# Towards a stem cell-based intraocular drug delivery system

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**Objective:** A continuous and long-lasting intraocular delivery of neuroprotective factors that attenuate the degeneration of photoreceptor cells is among the strategies aimed at developing mutation-independent treatments for hereditary retinal degenerations and other pathologies involving photoreceptor death. A continuous targeting of neuroprotective factors to the retina might be achieved by intraocular transplantations of genetically engineered stem cells. With the aim to establish a highly controlled stem cell-based delivery system, we have generated a panel of novel bicistronic lentiviral vectors (designated LeGO vectors) to express neurotrophic factors in adherently cultivated murine neural stem (NS) cells and their differentiated progeny. Intravitreal transplantations of engineered stem cells into mouse models of hereditary retinal dystrophies were performed to evaluate the therapeutic potential of this stem cell-based delivery system.

**Methods:** The lentiviral vectors used in this study encode a CMV early enhancer/chicken ß-actin (CAG) hybrid promoter, a "gene of interest", and a fusion gene composed of different combinations of reporter and resistance genes downstream of an internal ribosome entry site. Lentiviral particles were generated according to standard procedures and used to transduce NS cells by spinoculation. Positive cells were selected by application of antibiotics, and engineered clonally derived NS cell lines were established by FACS. To coexpress two or more "genes of interest", NS cells were transduced with several vectors each encoding a different reporter/resistance fusion gene and subsequently selected by simultaneous application of the respective antibiotics. Expression of transgenes in transduced NS cell cultures was verified by immunocytochemistry and immunoblot analysis of culture supernatants. Intravitreal transplantations of modified NS cells into mouse models of hereditary retinal dystrophies were performed to study the efficacy of this stem cell-based delivery system.

**Results:** The novel lentiviral vectors facilitated rapid derivation of engineered NS cell cultures and clonally derived NS cell lines. Immunocytochemistry and immunoblot analysis confirmed the expression of neuroprotective factors and their secretion into the culture supernatant. Coexpression of transgenes in NS cells was also successfully achieved, as indicated by the expression of two or more reporter genes in the same cell population. The expression of reporter genes allowed to track the intravitreally grafted NS cells in the host eyes, where they survived and expressed the transgenes for several months. When NS cells with forced expression of neuroprotective factors were grafted into the vitreous space of mouse models of hereditary retinal dystrophies, degeneration of photoreceptor cells was significantly attenuated. The neuroprotective effect was significantly increased when clonally derived NS cell lines selected for high expression levels of transgenes were used for transplantations.

**Conclusions:** We have developed a neural stem cell-based intraocular delivery system that enables us to target, in a highly controlled manner, functionally relevant quantities of neuroprotective factors to the murine retina. This stem cell-based delivery system will be of use to evaluate the therapeutic potential of known and novel neuroprotective factors, as well as factor combinations, in mouse models of photoreceptor degeneration.