

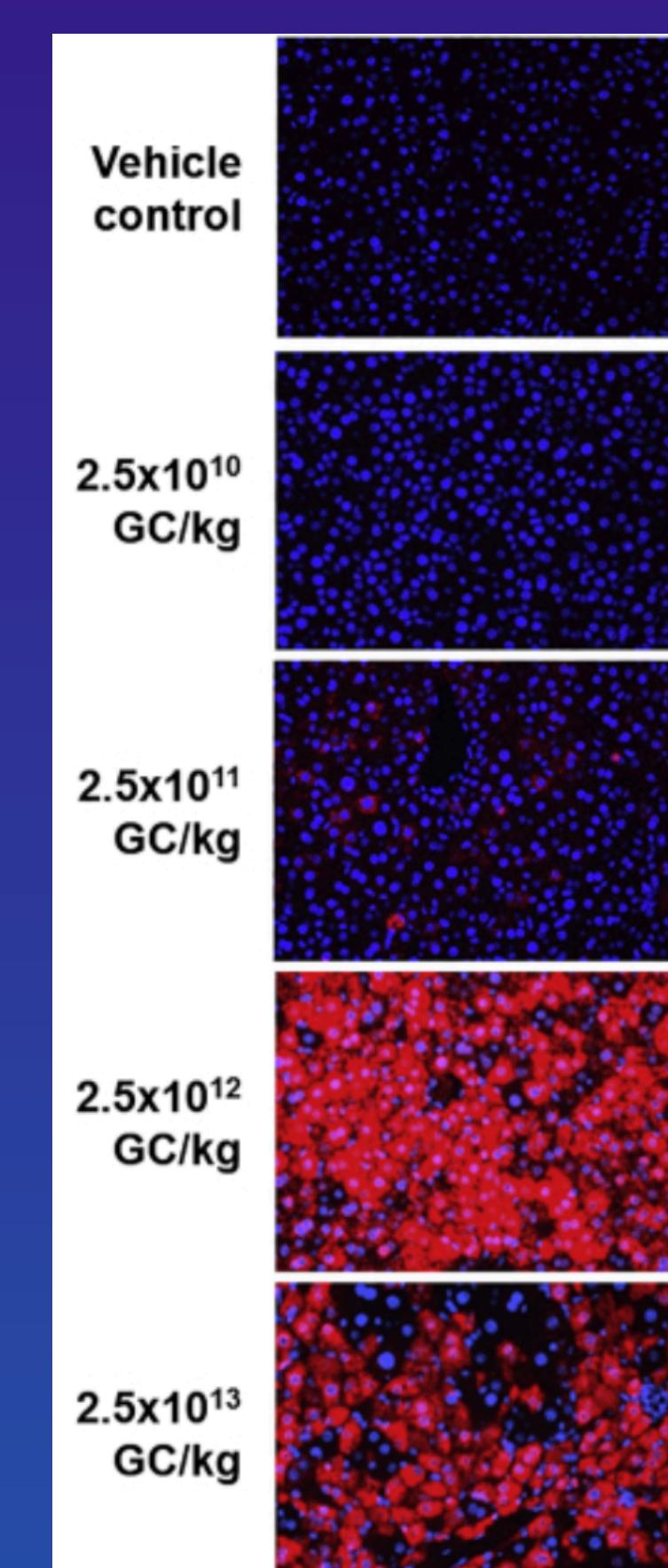
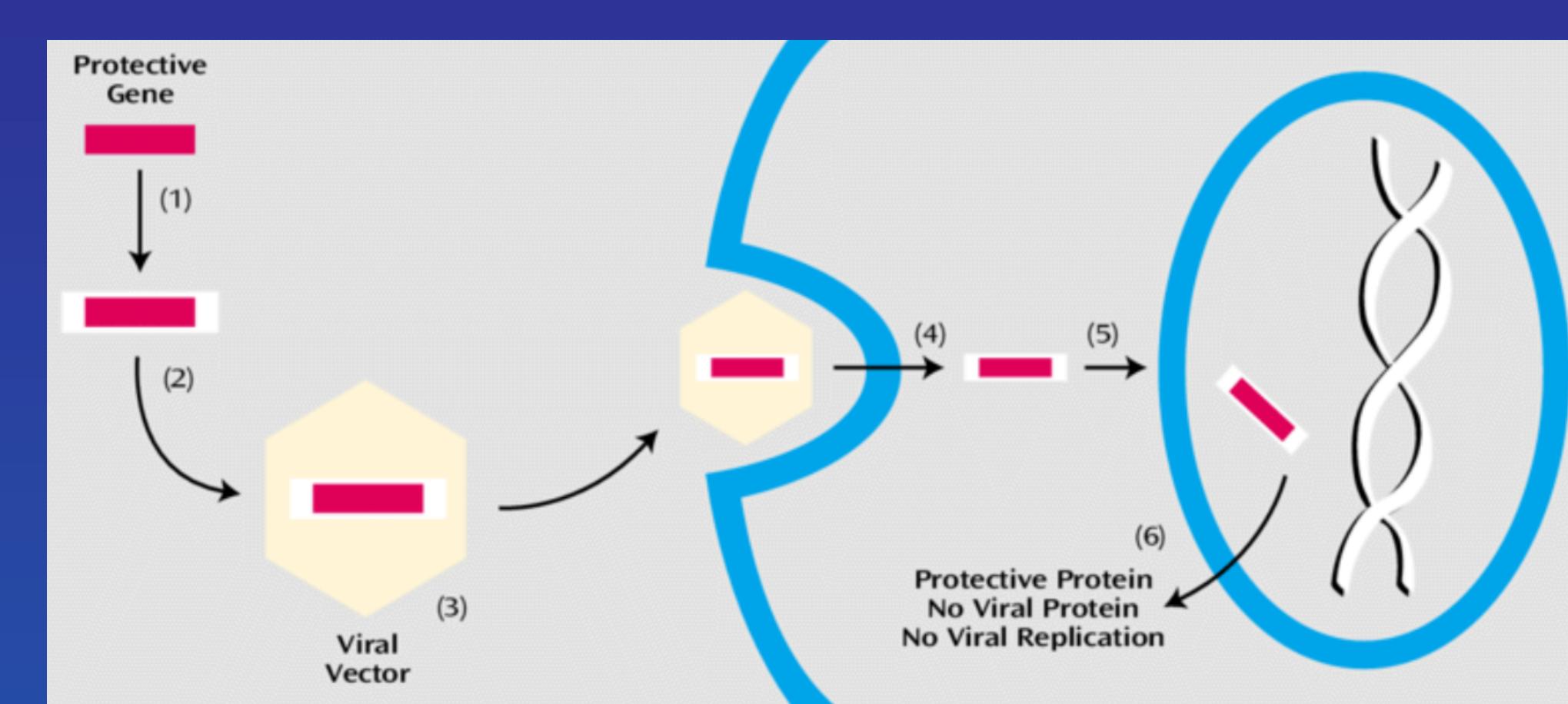
# VIRADOSE VD

