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 #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 #2: Mark as appropriate with an X:  
Yes [] No [X] N/A []  
Provide further comments here:*

**Comment:** *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.*

**Response:** We selected parameter ranges for LHS in an ad hoc manner to cover a realistic range of biologically relevant values. For example, for the viral burst size *p* we used 2500 as a baseline value and the range 500 to 5500 for LHS. We did not list the lower and upper bounds for each parameter but could if necessary, and we have rewritten parts of Section 3.1 to better explain our methodology.

**Comment:** *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.*

**Response:** We calculated PRCC values as Pearson correlation coefficients between the vector of sampled parameters and model outputs (i.e., , viral load, CD4+ counts), using MATLAB functions for LHS and calculating correlation coefficients. We have added additional writing to Section 3.1 explaining our process.

**Comment:** *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.*

**Response:** We agree that the original writing could be improved, so we have rewritten Section 3.5 almost entirely. The new writing more accurately reflects both the sampling procedure and method for calculating PRCCs that we used. Similar to Section 3.1, upper and lower bounds for each parameter were selected ad hoc to give a wide range of realistic values and PRCCs were calculated as Pearson correlation coefficients.

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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 #2: Mark as appropriate with an X:  
Yes [] No [X] N/A []  
Provide further comments here:***Comment:** *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.*

**Response:** We agree and have replaced the word significant with different language in the appropriate places. *--------------------  
  
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 #2: Yes, the manuscript would benefit from improving and removing existing figures.***Comment:** *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.*

**Response:** The reviewer is correct that is most sensitive to changes in escape ration *B* for low values, therefore we have replaced the heatmap in Figure 2 (now Figure 3) with a plot of *F* versus for several values of *B.* Like the heatmap, the new figure varies *F* from 0 to 1 and plots the corresponding threshold morphine on the y-axis, while still showing that does not change as much for large *B* values (greater than 10). We have also added the units for morphine on the left figure, and elsewhere in the manuscript.

**Comment:** *2. Fig 3a is difficult to interpret.  Please explain.*

**Response:** Figure 3a is meant to demonstrate how we obtain the numerical value of for the MOE. Each colored line in the figure represents a specific morphine concentration, and the x-intercept of each curve is the value at the MOE for that concentration. Figure 3b is an extension of 3a and shows the MOE morphine between 0 and 200 ug/l.

**Comment:** *3. Enlarge the font size in Fig 5cd as in Fig 5ab.*

**Response:** Font sizes are now consistent across Figure 5 and elsewhere in the manuscript.

**Comment:** *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.*

**Response:** [I don’t understand this comment, if we wanted to normalize we would have to pick M = 0 or M = 200 as the standard but that doesn’t make sense to me.]

**Comment:** *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.*

**Response:** We decided to use the reviewer’s alternative suggestion and significantly rewrite Section 3.5 to include more interpretation of the PRCCs rather than simply reporting them. The new writing emphasizes the difference in PRCCs between the morphine and no-morphine scenarios for viral load and CD4+ counts, particularly that *F* and *B* have increased sensitivity for because the mutant is the dominant strain. Each subfigure is now labeled to clearly show what each set of PRCCs are for. *--------------------  
  
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 #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:***Comment:** *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)?*

**Response:** We feel that the morphine induced change in viral dominance is the most significant result and have added writing to the conclusion section discussing further implications. In particular, through simulation we showed that morphine conditioning increases the time to reduce the infection below detection level during a course of ART, this gives quantitative evidence that morphine hinders treatment. The model also motivates further experimental study into the effect of drug use on viral evolution, which we now discuss in the conclusion.

**Comment:** *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?*

**Response:** The part of Section 3.3.1 discussing sensitivity to parameters has been extensively rewritten. The new writing goes into detail about the effects of *F* and *B* on and and other mechanisms, rather than simply reporting the values.

**Comment:** *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.*

**Response:** The purpose of both sensitivity analyses is to determine which parameters have the largest effect on the model output, having local and global analysis strengthens our conclusion about which parameters are most important and can inform experimental work. The sensitivities of *F* and *B* on the mutant reproduction number reflect that the mutant benefits from low fitness cost of mutation and high escape rate from immune response. This and other interpretations are discussed in the rewritten section 3.1.

