

# Two-Year Intraocular Delivery of Ciliary Neurotrophic Factor by Encapsulated Cell Technology Implants in Patients with Chronic Retinal Degenerative Diseases

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**PURPOSE.** To evaluate the pharmacokinetics of ciliary neurotrophic factor (CNTF) delivered over a period of up to 2 years by an intraocular encapsulated cell technology (ECT) implant in patients with retinitis pigmentosa (RP) and geographic atrophy (GA).

**METHODS.** Patients from phase 1 RP (CNTF1); phase 2 GA (CNTF2); and phase 2 late and early stage RP (CNTF3, and CNTF4) studies received an ECT-CNTF implant, designated as "NT-501," in one eye. Per protocol, all implants ( $n = 10$ ) were removed at 6 months from the CNTF1 study patients. Explant for the phase 2 studies was optional, but several patients were explanted at 12, 18, and 24 months post implant. A small amount of vitreous sample was collected at the time of explant. The rate of CNTF secretion from the explants and the corresponding vitreous CNTF levels were evaluated for each time point. Serum samples from these patients were evaluated for CNTF, anti-CNTF antibodies, and antibodies to the encapsulated cells.

**RESULTS.** NT-501 implants produced CNTF consistently over a 2-year period. The calculated half-life of CNTF in the vitreous continuously delivered by ECT implants was 51 months, with CNTF levels statistically equivalent between the 6- and 24-month implant period. CNTF, anti-CNTF antibodies, and antibodies to the encapsulated cells were not detected in the serum of patients.

**CONCLUSIONS.** This retrospective study demonstrated that the intraocular ECT implant has a favorable pharmacokinetic profile for the treatment of chronic retinal degenerative diseases without systemic exposure. (ClinicalTrials.gov numbers, NCT00063765, NCT00447954, NCT00447980, NCT00447993.) (*Invest Ophthalmol Vis Sci.* 2012;53:7484-7491) DOI:10.1167/iov.12-9970

Ciliary neurotrophic factor (CNTF) is a member of the IL-6 family of neurotrophic cytokines. Its biological activities are mediated through a heterotrimeric complex consisting of CNTF receptor alpha (CNTFR $\alpha$ ), gp130, and LIF receptor beta

(LIFR $\beta$ ), as well as downstream signal transduction pathways.<sup>1-4</sup> Although the intrinsic function of CNTF in adult animals is not fully understood, exogenous CNTF affects the survival and differentiation of cells in the nervous system, including retinal cells. The protective effect of CNTF on photoreceptors has been confirmed in a broad range of animal models of retinal degeneration.<sup>5-7</sup>

While CNTF and other proteins and polypeptides may offer therapeutic potential in the treatment of retinal degenerative diseases, localized treatment of retinal diseases is complicated by the blood-retinal barrier (BRB). The BRB prevents the penetration of a variety of molecules from the systemic circulation to the neurosensory retina<sup>8,9</sup> and makes the delivery of CNTF and other therapeutic proteins to the retina a significant challenge.<sup>8</sup> This is a particular issue in chronic conditions where repeated treatments may be necessary. To overcome this challenge, encapsulated cell technology (ECT), specifically the NT-501 implant, was developed to facilitate controlled, sustained delivery of therapeutic agents directly into the vitreous cavity, circumventing the restrictions imposed by the BRB and thus delivering the drug directly to the retina over a sustained period of time.

ECT is a novel delivery system consisting of an immortalized, human cell line<sup>10</sup> that is genetically engineered to endogenously express a select therapeutic protein at a regulated delivery rate. The recombinant protein expression of the cell line is initially achieved using standard DNA transfection methods, allowing for a high efficiency of expression, followed by selection of cell lines exhibiting long-term viability, normal growth characteristics, and stable expression.<sup>11</sup> After cell engineering and the establishment of cell lines with therapeutic protein expression levels, the cells are encapsulated in semipermeable polymer membrane capsules and implanted into the vitreous cavity.

There are several distinct advantages to ECT. Foremost, it offers the potential for any gene encoding a therapeutic protein to be engineered into a cell and therefore has a broad range of applications. In addition, the therapeutic protein is freshly synthesized and released in situ; thus, a relatively small amount of the protein is needed to achieve a therapeutic effect. Stable, endogenous secretion of the protein assures that the availability of the protein at the target site is not only continuous but also long-term. Furthermore, the output of an ECT implant can be controlled to achieve the optimal dose for treatment. Finally, treatment by ECT can be terminated if necessary by simply retrieving the implant. Thus, ECT is a potentially effective means of long-term delivery of proteins and polypeptides to the retina.