## INTRODUCTION

Gene therapy is a treatment method that involves delivering missing or correctional genes to cells in order to improve genetic disorders. The delivery of genes is accomplished by a vector. Viral vectors are especially effective at delivering genetic material directly into targeted body cells due to their natural impulse to infect. Viral vectors are very commonly used in gene therapy. However, high doses of these vectors trigger immune responses that may be dangerous to the patients.

ViraDose, the app created in this project, aims to assist with dosing Adeno-Associated Viruses (AAVs). AAVs are one of the most actively investigated vectors as well as one of the most promising candidates for gene therapy for many reasons. The most prominent of these reasons is their lack of pathogenicity. Since wild-type AAV cannot enter the lytic cycle in the body without the presence of other viruses, AAV does not trigger a significant immune response in humans. This factor allows them to be safe and predictable. ViraDose aims to advise a starting dose for AAV viral vectors in human gene therapy clinical trials and predict the person's immune response.

The purpose of this project was to create an application that makes gene therapy dosing easier, safer and more predictable. The target users for this application are professionals who are conducting gene therapy clinical trials using AAV vectors.



## METHODS AND PROCEDURE

### Calculating Starting Dose

ViraDose takes in user inputs relating to animal studies used to test dosing and patient-specific information. When animals are used in trials to find the highest non-severely toxic dose (HNSTD), different animals uniquely scale up to humans. The app contains a list of common lab animals for the user to choose from so that the HNSTD can first be scaled up to a human dose using allometry. Another component that had to be considered was a safety factor, a number that the initial dose is divided by to further ensure that no toxicity occurs. The user can choose from default safety factors based on the disease being treated or input their own based on their own research. The dose is also adjusted to flat dosing, which should only be used when there are studies to verify that clearance does not significantly vary with weight. Otherwise, the weight can be adjusted. After these calculations, ViraDose generates the recommended starting dose in genome copies.

Species	Reference body weight (kg)	To convert dose in mg/kg to dose in mg/m <sup>2</sup> , divide by K <sub>m</sub>		To convert human dose in mg/kg to AED in mg/kg, either multiply human dose by	
		Multiple	Divide	human dose by	human dose by
Human	60	37			
Mouse	0.02	3	12.3	0.081	
Hamster	0.08	5	7.4	0.135	
Rat	0.15	6	6.2	0.162	
Ferret	0.30	7	5.3	0.189	
Guinea pig	0.40	8	4.6	0.216	
Rabbit	1.8	12	3.1	0.324	
Dog	10	20	1.8	0.541	
Monkey (rhesus)	3	12	3.1	0.324	
Marmoset	0.35	6	6.2	0.162	
Squirrel monkey	0.60	7	5.3	0.189	
Baboon	12	20	1.8	0.541	
Micro pig	12	27	1.4	0.730	
Mini pig	40	35	1.1	0.946	

