

# Ocular Sublocalization and Pharmacokinetics of Expression of Pigment Epithelium-Derived Factor in Murine Eyes Following Adenovirus-Based Intravitreal Gene Delivery

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## Purpose:

Intravitreal delivery of AdPEDF.11D has been shown to block and cause regression of neovascular lesions in multiple animal models. In order to determine the levels of pigment epithelium-derived factor (PEDF) associated with this activity, we set out to assess the pharmacokinetics of expression and ocular sub-localization of this anti-angiogenic protein.

## Materials and Methods:

**Vector preparation.** Adenoviral vectors were constructed and produced as previously described (Brough et al., 1996). Briefly, an adenovirus vector deleted for E1A, E1B, partially deleted for E3, and deleted for the E4 region was constructed to express human PEDF from a cytomegalovirus (CMV) immediate early promoter. The human PEDF cDNA used for the construct was obtained from Dr. Patricia Becerra and licensed from the National Eye Institute (Bethesda, MD), and has been previously described (Steele et al., 1993). The production and quantification of the vector has been previously described (Brough et al., 1996).

**Vector delivery.** Adult female C57BL/6 mice were given an intravitreal injection of either 1e9, 1e8, or 1e7 total particle units (pu) of AdPEDF.11D with a Harvard pump microinjection apparatus and pulled glass micropipettes. Control animals were given buffer vehicle or were naïve controls. Each micropipette was calibrated to deliver ~ 2 µl of vector or vehicle upon depression of a foot switch. The mice were anesthetized, and under a dissecting microscope, the sharpened tip of the micropipette was passed through the sclera just behind the limbus and into the vitreous cavity to deliver the vector.

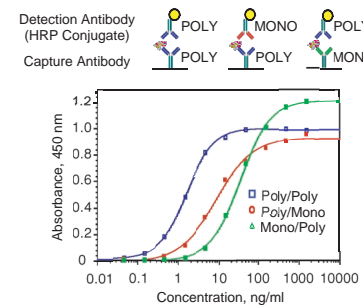
**Sample preparation.** At given intervals the mice were sacrificed and the eyes removed for analysis. These eyes were either snap-frozen in dry-ice or further dissected to cornea, aqueous humor, iris/ciliary body, lens, retina, RPE/choroid, sclera, and conjunctiva, and then frozen. Whole eyes were pulverized while frozen using a mortar and pestle and stored at -80°C until the time of analysis. Extraction of proteins from pulverized eyes and sub-fractionated ocular compartments was accomplished by re-suspension in PBS containing detergent and incubating at RT for 2 hours.

**Quantification of human PEDF.** A sensitive polyclonal antibody (PAb) sandwich enzyme-linked immunosorbent assay (ELISA) was developed in-house for the quantification of human PEDF. Rabbit polyclonal antibodies were raised against human PEDF expressed in HEK293 ORF6 cells and purified to homogeneity from cell culture supernatants. Three possible antibody combinations were tested for optimum sensitivity and specificity: a) polyclonal capture and polyclonal detection; b) polyclonal capture and monoclonal detection; and c) monoclonal capture and polyclonal detection. Mouse monoclonal anti-GST-hPEDF fusion protein is commercially available from Chemicon International (Temecula, CA). All detection antibodies were conjugated in-house to horse radish peroxidase (HRP). Optimizations were performed for all steps of the ELISA assay.

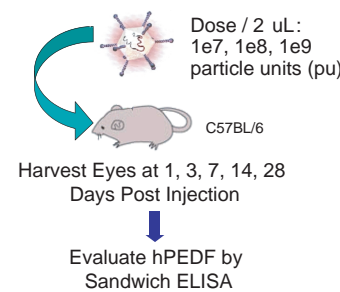
## Results:

