Choroideraemia trial shows promise and challenges of viral gene replacement approach

by Howard Larkin in Milan

A clinical trial of gene therapy for choroideraemia now under way in the UK provides an opportunity for ophthalmologists to contribute to what could be a revolution in treating genetic retinal conditions, Robert E MacLaren, DPhil, FRCS, FRCOphth, of Oxford University, Oxford Eye Hospital and Moorfields Eye Hospital, London, UK, told the 12th EURETINA Congress.

Viral-vector gene therapy holds the promise of curing life-long genetic retinal disorders with a single injection of replacement genetic material, Prof MacLaren said. The treatment he developed with Prof Miguel Seabra, Imperial College, London, is the first for choroideraemia, an x chromosome-linked degenerative retinal disease leading to progressive vision loss throughout life.

But many challenges remain, Prof MacLaren noted. These include identifying quantifiable treatment endpoints, and developing safe and effective surgical techniques for injecting viral vectors into delicate retinal structures.

Gene therapy “has reached a stage where, as vitreoretinal surgeons, we need to look at how to use it effectively and improve the techniques of gene delivery,” Prof MacLaren said.

In addition to testing the safety and efficacy of viral-vector approach, the multicentre trial is also designed to involve many ophthalmologists in the technology and objectively assessing outcomes and delivery techniques.

**Ideal candidate**

Prof Frans Cremers originally established that choroideraemia results from a genetic defect in which the Rab escort protein 1 is missing and Prof Seabra identified Rep1 as a chaperone for Rab27, which prenylates proteins involved in the movement of melanosomes within cells. This prenylation is essential to retinal pigment epithelium health and homeostasis. Without Rep1, the retinal pigment epithelium cannot function properly.

While choroideraemia is relatively rare, afflicting about one in 50,000 in northern Europe with the highest incidence in Finland, it is a promising disease entity for gene therapy for several reasons, Prof MacLaren noted.

For one, the choroideraemia phenotype is specific and instantly recognisable, whereas the appearance of the retina in other types of retinitis pigmentosa is similar regardless of the gene involved. This makes possible early identification of choroideraemia patients. Also, the gene involved, CHM, is 1.9 kilobases long, making it an ideal size for delivery by adeno-associated viral vectors, which have been shown safe and effective for retinal applications, Prof MacLaren said.

In addition, the condition typically progresses slowly, from early night vision loss to progressive peripheral vision loss with central vision often maintained until middle age or later, Prof MacLaren added.

Gradual and symmetrical degeneration in both eyes makes it possible to establish a measurable structural endpoint, Prof MacLaren said. Using auto fluorescence, the surviving area of the retina can be seen to shrink by about 10 per cent annually in late disease. Beyond demonstrating safety, the current trial seeks to document a slowing of shrinkage in treated eyes over two years.

Functional retina over the area of the surviving retina also has been plotted using microperimetry, which may provide a measurable functional outcome, Prof MacLaren said. Choroideraemia patients with a normal functional distribution typically have sensitivity which is below age group norms.

“One might expect that following gene transfer, we may see some functional retina pushed back to normal sensitivity.”

Biochemical evidence suggests that Rep1 functions primarily in the retinal pigment epithelium, rather than the choroid. Retinal appearance also suggests that most degeneration begins in the RPE with choroid loss secondary, Prof MacLaren said. Also, the gene affects the RPE, but not the choroid, in female carriers.

The RPE is the easiest layer to target with adeno-associated vectors, Prof MacLaren said. The treatment uses adeno-associated virus type 2 with the wild genome replaced with the Rep1 coding sequence and regulatory sequences to maximise efficiency of the viral vector. These include a CBA promoter, which switches genes on and off in cells, and a woodchuck hepatitis post-translational regulator, which works with messenger RNA to enhance expression by directing RNA out of the cell. “This is a new development to use an additional viral sequence from another virus to augment expression,” Prof MacLaren said. For safety reasons, regulatory bodies required that no protein other than Rep1 is translated by the woodchuck sequence.

The viral vector is delivered to the subretinal space, where it infects photoreceptors and the RPE. The trial involves a low dose of 1x1010 in the first six patients and a large dose of 1x1011 in six more, all given in a 0.1ml suspension.

Reaching the subretinal space requires detaching the retina, which is accomplished in a separate step by injecting balanced salt solution in as many operations as necessary before injecting the gene therapy. Prof MacLaren was initially concerned about patients’ ability to recover from the detached retina, but it has not been an issue so far. One patient with 20/60 vision and retinal oedema one day after injection recovered to 20/20 at five weeks, he said.

“Because we detach the fovea in patients with relatively good vision, we need to be sure there is no toxic effect of the viral vector.”

The operation involves a 23-gauge vitrectomy, and the therapy is delivered via a 23-gauge cannula with a 41-gauge Teflon tip. The internal limiting membrane must be visualised, using dye if necessary, and avoided. It is very difficult to insert the cannula through the ILM. The fovea also must be avoided to prevent macular holes, Prof MacLaren said.

The first patient in the trial was recruited in October, 2011. “We will report results in due course,” Prof MacLaren said.