Data adapted and modified from FDA draft guidelines.<sup>30</sup> FDA: Food and Drug Administration, AED: Animal equivalent dose

### Modeling Immune Response

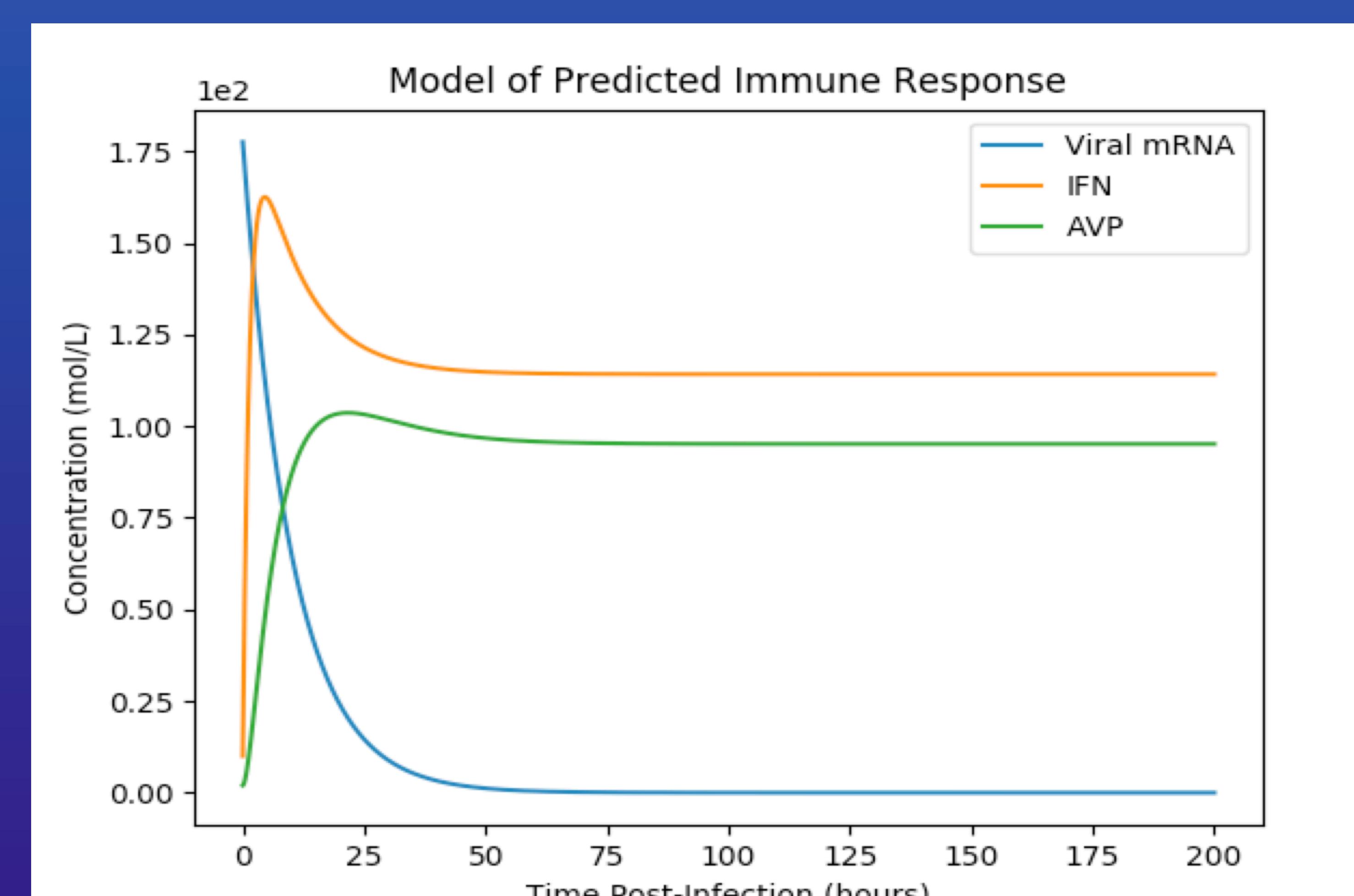
The first model is adapted from a study which shows a basic representation of a person's innate immune response to a virus using a system of differential equations. It measures the concentration of the virus, interferon (IFN), and antiviral proteins (AVP) over time. For the purposes of adapting it to AAV immune response, the replication rate of the virus is set to zero. Using rough estimations, the recommended initial starting dose generated by the app is converted to mol/L in order to implement it into this model. This mathematical model helps to determine the point in time at which the virus is cleared from the body, as well as the levels of IFN and AVP that remain in the body after the virus is cleared, indicating what the conditions of the immune system will be at readministration.

