Three-Year Follow-Up of Phase 1 and 2a rAAV.sFLT-1 Subretinal Gene Therapy Trials for Exudative Age-Related Macular Degeneration

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• PURPOSE: To assess the safety and the 3-year results of combined phase 1 and 2a randomized controlled trials of rAAV.sFLT-1 gene therapy (GT) for wet age-related macular degeneration.

• DESIGN: Phase 1/2a clinical trial.

• METHODS: Patients were prospectively randomized into control (n = 13) and GT (n = 24) groups. GT patients received $1X10^{11}$vg rAAV.sFLT-1 and were seen every month for 1 year then as needed every 1 to 2 months. They were given retreatment anti–vascular endothelial growth factor injections according to predetermined criteria. At 12 months, GT patients were divided into 2 groups: HD-1 (n = 14), requiring < 2, and HD-2 (n = 10), requiring > 2 retreatments.

• RESULTS: Between 1 year and 3 years there were 3 adverse events (AEs) and 33 serious AEs reported. Of these, 15 occurred in the 13 control subjects and 21 in the 24 GT patients. Except for 1 case of transient choroiditis in a control patient, serious AEs were deemed to be unrelated to the study. Control patients received a median of 7.0 retreatments and lost a median of 7.0 Early Treatment Diabetic Retinopathy Study (ETDRS) letters, HD-1 patients received a median of 2.5 retreatments and lost a median of 4.0 ETDRS letters, and HD-2 patients received a median of 11.0 retreatments and lost a median of 7.0 ETDRS letters over 3 years. Center point thickness fluctuated. Thirty-three percent of control subjects, 44% of HD-2 patients, and 51% of HD-1 patients showed maintenance of baseline visual acuity. Four HD-1 patients (34%) maintained significant visual improvement at 3 years. None of these observations were statistically significant.

• CONCLUSIONS: Given the small number of patients, this study was unable to unequivocally confirm the existence of a biologic efficacy signal; however, it confirmed that rAAV.sFLT-1 gene delivery was well tolerated among the elderly. (Am J Ophthalmol 2019;204:113–123. © 2019 Published by Elsevier Inc.)

EXUDATIVE (WET) AGE-RELATED MACULAR DEGENERation (wet AMD) before the widespread adoption of intravitreal anti–vascular endothelial growth factor (VEGF) injections has historically been the major cause of blindness in the developed world.[1] Current treatment for wet AMD aims to control choroidal neovascularization initiated by overexpression of VEGF through the injection of antagonists (bevacizumab, ranibizumab, and aflibercept). Although anti-VEGF treatments have been successful in maintaining and improving the vision of patients with wet AMD, most of the benefit is seen in the first year. Chronic management with frequent injections places a heavy burden on patients and the health care system in the longer term.

Unlike Lebers congenital amaurosis, with a specific monogenic defect, wet AMD is a complex, chronic disease with multiple contributing causal factors, and pathology that includes neovascularization, hemorrhage, and subretinal scar tissue formation. Thus, it was in the past not considered likely to become a candidate for gene therapy (GT). However, recombinant virus-mediated gene delivery has demonstrated the long-term production of therapeutic proteins in the eye making it a potentially useful treatment proposition for chronic diseases as a specialized form of extended drug delivery.[2] After injection, ocular cells can become "biofactories" producing the required therapeutic molecules in situ. This longevity raises the hope that GT for wet AMD, by producing a VEGF antagonist in the eye, could deliver a significant improvement in treatment burden for both the patients and the providers by reducing the number of injections required to limit disease progression and potentially evening out the peaks and valleys of drug levels after intravitreal drug injections.

Recent results demonstrated that rAAV2-mediated gene delivery was viable and safe in the elderly patient population, and that it could be administered in conjunction with anti-VEGF injections without any complications. In our phase 1 and 2a studies of rAAV.sFLT-1 at 1 year post–subretinal injection, 5 (83%)[3] and 12 (57%)[4] of the

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GT patients demonstrated maintenance/improvement in best-corrected visual acuity (BCVA), respectively. In another trial using the chimeric form, rAAV.sFLT01, the vector was administered by intravitreal rather than subretinal injection and there was little change in BCVA at 1 year.[5] Although these data in the aggregate suggested a biologic signal supportive of a therapeutic response, some were disappointed with the magnitude of the results for several reasons. The number of antagonist injections was not eliminated in either of the rAAV.sFLT-1 trials. In the rAAV.sFLT01 intravitreal study, the correlation between anatomic response and dose was poor and the presence of AAV2 serum antibodies at baseline appeared to have a potentially negative effect on transgene expression.[5]

However, in light of recent data showing that more frequent treatment with traditional anti-VEGF injections seems to be associated with better long-term outcomes, and that many patients required continuous treatment beyond 5 years,[6,7] the need for new treatments with lower treatment burden that are more suitable for the long-term management of wet AMD is clear. GT is one of a few technologies available at present that has the potential to deliver sustained VEGF antagonist levels at the required therapeutic dose and at the location of the neovascularization. Therefore, further detailed analysis of the long-term data obtained from the patients treated in these early trials is critical because it might provide further insight for the best way of deploying these new treatments. In this article, we report on the long-term (36 month) follow-up results of the study population who participated in the high dose subretinal rAAV.sFLT-1 trials.