Other options for delivering protein drugs to the retina include bolus intraocular injection of purified recombinant proteins, biodegradable or polymer-release systems, and gene therapy. Although ocular injections are potentially traumatic to

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the patient and pose an elevated risk of clinical complications, including endophthalmitis, retinal hemorrhage, and cataracts, the success of ranibizumab (Lucentis; Genentech, San Francisco, CA) and bevacizumab (Avastin; Genentech) has made intravitreal injection a standard of care for wet, ARMD.<sup>12</sup> A bolus injection of a molecule such as CNTF, however, would be prohibitive due to the extremely short intraocular half-life, which has been reported to be between 1 and 3 minutes,<sup>13</sup> and its potential toxicity due to injection levels of this potent cytokine that could exceed the maximum therapeutic threshold.<sup>14</sup> While polymer release systems, such as ganciclovir (Vitrasert; Bausch & Lomb, Rochester, NY) and fluocinolone (Retisert; Bausch & Lomb),<sup>15–17</sup> have proven effective in the treatment of select retinal diseases, these systems are designed for small molecule delivery and are not appropriate for the continuous delivery of large proteins over extended periods of time.<sup>18</sup> Gene therapies can achieve sustained expression of a given protein. However, the doses of therapeutic protein are difficult to control because there are no reliable means to regulate the expression levels of the transgene.<sup>19</sup> Furthermore, it is impossible to reverse the treatment once the gene is intergraded into the host genome.

The limitations of existing delivery systems to effectively and safely administer intraocular therapeutic molecules for sustained periods required for efficacious treatment of chronic retinal disorders emphasize the clinical need for the development and testing of improved drug delivery systems. An intraocular sustained-release system capable of maintaining appropriately safe, efficacious, and relevant drug levels in the retina for periods of years would eliminate the requirement of frequent injections, associated side effects, and improve patient compliance.<sup>20</sup> Preliminary results involving the NT-501 ECT implant appear very promising, demonstrating sustained, safe, and efficacious delivery of the therapeutic protein CNTF for periods of up to several years in the eye.<sup>21–24</sup>

The next step in the proposed development of ECT therapy toward a standard-of-care treatment is to incrementally establish the pharmacokinetics, safety, and efficacy of intraocular delivery in controlled clinical trials in human patients diagnosed with chronic retinal disorders, including RP and GA. To this end, the objective of the current study was to retrospectively evaluate the intraocular and systemic pharmacokinetics of CNTF in patients with retinal degeneration who received treatment with NT-501 intraocular implants in four clinical trial studies. Data from patients with RP who were treated with NT-501 implants during the phase 1 safety trial and patients with RP or GA who were treated in phase 2 clinical trials, were evaluated over a 24-month period to establish the kinetic and safety profiles of intraocular CNTF delivery in patients with chronic, degenerative retinal disease.

The establishment of a safe and long-term delivery profile for CNTF at clinically relevant levels in patients will help to facilitate the design of appropriate dosing strategies and duration for subsequent pivotal clinical trials of NT-501. The data collected for this purpose will also be useful for the future design of ECT therapeutic modalities for other chronic disorders, including diabetic retinopathy and choroidal neovascularization.

## MATERIALS AND METHODS

### Study Design

The studies were conducted according to the tenets of the Declaration of Helsinki. Informed consent was obtained from the subjects after explanation of the nature and possible consequences of the study, and

prior to study initiation, approvals were obtained from the institutional review board (IRB) of each clinical site.

Patients were enrolled in the CNTF1 (phase 1 study of NT-501 in RP, ClinicalTrials.gov Identifier: NCT00063765); CNTF2 (phase 2 study of NT-501 in GA, ClinicalTrials.gov Identifier: NCT00447954); CNTF3 (phase 2 study of NT-501 in late-stage RP, ClinicalTrials.gov Identifier: NCT00447993); and CNTF4 (phase 2 study of NT-501 in early stage RP, ClinicalTrials.gov Identifier: NCT00447980) trials and received an NT-501 implant in one eye. CNTF1 was a 6-month study with 10 patients (five high dose and five low dose), and patients were required to explant at the end of the study period. CNTF2 and CNTF3 were 12-month studies, and the patients were given the option to either explant at 12 months or to keep the implant in place (not to explant). CNTF4 was a 24-month study, and the patients were given the option to either explant at 24 months or keep the implant in place (not to explant). In the CNTF2 study, 27 patients received the high-dose implants, 12 patients received the low-dose implant, and 12 patients received the sham treatment. In the CNTF3 study, 43 patients received the high-dose implants and 22 patients received the low-dose implants. In the CNTF4 study, 48 patients received the high-dose implants and 20 patients received the low-dose implants. A majority of the CNTF2, CNTF3, and CNTF4 patients chose to keep their implants, and only 50 decided to have the device explanted. The data from the explanted cohort was used in the current study to generate the pharmacokinetic models.