$$\frac{dV}{dt} = k_1 V(t - \tau_1) \frac{b_1 K_1^{n_1}}{K_1^{n_1} + A^{n_1}(t - \tau_5)} - d_1 V(t)$$

$$\frac{dI}{dt} = k_2 V(t - \tau_2) + \frac{b_2 I^{n_2}(t - \tau_4)}{K_2^{n_2} + I^{n_2}(t - \tau_4)} - d_2 I(t)$$

$$\frac{dA}{dt} = k_3 I(t - \tau_3) - d_3 A(t)$$

Parameter	Description	Value
k1	Kinetic rate constant of viral replication	0
k2	Activation rate constant of IFN	0.3
k3	Activation rate constant of AVP	0.1
d1	Degradation rate of viral mRNA	0.1
d2	Degradation rate of IFN	0.7
d3	Degradation rate of AVP	0.12
b1	Maximal production rate of Hill function of AVP on virus	10
b2	Maximal production rate of Hill function of IFN	80
K1	Inhibition coefficient of Hill function of AVP on virus	33
K2	Activation coefficient of Hill function of IFN	0.1
n1	Hill coefficient	1
n2	Hill coefficient	1



The figure to the far left shows an example of the graph that this mathematical model produces using its original parameters and starting values. This graph represents a more typical viral infection in which the virus replicates and the body has a more intense immune response to it.

The figure to the left shows an example of the type of graph that this mathematical model produces when the replication rate of the virus is set to zero. This is a more accurate representation of how the immune system would respond to gene therapy vectors in the body. The initial concentration of the virus, as well as the peak concentrations of IFN and AVP are significantly smaller than they would be during a normal infection.

