Driving Factors in Cytotoxic Cell Stimulation as Treatment for HIV: Insight from Mathematical Models

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Background

Immunomodulation via cytokines is a potential alternative or complementary treatment for HIV. The cytokine IL-15 promotes the proliferation and activation of CD8+ T cell and NK cells, which respond to viral infection.

N-803* (formerly ALT-803) is an IL-15 superagonist that combines an IL-15 mutant with improved bioactivity [1] with an IL-15Rα-Fc complex to improve serum retention [2]. N-803 has been shown to induce proliferation of CD8+ T cells and NK cells in human cancer trials [3-4], as well as transiently reduce the viral population in SIV-infected macaques [5], an animal model of HIV.

Though initially responsive, the SIV population rebounded as treatment continued, despite elevation of bulk CD8+ T cell and NK cell over pretreatment values [5]. Response of SIV returned somewhat after an extended break in treatment [5]. This work uses a mechanistic mathematical model to probe possible explanations of these observed dynamics.

Mechanistic Mathematical Model

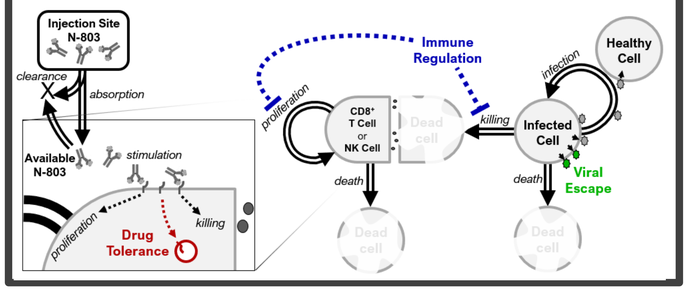
The ordinary differential equation model includes:

→ **CD8⁺ T-cell** and **NK cell** effector populations, with N-803 stimulation of proliferation and stimulation of killing, the latter convolving increased activation and migration evidenced by enhanced CD8⁺ T-cell presence in germinal centers [6]

→ **Drug tolerance** to N-803 evidenced by IL-15 receptor downregulation [5]

→ **Immune regulation** of effector cell killing and proliferation representing regulatory T-cell expansion, effector cell PD-1 and CD39 expression [5], and NK cell suppression of CD8⁺ T-cells via IL-10 [7]

→ **Viral escape** from CD8⁺ T-cell response representing a shift in strain frequencies [5]



Non-Human Primate Data

Models were calibrated to published NHP data [5]. Three rhesus macaques, chronically infected with SIVmac239 for at least 1.5 years, were given weekly 0.1 mg/kg subcutaneous doses of N-803. The regimen consisted of three cycles of four treatments each, with a 2 week break between the first and second cycles and a 29 week break between the second and third cycles (Fig. 1H). Peripheral blood was routinely assayed for SIV viral RNA, CD4+ T cells, CD8+ T cells, and NK cells. All three animals had been vaccinated against SIV epitopes prior to infection in a previous study [8].

References & Acknowledgments

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Model Comparison

The mechanisms required to explain the viral and cell dynamics were evaluated by:

→ **Calibrating** different versions of the model to the data, where each had a different combination of mechanisms (Fig. 1A, left)

→ **Comparing** models using the Negative Log-Likelihood (NLL) to measure goodness-of-fit and Akaike Information Criterion (AIC) to account for model complexity (Fig. 1A, middle).

→ **Evaluating** key quality criteria for each model, which are based on the distinct viral responses observed during each N-803 treatment cycle (Fig. 1A, right)

Observations from model comparison (Fig. 1A-D)

→ **Model #2** (no immune regulation) did not replicate the drop and rebound of the virus during treatment cycle 1

→ **Model #4** (*only* immune regulation) did not replicate the weaker viral response of cycle 3 (as compared to cycle 1)

→ **Model #1** (immune regulation + viral escape) and **model #3** (immune regulation + drug tolerance) both met all three qualitative criteria

→ **Model #3** (immune regulation + drug tolerance) was quantitatively superior as measured by low NLL and AIC, in part because it allowed a better fit to the CD8⁺ T cell and NK cell response (Fig. 1C-D)

→ *Note: The **full model** was comparable to model #3.*

Observations from model #1 & model #3 (Fig. 1E-G)

→ The **per-cell killing** is the effective killing rate constant averaged over both cytotoxic cells and viral strains (Fig. 1E)