Expression of PEDF following intravitreal administration of AdPEDF.11D was evaluated at 1, 3, 7, 14, and 28 days in murine whole eyes. When compared to vehicle-injected animals, a dose of 1e7 pu, results in a 3 fold increase (p= 0.01) in PEDF levels and is only detectable at day 1. This dose does not inhibit retinal and choroidal neovascularization (personal communications, K. Mori) in a small eye model. Also at day 1, a dose of 1e8 pu shows a 17- fold increase (p< 0.001) in PEDF, and 1e9 pu gives a 19- fold increase in PEDF levels (p< 0.001). At day 7, only doses of 1e8 and 1e9 pu give levels of PEDF 2-3 fold above vehicle (p= 0.001, for both). At day 14, only animals given 1e9 pu have measurable levels of PEDF (p= 0.01) above vehicle. No detectable levels of PEDF were observed above vehicle at day 28 for any of the vector doses used. Sub-fractionated eyes (cornea, aqueous humor, iris/ciliary body, lens, retina, RPE/choroid, sclera, and conjunctiva) were evaluated at days 1, 4, 7, and 14. In this study, measurable levels of hPEDF were detected at day 1 in every fraction except the lens. The retina and RPE/choroid show a different profile than the whole eye and other ocular regions, with a peak hPEDF concentration at day 4.

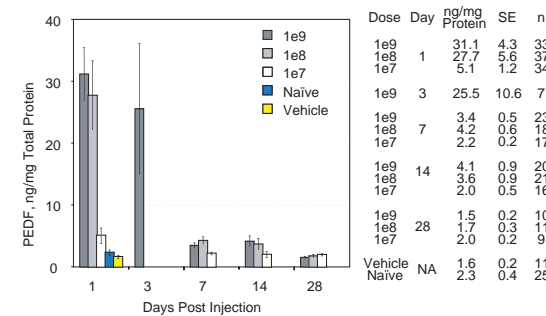
**Figure 1.** Polyclonal/Polyclonal System Provides Greatest Sensitivity



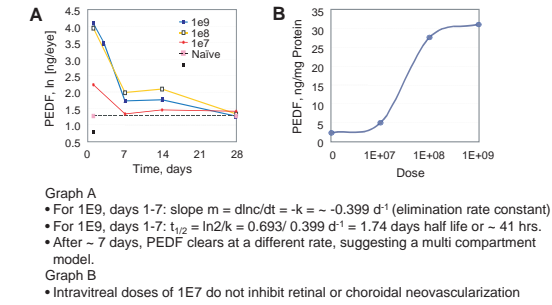
**Intravitreal Administration of AdPEDF.11D**



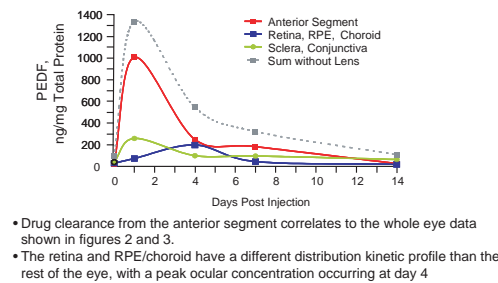
**Figure 2.** Expression of hPEDF Following Intravitreal Delivery of AdPEDF.11D



**Figure 3.** Pharmacokinetics of hPEDF After a Single Dose of AdPEDF.11D



**Figure 4.** Sublocalization of hPEDF Following Intravitreal Delivery of 1e9 pu of AdPEDF.11D



## References:

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## Conclusions:

- Intravitreal delivery of 1e9 particle units (pu) of AdPEDF.11D results in measurable levels of PEDF protein for 14 days in whole eyes (with a peak ocular concentration at day one).
- Sublocalization studies show similar profiles for most ocular regions except the retina and RPE/choroid, which show a peak PEDF level at day 4. Although the retina and RPE/choroid values represent only a fraction of the whole eye number, it may be sufficient to achieve regression of ocular neovascularization as shown by Mori et al. (Invest Ophthalmol Vis Sci. 2002)
- The concentration versus time curve for whole eyes (figure 3) exhibit distinct exponential phases of different slopes suggesting a multi compartment kinetic profile, one for absorption (not graphed), one for distribution and one for elimination. This is also true for the sub-localization studies shown in figure 4.
- Further studies of the pharmacokinetics of the retina and RPE/choroid after delivery of AdPEDF.11D could prove very useful toward understanding the dose requirements and optimal delivery modality (intraocular vs. periocular) for functional activity of PEDF.