# Gene Expression and Toxicity Model

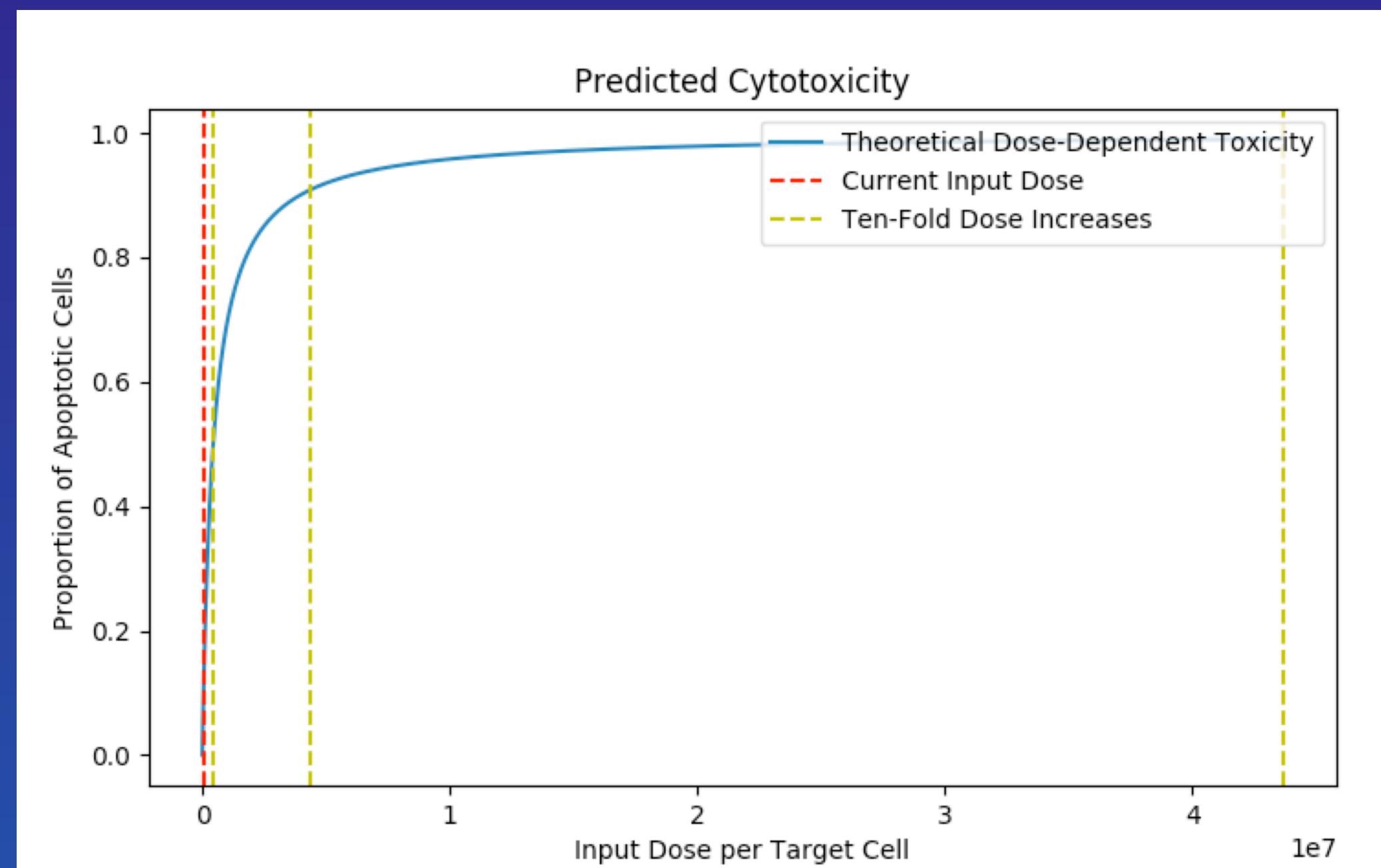
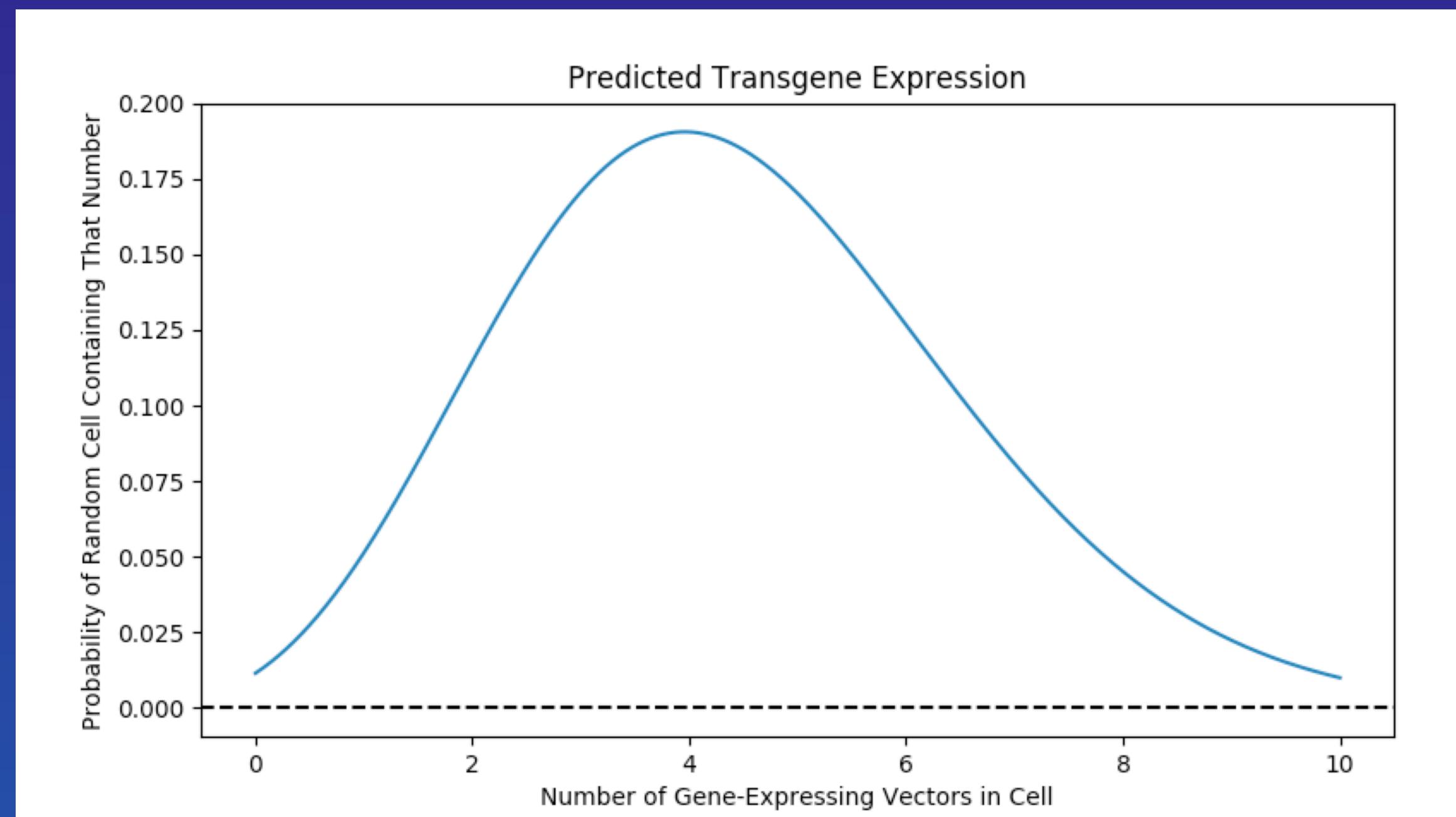
The second model is adapted from a study which uses functions to determine the amount of gene expression and toxicity through apoptosis that occurs based on AAV dose administered in gene therapy. These functions use different parameters based on the type of AAV vector used. Although these functions were used in the context of *in vitro* experiments, they can be adapted to ViraDose's experimental model and not only provide an indication as to how effective the dose will be, but also the chances of toxicity occurring.

