BRAIN COMMUNICATIONS

Intracerebroventricular delivery of vascular endothelial growth factor in patients with amyotrophic lateral sclerosis, a phase I study

Philip Van Damme,[1,2,3] Petra Tilkin,[3] Katarina Jansson Mercer,[4] Joke Terryn,[3] Ann D'Hondt,[3] Nina Herne,[4,*] Thomas Tousseyn,[5] Kristl G. Claeys,[3,6] Dietmar R. Thal,[5,7] Olof Zachrisson,[4,†] Per Almqvist,[4,8] Bart Nuttin,[9] Markus Jerling,[4] Folke Bernadotte,[4] Anders Haegerstrand[4,‡] and Wim Robberecht[1,3]

*Present address: Chiesi Pharma AB, Stockholm, Sweden.

†Present address: BioArctic AB, Stockholm, Sweden.

‡Present address: Annexin Pharmaceuticals AB, Stockholm, Sweden.

We studied the feasibility, safety, tolerability and pharmacokinetics of intracerebroventricular delivery of recombinant human vascular endothelial growth factor in patients with amyotrophic lateral sclerosis. In this phase I study in patients with amyotrophic lateral sclerosis, the study drug was delivered using an implantable programmable pump connected to a catheter inserted in the frontal horn of the lateral cerebral ventricle. A first cohort received open label vascular endothelial growth factor (0.2, 0.8 and 2 μg/day), a second cohort received placebo, 0.8 or 2 μg/day of study dug. After the 3-month study period, all patients could participate in an open label extension study. In total, 18 patients with amyotrophic lateral sclerosis, seen at the University Hospitals in Leuven were included. The surgical procedure was well tolerated in most patients. One patient had transient postoperative seizures, due to an ischemic lesion along the catheter tract. The first 3-month study period was completed by 15/18 patients. Administration of 2 μg/day vascular endothelial growth factor resulted in sustained detectable levels in cerebrospinal fluid. A pulmonary embolus occurred in 3 patients, in 1 patient in the first 3-month study, and in 2 patients during the open label extension study. The study drug was well tolerated in the other patients, for up to 6 years in the open label extension study. Our study shows that intracerebroventricular administration of 2 μg/day of vascular endothelial growth factor to patients with amyotrophic lateral sclerosis is feasible, results in detectable cerebrospinal fluid levels and is well tolerated in most patients. The most common serious adverse event was a pulmonary embolus.

1 Department of Neurosciences, KU Leuven – University of Leuven, Leuven, Belgium
2 Laboratory of Neurobiology, VIB, Center for Brain & Disease Research, Leuven, Belgium
3 Department of Neurology, University Hospitals Leuven, Leuven, Belgium
4 Newron Sweden AB
5 Department of Pathology, University Hospitals Leuven, Belgium
6 Laboratory for Muscle Diseases and Neuropathies, Department of Neurosciences, Experimental Neurology, KU Leuven – University of Leuven, Leuven, Belgium
7 Laboratory of Neuropathology, Department of Imaging and Pathology, KU Leuven – University of Leuven, Leuven, Belgium
8 Department of Clinical Neuroscience, Karolinska Instituted, Stockholm, Sweden
9 Department of Neurosurgery, University Hospitals Leuven, Leuven, Belgium

Received March 21, 2020. Revised August 11, 2020. Accepted August 17, 2020. Advance Access publication September 29, 2020