### CNTF Implant

The CNTF-secreting, encapsulated cell implants, which are designated NT-501 (Neurotech, Lincoln, RI), are 1 mm in diameter and 6 mm long (with the exception of the CNTF low-dose devices that were 11 mm long and used for the CNTF1 study and secreted 2-fold higher CNTF than the 6-mm low-dose devices used in all subsequent CNTF2, 3, and 4 studies) and are constructed of a semipermeable polymer outer membrane, medical-grade sealant, and a titanium anchor at one end of each device to facilitate suturing to the sclera. Each implant contains an internal polyethylene terephthalate (PET) yarn scaffold that supports human cells. These cells (designated NTC-201) were derived originally from the human RPE cell line and were genetically engineered to produce human CNTF. Two separate transfections yielded two independent cell lines that released CNTF at different output rates. The two lines were designated NTC-201-10 (low dose) and NTC-201-6A (high dose). The 6-mm devices were loaded with 203,000 cells from the lower or higher CNTF-expressing lines and resulted in devices that secreted CNTF at  $5 \pm 0.8$  ng/day or  $20 \pm 3.0$  ng/day; the 6-mm low-dose device was designated NT-501-10.02 and the 6-mm high-dose device was designated NT-501-6A.02. The 11-mm low-dose device was designated NT-501-10, loaded with 400,000 cells, and secreted 2-fold of CNTF as the 6-mm low-dose device. The CNTF output was established empirically and involved the intrinsic CNTF delivery rate of the cell line and the number of CNTF-secreting cells loaded into the capsule.

### Implantation and Explantation Procedures

Patients received either high or low dose NT-501 implants in one eye. The high-dose implant used in the studies was selected based on the dose-response effect of CNTF in the *rcd1* model of retinal degeneration<sup>22</sup> and was the maximum effective dose. The low dose (6-mm device) was 50% of the minimum effective dose in the *rcd1* dog model. On average, intraocular implantation required 15 minutes to perform under retrobulbar anesthesia using 0.75% bupivacaine at 1:1 mixture with 4% lidocaine. The implant was inserted through a 2.0-mm sclerotomy made 3.75 mm posterior to the limbus in the inferotemporal quadrant and anchored with a single suture. Two additional sutures were applied to facilitate the wound closure. A subconjunctival antibiotic injection of 100 mg of cefazolin was given at the conclusion of surgery, and topical 1% prednisolone acetate and ciprofloxacin drops were given daily over the following week. The implants were

**TABLE 1.** Sample Distribution of Patients Electing to Remove NT-501 Implants over Course of Combined CNTF1, 2, 3, and 4 Clinical Trials

Devices	6 Months	12 Months	18 Months	24 Months	Total Explant No.*
High dose	5	11	7	11	34
CNTF1	5				
CNTF2		1	2		
CNTF3		9	5	1	
CNTF4		1		10	
Low dose	5	3	3	5	16
CNTF1	5				
CNTF2		1	1		
CNTF3		2	2		
CNTF4				5	

\* The number of patients explanted at 6, 12, 18, and 24 months is shown for both the high-and low-dose groups.

surgically removed at 6 months (CNTF1 study); 12 and 18 months (CNTF2 and CNTF3 studies, respectively); and 24 months (CNTF4 study). In most explant cases, a small amount of vitreous sample (100  $\mu$ L) was collected at this time. Clinical care after removing the CNTF implant was similar to that following the implant procedure.

### Serum CNTF Levels and Antibodies to CNTF and NTC-201 Cells

Participant serum samples were collected at preimplantation (baseline) and at 6, 12, 18, and 24 months after implantation. The serum CNTF levels were determined by a CNTF ELISA (R&D Systems, Minneapolis, MN). Specific anti-hCNTF antibody titer was determined by an ELISA by incubating participant serum on a plate coated with hCNTF (R&D Systems), and the signal was detected using a secondary antibody, an HRP-conjugated donkey anti-human IgG (Jackson ImmunoResearch, West Grove, PA). Titers for serum antibodies against the NTC-201 cells were determined using ELISA by incubating the participant's serum on a plate coated with NTC-201 cells for 16 hours and then probing with HRP-conjugated donkey anti-human IgG (Jackson ImmunoResearch).

### Evaluation of the NT-501 Implant after Removal

The implants were removed from the eyes at 6, 12, 18, and 24 months and evaluated by functional (CNTF secretion levels) and morphological (histology) analysis. Immediately upon removal, the devices were placed into Endo-SFM conditioned medium (GIBCO BRL, Gaithersburg, MD) at 37°C, 5% CO<sub>2</sub>, and 95% humidity for 24 hours, and the rate of CNTF secretion was determined using a commercial ELISA kit (R&D Systems). The CNTF standard was prepared according to the package insert, and all standard and sample dilutions were performed in duplicate. Capsules were then fixed in 4% paraformaldehyde for 1 hour and embedded in methacrylate. Ten consecutive longitudinal sections, 4 microns thick, were cut from the center of each capsule and stained with hematoxylin and eosin (H&E). Cells were counted using light microscopy images at 10 $\times$  magnification by two independent observers who determined the viability, distribution, and cell number on a scale of 0 to 5 compared with preimplant NT-501 cohorts.

