

# Adenovirus Gene Delivery for the Treatment of Ocular Diseases: Promoter Expression and Genome Stability Analysis

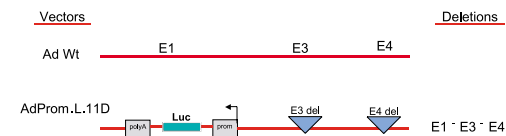
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## Introduction

Adenoviral vectors are currently being tested as a gene therapy platform for their utility in treating age-related macular degeneration (AMD). This disease can lead to blindness with aberrant blood vessel growth. Delivery of an adenovector that expresses Pigment Epithelium-Derived Factor (PEDF) from the human CMV (hCMV) promoter after intravitreal administration has been efficacious in mouse models of this disease (Mori et al, 2001; Mori et al, 2002). PEDF has been shown to be a potent anti-angiogenic factor.

After intravitreal administration adenovirus vectors transduce a number of cells in the eye. In the posterior portion of the eye, Muller cells and other ocular cells are transduced. The majority of transduction however, occurs in the anterior portion of the eye and includes the cornea endothelial, iris epithelial, ciliary epithelial and trabecular meshwork cells. Expression from the hCMV promoter is considered transient in nature lasting approximately 2 weeks in immune-competent mice. The decrease in transgene expression could be due to loss of vector genome or promoter shut off. To distinguish between these two possibilities the stability of the vector genome and expression from the hCMV promoter in an E1, E3, E4 deleted adenovector (AdL.11D) was determined over time. The results of these studies show genomes are more stable than expression and suggest promoter shut off as the cause for the decrease in transgene expression. Furthermore, loss of expression does not require an adaptive immune response. The substitution of alternative promoters for the hCMV promoter identified promoters that can remain relatively active even for 90 days. Together these data demonstrate that adenovectors can have a broad application for diseases in the eye.

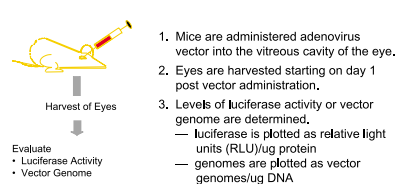
## Vector Configurations



## Figure 1. Adenovirus Vector Configurations

The viral vectors are derived from adenovirus 5. The vectors carry deletions in the viral E1, E3, and E4 regions rendering them replication defective. The E1 region of the virus has been replaced by a luciferase expression cassette in which the promoter has been varied. The AdL.11D vector contains the hCMV promoter.

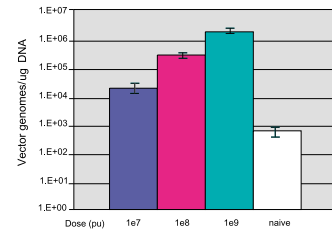
## Experimental Method



## Figure 2. Outline of Experimental Method

Mice were anesthetized and pupils dilated. Intravitreal injections were carried out with a Harvard pump microinjection apparatus and glass pulled micropipettes. Vector was delivered in 2 ul by passing the micropipette through the sclera just behind the limbus into the vitreous cavity. C57BL6 mice were used except where indicated. Vector dose is given in particle units (pu). Error bars are standard error of the mean.

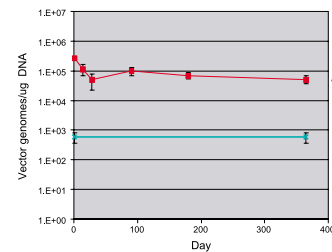
## Intravitreal Dose Response Curve: Vector Genome Levels Day 1



## Figure 3. Quantitative Determination of Vector Delivered to the Eye

**Result:** Vector is efficiently delivered to the eye. The relative levels of vector delivered to the eye are as expected. With each 10 fold increase in vector dose there is a 10 fold increase in levels of vector genome detected in the eye. N = 5 (Day: 1)

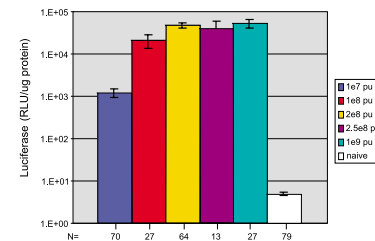
## Vector Genomes Persist 1 Year in the Eye