METHODS

• **STUDY DESIGN AND PARTICIPANTS:** This single-center, randomized controlled trial investigated the safety and potential biologic signals of a single administration of 2 different dose concentrations of a rAAV vector of serotype 2 encoding sFLT-1 in patients with advanced wet AMD. Patients were enrolled under Clinical Protocol 2008-135 version 1.4 registered with ClinicalTrials.gov (NCT01494805). The protocol was approved by the Australian Therapeutic Goods Administration. Appropriate approvals were obtained from The University of Western Australia Institutional Biosafety Committee and the Sir Charles Gairdner Hospital Human Ethics Committee. The trial was performed at the Lions Eye Institute and the Sir Charles Gairdner Hospital in Nedlands, Australia. Throughout the trial, the tenets of the Declaration of Helsinki were followed. All patients gave written informed consent.

• **RANDOMIZATION AND MASKING:** Randomization was accomplished by sequential study group assignment according to a randomization list that was computer-generated before the study and held off-site as described previously.[3] Technical staff performing the assessments were masked to the study group patients but not the surgeons.

The subretinal GT delivery procedure has been described previously.[3] All study patients received intravitreal ranibizumab 0.5 mg in the study eye at baseline (day 0) and at the 4-week visit. Patients from the phase 1 and 2a trials rAAV.sFLT-1 treatment groups who received subretinal injection of 100 μL rAAV.sFLT-1 [1×10^{11} vector genomes (vg)] at the time of vitrectomy at day 7 were included in this analysis. Rescue treatment with traditional anti-VEGF treatment was given when there was: (1) loss of ≥10 letters on the Early Treatment Diabetic Retinopathy Study (ETDRS) scale from previous visit, or loss of >5 ETDRS letters in conjunction with patient perception of functional loss; (2) increase in subsensory, intraretinal, or subretinal pigment epithelial (RPE) fluid on optical coherence tomography (OCT); or (3) signs of increased leakage on fluorescein angiography.

• **OUTCOMES:** The primary endpoints were measured using ophthalmic examinations, vital signs, and clinical laboratory testing according to the schedule of assessments. After the 12-month visit, patients were monitored at the 18- and 36-month scheduled visits, as well as at multiple unscheduled visits, as required clinically. Signs of visual loss, infection, inflammation, and other safety events, including cataract formation and retinal detachment, were closely monitored. Study data and adverse events (AEs) were monitored by a data safety monitoring committee with expertise in retinal diseases and GT vectors. Secondary endpoints included the requirement for VEGF antagonist retreatment injections, BCVA, and center point thickness (CPT) of the retina, measured by optical OCT as previously described.[3]

• **STATISTICAL ANALYSES:** The purpose of this study was to address the safety of rAAV.sFLT-1 GT and to provide a longer-term dataset of human treatment results to aid in the design of a larger study. We did not perform any sample size calculations. Comparisons between groups were performed using *t* tests.

• **ROLE OF THE FUNDING SOURCE:** Adverum Biotechnologies Inc. had no role in this phase of later data collection and interpretation, and the corresponding author had full access to all the data in the study and takes full responsibility for the decision to publish the results.

RESULTS

FROM DECEMBER 16, 2011 TO MARCH 26, 2014 WE SCREENED 44 patients, of whom 40 were enrolled and randomly