### Evaluation of Vitreous CNTF Levels

Vitreous samples were collected and flash frozen until the time of assay. To quantify the amount of CNTF in the vitreous, the liquid portion was separated by homogenization and centrifugation at 4°C. The supernatant containing the CNTF protein was collected and analyzed using the same CNTF ELISA kit and methods described above to quantify the device explant secretion levels. Previous qualification studies of the method to quantify CNTF from the vitreous by extracting known concentrations of CNTF from sample vitreous indicate that the CNTF recovery method from the vitreous is approximately 80% efficient with reported recovery concentrations 20% lower than the actual amount. The results reported in this study, however, were based

upon the raw concentration levels determined by ELISA and were not adjusted for potential loss due to the recovery procedure.

### Pharmacokinetic Methods

CNTF secretion rates from explanted NT-501 devices, CNTF levels in the vitreous and serum levels of CNTF, if detected, were each fit to a standard noncompartmental model to evaluate the half-life ( $t_{1/2}$ ), AUC<sub>0- $\infty$</sub> , and mean residence time (MRT, time to reduce drug levels by 63.2%) of CNTF using pharmacokinetic analysis software (PK Solutions, version 2.0; Summit Research Services, Montrose, CO). Statistical analysis of inter- and intratime point and group data using either ANOVA or Student's *t*-test was performed using statistical software (JMP, version 6.0; SAS, Cary, NC).  $P < 0.05$  was considered statistically significant.

## RESULTS

### Sample Distribution

All CNTF1 patients were explanted at 6 months post implantation as per protocol (5/5 for the high dose and 5/5 for the low dose). In the subsequent studies, the NT-501 implant was not required to be explanted at study completion, and the device samples were obtained only when patients elected to have the device removed. The sample distribution of explanted patients included in this study is presented in Table 1. At 12 months post implantation, a total of 11 patients from the high-dose group elected to explant (1/27 in CNTF2, 9/43 in CNTF3, and 1/48 in CNTF4), and 3 patients from the low-dose group elected to explant (1/12 in CNTF2 and 2/22 in CNTF3). At 18 months post implantation, seven patients from the high-dose group elected to explant (2/27 in CNTF2 and 5/43 in CNTF3), and three patients from the low-dose group elected to explant (1/12 in CNTF2 and 2/22 in CNTF3). At 24 months post implantation, 11 patients from the high-dose group elected to explant (1/43 in CNTF3 and 10/48 in CNTF4), and five CNTF4 patients in the low-dose group (5/20) elected to explant. In total, 34 high-dose implants and 16 low-dose implants were assessed in the current study. No NT-501 device was explanted during the course of the studies due to safety concerns or as the result of an adverse event.

### CNTF Pharmacokinetics from Explanted Devices

Over the course of the 24-month implant period, the mean release rate of CNTF from the explanted NT-501 high-dose device was  $1.6 \pm 0.7$  ng/day, while the mean release rate of CNTF from the NT-501 low dose device was  $0.19 \pm 0.12$  ng/day ( $P < 0.0001$ ). The range of CNTF secretion at explant was 0.6 to 2.8 ng/day for the high-dose group and 0.1 to 0.4 ng/day

TABLE 2. Concentration Profiles of Explanted ECT Capsules Delivering CNTF over 24 Months in the Human Eye

Explanted Dose	Rate Constant, $k$ ( $\text{mo}^{-1}$ )	$t_{1/2}$ , mo	MRT*, mo	AUC $_{0-\infty}$ , ng-mo/mL
High dose	0.020	35	47	106
Low dose	0.034	20	30	9

\* MRT (37% CNTF levels compared with 6 months are still present).

for the low-dose group from 6 to 24 months. The mean CNTF production rate of the explanted devices over the 24-month study for both the high- and low-implant groups is shown in Figure 1. CNTF secretion from the high-dose group at 6 months was  $2.1 \pm 0.6$  ng/day and declined to  $1.1 \pm 0.5$  ng/day at the 24-month explant time point. Low-dose explants delivered  $0.28 \pm 0.07$  ng CNTF/day at 6 months and  $0.15 \pm 0.17$  ng CNTF/day at 24 months. Table 2 shows the regression analysis of the high- and low-explant device groups for the 6- to 24-month period of time, which resulted in implant-produced CNTF half-lives of 35 months and 20 months, respectively. The pharmacokinetic model predicts that the mean residence time (MRT) of CNTF, or the time that the devices are calculated to still produce approximately 36% of the levels compared with that of the 6-month device, would occur at approximately 47 months following implantation of the high-dose device, while the MRT for the low-dose device group would be reached at 30 months, or nearly 36% sooner than that of the high-dose group. An intergroup analysis of the mean explant CNTF secretion levels of the NT-501 high dose devices after 12, 18, and 24 months to the levels produced at 6 months indicates statistical equivalence ( $P = 0.1000, 0.4909, \text{ and } 0.1886$ , respectively). A similar intergroup analysis of the low-dose device explants was also statistically equivalent when 12, 18, and 24-month explants were compared with 6-month explants ( $P = 0.1968, 0.1168, \text{ and } 0.1005$ , respectively).