**Comment:** *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.*

**Response:** Section 3.3.5 and Figure 5 characterize the stability of the three equilibria in terms of *M,* *F,* and *B*. High morphine tends to favor the wild-type virus and lead to the coexistence equilibrium, lower morphine can stabilize either the infection-free or mutant-only equilibria depending on the fitness of the mutant virus. We have added a summary paragraph to the end of Section 3.3.5 clearly stating these results, however the most surprising result is the more fit wild-type virus in the presence of morphine and language to that effect has been added throughout the paper.

*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 #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 #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 #2: Yes, reorganization is needed.***Comment:** *1. To improve the flow of the paper, Figure 5 should be Figure 1.*

**Response:** [Not sure how to move this and keep the flow of the paper correct, also contradicts comment 3 below]

**Comment:** *2. Rearrange the order of 3.3.3 and 3.3.2.*

**Response:** The order of Sections 3.3.3 and 3.3.2 have been rearranged. The order of Sections 3.3.1-3 is now Infection-free equilibrium, Wild-type only equilibrium, and Mutant-only equilibrium.

**Comment:** *3. The paper would flow better if first, the analytical results are provided, and then afterwards, the numerical results, as in most papers.*

**Response:** [Not sure about this. The R0w/R0w result is the main analytical result and its first. Moving Figure 5 to the beginning would also be putting numerical results first, so this seems like a contradiction.] *--------------------  
  
9. Could the manuscript benefit from language editing?  
  
Reviewer #2: No  
  
  
  
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:***Comment:** *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?*

**Response:** The constant term in the CTL equation is primarily to create a non-zero steady-state for the infection-free equilibrium. However, this term can be interpreted as background CTL production or other uncertainties and can be neglected by taking . We have expanded on this in the model description section and cited additional references that include this term when modeling CTLs in HIV infections.

**Comment:** *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.*

**Response:** While we did not give eigenvalues explicitly in the manuscript, we computed eigenvalues for morphine between 0 and 200 ug/l to determine stability for the MOE and CE. The real part of one eigenvalue of the MOE becomes positive as *M* increases through and the CE becomes locally stable. This has been clarified in the writing of Section 3.3.3.

**Comment:** *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?*

**Response:** We originally intended for the section on parameter spaces and stability to fulfil this need. In that section we discuss conditions on morphine, fitness cost, and immune escape which stabilize the three equilibria. However, we agree with the review that a section devoted to the coexistence equilibrium should be included for completeness and have added a short section that discusses it and its stability.

**Comment:** *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.*

**Response:** We agree with the reviewer and have changed the language accordingly.

**Comment:** *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?*

**Response:** This is a good point, and we added more citations to these papers and cited additional HIV modeling studies that investigate wild-type/mutant dynamics. One of the references we cite is a model featuring viral mutation and cellular immune responses. An additional study investigating treatment optimization with a drug resistant mutant is also now discussed in the conclusion section.

**Comment:** *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).*

**Response:** We appreciate this feedback and have added a schematic of the model as a new Figure 1.

*Other comments/edits/typos:***Comment:** *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).*

**Response:** These typose have been corrected where appropriate.

**Comment:** *2. Ref 35 is incomplete*

**Response:** We have added a publication date for Ref 35.

**Comment:** *3. Ref 39 - should it say post-operative?  Why IV morphine in children rather than adults?*

**Response:** This has been corrected. We chose this reference because it includes intravenous blood-plasma concentration data relevant for our modeling.

**Comment:** *4. Section 2.2:  40980 cells/ml and 959020 cells/ml*

**Response:** This has been corrected.

**Comment:** *5. 3.3.2: typo 'from' should be 'form'. Last line page 11.*

**Response:** This has been corrected.

**Comment:** *6. P.9: 'Sensitiveness' should be 'sensitivity'*

**Response:** This has been corrected.

**Comment:** *7. There should be stars in (13) since these equations have been set to 0 and are no longer varying*

**Response:** This has been corrected.