

**rAAV Gene Therapy for Neovascular Age-Related Macular Degeneration:
One year follow up of a Phase 1 randomised Clinical Trial**

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ABSTRACT

Background: Neovascular or wet AMD causes central vision loss and represents a major health problem among the elderly. Wet AMD is currently treated with frequent intraocular injections of anti-VEGF protein, which is a significant burden. Gene therapy may enable long-term anti-VEGF therapy from a single treatment. We report results of a Phase 1 trial with rAAV.sFLT-1 to treat wet AMD with a single subretinal (SR) injection.

Methods: Eight patients with wet AMD were randomized by sequential group assignment to receive 1E10 “low” vector genomes (vg) (n=3) or 1E11 “high” vg (n=3) of rAAV.sFLT-1, or a control regimen (n=2). All patients were unmasked, received ranibizumab at Baseline and Week 4, and rescue treatment during follow up based on pre-specified criteria including best-corrected visual acuity (BCVA) measured on the Early Treatment Diabetic Retinopathy Study (EDTRS) scale, optical coherence tomography, and fluorescein angiography. The primary endpoint was ocular and systemic safety. Secondary endpoints, assessed on low and high dose groups combined, included the requirement for rescue therapy, BCVA and centre point thickness (CPT).

Findings: SR injection of rAAV.sFLT-1 was highly reproducible. No drug-related adverse events were observed; procedure-related adverse events were generally mild and self-resolving. There was no evidence of chorioretinal atrophy. Clinical laboratory assessments generally remained unchanged from Baseline. Four of six (67%) Treatment group patients required zero rescue injections, and the other two (33%) required only one rescue injection. Median BCVA at Baseline was 40 EDTRS letters (range 28-56), and improved to 49 EDTRS letters (range 40-64) at Week 52. The median CPT was 549 μm at Baseline (range 193-1094 μm), decreasing to 311 μm (range 236-597 μm) at Week 52.

Interpretation: In this phase 1 study, rAAV.sFLT-1 was safe and well tolerated. A decreased need for anti-VEGF treatment and the maintenance of BCVA were observed.

These results support ocular gene therapy as a potential long-term treatment option for wet AMD.

Trial Registration: NCT01494805; <https://register.clinicaltrials.gov/>

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INTRODUCTION

Age-related macular degeneration (AMD) is the most common cause of visual impairment in the developed world.¹ The neovascular or wet form of the disease is characterized by abnormal choroidal blood vessel growth beneath the macula, which is responsible for high-resolution vision.² Wet AMD, leads to rapid vision loss and, when left untreated, central blindness.³ Excessive secretion of vascular endothelial growth factor (VEGF) plays a central role in promoting neovascularization in human wet AMD.^{4, 5} In addition, downregulation of anti-angiogenic factors, such as the naturally-occurring VEGF-blockers sFLT-1⁶ and pigment epithelium derived factor (PEDF),⁷ may also promote wet AMD, contributing to an imbalance between pro-angiogenic and anti-angiogenic factors potentially underlying the pathophysiology of neovascularization.⁸

VEGF inhibitors, including pegaptanib sodium,⁹ ranibizumab,^{10, 11} and aflibercept¹² are clinically useful for slowing disease progression and, in some cases, improving vision. However, such therapies must be administered frequently via intravitreal injection (IVI); treatment intervals greater than every 4-8 weeks may result in rapid vision decline.¹²⁻¹⁷ When given pro re nata (PRN) or as needed according to a flexible dosing regimen, patients required seven or more injections per year.^{14, 18} Frequent IVI are associated with increased cumulative risk of vision threatening adverse events, including endophthalmitis,^{19, 20} and intraocular pressure (IOP) elevation,²¹⁻²³ and may increase the risk of arterial thromboembolic events.^{24, 25} In addition, the treatment regimen places a substantial burden on patients, physicians, and the healthcare system,²⁶ and patients are systematically undertreated due to difficulties with compliance.^{27, 28}

Intraocular gene therapy has the potential to enable long-term, stable delivery of therapeutic proteins to treat retinal diseases.²⁹ Previous studies have demonstrated the use of recombinant adeno-associated vectors (rAAV) to treat Leber's Congenital Amaurosis (LCA)³⁰⁻³² and choroideremia,³³ two rare congenital disorders characterized by loss-of-function mutations in intracellular proteins. To treat wet AMD, we developed a strategy to transduce retinal cells with rAAV encoding sFLT-1, a highly potent ($K_d \approx 10$ pM), naturally-occurring VEGF inhibitor,³⁴ thus creating a "biofactory" to locally deliver therapeutic levels of anti-VEGF to the macula.

rAAV.sFLT-1 is administered via subretinal (SR) injection, which places the vector in direct contact with retinal pigment epithelial (RPE) cells and photoreceptors of the outer retina. Although other approaches involve intravitreal administration of rAAV vectors (e.g., rAAV2-sFLT01,^{35, 36} registered with clinicaltrials.gov as identifier NCT01024998), we chose SR injection of rAAV because of the highly efficient expression and more limited biodistribution.³⁷⁻³⁹ The protein that is produced, sFLT-1, is the soluble form of the VEGFR1 receptor, which binds and inactivates VEGF-A, VEGF-B, and PlGF through its domain 2, the same domain used by the currently marketed therapy aflibercept.⁶ We previously demonstrated that rAAV.sFLT-1 enables long-term sFLT-1 secretion⁴⁰ and is safe and efficacious in animal models of retinal and choroidal neovascularization (CNV).⁴¹⁻⁴³ This study reports the first-in-human results with rAAV.sFLT-1 in a clinical trial for wet AMD, and is the first example of rAAV-mediated gene therapy to treat a highly prevalent condition.