### CNTF Vitreous Pharmacokinetics

The steady-state vitreous levels for the high-dose implants over the 24-month period were compared with their corresponding high-dose explanted device expression levels and are shown in Figure 2. The mean vitreous concentration of CNTF for the high-dose implants over the study period was  $0.051 \pm 0.018$

ng/mL, with levels ranging between 0.046 to 0.054 ng/mL. Intergroup mean vitreous CNTF levels detected at the 6-, 12-, 18-, and 24-month endpoints were statistically equivalent as a group ( $P = 0.1215$ ). Vitreous CNTF levels for the NT-501 low-dose implant groups were only detectable at 6 months and dropped to levels below the limit of detection at all subsequent endpoints. Table 3 shows the regression coefficients and the pharmacokinetic data for CNTF levels in the vitreous from NT-501 high-dose implant secretion over the course of the of a 24-month period. The vitreous half-life of CNTF delivered continuously by the high-dose implants was calculated to be 51 months in human patients, with up to 37% of the levels detected at 6 months remaining at 73 months (MRT).

### Viability of Encapsulated Cells over Course of Implant Period

The number of viable cells remaining within each explanted device for both the low- and high-dose groups was quantified by subjective grading of H&E-stained histologic sections and careful comparison to preimplant controls. Data for this analysis is found in Table 4 and representative images of H&E-stained explant device sections from each time point from the high-dose groups are found in Figure 3. Statistically, the number of viable, encapsulated cells remaining at explants indicated intergroup equivalence for the comparison between the 12-, 18-, and 24-month explant endpoints and the preimplant controls for both the high and low NT-501-explanted device groups ( $P = 0.9255 \text{ and } 0.7442$ , respectively). The 6-month cell number was slightly lower compared with all other endpoints, including the preimplant group, for both the high and low NT-501-explanted device groups. Overall cell number and viability of cells within the capsule remains stable over the

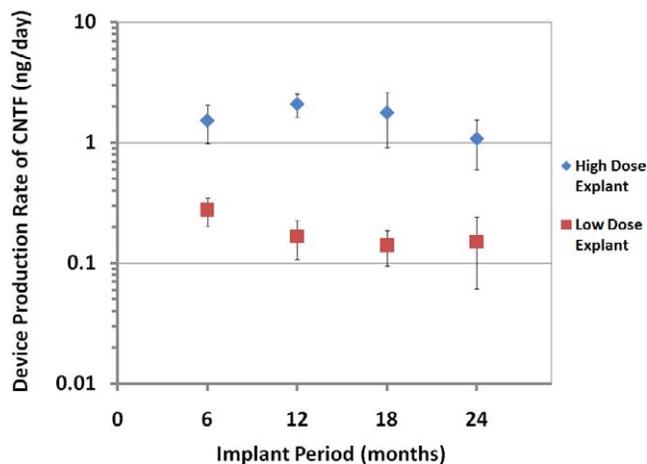


FIGURE 1. CNTF production rate from explanted high-dose and low-dose devices from human vitreous following 6-, 12-, 18-, and 24-month implant period. The mean release rate from explanted devices over the course of a 24-month intraocular implant period was  $1.6 \pm 0.7$  ng/day and  $0.19 \pm 0.12$  ng/day for NT-501 high dose (triangles) and NT-501 low dose (squares), respectively. Data represents mean  $\pm$  SD.

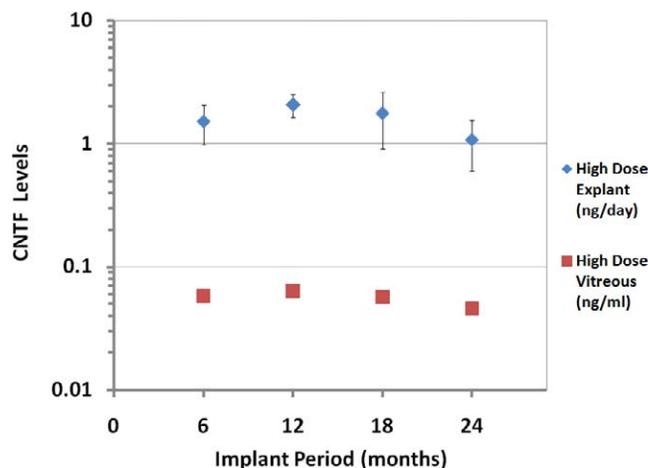


FIGURE 2. CNTF levels detected in human vitreous over 6, 12, 18, and 24 months from high-dose implants compared with corresponding explanted device CNTF production rates. The high-dose implant mean rate of production (triangles) over the course of 24 months was  $1.6 \pm 0.7$  ng/day and the corresponding mean levels of CNTF detected in the vitreous (squares) were  $0.051 \pm 0.018$  ng/ml. Data represents mean  $\pm$  SD.