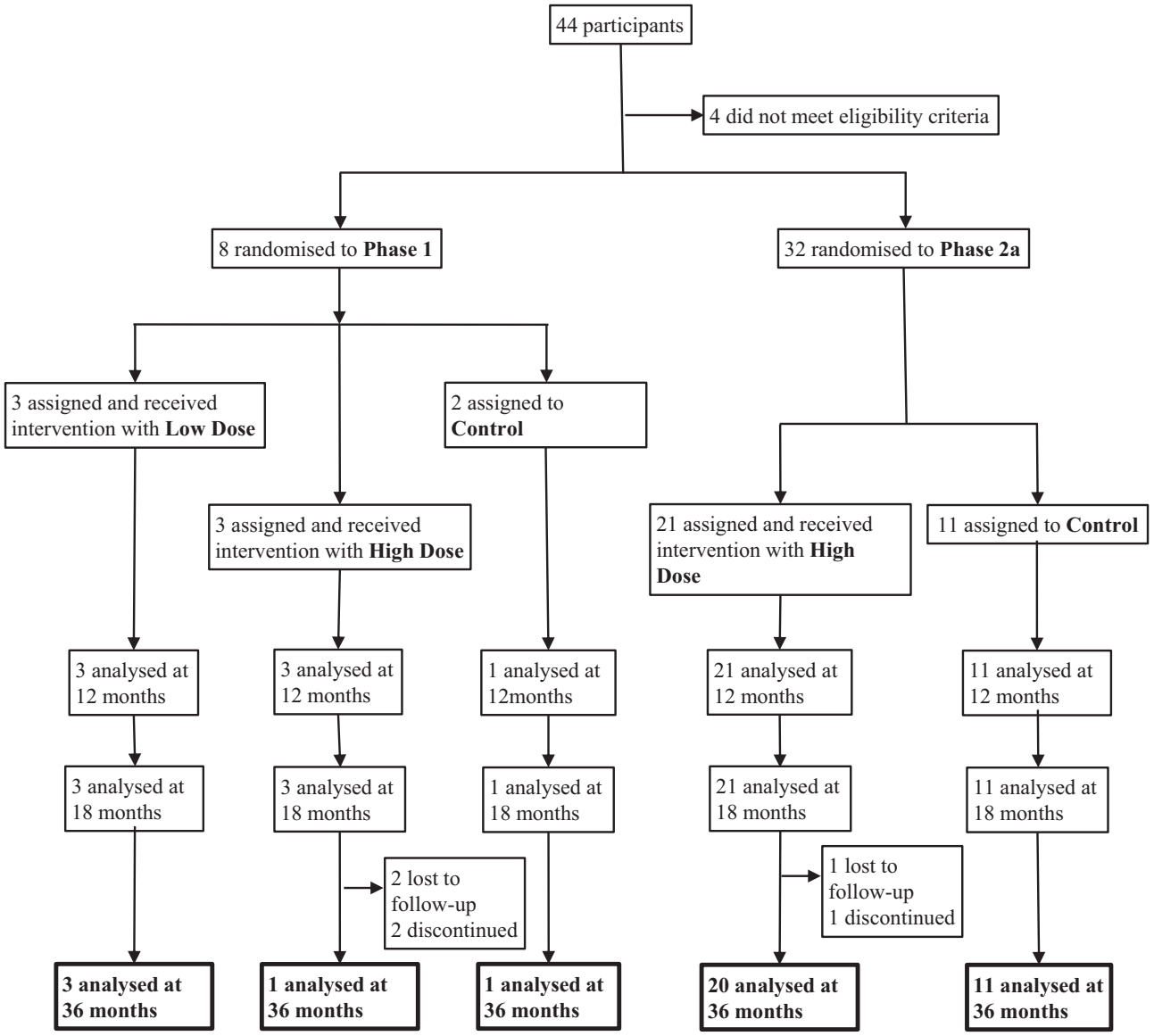

FIGURE 1. Trial profile for the phase 1 and 2a clinical trials. A total of 3 gene therapy patients exited the trial before month 36. One enrolled control patient with atypical wet AMD was excluded from analysis.[3]

assigned to receive either intervention (low-dose rAAV.sFLT-1 [n = 3] and high-dose rAAV.sFLT-1 [n = 24]) or a control regimen (n = 13; Figure 1). The 36-month results of the low-dose rAAV.sFLT-1 group have been previously reported[8] and are not included in this analysis. Patient demographics at baseline are shown in Supplemental Table 1. Three patients withdrew for reasons unrelated to the trial, including increasing infirmity related to carcinomatosis, Alzheimer disease, or age after the 18-month visit. One control patient was excluded from data analysis because of a pre-existing epiretinal membrane. The median number of unscheduled visits for the control subjects and GT patients was 11.0 and 9.8 visits, respectively, between the 12- and 36-month follow-up period.

• **ADVERSE EVENTS:** There were 3 AEs observed in 2 GT patients between the 12- and 36-month visits (Supplemental Table 2). In 1 patient, a decrease in immunoglobulins (deemed not related to the study) and eye irritation (deemed probably related to study procedures) were recorded. The third AE was a decrease in natural killer cell count, which was deemed to represent natural fluctuation and was considered unlikely to be related to GT. In the same period, 33 serious AEs (SAEs) occurred, 15 in control subjects and 18 in GT patients (Supplemental Table 2). Except for 1 case of choroiditis in a control subject and 1 eye with chronic irritation in a GT patient there were no eye disorders reported.

Two patients from the GT group (n = 24) had cancer at the point of enrollment. One patient who had breast cancer

underwent mastectomy and chemotherapy 1 year before entering the trial and subsequently died from metastatic cancer after the closure of the trial. The other patient was diagnosed with suspected lung cancer before enrollment, the diagnosis was confirmed 15 months after GT, and the patient died 6 months later. Three GT patients encountered a diagnosis of cancer during the trial. One patient developed breast cancer 1 year after entering the trial and was successfully treated. One GT patient developed bowel cancer 18 months after GT and had successful hemicolectomy. One patient (a smoker) developed a lung neoplasm during the trial. One patient developed colorectal cancer after the study closure date. None of the patients in the control group (n = 13) reported cancer/neoplasia at enrollment, and none of them were diagnosed with cancer during the trial. Two significant nonlethal cardiac events occurred in GT patients, and 1 in a patient from the control group.

• **IMMUNE RESPONSE TO THE VECTOR:** Anti-AAV2 neutralising antibodies (nAbs) were detected at baseline in 12 (50%) of 24 GT patients.[4] Five (41%) GT patients who were negative at the time of recruitment developed anti-AAV2 nAbs within the first year after receiving the GT subretinal injection. Of these 5 patients, antibodies returned to baseline in 3 by the end of the study period.