MATERIALS AND METHODS

Study Design

This single centre, randomized, clinical trial investigates the safety and efficacy of two different dose levels of a rAAV vector of serotype 2 encoding sFLT-1 in patients with wet AMD. Step one randomized three patients to low dose (LD) rAAV.sFLT-1 (1E10 vector genomes, or vg) and one patient to the Control (untreated) arm (Figure 1A). After allowing eight weeks to observe any adverse effects from the LD rAAV.sFLT-1 (1E10), step two randomized an additional three patients to treatment with high dose (HD) rAAV.sFLT-1 (1E11 vg) and one patient to the Control arm. Unequal randomization of patients to the study groups was implemented primarily to counteract selection bias during study execution, since this phase 1 trial was not powered to detect statistically significant differences in outcome measures between the groups. Randomization was accomplished by sequential study group assignment according to a randomization list generated prior to the study and held off-site. Patients and procedure staff were not masked to treatment received. Staff performing the assessments was masked to the study group of patients at study visits. The purpose of this study was to address the safety of the rAAV.sFLT-1 gene therapy treatment and to provide a set of human treatment results from which estimates of efficacy can then form the hypothesis of a second larger study. Therefore no sample size calculations were performed.

Eligible patients underwent an informed consent process and were enrolled in the study. All patients received 0.5 mg ranibizumab IVI at Baseline (Day 0) and Week 4 (Figure 1B). On study Day 7 (Week 1 visit), patients randomized to HD or LD received a 100 µL SR injection of the appropriate dose of rAAV.sFLT-1. An 8-week observation period was used between LD and HD treatments to assess dose dependent safety. This period of time provided an adequate window to allow time for the start of sFLT-1 protein expression and for any

potential early immune response to develop. Beginning at Week 8, subsequent study visits occurred every four weeks through to Week 52 (total 12 visits). During the follow-up period, patients were permitted retreatment with ranibizumab according to pre-specified criteria based on best corrected visual acuity (BCVA), spectral domain optical coherence tomography (SD-OCT) and fluorescein angiography (FA). Laboratory tests included haematology, renal and hepatic function, electrolytes, urine protein and IgM, IgG, IgA and lymphocyte subset analysis. In addition, assays for AAV vector shedding, anti-AAV antibodies, neutralizing antibodies, and cellular immunity were also performed (see Supplementary Methods). Adverse event information was solicited from patients at each visit.

The protocol was approved by the Australian Therapeutic Goods Administration. Appropriate approvals were obtained from The University of Western Australia Institutional Biosafety Committee and Sir Charles Gairdner Hospital (SCGH) Human Ethics Committee. The trial was performed at the Lions Eye Institute (LEI) in Perth, Australia. Throughout the trial the tenets of the Declaration of Helsinki were followed. The trial was registered with ClinicalTrials.gov under NCT01494805.

Study Population

Patients were enrolled under Clinical Protocol 2008-135 version 1.4. Patients were selected from those seen at the LEI and SCGH who were aged 65 or above, of either gender, and had been diagnosed with wet AMD meeting the inclusion criteria. Eligibility criteria included AMD secondary to active subfoveal CNV as evidenced by leakage of FA and fluid on OCT, with BCVA of 3/60 – 6/24 and 6/60 better in the other eye. Patients with previous anti-VEGF therapy were not excluded, and there was no washout period for anti-VEGF therapy prior to the Baseline visit.

Study Treatments

In all patients randomized to receive rAAV.sFLT-1, the SR administration procedure was initiated with a standard 23 gauge 3-port pars plana approach. A core vitrectomy and induction of posterior vitreous separation from the optic nerve head (if not already present) was performed to prevent potential complications. LD or HD rAAV.sFLT-1 (see Supplementary Methods) was delivered into the SR space via 41G Cannula (23G/0.6 mm, DORC International B.V., Zuidaland, The Netherlands) in a volume of 100 μ L. The injection site was chosen to avoid detachment of the fovea. All gene therapy patients received topical eye drops of Prednefrin[®] Forte and Chlorsig[®] four times per day post-operatively for three days in the study eye. No other anti-inflammatory or immunosuppressive medications were used.