→ Both models predict an early increase in cytotoxic activation that is followed by a **reduction in per-cell killing** below pre-treatment levels during each N-803 treatment cycle

→ Both models predict a **recovery of per-cell killing** after each treatment cycle

→ **Model #1** predicted a more severe reduction in per-cell killing that did not fully recover after treatment (Fig. 1E) and was accompanied by a reduction in **viral fitness** (Fig. 1F)

→ **Model #3** showed a weaker CD8⁺ T cell and NK cell expansion in cycle 3 (as compared to cycle 1) (Fig. 1C-D) which was enabled by long-term reduction in **drug efficacy** (Fig. 1G)

Summary of observations

→ **Immune regulation** was necessary, but not sufficient, to explain the short-term viral dynamics during the N-803 regimen

→ Either **drug tolerance** or **viral escape** were necessary to explain the long-term viral dynamics across multiple N-803 regimens

→ **Drug tolerance** reduced viral suppression indirectly by influencing the response of CD8⁺ T cells and NK cells to N-803

→ **Viral escape** reduced viral suppression directly by influencing the response of the virus to CD8⁺ T cells

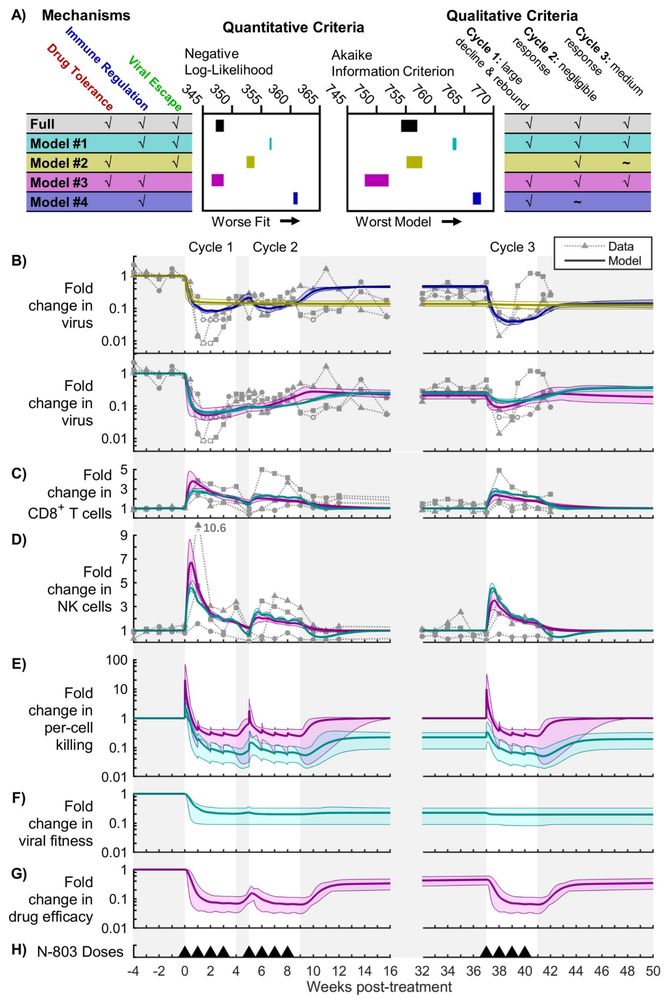


Figure 1. **A)** Summary table for model comparison; **B-H)** fitted outputs for models, shown as mean and range of outputs from top 20 fitted parameter sets for each model

Sensitivity Analysis

The relative influence of model mechanisms were analyzed by:

→ **Sampling** parameter values across 2+ orders of magnitude and evaluating model results for each parameter set

→ **Calculating** the Partial Rank Correlation Coefficients (PRCC) between parameter values and measures of treatment efficacy (Fig 2), considering those where p<=0.00001 across 3 repetitions of 10,000 samples

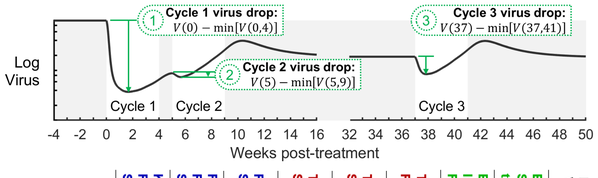
Observations from sensitivity analysis (Fig. 2)

→ **Killing Regulation Strength** had strong negative correlations to treatment efficacy, highlighting regulation as a target for intervention

→ **Killing Regulation Speed** had a positive correlation to treatment efficacy in cycle 2, suggesting that administering a dose after regulatory signals have normalized could improve viral suppression