• **EXPLORATORY ENDPOINTS:** The number of intravitreal anti-VEGF retreatment injections received by the patients is summarized in Table and Figure 2. In addition to the initial GT administration, the protocol required retreatments whenever the presence of fluid was detected on OCT or there was increased fluorescein leakage.[3,4] At the end of 12 months for the purposes of post hoc analysis, we grouped GT patients into 2 categories, as determined by the need for reinjection with ranibizumab. The first group HD-1 (n = 14) received ≤2 anti-VEGF retreatment injections in their first year.[4] Of these, 7 did not require any reinjection during the first 12 months. Most of these patients did not require retreatments between 12 and 36 months and received a median of 2.5 (interquartile range [IQR] 1.0-5.0) injections over 36 months. The subgroup of GT patients designated HD-2 (n = 10) had >2 anti-VEGF retreatment injections in their first year and received a median of 11.0 (IQR 9.0-13.0) retreatment injections over 36 months. Control subjects (n = 12) received a median of 7.0 (IQR 6.0-11.0) anti-VEGF injections during the trial. During the first 12 months, all control subjects required >3 retreatments except for 1 who developed a dry fibrovascular scar. The mean number of anti-VEGF retreatment injections per patient per year was 2.6 ± 1.5 per year for the control subjects, 1.1 ± 0.9 per year for the HD-1 patients, and 3.7 ± 0.9 per year for the HD-2 patients. Notably, of the 12 HD-1 patients, 10 were seropositive for anti-AAV2 antibodies at baseline.

The BCVA of the trial patients are summarized in Figure 3 and Supplemental Table 3. A summary of each patient's individual BCVA data, OCT images, and anti-VEGF retreatment injections received are presented in Table and Supplemental Figure 1. The median changes of BCVA of all 3 groups over 3 years is shown in Figure 3. There were no statistically significant differences between any of the data points at any point of time. The median BCVA in ETDRS letters changed from 61.5 to 41.0 for the control subjects, 63.5 to 54.0 for HD-1 and 53.5 to 36.0 for HD-2 patients. Three control, two HD-1, and two HD-2 patients had lost more than 30 ETDRS letters from baseline by their final visit due to retinal bleed or atrophy associated with the progression of AMD. Only HD-1 patients (34%) maintained large degrees of improvement in visual acuity (12-22 ETDRS letters) from baseline at the 36-month visit (Figure 4).

CPT measurements for trial patients are summarized in Supplemental Table 3. The median CPT for control subjects and GT patients fluctuated. Nevertheless, compared with median baseline CPT, an average decrease was seen at the 36-month visit for the control subjects and the HD-1 group. The HD-2 group showed an increase in the median CPT (Figure 3). None of these changes were statistically significant.

Among the control subjects, 1 (7.7%) had subretinal fluid and 4 (31%) had intraretinal fluid at 36 months (Table and Supplemental Figure 1). In the GT group, 3 (12.5%) had subretinal fluid (2 with RPE detachment) and 6 (25%) had intraretinal fluid (1 with RPE detachment) at the end of the study. At 36 months, OCT showed that 7 (58.3%) of the control subjects and 12 (57.1%) of the GT patients were dry. Subretinal scar is thought to be an important factor in terms of visual outcome. We found that 9 (75.0%) control subjects and 14 (58.3%) GT patients had subretinal scar tissue present at baseline. At 36 months, 12 (100%) of the control subjects and 17 (81%) GT patients had scar tissue present and the thickness of scar tissue increased in 5 control subjects and 8 GT patients. At baseline, 1 (7.7%) control subject and 2 (8.3%) GT patients had geographic atrophy (GA).[9] By 36 months, 6 (60%) control subjects and 6 (30%) GT patients had developed some GA. Of these, 2 (16.4%) belonged to the HD-1 group.

The median sFLT-1 protein levels at baseline and at the 36-month visits are summarized in Supplemental Table 3. At baseline, there was no statistically significant difference ($P \geq .05$) between control subjects and GT patients in sFLT-1 protein levels from serum, urine, and saliva. In GT patients, there were also no statistically significant changes ($P \geq .05$) in sFLT-1protein levels from serum or urine sampled at the baseline and 36-month visits. However, in saliva there was a statistically significant decrease ($P \leq .05$) in sFLT-1 protein levels at the 36-month visit compared with baseline, possibly because of difficulties in