Rescue therapy with ranibizumab was given when active CNV was detected, as measured by: 1) >10 letter loss on Early Treatment Diabetic Retinopathy Study (ETDRS) scale, or >5 letter loss on ETDRS scale in conjunction with patient perception of functional loss, compared to the previous visit and attributable to retinal causes; 2) CNV-related increased sub-RPE fluid; or any increased, new, or persistent subsensory or intraretinal fluid on SD-OCT; or 3) signs of increased CNV leakage on FA.

Assessments

The primary endpoint was ocular and systemic safety. Ocular safety was monitored at each monthly visit with BCVA, IOP, slit lamp biomicroscopy, indirect ophthalmoscopy, and SD-OCT according to the schedule of assessments, with Baseline visit considered as Week 0 and day of surgery as Week 1. Signs of visual loss, infection, inflammation, and other safety events including cataract formation and retinal detachment were closely monitored. Patient

safety was monitored by periodic physical examinations, vital sign assessment, and routine clinical laboratory testing (complete blood count, comprehensive metabolic panel, lipid panel, and serum electrophoresis measurement). Study data and adverse events were monitored by a data safety monitoring committee with expertise in retinal diseases and gene therapy vectors. Secondary endpoints, assessed on LD and HD groups combined, included the requirement for rescue therapy, BCVA, and centre point thickness (CPT). SD-OCT was performed using the HRA2 (Heidelberg Engineering, Heidelberg, Germany). Images were acquired with raster-scanning of 37 sections of the central portion of the retina and stored for later analysis. The Heidelberg SD-OCT used measures both CPT and surrounding 1mm subfield thickness automatically. An earlier study⁴⁴ found a reasonable correlation between the two. Since CPT better represents foveal function and BCVA was an important secondary endpoint, we manually confirmed the central foveal slice for re-measurement on each monthly visit.

RESULTS

Eight patients meeting the inclusion criteria were randomized (Treatment group: 1,2 and 4 LD; 5,6 and 8 HD; Controls:3 and 5) (Supplementary Table S1). The Treatment group median age was 82 years (Range 74-86 years), and all Treatment group patients had a confirmed diagnosis of wet AMD with median of 20.5 prior anti-VEGF injections (range 3-29, Supplementary Table S1). Since study eyes had been previously treated, lesion classification into categories such as occult or classic was not performed. The median Treatment group BCVA at Baseline was 40 ETDRS letters (range 28-56) and their median Baseline CPT was 549 μm (range 193-1094 μm).

SR injection was successfully performed in all six rAAV.sFLT-1 treated patients, and was confirmed by direct visualization. The investigator made a determination intraoperatively of the safest location for the SR injection, considering accessibility and other factors. The procedure was well tolerated in all treated patients, and the SR bleb was absorbed within 24 hours (Figure S1). No ocular or systemic adverse events attributed to rAAV.sFLT-1 were observed. Multiple assessments up to and including Week 52 showed no evidence of ocular inflammation in the anterior segment or posterior segment and no significant IOP elevation, or retinal detachment. OCT scans at Baseline and at Week 52 show that for each patient retinal thickness either visibly improved (Figure 2: patients 1, 2, 6, and 8) or remained stable (Figure 2: patients 4 and 5).

No cardiovascular or other systemic adverse events were observed. Adverse events related to the study procedures were noted, including subconjunctival haemorrhage (3/6 patients), subretinal haemorrhage at the injection site (1/6), and mild cell debris in the anterior vitreous (1/6); these adverse events were minor and transient, and did not impact vision. Of the six rAAV.sFLT-1 treated patients, two were phakic with early-stage cataracts and the remaining four were pseudophakic at Baseline. The two phakic patients developed progression of nuclear cataracts, as expected following vitrectomy in this age group. Cataracts were removed at Week 44 (Patient 1) and Week 36 (Patient 4) post-injection, with no discernible effect on the macula. Prior to cataract removal, Patient 1 improved from 33 ETDRS letters to 40 letters, then due to cataract progression decreased to 29 letters; following cataract removal, Patient 1 returned to 40 letters at Week 52 (Figure 3). Similarly, Patient 4 improved from 46 letters to 57 letters, then due to cataract progression decreased to 45 letters; following cataract removal, Patient 4 improved to 58 letters (Figure 3). Therefore cataract removal restored patients to their full visual potential, but was not solely responsible for the vision gain observed during

the course of the trial. A number of other adverse events, including two serious adverse events (confusion post urinary tract infection and sinus bradycardia) were deemed unrelated to gene therapy or study procedures.

Vector shedding was assayed, both for vector DNA and viral capsid proteins, in samples of serum, urine, saliva, and tears (from both study and fellow eyes). The rAAV.sFLT-1 vector DNA copy number was assessed by quantitative PCR (qPCR) and detected only in tear specimens collected 1 day post-injection from the injected eye of Patients 1 and 5 (Supplementary Table S2). The vector was undetectable by the next time-point at Week 3 and no other qPCR samples were positive. AAV capsid ELISA did not reveal any evidence of capsid proteins (data not shown). Serum VEGF levels, measured by ELISA, remained unchanged over the course of the study. The median circulating VEGF level in the Treatment group at Baseline was 296 pg/mL (range 169-471 pg/mL) and 267 pg/mL (range 73-541 pg/mL) at Week 52.