## Figure 4. Vector Genome Persistence Profile in the Eye

**Result:** Vector genome is stable for at least 1 year in the eye. Genome levels decrease approximately 4-5 fold by day 28 and then remain stable for a year. This suggests that it may be possible to express a gene product from an adenovector for an extended period of time in the eye. N = 5 (Day: 1, 14, 180), 4 (Day: 28, 90, 365)

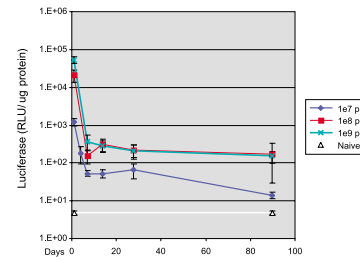
## Intravitreal Dose Response Curve: Expression Levels Day 1



## Figure 5. Dose Response Curve of AdL.11D Following Intravitreal Delivery

**Result:** Expression increases in a dose dependent fashion up to a 2e8 pu dose. Above a 2e8 pu dose there is no further increase in expression, suggesting saturation of promoter activity. N ≥ 13, multiple experiments (Day: 1)

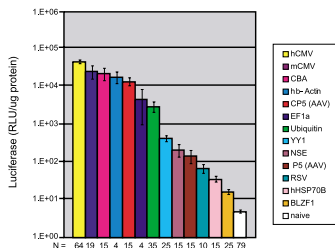
## Expression Lasts at Least 3 Months



## Figure 6. Expression Profile of AdL.11D at 3 Different Doses Over Time

**Result:** Expression at all doses tested persist for at least 3 months. Initial levels of expression decrease from day 1 levels to 3 month levels by 100x. The loss of expression (~100 fold) is substantially more than what was observed for the 4-5 fold loss of genomes over the same time (figure 4). The loss of expression is therefore not entirely the result of immune clearance of transduced cells but suggests a silencing mechanism of the promoter on the vector. The use of an alternative promoter may circumvent this silencing event. N ≥ 10, multiple experiments (Day: 1, 7, 14, 28, 90)

## Promoter Choice Determines Initial Expression Level

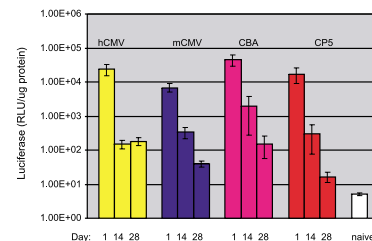


## Figure 7. Expression Level is Promoter Dependent

**Promoters Assayed:** hCMV = human Cytomegalovirus major immediate-early, mCMV = mouse Cytomegalovirus major immediate-early, CBA = human hCMV enhancer + chicken b-Actin chimeric, hb-Actin = human β-actin\*, CP5 = human hCMV enhancer + AAV P5 chimeric, EF1a = Elongation Factor 1α\*, Ubiquitin = Ubiquitin C, YY1 = Ying Yang 1, NSE = Neuron Specific Enolase, P5 = AAV P5\*, RSV = Rous Sarcoma Virus ITR\*, hHSP70B = human Heat Shock Promoter 70B, BLZF1 = BLZF1. \* Vectors contain wild type E4 region. (Day: 1) Dose = 2e8 pu

**Result:** The expression level of transgene in the eye is determined by the promoter. The dynamic range is ~3000 fold.

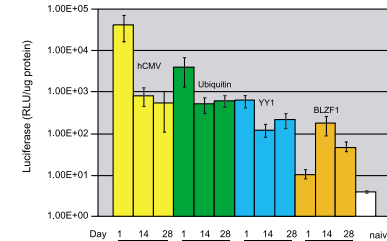
## Decreasing Expression Profiles



## Figure 8. Vectors With Expression Profiles Similar to the hCMV Promoter

**Result:** One class of promoters provides an expression profile similar to that directed by the hCMV promoter found in AdL.11D in which expression decreases ~100 fold within a month. N = 5, Dose = 2e8 pu