TABLE. Data from Each Patient

	Anti-VEGF Injections		BCVA (ETDRS)		CPT (μm)		Fluid Type		Subretinal Scar		Geographic Atrophy	
Patient No.	Prior	Retreatments	Baseline	Month 36	Baseline	Month 36	Baseline	Month 36	Baseline	Month 36	Baseline	Month 36
Control subjects												
3	1	6	28	22	896	462	IRF	Dry	++	++	0	++
10	0	1	68	65	552	424	SRF	Dry	++	+++	0	+
13	25	11	45	2	179	1110	IRF	IRF	++	+++ SR bleed	+	++
17	0	11	63	64	671	308	IRF	IRF	+	+	0	0
20	23	7	70	73	703	353	IRF	Dry	++	+++	0	+
21	8	13	50	43	508	535	IRF	IRF	+	++	0	0
24	1	5	39	15	451	274	SRF + RPED	Dry	0	++	0	+
28	24	16	63	32	314	498	SRF	IRF	+	++	+	++
32	5	10	75	55	334	293	SRF	SRF	+	+	0	0
33	7	7	60	38	349	301	SRF	Dry	0	+++	0	++
36	23	6	56	56	299	224	IRF	Dry	+	+	0	0
39	9	6	78	39	268	155	SRF + RPED	Dry	0	+	0	+
Median	7.5	7	61.5	41.0	400.0	330.5						
(IQR1-IQR3)	(1.0-23.0)	(6.0-11.0)	(48.8-68.5)	(29.5-58.0)	(310.3-581.8)	(288.3-471.0)						
Gene therapy												
HD-1 group												
5	16	0	56	47	268	246	SRF	SRF	+	++	0	0
6[a]	4	NA	54	NA	601	NA	RPED	NA	0	NA	0	NA
8[a]	20	NA	34	NA	1094	NA	IRF	NA	0	NA	+	NA
12	5	9	71	47	353	464	IRF	IRF + RPED	+	+	0	0
14	13	1	78	36	331	489	SRF	Dry	+	+++ SR bleed 2.5 years	0	0
15	13	1	78	79	267	340	SRF + RPED	SRF + RPED	0	+	0	0
18	9	7	75	51	646	524	SRF + RPED	Dry	++	+++	0	0
25	0	2	67	79	234	173	IRF	Dry	0	0	0	0
26	16	5	64	10	315	434	IRF	Dry	++	+++ SR bleed 1.5 years	0	0
27	9	5	67	39	420	253	IRF	Dry	+	++	0	+
29	21	1	49	71	252	440	IRF	Dry	0	0	0	++
35	11	4	63	67	285	264	SRF	Dry	0	+	0	0
37	7	2	42	57	299	196	IRF	Dry	+	+	0	0
40	0	3	56	72	314	242	SRF	Dry	++	+++	0	0
Median	10.0	2.5	63.5	54.0	314.5	302.0						
(IQR1-IQR3)	(5.5-15.3)	(1.0-5.0)	(54.5-70.0)	(45.0-71.3)	(272.3-403.3)	(245.0-446.0)						
HD-2 group												
9	10	11	50	52	504	314	IRF	IRF	++	++	0	0

Continued on next page

TABLE. Data from Each Patient (*Continued*)

	Anti-VEGF Injections		BCVA (ETDRS)		CPT (μm)		Fluid Type		Subretinal Scar		Geographic Atrophy	
Patient No.	Prior	Retreatments	Baseline	Month 36	Baseline	Month 36	Baseline	Month 36	Baseline	Month 36	Baseline	Month 36
11	11	10	38	31	816	408	IRF + RPED	IRF	+	++	0	++
16	4	8	35	7	356	403	IRF	Dry	++	+++ SR bleed 1.5 years	0	++
19	13	8	69	71	349	275	IRF	IRF	++	++	0	0
22	23	9	37	35	312	414	SRF	SRF + RPED	0	0	0	0
23	8	13	61	36	398	252	SRF + RPED	Dry	0	++	0	+
30	24	12	53	59	328	344	IRF	Dry	+	+	0	0
31	11	15	74	35	282	698	SRF + RPED	IRF	+	+	0	+
34[a]	5	NA	54	NA	486	NA	SRF + RPED	NA	0	NA	0	NA
38	2	14	77	46	235	422	IRF	IRF	0	0	+	+
Median	10.5	11	53.5	36.0	352.5	403.0						
(IQR1-IQR3)	(5.8-12.5)	(9.0-13.0)	(41.0-67.0)	(35.0-52.0)	(316.0-464.0)	(314.0-414.0)						

The data include the total number of anti-VEGF retreatments over the 36-month trial (excluding the 2 mandatory ranibizumab injections at baseline and week 4), BCVA, type of fluid (IRF, SRF, or RPED) and grading of subretinal scar tissue (+, any seen on optical coherence tomography; ++, <200 μm thick; +++, >200 μm thick) from optical coherence tomography analysis, and grading of geographic atrophy from fundus photography (+, any seen on fundus images; ++, patch >2 disc diameters; +++, geographic atrophy extending under most of the macula) at baseline and at month 36.

BCVA = best-corrected visual acuity; CPT = center point thickness; ETDRS = Early Treatment Diabetic Retinopathy Study; IQR = interquartile range; IRF = intraretinal fluid; RPED = retinal pigment epithelial detachment; SRF = subretinal fluid; VEGF = vascular endothelial growth factor.

[a]GT patients who exited before the end of the study.

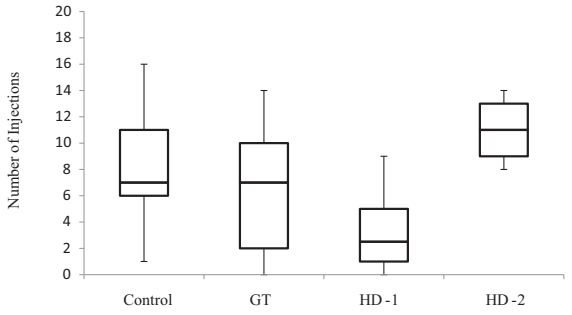

FIGURE 2. Box and whisker plot showing the total number of anti–vascular endothelial growth factor (VEGF) injections received by the control subjects and gene therapy (GT) patients over the 36-month duration of the clinical trial. The GT group was subdivided into 2 subgroups. HD-1 subgroup patients required ≤2 anti-VEGF retreatments and HD-2 subgroup patients required >2 retreatments during the first year of the study. The median number of anti-VEGF retreatment injections received by HD-1 patients was 2.5 (1.0-5.0) and by HD-2 patients was 11 (9.0-13.0) over 36 months.

handling and diluting samples of high viscosity at these time points.

