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Predictive pharmacodynamics model of transgene delivery for curative HIV gene therapy

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Background: Our group is developing a gene therapy approach for the cure of HIV, which relies on the efficient delivery and expression of DNA cleavage enzymes within infected cells. These enzymes are engineered to bind and mutate specific target sequences within latent HIV genomes, rendering the virus replication incompetent. Delivery is achieved with viral vectors that contain the enzymes as a transgene payload and assessed by quantifying expression of a fluorescent reporter gene using flow cytometry (FCM).

Methods: We have developed a mechanistic model that predicts quantitative transgene expression in target cells as a function of vector dosage. We fit the model to FCM data from three experiments aimed at optimizing transgene delivery to HIV-permissive CD4+ memory T-cells in culture using self-complementary adeno-associated virus (scAAV) vectors.

Results: We identify that delivery follows a sigmoidal dose-response relationship and that the level of saturation of gene expression depends on the serotype, promoter and experimental conditions. Delivery saturates at a maximum of ~30 vector genome copies per cell (vg/cell) when using unpurified scAAV stocks or ~9 vg/cell with purified scAAV. Of the different serotypes and promoters, scAAV1 vectors with the EF1 alpha short promoter have the lowest particle to cell ratio required for saturation. We identified differences in half-maximal dose (EC50) and maximal predicted delivery (t) that explain the variation in expression among serotypes and promoters. In co-transduction experiments, we find that cells that express one reporter gene at high levels have a greater than random chance of expressing the other reporter. In order to obtain more than 95% co-transduction, vectors need to be added at ratios of at least 50000.

Conclusions: For a given serotype and promoter, the model accurately predicts the minimum dose needed to obtain a desired level of transduction. Our model provides a quantitative method of dose, serotype and promoter optimization that can be applied to the cure of HIV as well as other gene therapy applications.