→ **Drug Tolerance Strength** had a strong correlation to reduced efficacy in cycle 2, reflecting how tolerance acts indirectly through CD8⁺ T cell and NK cell contraction

→ **Escape Strain Susceptibility** had the same strength of correlation in cycle 1 and cycle 3, reflecting the long-term nature of viral escape



Measure of treatment efficacy	Killing Regulation Strength	Killing Regulation Speed	Proliferation Regulation Strength	Regulation Speed	Tolerance Strength	Tolerance Speed	Tolerance Recovery	Escape Strain Initial Frequency	Escape Strain Susceptibility to T cells	Dummy Variable
Cycle 1 virus drop	**−0.60**	**−0.13**	**−0.50**	**−0.21**	**−0.23**	-	**−0.30**	**+0.25**	-	
Cycle 2 virus drop	**−0.49**	**−0.36**	**+0.07**	**−0.47**	**−0.07**	-	-	**+0.13**	-	
Cycle 3 virus drop	**−0.35**	**−0.08**	**−0.39**	**−0.17**	**−0.07**	**+0.07**	**−0.06**	**+0.23**	-	

Figure 2. **top)** measures of treatment efficacy; **bottom)** partial rank correlation coefficients (PRCC) between efficacy measures and select parameters

Treatment Exploration

Both **model #1** (immune regulation + viral escape) and **model #3** (immune regulation + drug tolerance) were used to explore potential treatment improvements and alternatives:

→ **Increasing the dosing period** could achieve the strong initial killing rate more often. The effect of a 4-week dosing regimen is shown (Fig. 3).

→ **Limitation of regulation** could be achieved by simultaneously blocking the PD-1/PD-L1 pathway. For example, N-809* combines IL-15 with an anti-PD-L1 agonist [9]. The effect of reducing killing regulation strength by 40% is shown (Fig. 3).

Observations from treatment exploration (Fig. 3)

→ **Regulation blockade** resulted in a stronger viral suppression with the first dose and a weaker viral rebound during subsequent doses (Fig. 3A)

→ **Dose spacing** resulted in more consistent cytotoxic activation (Fig. 3B) and viral suppression (Fig. 3A) with each dose

→ Combining both regulation blockade and dose spacing had the strongest effect.

→ **Model #1** was more resistant to treatment improvement, suggesting that viral escape may ultimately limit N-803 therapy

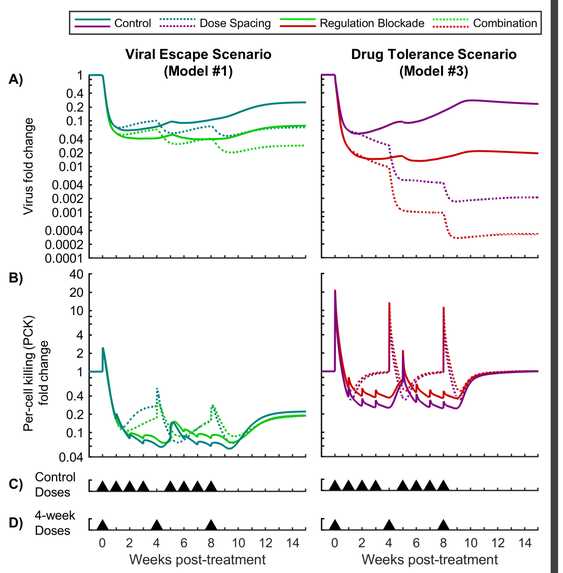


Figure 3. Result of treatment exploration for model #1 and model #3, shown as mean of outputs from top 20 parameter sets for each model

Conclusions & Future Directions

Primary conclusions from this project are:

→ **Model Comparison** demonstrated that **immune regulation** reduces cytotoxic activity during weekly N-803 doses, while either **drug tolerance** or **viral escape** may reduce N-803 efficacy across breaks in treatment.

→ **Sensitivity Analysis** highlighted regulation strength and timing as targets for interventions to improve N-803 treatment outcomes.

→ **Treatment Exploration** showed that combining an altered dosing regimen with regulation blockade could improve outcomes, but this may be limited by immune escape by the virus.

Future directions related to this project include:

→ Analysis of the effects **latency reversal** [10] on viral dynamics during N-803 treatment with a latent infection model

→ Analysis of the effects of **enhanced migration** of CD8⁺ T cells into germinal centers [6] with a multi-compartment model and new data from a currently ongoing SIV experiment