One patient in the Treatment group (Patient 2) tested positive at Baseline for neutralizing antibodies against AAV2 (Table 1). In 5 of 6 Treatment group patients there were no changes in anti-AAV2 antibody titres. In Patient 6, there was an increase in anti-AAV2 neutralizing antibodies at the Week 3 visit; neutralizing antibodies remained elevated compared to Baseline, but were not associated with clinical observations related to safety or efficacy. The frequency of AAV-specific T cells was determined by IFN- γ ELISpot assay.⁴⁵ Whereas Patient 4 showed a transient elevation at Week 4, reactive T cells in the other patients did not exceed the specificity threshold, or were not significantly increased compared to Baseline (See Supplementary Results and Supplementary Figure S2).

Prior to enrolment, the majority of patients required extensive treatment with anti-VEGF (Supplementary Table S1). During the 52 week follow-up, each patient was examined monthly for need to rescue with ranibizumab. Pre-specified rescue criteria were based on BCVA, OCT, and FA as evaluated by personnel masked to the patient's Treatment group assignment. Of the six patients in the Treatment group, four required no ranibizumab injections and the other two required a single injection each (Table S3). Thus, the Treatment group had two injections in 6 patients over the course of a year, for an annualized per patient average of 0.33 rescue injection.

The Week 52 assessment in the rAAV.sFLT-1 treated patients showed no evidence of vision loss, retinal or pigment epithelial atrophy in the paramacular area of the bleb site, and no evidence of retinal thinning when assessed by OCT. The median CPT was 549 μm at Baseline (range 193-1094 μm), decreasing to 311 μm (range 236-597 μm) at Week 52 (Supplementary Figure S3). FA assessment showed no recurrence of leakage during the year in any of the rAAV.sFLT-1 treated patients.

BCVA scores improved in five of the six patients treated with rAAV.sFLT-1 compared to the Baseline visit, with 3/6 gaining ≥ 10 letters and 1/6 gaining ≥ 15 letters (Table 2 and Figure 3). Patient 5 had fibrovascular scar tissue that included the subfoveal region at Baseline. This patient improved anatomically, recovered BCVA to Baseline levels at Week 52, remained fluid-free on SD-OCT, and had decreased CNV leakage on FA. However, the retinal health at the fovea may have limited the patient's ability to improve in visual acuity. The BCVA in the Treatment group improved from a median of 40 EDTRS letters (range 28-56) at Baseline to 49 EDTRS letters (range 40-64) at Week 52.

DISCUSSION

Previous studies with rAAV vectors have shown promising results in children and young adults with LCA³⁰⁻³² and choroideremia.³³ Evidence from these studies suggests that SR rAAV is well tolerated, has the capability to provide multi-year benefit following a single treatment, and can be safely re-administered to the fellow eye. This study extends the application of rAAV-mediated gene therapy to wet AMD, a major and complex retinal disease affecting elderly patients and representing a major public health concern. The therapeutic strategy employed expression of an endogenously secreted VEGF-inhibitor, sFLT-1, to neutralize the detrimental effect of VEGF. A major advantage to this strategy may be greatly extended therapeutic benefit following a single intervention, and elimination of the bolus-decay pharmacokinetic profile of current IVI strategies. This, in turn, should reduce morbidity from repeated IVI, reduce systemic safety concerns at peak levels and ocular efficacy concerns at trough levels, and, ultimately, reduce treatment burden on patients, physicians, and the healthcare system.

This phase 1 study in six patients treated with rAAV.sFLT-1 supports the safety and tolerability of SR rAAV for the treatment of wet AMD. As seen here, SR injection resulted in a temporary partial detachment of the retina at the site of injection that healed within a day in all patients. The safety data reported here are consistent with previous reports using rAAV for gene therapy in the retina.

As reported in other studies,³⁰⁻³² only transient, mild intraocular inflammation following surgery was observed and no dose limiting toxicities were encountered. Also consistent with previous studies, the presence of vector was limited to the injected eye one day post-treatment. Similarly, the immune response observed was limited, with no clear relationship to

any clinical observations. The immunological safety of rAAV vectors observed here is reinforced by the recent report of lack of an immune response following a second administration of rAAV vectors to a contralateral eye,³¹ where the potential for significant immune responses based on previous exposure might be enhanced. This study provides additional reassurance for the positive immunological safety profile of rAAV as a gene therapy vector.