DISCUSSION

WE PRESENT THE LONG-TERM PATIENT OUTCOME FOCUSED analysis of data from our rAAV.sFLT-1 GT trial for exudative AMD, which is to the best of our knowledge the largest clinical trial using AAV2 as the vector to treat any ocular disease to date. The safety data for the first 12 months of treatment have been previously reported,[3,4] and there were no AEs observed for the remaining 2 years of the trial that were considered likely to be associated with the GT. This is consistent with other clinical trials examining AAV2 vectors to treat ocular diseases.[9] This trial was conducted in aged patients, and it is worth noting that 3 of 24 patients in the GT group were diagnosed with various carcinomas during the 3-year study period compared with no cancer diagnoses in the 13 control subjects. One GT patient was diagnosed with cancer after the study closure date. In addition, in the GT group, 2 patients reported a pre-existing cancer diagnosis at enrollment that progressed during the trial. Statistical comparisons did not show any statistically significant evidence of a difference in the proportion of cancers diagnosed during the trial between the GT and control groups. At 80 years of age, the incidence of clinically detected cancer in Australia is reported to be 1 in 5.[10] Although more cases of cancers were observed in the GT group than in control subjects during the follow-up period, the numbers were small and there was no statistical evidence of a difference in the proportion of cancers diagnosed during follow-up. Furthermore, cancer diagnosis was not an exclusion criterion in this trial. For these reasons, this study is unable to comment on cancer risk related to intervention. Future large clinical trials should consider incorporating cancer diagnosis into the trial design.

The exploratory endpoints that were followed over the 36-month clinical trial included the number of intravitreal anti-VEGF retreatment injections, BCVA, and CPT. Amongst the GT patients in our clinical trial, we did not observe close correlation between the patients' BCVA and CPT. This is reflective of data from multiple clinical trials[11,12] that have shown no close correlation between CPT and visual improvement. Those studies found that a percentage of wet AMD patients continued to lose visual acuity over time while maintaining or improving CPT. Although CPT measurement is an adequate marker for retinal swelling, it is a less reliable indicator for advanced wet AMD because of the variable degree of subretinal scar tissue formation and overlying subretinal fluid presence, as well as the fact that decreasing CPT may also signify increasing GA of the pigment epithelium and retinal atrophy. We found that rescue anti-VEGF treatments were partly effective in removing subretinal fluid and that GT patients might have performed better in terms of GA formation. Considering these observations, and that improvement of vision is the most important outcome for the patients, we focused our analysis on BCVA maintenance/improvement.

It should be noted that the patients in this study were suffering from chronic advanced exudative AMD and were not anti-VEGF treatment-naïve, with a mean of 12 anti-VEGF treatment injections before entering the trial. Other clinical trials have suggested that treatment-naïve patients with earlier disease parameters have a higher capacity to respond to anti-VEGF therapy.[13] Our data were not dissimilar to other trials.[7,14,15] However, there was no statistically significant difference between GT patients and control subjects. These results suggest that because the underlying cause of the disease (genetic mutations or lifestyle) are not treated by VEGF antagonists, the slow progression of AMD continues. We propose that this leads to an evolution to a chronic less responsive state, characterized by more histologically mature choroidal neovascularization and subretinal scar tissue. Indeed, we observed more scar tissue formation and an increase in scar area and thickness during the study. Most of our patients (75.0% control subjects and 58.3% GT patients) had scar tissue present at baseline, which is reflective of the advanced stage of wet AMD at the time of enrollment in our patient trial population.[16] At 36 months, all of our control subjects and 81% of our GT patients demonstrated the presence of subretinal scar tissue.

Antibodies to AAV2, generated in response to previous natural infection, hamper the successful delivery of rAAV2 vectors used for GT when the therapy is administered systemically.[17] Similarly, suboptimal expression of a