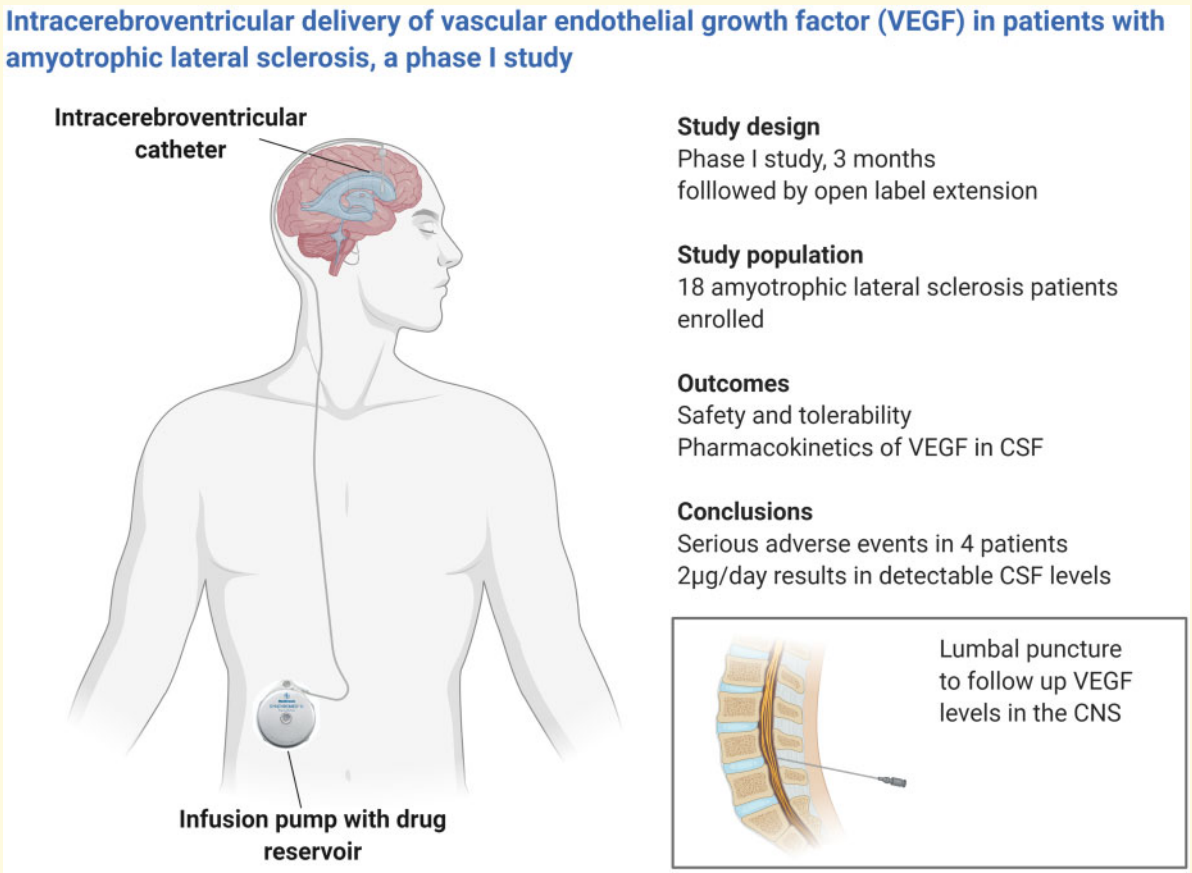
Correspondence to: Philip Van Damme, MD, PhD Neurology Department UZ Leuven, Herestraat 49, 3000 Leuven, Belgium
E-mail: philip.vandamme@uzleuven.be

Correspondence may also be addressed to: Wim Robberecht, MD, PhD. E-mail: wim.robberecht@uzleuven.be and Anders Haegerstrand, PhD, Newron Sweden. E-mail: anders.haegerstrand@newronsweden.com

Keywords: amyotrophic lateral sclerosis; ALS; VEGF; intracerebroventricular; SynchroMed® II programmable pump

Abbreviations: ALS =amyotrophic lateral sclerosis; ALS FRS-R = ALS functional rating scale revised; FUS = fused in sarcoma; ICV = intracerebroventricular; SOD1 = superoxide dismutase 1; SVC = slow vital capacity; VEGF = vascular endothelial growth factor

Graphical Abstract



Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder that primarily affects the motor system. A progressive death of upper and lower motor neurons results in muscular weakness, wasting and spasticity. Patients are gradually incapacitated and usually die of respiratory failure with a median survival of 36 months after disease onset (Brown and Al-Chalabi, 2017; Hardiman *et al.*, 2017; van Es *et al.*, 2017; Masrori and Van Damme, 2020). About 10% of ALS is hereditary, with mutations in *superoxide dismutase 1* (*SOD1*), *chromosome 9 open reading frame 72* (*C9orf72*), *TAR DNA-binding protein 43* and *fused in sarcoma* (*FUS*) being the most common causes (Renton *et al.*, 2014).

Despite advances in our understanding of the genetic causes and disease mechanisms in ALS (Robberecht and Philips, 2013; Taylor *et al.*, 2016), effective therapies are still lacking. In 2001, vascular endothelial growth factor (VEGF-A or briefly VEGF), well known for its role in angiogenesis and cancer (Carmeliet, 2000; Carmeliet and Jain, 2000) was identified as a molecular player in motor neuron degeneration (Oosthuyse *et al.*, 2001). Transgenic mice with reduced VEGF levels, due to a deletion of the hypoxia responsive element in the VEGF promoter, were shown to suffer from a slowly progressive form of motor