TABLE 3. Concentration Profile of Vitreous CNTF Levels Produced by High-Dose ECT Capsules over 24 Months in the Human Eye

Vitreous Compartment	Rate Constant, $k$ ( $\text{mo}^{-1}$ )	$t_{1/2}$ , mo	MRT*, mo	AUC $_{0-\infty}$ , ng-mo/mL
High dose	0.014	51	73	5

\* MRT (37% secretion levels compared with 6 months are still present).

course of a 24 month implant period, which was consistent with the stable delivery levels of CNTF from explanted devices evaluated at all endpoints of the study.

### CNTF Serum Levels and Immunological Response

Serum CNTF levels were not detected for either implant group at any time point over the duration of the study period (Lower Limit of Quantification, LLOQ, was 30 picograms/mL). Circulating antibody titers to either CNTF or the encapsulated cells for both high- and low-dose groups over the study period were unchanged relative to preimplant baseline serum levels.

### DISCUSSION

In this retrospective study, we evaluated CNTF levels accumulated in the vitreous and production rate from explanted NT-501 devices over a 24-month clinical trial using the half-life pharmacokinetic parameter. The rationale for use of this calculated kinetic constant was to establish a method to quantify the stability profile of CNTF delivery by ECT over time in patients. Studies investigating the half-life of injected CNTF report the residence time on the order of minutes, a time frame which may severely limit the therapeutic potential of this molecule using bolus injection. Compared with a bolus injection, delivery of CNTF by adenovirus-mediated expression showed improved photoreceptor protection in several studies demonstrating the therapeutic benefit of a sustained delivery approach.<sup>6,25</sup> Our intent in the current study was to evaluate the rate of CNTF delivery and the accumulation of CNTF in the human vitreous following an extended implant of NT-501, and to establish the preliminary implant longevity and drug delivery characteristics necessary to effectively treat patients with chronic retinal degenerative disease by means of the ECT technology.

Data from the explants of NT-501 demonstrates that steady-state expression of CNTF is maintained over 24 months from a high-dose implant, resulting in a calculated CNTF half-life of 35 months or 51 months based upon the device production rate, or the vitreous CNTF levels, respectively. The half-life values for CNTF from an ECT high-dose device implanted in human patients, whether using the device production rate or the vitreous levels, suggests a significant long-term delivery of CNTF by ECT. The low-dose implant CNTF production rate resulted in a calculated half-life of 20 months. However, there were no detectable levels of CNTF in the vitreous beyond the 6-month evaluation period. Therefore, half-life estimates of the low dose vitreous group were not possible.

Comparing the nutrient-poor conditions of the vitreous to the nutrient-rich culture conditions in which the encapsulated cells are maintained prior to implant, the observed difference in 10-fold CNTF output is not surprising. However, neither the health of the implant nor the longevity of stable secretion in the human vitreous appears to have been compromised by the vitreous environment during the course of the study. In fact, the initial preclinical studies in rabbits, pigs, and dogs, and the subsequent studies in humans, suggest that the vitreous milieu of multiple species, including humans,<sup>26</sup> is not only capable of maintaining a healthy environment that can support the survival of NT-501 implants, but is also capable of promoting a stable secretion of CNTF from the encapsulated cells over a sustained period of time.

Robust viability of encapsulated cells over the 24-month implant period in the human eye coincided with the NT-501 high-dose capsules continuously secreting CNTF at a rate of  $1.6 \pm 0.7$  ng/day, providing a range of 0.6 to 2.8 ng/day. In the *rcd1* dog model of retinal degeneration,<sup>24,27</sup> NT-501 explanted devices secreting CNTF at levels exceeding 0.1 ng/day resulted in a dose-dependent increase in photoreceptor protection. However, delivery below 0.1 ng/day was ineffective. The NT-501 high dose implant was selected for study because it continuously secreted CNTF at levels that have previously been shown to support photoreceptor protection in animal models of retinal degeneration. In contrast to the delivery rates of the NT-501 high-dose implant, the NT-501 low-dose implants delivered CNTF near the lower limit of the known efficacious dose (50% of the minimum effective dose). In addition, the levels of CNTF in the vitreous were not detectable in the low-dose group after 6 months. This suggests the low dose CNTF was cleared at an equivalent or faster rate as that produced by the ECT implant; therefore, it is unlikely to have achieved levels necessary to provide therapeutic efficacy.