$$v(m) = \frac{t}{1 + (c/m)^h}$$

$$P(k) = \frac{v^k e^{-v}}{k!}$$

$$P_d(m) = \frac{1}{1 + (c_1/m)^{h_1}}$$

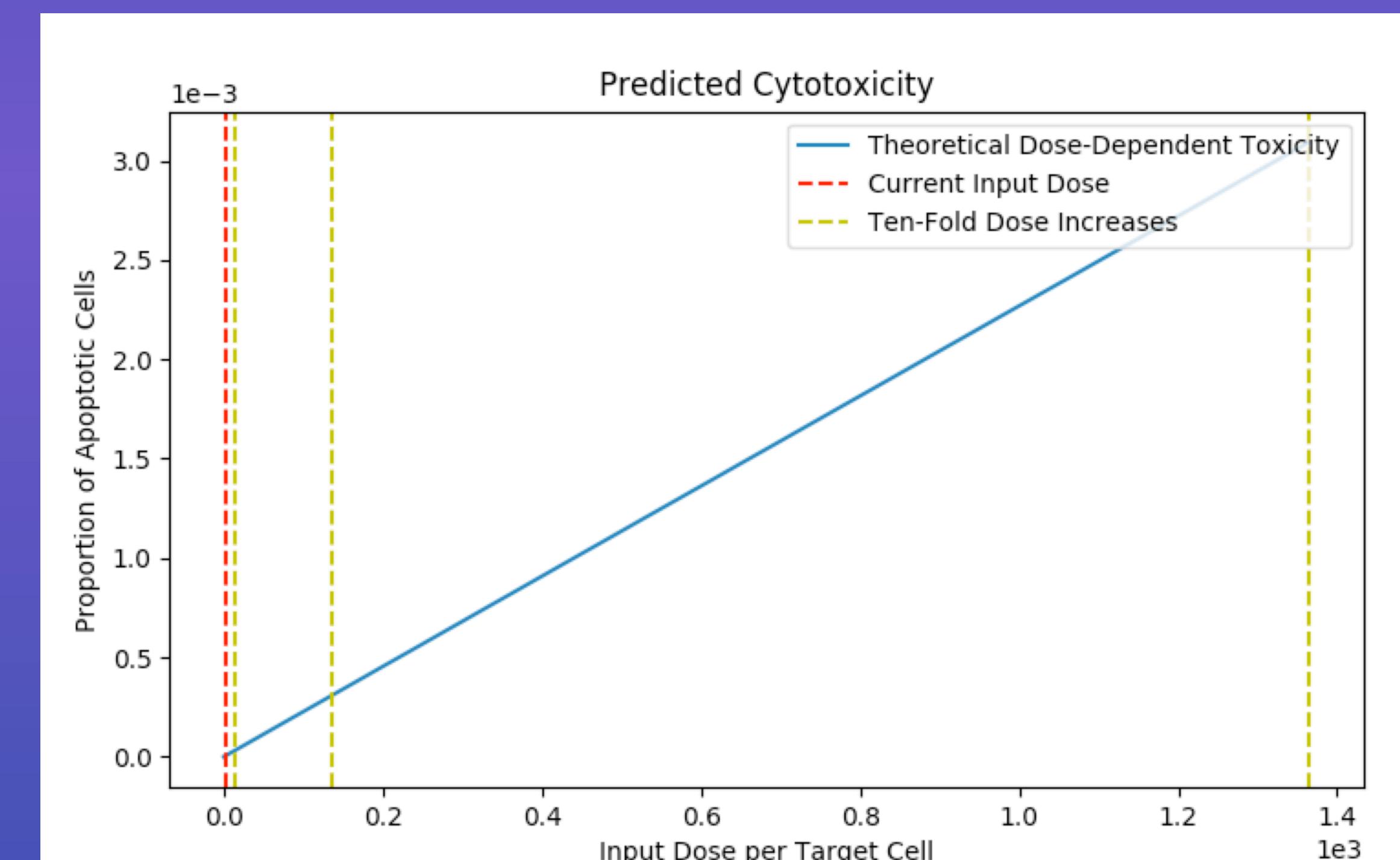
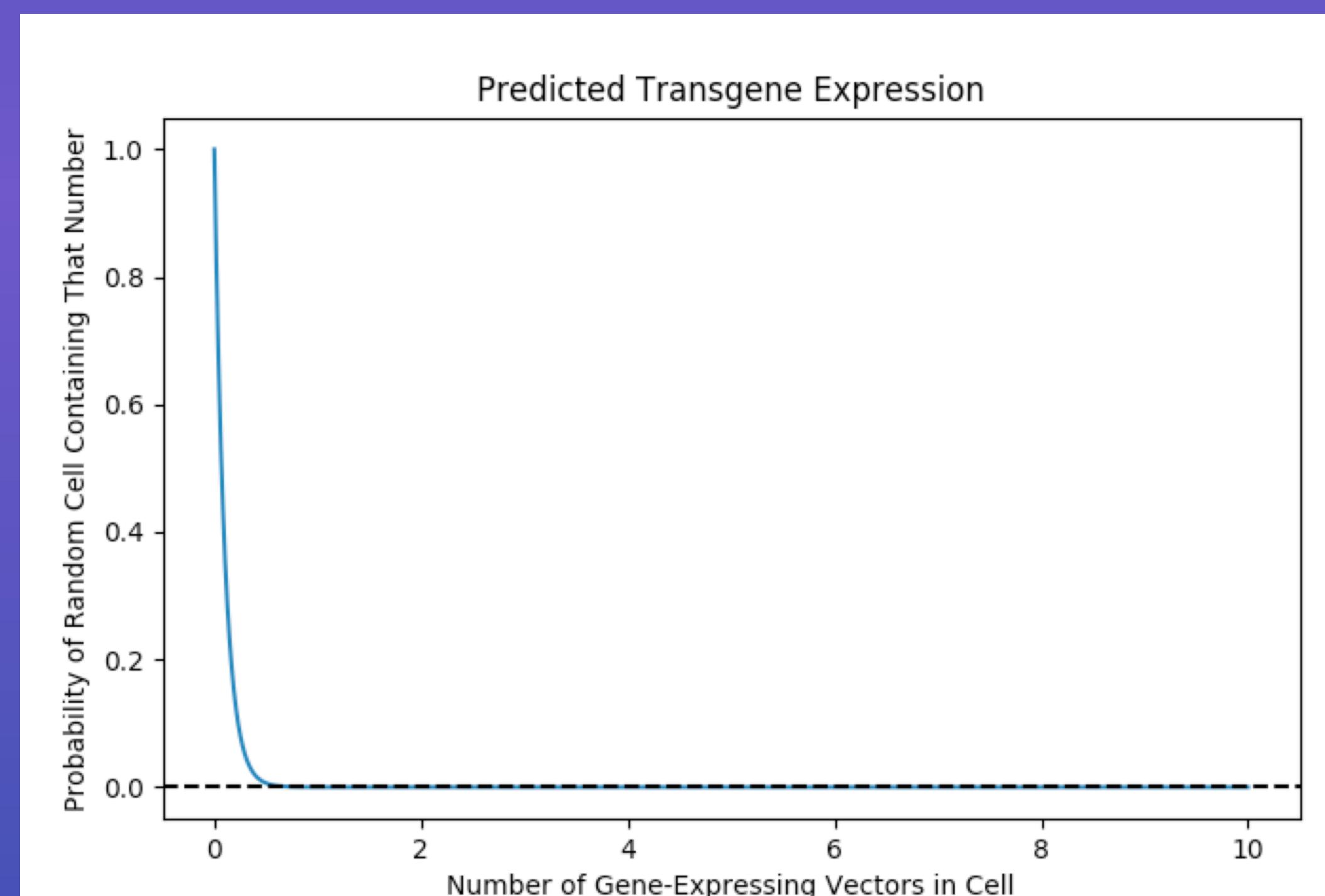
