremely important and relevant topic!  The model analysis is well-executed and addresses key questions, both mathematically and biologically. However the main issue in this paper is not that the interpretation of the results is unsupported; it is simply the lack of interpretation of the model's results. Examples of this are present throughout the paper, some of which are listed below:

1. What are the implications of the results? What does this study tell us that we didn’t know before, that we can use going forward? It is clear that the authors’ aim is to quantify the effects of opiate use on HIV infection, but what does this help us do - Does it give better treatment options?  Does it shed light on behavior change (i.e., discourage drug use)?

*Added a paragraph to the conclusion section discussing implications.*

~~2. Figure 1 is delivered with no explanation of results, implications, or useful conclusions.  The results state that certain parameters (given by parameter name only) correlate with the R naughts, but not what that indicates or why that is interesting/relevant.  The results need to be interpreted (throughout the paper), not just reported.  Do the results make sense given previous knowledge?  Are they consistent with what would be expected, or are they unexpected, and why? How do they relate to the biology being modeled?~~

*3.1 has been rewritten extensively to discuss interpretations of sensitivities in terms of mechanisms of the model.*

~~3. Why are the local sensitivity indices and the prcc results so different in magnitude? Why are both measures of sensitivity needed?  Is the point here to compare the methods to one another, and if so, what does the comparison reveal?  What is the interpretation that of the finding that Rm0 is positively related to B and negatively related to F, and what does this mean in terms of the meanings of these thresholds and parameters?  The key sentences that tie the results together with the questions that the authors set out to answer are absent.~~

*Addressed in 3.1, language added to compare local and global sensitivities, and interpretation of F and B with Rm0. Further discussion of F and B in included in 3.3.5 in terms of viability of the mutant and stability.*

4. What is the interpretation of the finding that morphine affects the long-term dynamics in terms of which species, if any, survive, given different values of the mutant fitness and the propensity for the mutant to escape from the CTLs?  What does the model conclude about the short and long-term outcome of an HIV infection in the presence of morphine that we did not already know from clinical data?  What can be concluded about the characterization of stability of the equilibria - does lower B or higher F correspond with stability of an equilibrium and if so which one?  Clearly state which equilibrium is stabilized by morphine. The M\_thresh is interesting in terms of the dynamics, but why is it important - does this help contain the infection,  stop progression, improve ART effectiveness?  Should it be monitored for treatment or to reach a better outcome?  Etc.

*Partly addressed in 3.3.5 with new paragraph*  
  
~~The conclusion of section 3.3.3 is excellent because it says “This is expected because…”  The other results need to be followed by this type of conclusion; otherwise the paper is unfinished.~~  
  
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**~~6.~~** ~~Have the authors clearly emphasized the strengths of their study/theory/methods/argument?<br><br>Please provide suggestions to the author(s) on how to better emphasize the strengths of their study. Please number each suggestion so that the author(s) can more easily respond.  
  
Reviewer #1: Yes  
  
Reviewer #2: Yes~~  
  
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**~~7.~~** ~~Have the authors clearly stated the limitations of their study/theory/methods/argument?<br><br>Please list the limitations that the author(s) need to add or emphasize. Please number each limitation so that author(s) can more easily respond.  
  
Reviewer #1: Yes  
  
Reviewer #2: Yes~~  
  
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8. Does the manuscript structure, flow or writing need improving (e.g., the addition of subheadings, shortening of text, reorganization of sections, or moving details from one section to another)?<br><br>Please provide suggestions to the author(s) on how to improve the manuscript structure and flow. Please number each suggestion so that author(s) can more easily respond.  
  
~~Reviewer #1: No~~  
  
Reviewer #2: Yes, reorganization is needed.   
1. To improve the flow of the paper, Figure 5 should be Figure 1.

~~2. Rearrange the order of 3.3.3 and 3.3.2.~~

*Done.*

3. The paper would flow better if first, the analytical results are provided, and then afterwards, the numerical results, as in most papers.  
  
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**~~9.~~** ~~Could the manuscript benefit from language editing?  
  
Reviewer #1: No  
  
Reviewer #2: No~~  
  
  
**Other**  
Reviewer #1: The manuscript describes a mathematical model that incorporates the effect of morphine on HIV infections. The authors use mathematical analysis, computer simulation, and sensitivity analysis to study the role of morphine in HIV infections. The manuscript is well-written and the analysis is thorough. I have a few minor comments:

- I don't believe the authors need to change how mutation is incorporated in the model, but they could perhaps discuss/justify the following choices. Why is it assumed that there is a fitness "cost" (0<F<1) for mutations --- sometimes mutations result in a competitive advantage, particularly in the presence of drug treatment. Any thoughts on how a more competitive mutant might change the analysis? Why is there no back-mutation? Mutation in viruses is caused by random changes in the amino acid sequence --- surely it's just as likely to switch one way as it is to switch back?

~~- Units are sometimes italicized, sometimes not. This should be consistent.~~

*Done.*

- In the second point of the highlights, "outcome" should be "outcompete."   
  
  
Reviewer #2: This field is optional. If you have any additional suggestions beyond those relevant to the questions above, please number and list them here.  
  
This has the workings of a really excellent paper.  The model is quite complex but it was clearly designed carefully.  
The mathematical analysis was carried out well, especially considering the effort involved given the number of parameters.  
The motivation for the numerics and figures shown is sound.  However the study is incomplete because the results are not interpreted in light of the aims of the paper (as mentioned in 5.).  
  
Further improvements:  
~~1. Justify the first term of dC/dt.  Why are the CTLs recruited at a constant rate?  In the IFE, if there is no infection, then why is C\*>0?~~

*Addressed in 2.2*

~~2. Was the MOE shown to be stable for M<M\_thresh?  Are all eigenvalues negative in their real parts?  This work was hard to locate in the paper.~~

*Addressed in 3.3.3, clarified writing about eigenvalues*

~~3. I could not find the section on the coexistence equilibrium.  There is mention of the “three biological relevant equilibria,” but after the IFE and MOE,  
the paragraph introducing the coexistence equilibrium was perhaps unintentionally deleted?~~

*Short new section added, Coexistence equilibrium*

~~4. Since the model includes only 1 step of mutation and no back-mutation, use of the word "mutation" instead of "evolution" would be a bit more reasonable.~~

*We have changed the word evolution to mutation where appropriate.*

~~5. There many more modeling papers in the literature that look at a wild type strain, a mutant strain, and various factors that affect the dynamics.  More of these  
should be cited.  Where do the results of this study fall among the results of the many others?~~

*Added references to other HIV modeling papers, discussed their strategies and compared with ours in the conclusion.*

~~6. Adding a schematic diagram for the model would help. There may be such a diagram in Ref 35, but it is unclear if this paper is published yet (no date).~~

*Done.*  
  
Other comments/edits/typos:  
1. Some words like "a" or "the" are missing in several locations on page 16, and there is a noun/verb disagreement (boundary is, not are).  
~~2. Ref 35 is incomplete~~  
3. ~~Ref 39 - should it say post-operative?~~  Why IV morphine in children rather than adults?  
~~4. Section 2.2:  40980 cells/ml and 959020 cells/ml~~  
~~5. 3.3.2: typo 'from' should be 'form'. Last line page 11.~~  
~~6. P.9: 'Sensitiveness' should be 'sensitivity'~~  
~~7. There should be stars in (13) since these equations have been set to 0 and are no longer varying~~