According to the Alliance for Eye and Vision Research, 16% of the injuries sustained by soldiers in the recent Iraq and Afghanistan operations affect the eyes. This amounts to nearly 58,000 soldiers who have sustained significant eye injuries. Importantly, only 20% of soldiers with eye injuries return to active service. Of the soldiers that sustain a traumatic brain injury, 75% also suffer from vision problems. Taken together, vision loss due to traumatic injuries is a major cause of disability in our veterans.

A portion of these injured soldiers permanently lose their vision because of damage to the retina; the light-sensing tissue that lines the back of the eye. The retina contains two types of light-sensing cells, rods and cones. Rod photoreceptors are responsible for low-light (nighttime) vision. Cone photoreceptors are 20 times less abundant than rods, yet they are much more important in our daily lives. This is because cones mediate color and high-acuity vision. Loss of cones is devastating, as these cells are needed for everyday tasks like reading, driving, and face recognition. Unfortunately, our retinas do not have an inherent capacity to repair themselves. Once the cone photoreceptors are lost to damage/injuries, they do not regenerate. There are currently no treatments that can restore sight in these people. Regenerative medicine approaches seek to restore vision by transplanting new cone photoreceptors generated from unlimited stem cell sources into the damaged eye. Proof-of-concept animal model studies have shown that this "cell replacement therapy" is safe, but not very efficient. This is mostly due to our inability to create a large enough number of cones in culture. To reach our goal of restoring high-acuity cone vision in injured service men and women, we must first learn how to efficiently program stem cells into cone photoreceptors.

We and others have shown that exposing human embryonic stem cells to the same signals that initiate eye development forces them to become retinal cells at a high rate. We hypothesize that these retinal stem cells can be further programmed into cones if they are exposed to the same factors that normally promote cone development. This hypothesis has been difficult to test because the genes that control cone development are poorly understood. Recently, some important genes have been characterized. Otx2 is the earliest known gene made by cones. However, it is not cone-specific as Otx2 is also expressed by rods and some other retinal neurons. Onecut1 and Blimp1 are other recently identified genes present in cone photoreceptors. We recently showed that as cones develop, these three genes are expressed sequentially (Otx2-Onecut1-Blimp1). While each of these three genes plays a unique role in cone development, none of them can promote cone formation on their own.

In the first aim of our proposal, we will conduct next-generation sequencing on developing mouse retinas to identify new genes that play a role in cone development. We will then rapidly evaluate how these genes control Otx2 expression and cone formation by either forcing or removing their expression in developing retinas. This will reveal whether these genes control the fraction of retinal stem cells that become...
cones. In the second aim of this proposal, we will test whether forcing cultured human retinal stem cells to express OTX2, BLIMP1, and ONECUT1 either together or in specific sequences will efficiently program them into cones. We will also test whether newly identified genes from the next-generation sequencing analysis (above) can promote cone formation in the retinal stem cell cultures. These stem cell-derived cones will then be transplanted into the eyes of mice to gauge their ability to successfully incorporate into the retina. These experiments will show whether briefly exposing human retinal stem cells to a combination of photoreceptor genes is sufficient to create a large population of cones that can be successfully transplanted into the eye.

By discovering how to program retinal stem cells into transplantable cones, we will greatly improve the chances that cone cell replacement can soon become a viable therapy. Restoring high-acuity vision in these injured veterans will substantially improve the rate at which they return to active duty or enter the private work force. It will also significantly lower the long-term costs of caring for these disabled veterans. Perhaps most importantly, reversing vision loss will profoundly improve the quality of life of both our wounded soldiers and their families.