Consistent with the CNTF levels found to protect photoreceptors in the *rcd1* dog model, patients with GA treated with the NT-501 high-dose capsule demonstrated both structural and functional improvements.<sup>23</sup> In those patients treated with the high-dose NT-501 implant in the CNTF2 trial, a statistically significant increase in macular volume was documented between 4 and 12 months (study completion) using optical coherence tomography (OCT). The macular volume increase was also associated with a trend in stabilization of vision (loss of less than 15 letters) from baseline over 12 months. In a subgroup analysis of patients who started the trial with a visual acuity of 20/63 or better, the high-dose treatment group ( $n = 10$ ) gained a mean of 0.8 letters over the 12-month treatment

TABLE 4. Cell Viability and Remaining Cell Number in Explanted Devices at 6-, 12-, 18-, and 24-Month Endpoint for Both NT-501-10.02 and NT-501-6A.02 Implants

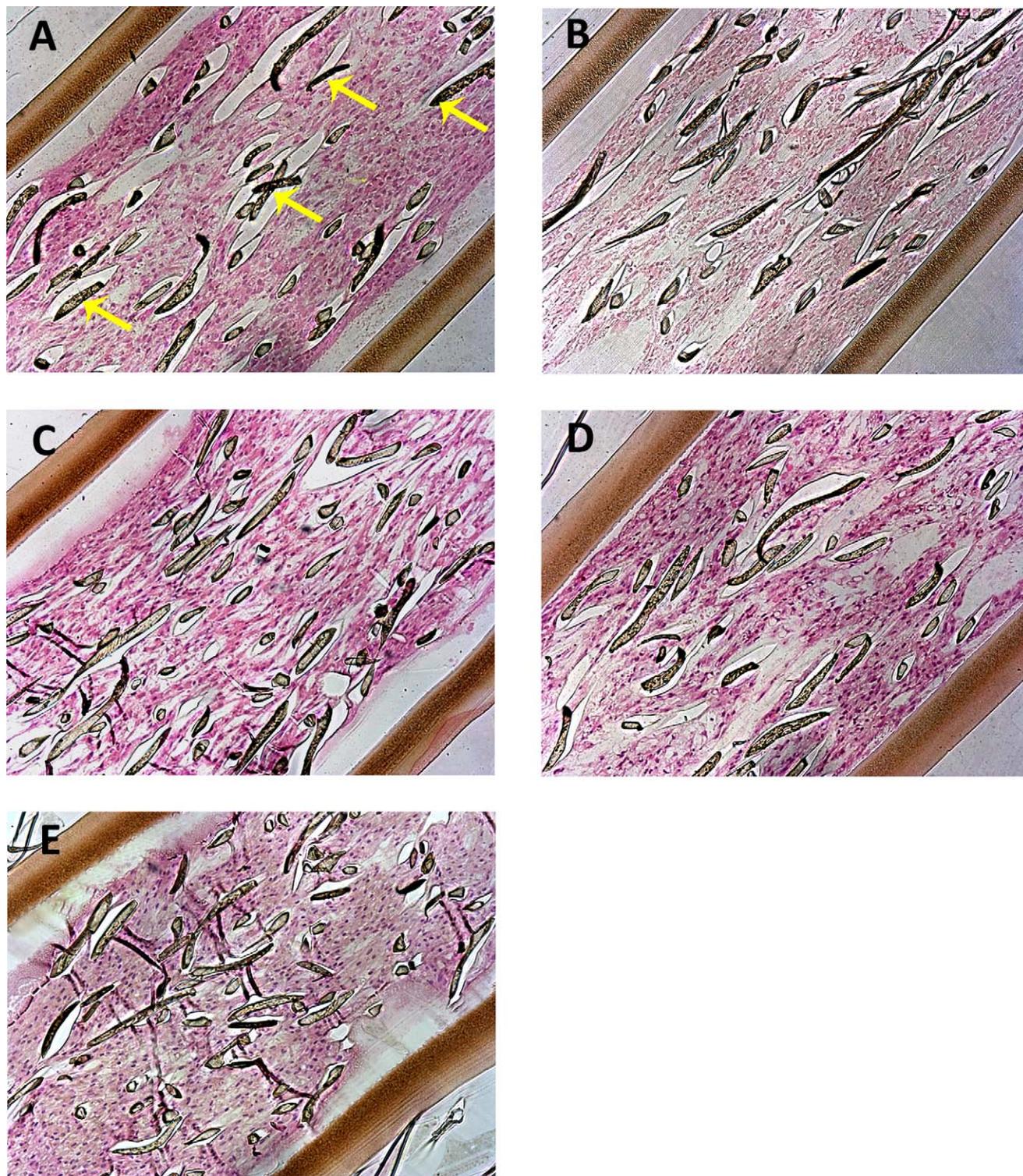
Dose	Preimplant	6-Month	12-Month	18-Month	24-Month	P Value*	P Value†
High dose	$4.7 \pm 0.6$	$3.2 \pm 0.8$	$4.4 \pm 0.7$	$4.4 \pm 0.8$	$4.4 \pm 0.6$	0.0324	0.9255
Low dose	$4.7 \pm 0.6$	$2.5 \pm 0.7\ddagger$	$4.0 \pm 1.0$	$4.5 \pm 0.5$	$4.6 \pm 0.8$	0.0053	0.7442

Two independent graders, blind to the groups, evaluated all device sections and rated the implant on a scale from 0 to 5.

\* ANOVA analysis compared the 6-, 12-, 18-, and 24-month groups to the preimplant control group.