While an association between monthly anti-VEGF treatment and geographic atrophy (GA) has been suggested by retrospective analysis of data from large anti-VEGF trials such as IVAN, CATT, HARBOR⁴⁶⁻⁴⁹ there is no evidence that this is caused by anti-VEGF therapy as opposed to natural AMD progression or potential artefact^{47, 50}, and no study to date has formally evaluated this relationship. Importantly, the best visual outcomes in these studies, as well as several other studies of alternative dosing regimens,¹²⁻¹⁷ have consistently been achieved with monthly anti-VEGF therapy, and GA growth is not accelerated with monthly therapy compared to PRN therapy⁵¹ or the natural history seen in AMD patients.⁵² Concerns have been raised regarding systemic effects of anti-VEGF agents on the cardiovascular system following intraocular administration.²⁴ In this study, however, we did not detect any change in circulating levels of VEGF in Treatment group patients, suggesting that SR rAAV.sFLT-1 does not influence VEGF levels systemically. Together, the safety data in this phase 1 study support further investigation of rAAV.sFLT-1 as a potential long-term therapy for wet AMD.

We also observed encouraging preliminary signs of improved clinical outcome measures. During the 1-year follow up period, four of six treated patients (67%) did not require any anti-VEGF rescue injections and two of six (33%) required only one anti-VEGF rescue

injection, for an annualized average of 0.33 injection per patient. Although there are limitations of cross-trial comparisons, it is instructive to view these data in light of the large body of data from patients on anti-VEGF therapy, including the CATT study. In these published studies patients treated with ranibizumab under PRN, regimen using rescue criteria similar to those used in this study, required frequent injections. Number of rescue injections was consistent irrespective of whether patients were treatment naïve (6.9 injections), were treated PRN during the first year (5.7 injections), or were treated during the first year with monthly injections (5.0 injections).⁴⁹ In rAAV.sFLT-1 treated patients, CPT, a highly sensitive measure of disease recurrence, improved and was maintained throughout the one-year follow-up period. CPT was selected as a practical secondary endpoint as it best describes the anatomical status of the area responsible for BCVA and also an acceptable correlation had been shown between the CPT and the subfield surrounding area in wet AMD.⁴⁴

Finally, BCVA was maintained or improved in all patients treated with rAAV.sFLT-1. In previous studies, such as the VIEW trial examining aflibercept given every 8 weeks, CPT had been shown to increase when anti-VEGF therapy was withheld beyond 4-week intervals.¹² In the CATT study, when patients were switched from monthly to PRN dosing CPT increased by 31 μm and visual acuity declined by an average of 1.8 letters.⁴⁹ Collectively, our data are consistent with prior observations of rAAV.sFLT-1 in animal models, including long-term expression and therapeutic effect,^{41, 43} as well as data from other human and animal studies investigating subretinal rAAV.

Patients enrolled in this study were not treatment naïve, and had received a number of anti-VEGF injections prior to enrolment. There was no washout period, since the goal of the study was to evaluate patients who are currently under treatment with standard anti-VEGF agents.

Due to the chronic nature of wet AMD, patients rarely stabilize and require regular injections even after extensive regular treatment. In the CATT study, for example, patients who were treated with monthly injections for one year and then transitioned to PRN therapy still required 5.0 injections in their second year.⁴⁹ Thus, a transition from active CNV following a number of prior anti-VEGF injections to injection independence without vision decline could provide a powerful means to evaluate the effectiveness of anti-VEGF gene therapy.

This study was designed as a phase 1 study to assess the safety of the SR procedure and rAAV.sFLT-1 hence, it was not powered to draw definitive conclusions about differences in efficacy between groups. rAAV.sFLT-1 was well tolerated at both doses tested, and there were no meaningful safety differences detected between groups. Results were also similar between treatment groups in terms of CPT, BCVA and number of rescue treatments. Future dose-ranging studies will provide more information about the relative safety and efficacy between doses. Because of its design as an early stage phase 1 clinical trial, outcomes such as microperimetry and fundus autofluorescence were not collected, but will be collected in future trials. The small Control group did not receive sham treatment, which limited its application to data analysis. Conduct of this study at a single centre with all surgeries performed by the same surgeon also impacts the generalizability of the results. Future assessments beyond Week 52 will be needed so that conclusions regarding the stability of response can be made.

In summary, SR delivery of rAAV.sFLT-1 was safe and well-tolerated in this population of patients with wet AMD. The results of this study support the concept that ocular gene therapy may be a viable long-term treatment option for wet AMD and add to the growing body of evidence for the viability of intraocular gene therapy to treat retinal disease.

Role of the funding source

Avalanche Biotechnologies, Inc. covered the cost of surgery and patient screening and provided funding for some staff involved in recruitment and data collection and continues to provide funding for patient safety monitoring up to 3 years post-injection. Avalanche Biotechnologies, Inc. had no role in data analysis and interpretation and the corresponding author had full access to all the data in the study. Dr Chalberg from Avalanche Biotechnologies, Inc. a co-author, made some contributions towards the discussion without interfering with data interpretation. The first author, Prof Rakoczy, takes responsibility for the decision to publish the results.

Conflicts of interest:

EPR received funding from Avalanche Biotechnologies, Inc. during the conduct of the study; In addition, EPR has a patent pending and acts as the Chair of the Scientific Advisory Board for Avalanche Biotechnologies, Inc.