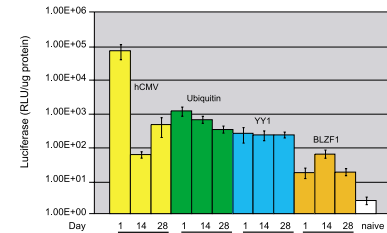
## Alternative Expression Profiles: Relatively Stable and Increasing



## Figure 9. Vectors With Expression Profiles Different from the hCMV Promoter.

**Result:** Vectors with the ubiquitin, YY1 and BLZF1 promoters gave expression profiles different from that with the hCMV promoter. Ubiquitin and YY1 represent a second class of promoters that remain relatively more active than the hCMV promoter when comparing levels of expression at day 1 and day 28. A third class of promoters is represented by BLZF1 in which onset of maximum expression is delayed. N = 5, Dose = 2e8 pu

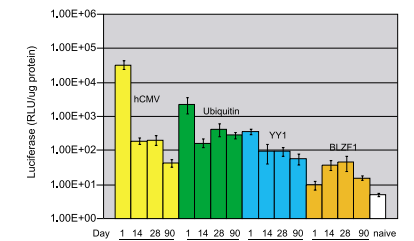
## Alternative Expression Is Not Mouse Strain Dependent



## Figure 10. Expression Analysis of Vectors in CD-1 nu/nu Mice

**Result:** The alternative expression profiles directed by the ubiquitin, YY1 and BLZF1 compared to the hCMV promoter found in C57BL6 mice were recapitulated in CD-1 nu/nu mice. This confirms the generality of the behavior of these promoters in the eye. Expression directed from the hCMV promoter decreased approximately 100 fold by day 28 while the ubiquitin, YY1 and BLZF1 promoters retained 25-100% of their activity. Once again the BLZF1 promoter showed an increase in expression compared to day 1 before maximum expression is obtained. The loss of activity directed by the hCMV promoter in this mouse strain demonstrates that an adaptive immune response is not required for this phenomenon. N = 5, Dose = 2e8 pu

## Relatively Stable Expression for 3 Months



## Figure 11. Three Month Expression Profiles

**Result:** Three different expression profiles are shown in the figure. The first class is represented by the hCMV promoter where a rapid decline in expression to ~1% or less of day 1 levels with in one month occurs and is then maintained for 3 months (also see figure 6). Relatively stable expression from vectors containing the ubiquitin and YY1 promoters which retain approximately 10-20% of their day 1 levels 3 months post vector administration. The BLZF1 promoter once again demonstrates a delayed onset followed by levels of expression that remain higher at 3 months that what is found on day 1. N = 5, Dose = 2e8 pu

## Conclusions

- Promoter Choice is an efficient means of regulating expression. At least 3 classes of promoters are available for use in the eye.

- Class 1: Expression levels decrease over time
- Class 2: Expression levels are relatively stable over time
- Class 3: Delayed onset of expression

- The decrease in expression found with Class 1 promoters appears to be determined by promoter down regulation, not the loss of vector genome nor due to an adaptive immune response.

- Adenovector genomes persist for at least 1 year at ~20% of day 1 levels. Safety studies show that although genomes are detectable, there are no effects on any parameters of clinical pathology, chemistry or histopathology. These genomes may represent a potential reservoir for long term gene expression.

## References:

- Mori K, Duh E, Gehlbach P, Ando A, Takahashi K, Pearlman J, Mori K, Yang HS, Zack DJ, Etyreddy D, Brough DE, Wei LL, Campochiaro PA. Pigment epithelium-derived factor inhibits retinal and choroidal neovascularization. J Cell Physiol. 2001 Aug; 188(2):253-63
- Mori K, Gehlbach P, Ando A, McVey D, Wei L, Campochiaro PA. Regression of ocular neovascularization in response to increased expression of pigment epithelium-derived factor. Invest Ophthalmol Vis Sci. 2002 Jul;43(7):2428-34.