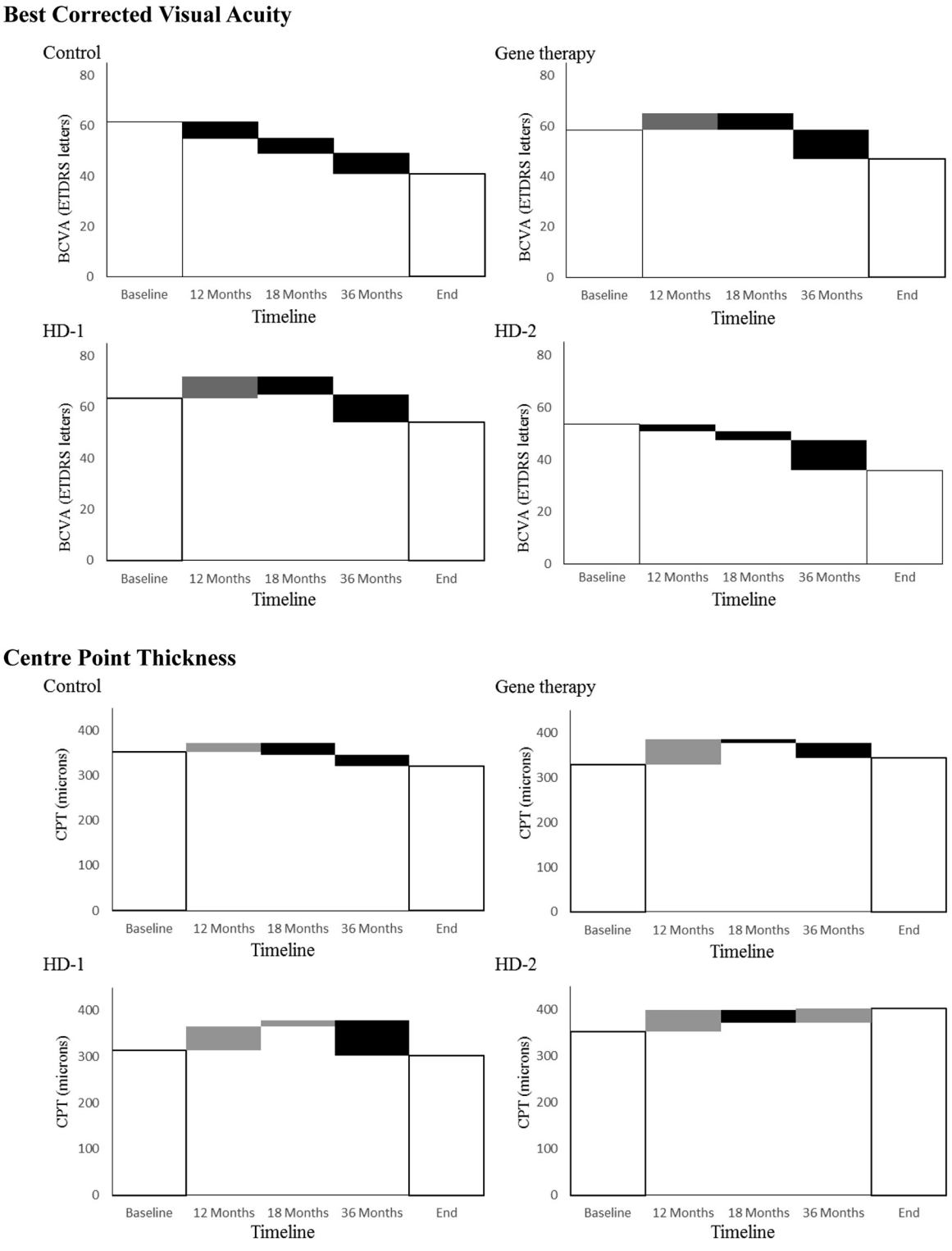

FIGURE 3. The top 4 waterfall plots show the median best-corrected visual acuity (BCVA) scores for control subjects and gene therapy (GT) patients, including the HD-1 and HD-2 subgroups, throughout the clinical trial. The GT group was subdivided into 2 subgroups. HD-1 subgroup patients required ≤2 anti–vascular endothelial growth factor retreatments and HD-2 subgroup patients required >2 retreatments during the first year of the study. Of all groups, HD-1 subgroup patients showed a modest loss of 9 Early Treatment Diabetic Retinopathy Study letters at 36 months. The bottom 4 waterfall plots show the median center point thickness measurements for control subjects and GT patients, including the HD-1 and HD-2 subgroups, throughout the clinical trial. With the exception of the HD-2 subgroup, center point thickness decreased in all groups. Light gray bars are a median increase. Black bars are a median decrease.