C-ML reports grants from National Health and Medical Research Council of Australia, other from Avalanche Biotechnologies, Inc., other from Richard Pearce Bequest, during the conduct of the study.

ALM reports personal fees and non-financial support from Avalanche Biotechnologies, Inc. during the conduct of the study.

MEW has nothing to disclose.

MAD-E has nothing to disclose.

MAF has nothing to disclose.

CMP has nothing to disclose.

SDS reports other from Avalanche Biotechnologies, Inc. during the conduct of the study.

MSB reports other from Avalanche Biotechnologies, Inc. other from Presbia, other from Oculeve Corporation, other from Digisight Corporation, outside the submitted work; In addition, Dr Blumenkranz has a patent on Ocular Gene Therapy using Avalanche-related transfection issued, and a patent on Ocular Gene Therapy using Avalanche-mediate transfection issued.

TWC reports personal fees from Avalanche Biotechnologies, Inc., outside the submitted work; In addition, Dr Chalberg has a patent on Treatment of AMD using AAV.sFLT-1 licensed to Avalanche Biotechnologies, Inc.

IJC reports funding from Avalanche Biotechnologies, Inc. during the conduct of the study; In addition, Prof Constable has a patent pending and Chairs the Avalanche Medical Advisory Board.

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Contributions of each author: Conceptual design: EPR; Study design: EPR, C-ML, MAD-E, SDS, MSB, TWC, IJC; Regulatory approval: EPR, C-ML, CMP, TWC, MAD-E, IJC; Data

collection: EPR, C-ML, ALM, CMP, MF, IJC; Surgery: IJC; Data analysis: EPR, C-ML, ALM, MEW, MAD-E, MF, IJC; Writing: EPR, C-ML, ALM, MEW, MAD-E, TWC, IJC

Panel: Research in context

Systematic review

A systematic review of the evidence published up to 24 July 2014 relevant to gene therapy of wet AMD in PubMed was executed. The following searches were conducted: clinical studies in which exudative AMD was the patient condition, gene therapy for diseases of the retina, and gene therapy for AMD. Articles considered excluded review articles, editorials, case studies, animal studies, and non-English language articles.

Reviewed wet AMD clinical trials covered a variety of therapies, including surgical, laser ablation, and, more recently, IVI of anti-VEGF therapies. Reports of gene therapies designed to treat eye disease have utilized a number of viral vectors, with rAAV vectors being the most common. No publications were identified that use a rAAV vector to treat wet AMD with a VEGF antagonist. A single report of a Phase I gene therapy clinical trial using an adenoviral vector designed to overexpress PEDF to treat exudative AMD was identified.⁵³ Patients experienced mild, transient intraocular inflammation and elevated IOP. In patients treated with IVI with less than 1E8 particle units of AdPEDF.11, decreases in visual acuity and increases in the size of CNV lesions were observed. The majority of patients treated with over 1E8 experienced no change to either their visual acuity or CNV lesion size.

Interpretation

Anti-VEGF therapy delivered IVI is standard of care for wet AMD. No other report of gene therapy using rAAV to deliver anti-VEGF therapy for wet AMD was identified. The results from the first year of this study confirm the excellent safety profile of rAAV delivered by SR and suggest efficacy for gene therapy to treat exudative AMD. This study represents an important next step toward gene therapy for chronic diseases of the eye.

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FIGURE LEGENDS

Figure 1. Patient Flow and Study Design

A. Trial profile. B. Study Design. BL-Baseline visit; SR Injection-subretinal injection; Ran IVI-Mandatory ranibizumab IVI; Ran IVI – According to Pre-Specified Rescue Criteria – ranibizumab rescue injection given if pre-specified re-treatment criteria were met, as judged by masked evaluators unaware of the patient’s treatment group. The Study Day 0 is at the BL visit. SR injection was performed 7 ± 1 days later. Patients were seen post-operatively on Day 8, Day 11-14 (Week 2 visit), and Day 20-24 (Week 3 visit). Week 4 and subsequent visits were designated in increments of weeks and occurred on 4-week intervals.

Figure 2: Comparative OCT Images of Treatment Group Patients

OCT images are shown for patients at the Baseline visit (BL) and the Week 52 visit. Location of each scan on the patient retina is shown.

Figure 3: Display of visual acuity over follow-up period. Visual acuity was assessed by measuring ETDRS letters at study visits for each patient. The median ETDRS letter at each time point for all 6 Treatment group patients. *: Patient 1 had a cataract removed from the study eye between the Week 44 and the Week 48 visits; ‡: Patient 4 had a cataract in the study eye removed one day before the Week 36 visit.

