Predictive pharmacodynamics model of DNA cleavage enzyme delivery for curative HIV gene therapy

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Background and objectives

DNA cleavage enzymes such as homing endonucleases, zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases and CRISPR/Cas9 proteins are a promising method to eliminate chronic viral infections such as HIV, hepatitis B and herpes simplex virus. These enzymes are engineered to bind and mutate specific target sequences within latent viral genomes. Since DNA repair via non-homologous end-joining is error-prone, it can induce mutations into viral DNA that can render the virus replication-incompetent. Delivery of cleavage enzymes to infected cells can be achieved via viral vectors to infected cells as a form of gene therapy. While detailed mathematical formulations exist for small molecular drug delivery (pharmacokinetics) and activity on a specific target (pharmacodynamics), and represent a fundamental step within the regulatory process of drug licensure, no similar sets of equations exist for gene therapy. Here we use data derived from delivery of adeno-associated virus (AAV) vectors that express fluorescent proteins to develop mathematical models linking vector delivery dose to quantitative gene expression.

Methods

In vitro assessment of delivery and efficacy

Predicting gene expression in a single cell

Mathematical model

\[ y = T(m) \]

* Given \( m \) input vector genomes per cell, let \( y \) be the number of genomes delivered and expressed per cell

\[ P(Y) = \text{probability that a cell contains } k \text{ delivered genomes} \]

\[ f(Y) = \text{fluence of cell containing } k \text{ delivered genomes, } f_0 = \text{baseline fluence, } n = \text{multiplicative effect of gene expression, } f = \text{fluence due to single successfully delivered genome} \]

Sigmoidal dose-response curve

\[ T(m) = \frac{t}{1 + \left( \frac{m}{c} \right)^n} \]

* \( t \) = max response

* \( c = EC_{50}, \) dose at half-max

* \( n = \) Hill slope

* \( m = \) dose

Model fitting and parameter estimation

\[ RSS = \sum (y_{\text{pred}} - y_{\text{exp}})^2 \]

I. Serotype screen

- Eight serotypes of self-complementary adeno-associated virus (scAAV) vectors used to transduce SupT1 cells at three doses, GFP reporter
- Quantification of gene expression using flow cytometry, \(~10000 \) cells per sample

Good model fits to fluorescence histograms

II. Promoter screen

- scAAV vectors with one of eight promoter constructs driving GFP expression in SupT1 cells at three doses
- Quantification of gene expression using flow cytometry, \(~10000 \) cells per sample

Predict delivery and percentage of cells transduced at different doses

III. Co-transduction assay

- Co-transduction of SupT1 cells with scAAV1-1FS-GFP and scAAV1-1FS-nCherry
- Quantification of gene expression using flow cytometry, \(~10000 \) cells per sample

IV. AAV- and ZFN-mediated toxicity

- PI and Annexin stains used to label apoptotic cells
- Six ZFN pairs, one GFP only and controls, at two doses on SupT1 cells
- Flow cytometry, \(~10000 \) cells per sample

Used clustering to distinguish between live and dead cells and to separate debris

Found slightly elevated amounts of debris (4-15% more) and apoptosis (1-4% more) in treatment groups compared to controls

Summary and future work

We have developed and validated a mechanistic model of transgene delivery and expression for anti-HIV gene therapy

Key results:

- Fluorescence per expressed transgene \( f \) depends mainly on fluorophore
- Serotypes and promoters are characterized by their dose-response curves, primarily \( f \) and \( c \)
- Experiments needed to set bounds on \( f \)
- Values of \( n \) suggest amplification of response after delivery
- Expression of multiple reporters is highly correlated

Next steps:

- Pharmacokinetic model, prediction of efficacy and off-target activity

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