Project Title: Reduction of Corneal Scarring Following Blast and Burn Injuries to Cornea Using siRNAs Targeting TGFβ and CTGF

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Background: Blast and burn injuries to the eye caused by explosions during combat operations or terrorist attacks are devastating injuries, and in eyes that can be saved, the major causes of vision impairment are excessive corneal scarring and neovascularization. This proposal will develop new drugs that use RNA-interference (RNAi) to reduce vision impairment following corneal injuries by accelerating corneal re-epithelialization, reducing formation of corneal scar (corneal haze), and reducing corneal neovascularization.

Hypothesis/Objective: Prolonged, elevated levels of transforming growth factor beta (TGFβ) and connective tissue growth factor (CTGF) in blast and burn wounds of the cornea cause excessive scarring that impairs vision. Our objective is to develop new drugs utilizing small interfering RNA (siRNA) technologies that will selectively reduce the level of TGFβ, TGFβ receptor (TGFβRII) and CTGF in these types of corneal wounds, and thereby, reduce vision loss. siRNAs are the most potent and selective of all gene-targeted, oligonucleotide-based drug approaches (better than ribozymes, antisense oligonucleotides (ASO), or microRNAs). Data from numerous laboratories, including our lab, and several clinical studies clearly demonstrate that TGFβ (isoforms 1&2) and CTGF regulate key phases of normal wound healing in tissues throughout the body, including the cornea. TGFβ is a potent chemo attractant for inflammatory cells and it stimulates synthesis of CTGF. TGFβ and CTGF are the most potent stimulators of collagen synthesis and transformation of fibroblasts into myofibroblasts, which are the two processes that scatter light and produce corneal haze. Furthermore, CTGF mediates the action of TGFβ on collagen synthesis and myofibroblast transformation. TGFβ also inhibits proliferation and migration of epithelial cells, which slows the rate of epithelial wound healing. In hypertrophic scarring in skin wounds, prolonged, elevated levels of TGFβ and CTGF cause excessive formation of disorganized scar tissue in skin. Similarly, researchers reported that injections of TGFβ into rabbit glaucoma surgery sites rapidly caused fibrosis of conjunctival tissue. Scientists also reported that injections of ASO targeting TGFβ or CTGF mRNA into rabbit glaucoma wounds or into ear skin wounds significantly reduced the amount of hypertrophic scar tissue. Repeated dosing with eye drops of neutralizing antibody to TGFβ reduced corneal scaring in a rabbit model, although the final effect was limited by the poor penetration of antibodies through corneal epithelial cells. These studies demonstrate that selectively reducing TGFβ or CTGF levels in wounds will reduce formation of scar in several tissues.

Specific Aims:
1. Design and evaluate siRNAs oligonucleotides that selectively target TGFβ, TGFβRII, and CTGF mRNA in cultures of human corneal fibroblasts and epithelial cells.
2. Evaluate the two most effective siRNA oligonucleotides for each TGFβ, TGFβRII, and CTGF in rabbit models of blast and burn corneal injuries for reduction of corneal scarring (haze).
3. Compare the effectiveness of siRNAs oligonucleotides and AAV-vectored siRNAs in rabbit models of blast and burn corneal injuries for reduction of corneal scarring (haze).

Study Design: The first phase of the research program will utilize in vitro cell culture techniques to identify the siRNA oligonucleotides sequences that most potently and selectively reduce TGFβ and CTGF expression in corneal stromal fibroblasts and epithelial cells. The second phase will progress to in vivo rabbit models of acute blast and burn injuries to the cornea and will assess both chemically stabilized siRNA oligonucleotides and AAV-vectored siRNAs.

Relevance: Development of a siRNA drug that reduces vision loss caused by corneal scarring and neovascularization following blast and burn injury will directly address a critical need area identified in the Program Announcement and have tremendous application in civilian patients who have corneal injuries caused by similar types of trauma.