† ANOVA analysis compared the 12-, 18-, and 24-month groups to the preimplant control group.  $P < 0.05$  is considered statistically significant.

‡ NT-501-10 6-month device was 11 mm in length.



**FIGURE 3.** Cross-section histology images of CNTF high-dose devices. (A) Prior to implant. (B-E) Following removal at 6, 12, 18, and 24 months, respectively. Viable cells are documented throughout the entire length and width of the internal compartment of all devices. NTC-201 cells are found to attach to the polyethylene terephthalate scaffold (*yellow arrows* shown for emphasis in image [A] only) while additionally forming cell-to-cell attachments and ultimately populating a majority of the internal space of the device between the scaffolding and the inner wall of the encapsulation membrane (outer bounding structure running parallel on both sides of capsule). All images shown are 4-micron-thick sections, cut approximately at center mass of device, embedded in glycol methacrylate, and stained with hematoxylin and eosin. Images shown are 10 $\times$  magnification.

period compared with the control group ( $n = 9$ ), which lost a mean of 9.7 letters ( $P < 0.033$ ).

Although retinitis pigmentosa patients enrolled in the CNTF4 study did not show significant changes in visual field, visual acuity or ERG responses in either NT-501 treated or the sham treated eye, it is likely that the conventional visual functional tests are not sensitive enough to detect any changes in the slow progression of this disease in the studied period. A subset of patients from this study, however, was additionally evaluated using adaptive optics scanning laser ophthalmoscopy (AOSLO), a relatively new and highly sensitive technique to measure individual cone photoreceptors. Cone preservation in CNTF-treated eyes compared to sham-treated eyes over a 2-year treatment period was demonstrated using AOSLO in these RP patients, suggesting that CNTF treatment may slow or halt progression of retinal degeneration in patients with retinitis pigmentosa.<sup>24</sup>

In both GA and RP clinical studies, no serious adverse events attributed to either the implant or the surgical procedures were reported over the course of either the 12- or 24-month study. Collectively, the clinical trial results provided evidence that sustained intraocular delivery of CNTF protected the retina from degeneration in humans in a safe manner and further support the therapeutic potential of CNTF delivery by encapsulated cell technology.

In addition to neurotrophic factors, such as CNTF, as potential therapeutic agents in the treatment of retinal diseases, the last decade has seen an unprecedented increase in therapeutic advances to treat a wide range of retinal diseases, including the advent of antivascular endothelial growth factor (anti-VEGF) to treat exudative age-related macular degeneration (wet AMD).<sup>28-31</sup> Although highly successful as a treatment option, the requirement of frequent, long-term, high-dose administration of anti-VEGF agents to the patient underscores the need for the development of improved delivery modalities for diseases such as wet AMD, with the goal of improving patient comfort and compliance, while reducing the risks associated with repeated intraocular injections. A drug delivery system such as ECT that is capable of delivering a continuous, de novo synthesized, anti-VEGF agent at microgram-per-day levels over several years would be a promising alternative.

Because of its potency and mechanism of action, the concentration required for a neurotrophic factor to provide neuroprotection is low.<sup>32,33</sup> As such, NT-501 implants in the present study were designed to continuously secrete nanogram levels of CNTF, levels appropriate for the target disease. Therapeutic target drug levels produced by the ECT system are capable of adjustment from picograms/day up to double-digit micrograms/day levels with concomitant stability of the protein secretion profile over time. The levels of protein produced, and the potency of the protein engineered, can be optimized with the ECT platform cell line as in many mammalian biologic production cell lines.<sup>34</sup> Optimization of other components of the ECT system, such as the permeability of the membrane and the internal volume of the ECT device, is also possible. To this end, we have designed ECT systems to produce anti-VEGF agents having recently demonstrated consistent microgram delivery over extended periods of time in preclinical animal models (Kauper K, et al. *IOVS* 2011;ARVO E-Abstract 3223 and Ling V, et al. *IOVS* 2011;ARVO E-Abstract 474), suggesting the possibility of utilizing ECT as a future therapy for wet AMD.

In summary, the data from this retrospective study of ECT implants removed from patients over the course of a 24-month study period, spanning four clinical trials, conclusively shows that ECT can continuously secrete CNTF at efficacious levels in the human eye over the duration of a 24-month implant period. In addition, encapsulated cells remained viable during the

entire implant period, and positive clinical safety profiles for all patients was supported by the absence of CNTF detection in the serum and the lack of circulating antibodies to CNTF or the cell line. The demonstration of steady-state delivery of CNTF, coinciding with an acceptable safety profile and potential efficacy observed in the study patients, support the continued clinical development of NT-501 as a therapy for the treatment of patients with RP and GA. Perhaps as important, the implant durability and the long-term delivery kinetics provided by encapsulated cell technology also justifies expansion of the platform technology to potentially improve standard-of-care treatment modalities for a variety of ocular diseases including wet AMD.

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