TABLES

Table 1: Analysis of Anti-AAV Antibodies

Patient ID		Scr/BL	Day 1	Week 3	Week 4	Week 8	Week 12	Week 16	Week 24	Week 36	Week 52
1	Neutralizing Ab†	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20
	Total Ab‡	0·03	0·29	0·36	0·62	0·42	0·59	0·46	0·15	0·14	0·14
2	Neutralizing Ab	1:20-1:100	1:20-1:100	1:20-1:100	1:20-1:100	1:20-1:100	1:20-1:100	1:20-1:100	1:20-1:100	1:20-1:100	1:20-1:100
	Total Ab	0·75	0·52	0·83	0·42	0·64	0·84	0·60	0·56	0·69	0·57
4	Neutralizing Ab	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20
	Total Ab	0·22	0·11	0·52	0·17	0·11	0·06	0·07	0·07	0·04	0·05
5	Neutralizing Ab	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20
	Total Ab	0·50	0·61	0·17	0·07	ND	0·26	0·30	0·14	0·21	0·55
6	Neutralizing Ab	<1:20	<1:20	1:20-1:100	1:20-1:100	ND	1:20-1:100	ND	1:20-1:100	1:20-1:100	1:20-1:100
	Total Ab	0·79	1·04	1·28	1·55	ND	1·69	ND	0·76	1·07	1·14
8	Neutralizing Ab	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20
	Total Ab	0·26	0·40	0·74	0·61	0·45	0·82	0·34	0·83	0·59	0·52

† Titre; ‡ Total Ab is total anti-AAV2 antibodies and values are presented as normalized absorbance at 450 nm;

Table 2: Best corrected visual acuity at Baseline and at Week 52

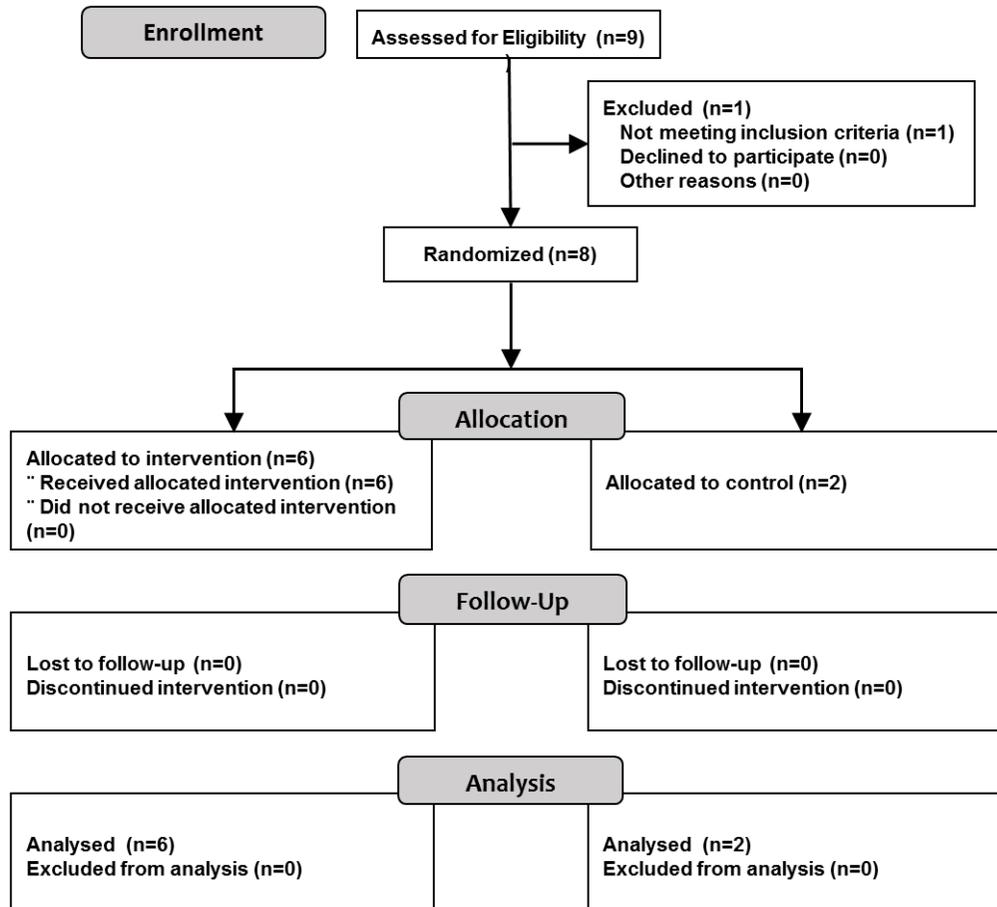
Patient ID	BCVA in ETDRS letters		
	Baseline	Week 52	Change
1	33	40	+7
2	28	41	+13
4	46	52	+6
5*	56	50	-6
6	54	64	+10
8	34	49	+15
Median (Range)	40 (28 - 56)	49.5 (40 - 64)	+8.5 (-6 - +15)

