**Point-by-point response to the reviewers’ comments**

We would like to thank the reviewers for their valuable comments and constructive suggestions, which have helped improve our manuscript significantly. Point-by-point responses to the reviewers’ comments are provided below. The changes made in the manuscript are highlighted in red.

**Response to Reviewer**

***Comment-1A:*** 1. *The authors over-interpret the results of morphine on virus dynamics. Measuring impact is extremely complicated and stating that all the impacts assumed in the model are "experimentally verified" is a bit naive. Here are specific details:*

*A) morphine makes T cells more susceptible to SIV/HIV infection. This is not fully established. For example, increasing CCR5 expression does not have to result in higher infection rate - for example, if a T cell expresses 10,000 CCR5 molecules and morphine increases that 2 fold but HIV needs 1,000 molecules for expression, this increase is irrelevant. Original data on CCR5 expression have been overinterpreted by Vaidya et al.*

**Response:** We apologize that we could not make our model formulation clear to the reviewer. Our model includes terms that account for transition between the lower- and higher-susceptibility CD4+ target cell populations. The transition rates between the two target cells populations are the morphine dependent Hill functions q and r which saturate at sufficiently high morphine concentrations. These functions saturate at sufficiently high or low morphine concentrations in a way that accounts for the hypothetical scenario the review suggests, so for example, an increase in morphine from 300 ug/L to 350 ug/L will not significantly change the predictions of our model.

***Comment-1B:*** *the fact that morphine impacts virus evolution is extremely imprecise. Evidence that morphine changes mutation rate of the virus (i.e., ability of RT to make errors when making DNA from RNA) has not been documented and is highly unlikely. There are many other reasons for why SIV may evolve slower in morphine-using monkeys. For example, lower rates of metabolism may result in all rates (T cell division/activation) to be lower, this, showing slower accumulation of mutations.*

**Response:** When introducing morphine dependent dynamics in our model we only consider the net effect morphine has on target cell susceptibility, viral mutation, and cellular immune response. The purpose of our model is not to determine the mechanisms of, e.g., viral mutation, but rather the overall change in dynamics induced by morphine. The experimental studies we cite in the manuscript are based on sequence analysis and clearly show that morphine use is associated with a decrease in viral mutation, we have simply incorporated this relationship into our model without attempting to identify the mechanisms responsible.

***Comment-1C:*** *How morphine impacts CTL response is not shown. How would morphine impacts CTL division rate? Is that naive T cell production from thymus? Is it CTL sensitivity to antigen? None of that has been thoroughly tested in experiments. Just a note - to detect a change in only 1 parameter requires a lot of specific experiments which have not been performed.*

**Response:** Again, the goal of this study was not to identify the physiological mechanism(s) responsible for decreased CTL production associated with morphine use. The experimental studies in animal models show that morphine use diminishes CTL production. We incorporated this into our model by making the net CTL production rate a decreasing function of morphine. We apologize that the formulation of our model was not clear to the reviewer.

***Comment-2:*** *Authors performed sensitivity analyses but the results are not properly interpreted. Ok, these parameters (XYZ) are "more important". So what? At present, it is pretty much useless unless we understand why these parameters are important, how does it relay to disease, etc.*

**Response:** We performed additional sensitivity analysis using Latin hypercube sampling at the suggestion of the reviewer and identified the most sensitive parameters. The results of our LHS procedure were also consistent with the local sensitivity analysis we had previously performed. We do not agree that the results are misinterpreted, the purpose of sensitivity analysis is to identify parameters that have the largest relative effects on the model predictions. Identifying these parameters, as we have done, can be useful in designing future experimental work.

***Comment-3:*** *I will still insist on my interpretation that all answers authors found are logical consequences of the assumptions. Why do we need the model? Precise predictions of this model will strongly depend on parameters which are largely unknown (reference 30 is not a good one because many parameters in that paper have not been rigorously estimated due to limited data, thus, were overfitted).*

**Response:** The utility of mathematical models is their ability to quantify biological results that are difficult to measure experimentally and to identify sensitive parameters. Results obtained via model simulation may be useful in developing future *in vivo* experiments. For example, our model prediction that wild-type virus dominates under morphine conditioning has not been studied experimentally. The net effect of the three morphine-altered mechanisms that we investigated provided a result that is a surprising consequence of morphine use in a quantitative manner.

***Comment-4:*** *"rapid pathogenesis" in abstract is jargon.*

**Response:** We have replaced this with “rapid disease progression”.

***Comment-5:*** *abstract: The conclusion that wild type virus outcompetes escape variant contradicts conclusion on 3 steady states listed.*

**Response:** We disagree that there is a contradiction here. The three steady states are determined by parameter values that we show in the main text. For particular parameter values the wild-type dominates the mutant, but a small amount of mutant persists. For different parameters the mutant is the dominant strain. These steady states are summarized in Figure 4.

***Comment-6:*** *Abstract: Higher set point with WT virus - not because of escape but because of morphine. This is obvious because you make morphine to make monkey more susceptible to SIV*

**Response:** We do not understand what the reviewer means by “you make morphine to make monkey more susceptible to SIV”. As we explain in the Section 2.1.2 of the manuscript, morphine increases target cell susceptibility in our model, but higher set point viral load due to wild-type virus is not an obvious result of this mechanism. Rather, higher viral load is a net result of increased susceptibility and decreased cellular immune response and mutation.

***Comment-7:*** *Page 2: "Many mutations due to RT" - this is imprecise. Because mutations occur during infection given RT mutation rate and HIV/SIV genome size, most RNA->DNA conversions are fully correct (you may want to verify this personally!)*

**Response:** [??? I don’t know what RT is or what on page 2 he’s referring to]

***Comment-8:*** *Page 2 - "increased CD4+ T cells" - this is jargon.*

**Response:** We agree that an abstract should not contain jargon, however CD4+ T cells are an integral component of HIV virology, and we frequently refer to CD4+ cells throughout the manuscript.

***Comment-9:*** *Page 5 - make sure you are clear which parameters are "estimates" vs. taken. Because none of the parameters in this paper are estimates, these should be listed as "taken from literature".*

**Response:** We feel that the term “estimate” is appropriate for in the context of our parameter values used in the model. While we did not estimate parameter values in this study, the values we used are from a combination of experimental measurements or data fitting where the values were, in fact, estimated by fitting to data. We feel there is now confusion about when we are taking a parameter value from a published source.

***Comment-10:*** *Why is the virus decline after ART is different in two sets of "monkeys? (Figure 6)*

**Response:** The objective of this simulation was to demonstrate the difference in ART effectiveness under different levels of morphine conditioning. A high level of morphine (M = 200) results in a 7 day increase in time needed for the virus to be reduced below the detection level. This increase is a result of a higher set-point viral load associated with morphine use and the more favorable conditions that morphine gives to the virus which cause more severe disease.

***Comment-11:*** *Page 19: 300 count decrease. What does that mean? Is that from 10^6 to 10^6-300 - which is irrelevant then.*

**Response: [**I didn’t understand the 300 either?]