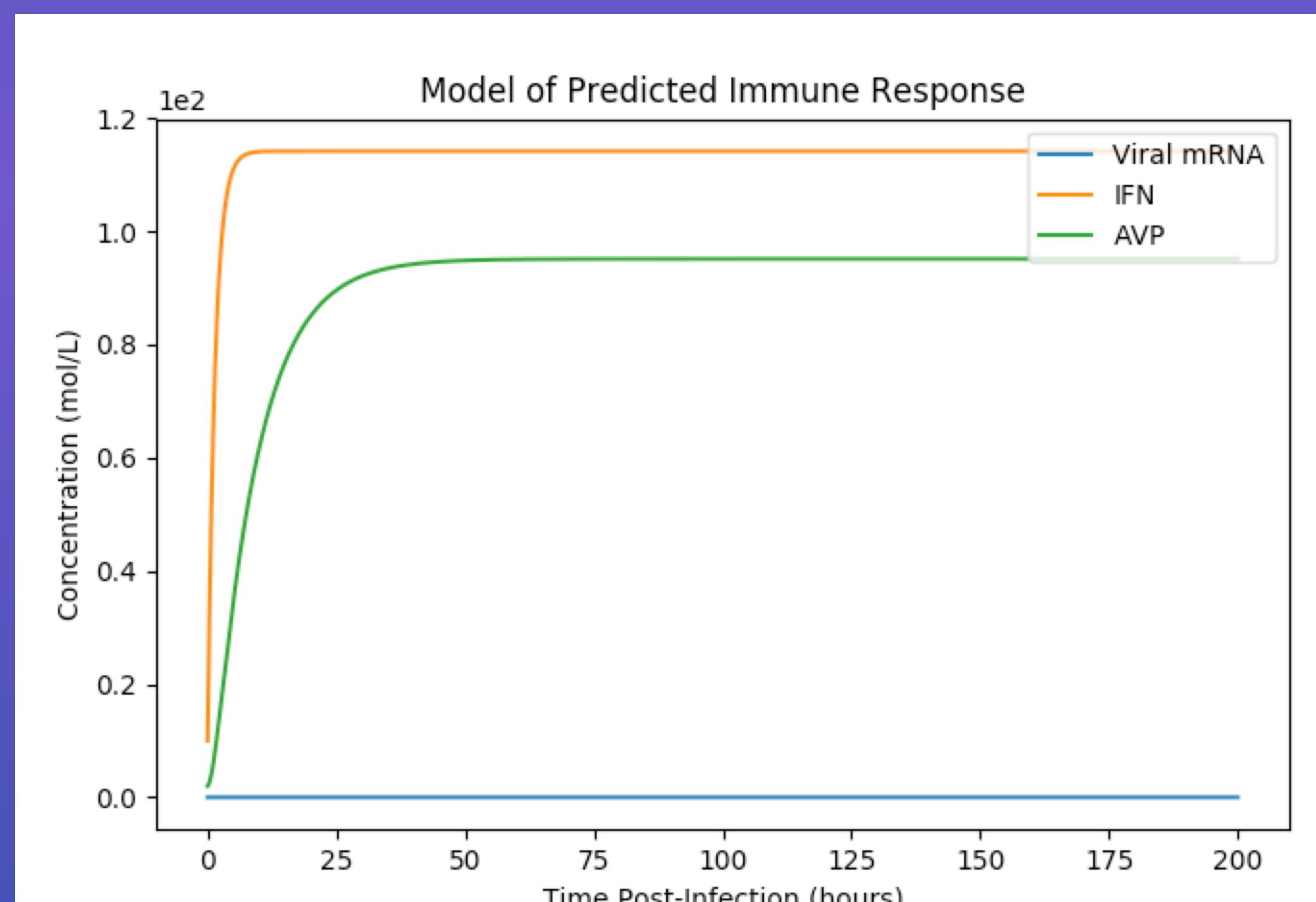
Serotype	t (average value)	log c (average value)
AAV1	4.47	5.46
AAV2	2.00	4.95
AAV4	3.81	14.34
AAV5	3.65	6.05
AAV6	6.65	5.68
AAV7	3.60	6.73
AAV8	14.55	13.44
AAV9	2.23	9.50