*Subfoveal fibrovascular scar

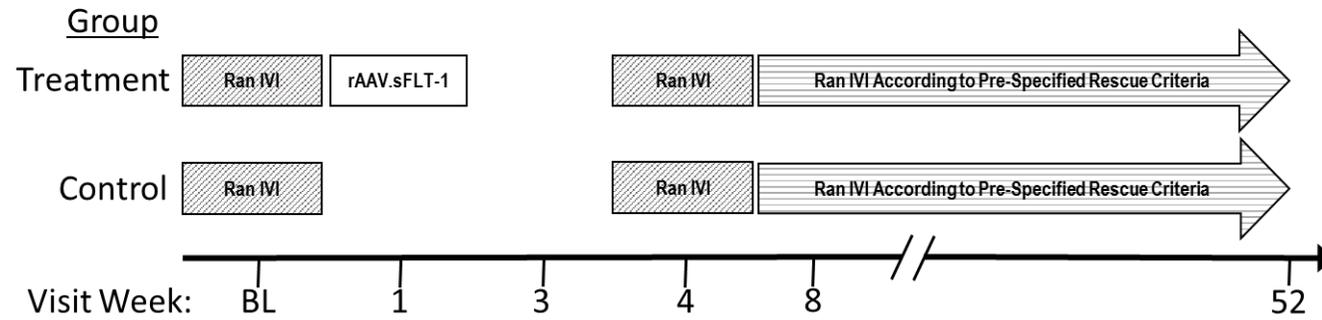
FIGURES

Figure 1: Study Design

A



B



BL-Baseline visit; SR Injection-subretinal injection; Ran IVI-Mandatory ranibizumab IVI; Ran IVI-ranibizumab IVI if disease progression detected based on pre-specified re-treatment criteria

Figure 2: Comparative OCT Images of Treatment Group Patients

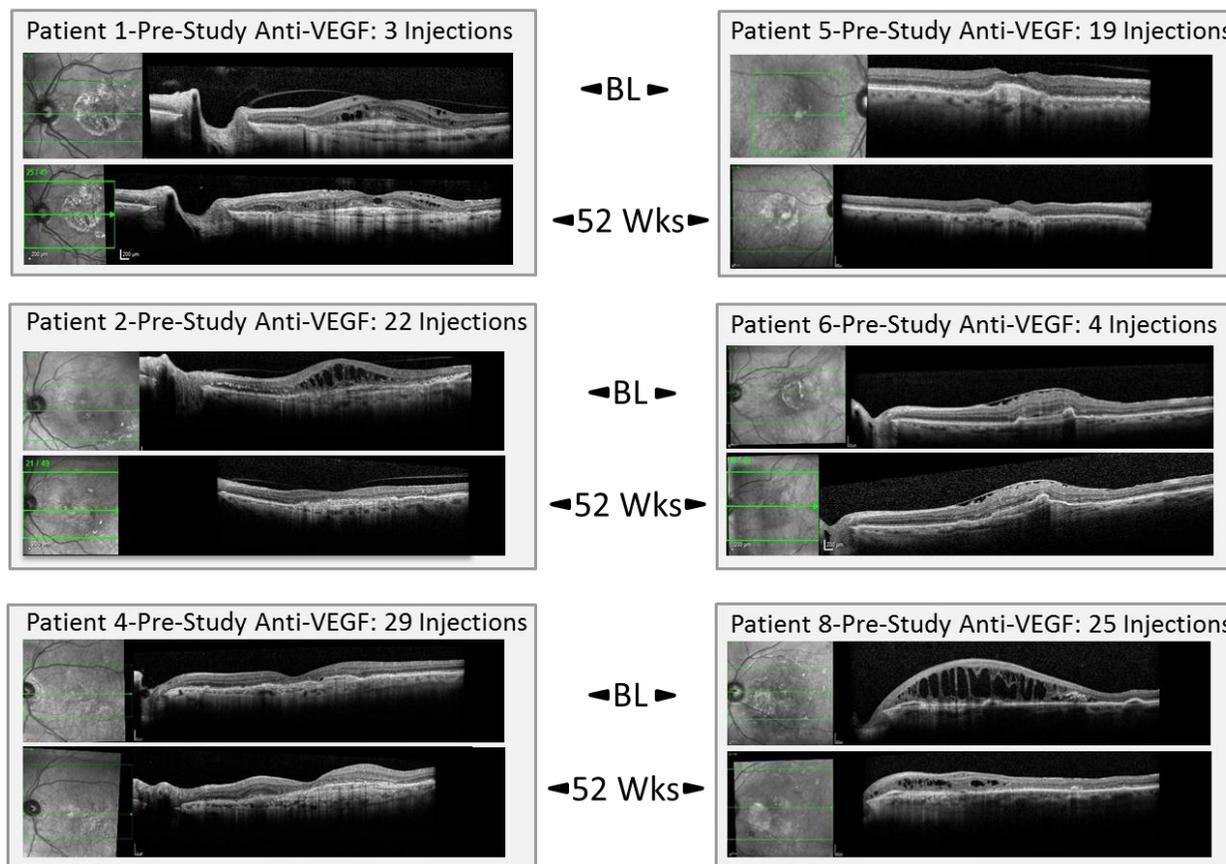


Figure 3: Visual Acuity