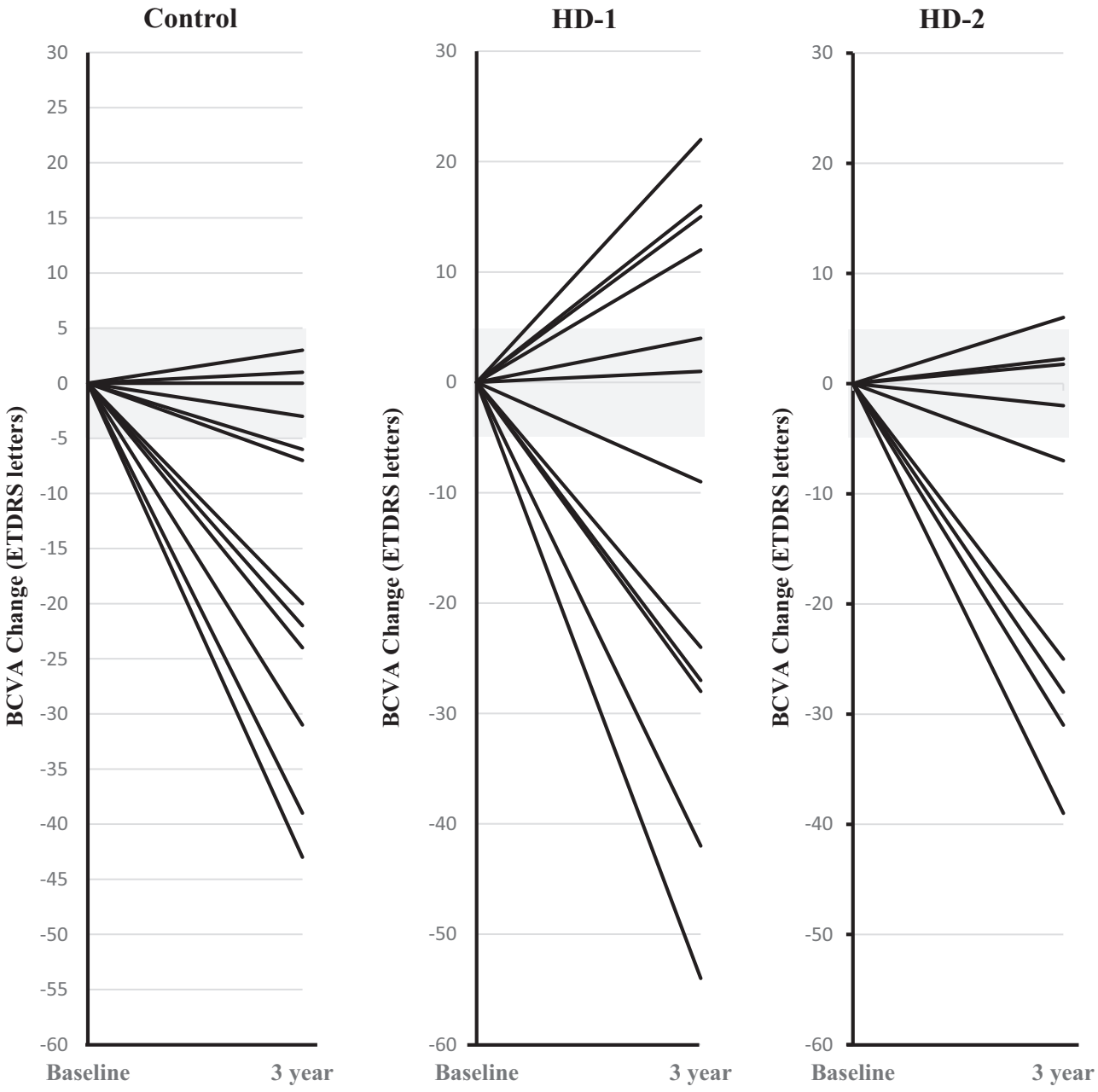

FIGURE 4. Best-corrected visual acuity (BCVA) of each patient at baseline (0) and BCVA change at 36 months. The left plot shows changes in BCVA for the control subjects (n = 12). The gene therapy (GT) group was subdivided into 2 subgroups. HD-1 subgroup patients required < 2 anti-VEGF retreatments and HD-2 subgroup patients required > 2 retreatments during the first year of the study. The middle plot shows changes in BCVA for the HD-1 (n = 12) patients and the right plot shows changes in BCVA for the HD-2 patients (n = 9). Two HD-1 patients and 1 HD-2 patient exited the trial before month 36. The shaded area covers BCVA changes of ≤5 9 Early Treatment Diabetic Retinopathy Study letters.

therapeutic molecule delivered by a rAAV2 vector has been noted after intravitreal delivery.[5] By contrast, rAAV2 delivery and transduction do not appear to be altered to the same degree by pre-existing humoral immunity when the vectors are delivered in the subretinal space, presumably related to its relative degree of immune privilege compared with the vascular compartments and vitreous cavity.[3,4,18–20] Consistent with this, we found that the presence of AAV2 antibodies at baseline did not interfere with the improvement of BCVA. Indeed, 10 of 12 HD-1 patients were AAV2 nAb-positive before enrollment. Thus, the establishment of the "biofactory" or the production of sFLT-1 did not appear to be hampered by pre-existing immunity to AAV2 in this study. In

4 AAV2-naïve patients, low-level AAV2 nAb were measurable in serum after rAAV.sFLT-1 treatment but were lost in 3 of these patients over the course of the study. Several factors might have contributed to this. In our study, the vector was administered in the subretinal space, thus systemically there would have been minimal exposure to rAAV2. In addition, our patients are an elderly population likely to mount only a suboptimal response to immune challenges.[21] Previous studies have shown that the presence of serum antibodies elicited by rAAV2 does not prevent efficient delivery and transduction in the retina.[18,22] Our results confirm and extend these findings and provide evidence that pre-existing AAV2 nAbs should not represent an exclusion criterion when rAVV2 is delivered subretinally, and that delivery to these patients does not appear to affect either the safety or potential efficacy of the therapy.

Subretinal injection is now a well-accepted surgical method used not only for GT for Lebers congenital amaurosis and choroideremia but for a variety of clinical conditions, including submacular hemorrhage and previously failed macular hole surgery. In principle, intravitreal delivery for wet AMD as opposed to surgical subretinal injection would be much preferred. Further studies of new vectors that facilitate intravitreal delivery as well as further investigations into the immune response after intravitreal injection for GT are underway.[23]

These phase 1 and 2a trials had serious limitations as far as meaningful analysis of efficacy is concerned. They were designed for safety; thus, the patient population was not treatment-naïve but rather represented a subset of patients with advanced wet AMD and active choroidal neovascularization including a mean of 12 previous anti-VEGF injections. Most already had some submacular scar tissue. The number of patients was low (n = 37) compared with other VEGF antagonist trials. In addition, this is an unusual analysis by retrospectively selecting the 2 groups by their greater or lesser need for rescue injections at 12 months. In theory it is possible that there is an underlying difference in disease responsiveness to anti-VEGF therapy between those 2 groups rather than a differential response to gene versus conventional anti-VEGF therapy. However, to the best of our knowledge, to date no distinctly different groups have been identified as far as anti-VEGF therapy response is concerned. Although the numbers are low, the use of the control group (n = 13) should help to allay that concern to some extent.

Although these trials were unable to unequivocally confirm the existence of a biologic efficacy signal, they confirmed that rAAV.sFLT-1 gene delivery was well tolerated among the elderly.

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