The figures to the left are examples of the mathematical models. The first graph, obtained from the first two equations, shows the probability of a certain number of vectors achieving gene expression in any given target cell. The second graph, obtained from the third equation, shows the predicted proportion of apoptotic cells as a function of the input dose. The red line represents the input dose used, and the yellow lines represent possible higher doses.

## USING VIRADOSE

Due to the lack of complete certainty of the effects of gene therapy treatments, gene therapy doses tend to be very small. This is to ensure safety in clinical trials. The graphs below are generated from entering an HNSTD of  $2.5 \times 10^{12}$  genome copies per kilogram into the ViraDose app. This example was obtained from a study done on mice with Crigler-Najjar Syndrome. For the graph on the left, the virus concentration may seem like it is at zero, but this is due to the very small quantity of the dose. The body's immune system still reacts to this dose. Based on the other two graphs, there is a low probability of gene expression, but also a very low probability of toxicity occurring. Based on the results that ViraDose gives using this example, it may be safe to administer a higher dose, but nevertheless, readministration would likely be recommended in order to increase gene expression.



## CONCLUSIONS

Currently, clinical trials are approached with a great amount of caution and the patient's immune response is observed after dose administration. However, many mathematical models exist to predict immune response based on specific parameters. The first model is only able to look at two specific components in the immune system which makes it difficult to accurately determine whether or not dangerous levels of inflammation will occur in the patient. However, it does give a sense of how quickly the function of the gene therapy vector will be inhibited by the body and the levels of antiviral proteins that will remain in the body after infection. The second model is based on data from *in vitro* experiments. Although it is specific to AAV gene therapy, this type of math is not yet used in actual human clinical trials. The ultimate goal of ViraDose is to eventually derive original mathematical models to very accurately describe the human immune response to AAV vectors. This application is in the very early stages of research and development. However, the essential idea of how it helps calculate and understand doses is very innovative to the field of gene therapy. If gene therapy is to become a more common treatment, these types of accurate, automatic predictions will be crucial to this field. For now, this type of application could ensure safer dosing and increase researcher accuracy within gene therapy clinical trials, improving the data needed to create these types of treatments.

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