neuron degeneration (Oosthuyse *et al.*, 2001). Human and animal studies have confirmed the importance of VEGF in the pathogenesis of motor neuron degeneration (Bogaert *et al.*, 2006). Genetically, a single nucleotide polymorphism in the *VEGF* gene that lowers VEGF expression was found to be associated with ALS, at least in males (Lambrechts *et al.*, 2003, 2009). Furthermore, decreased levels of VEGF have been reported in the cerebrospinal fluid of ALS patients (Devos *et al.*, 2004; Moreau *et al.*, 2006) and the expression of VEGF and VEGF receptor 2 was found to be reduced in the spinal cord of ALS patients (Brockington *et al.*, 2006) and mutant SOD1 mice (Lunn *et al.*, 2009). Similarly, hypoxia-induced VEGF expression has been reported to be attenuated in ALS patients and mouse models (Murakami *et al.*, 2003; Moreau *et al.*, 2006) and VEGF levels have been shown to be inversely correlated with disease progression in ALS (Gao *et al.*, 2014). A patient on long term anti-VEGF treatment developed ALS, but a clear association between such treatments and ALS is not established (Canosa *et al.*, 2015). Finally, VEGF (and also its homolog VEGF-B) has direct neurotrophic effects on motor neurons (Van Den Bosch *et al.*, 2004; Poesen *et al.*, 2008) and protects against excitotoxicity (Tovar *et al.*, 2007; Tolosa *et al.*, 2008; Bogaert *et al.*, 2010), a mechanism likely to play a role in the pathogenesis of ALS (Van Damme *et al.*, 2005).

Increasing VEGF in mutant SOD1 rodent models using a variety of genetic or pharmacological approaches consistently had a beneficial effect on the disease phenotype. Transgenic overexpression of VEGF (Wang *et al.*, 2007) or VEGF receptor 2[32], viral vector-mediated delivery of VEGF to motor neurons (Azzouz *et al.*, 2004; Dodge *et al.*, 2010), intrathecal transplantation of VEGF-overexpressing neural stem cells (Hwang *et al.*, 2009) and direct intraperitoneal (Zheng *et al.*, 2004) or intracerebroventricular (ICV) administration of recombinant VEGF (Storkebaum *et al.*, 2005), did significantly increase the life span of mutant SOD1 rodents with up to 40%.

ICV delivery of VEGF using subcutaneous implanted pumps allows for variable dosing and interruption of therapy, and thus appears to be a controlled strategy for drug administration. Encouraged by the evidence in the literature for this treatment paradigm, we established a safe and tolerable dose in animal toxicity studies and performed a phase I, first-in-human, placebo-controlled randomized trial with ICV delivery of recombinant VEGF in patients with ALS.

Materials and methods

Dose-finding, toxicity and dosing simulation studies

VEGF (recombinant human $VEGF_{165}$, telbermin, sNN0029, Genentech Inc.) was previously used in studies for coronary artery disease (IV administration) (Eppler *et al.*, 2002) and diabetic foot ulcers (topical administration) (Hanft *et al.*, 2008). Limited information about safety and tolerability of ICV VEGF was available from various animal studies using ICV administration of VEGF(Jin *et al.*, 2002; Schanzer *et al.*, 2006; Storkebaum *et al.*, 2005). Doses extrapolated from rodent models (0.6 µg/kg/day) were tested in Cynomolgus monkeys. An ICV dose of 0.34 µg per day delivered continuously using Alzet mini-pumps with a flow rate of 2.5 µl/h was found to be safe, but was accompanied by minor capillary changes in the brain, confirming the expected biological action of the administrated protein. The no observed adverse effect level was 0.2 µg per day, which roughly corresponds to 0.067 µg/kg/day. Based on available data regarding CSF turnover and volume of the central nervous system tissue and CSF containing compartment we selected a dose of 2 µg per day (which corresponds to a dose of 0.028 µg/kg/day for an individual of 70 kg) as the top dose for this first-in-human phase I study.

The delivery of VEGF by the implantable Medtronic SynchroMed® II Programmable Pump Model 8637-20 connected to the Medtronic® Model 8770-4 intracerebroventricular infusion catheter was simulated *in vitro*. A steady state drug delivery from the tip of the catheter was obtained after 4–7 days. VEGF was found to be stable and bioactive in the device at 37°C, over a time period of at least 30 days, which was the maximum time period between refills of the pump in the trial.

Study design and participants

These phase I studies were performed at the University Hospitals Leuven, Belgium. Patients with a diagnosis of definite or probable ALS according to the revised El Escorial criteria and that were judged fit to undergo general anesthesia could participate after signing an informed consent. None of the patients fulfilled the criteria of possible behavioral variant frontotemporal dementia (Rascovsky *et al.*, 2011). All patients underwent gene testing for *C9orf72*, *SOD1*, *FUS* and TAR DNA-binding protein after written informed consent, as part of a separate genetic study (Debray *et al.*, 2013). Patients were recruited between December 2008 and January 2011.

At screening, patients underwent a magnetic resonance imaging and magnetic resonance angiography of the brain to exclude structural lesions that could increase the risk of intracerebral bleeding. Values of coagulation parameters such as platelet count, international normalized ratio, activated partial thromboplastin time had to be within normal ranges. A complete list of the inclusion and exclusion criteria is given in Supplementary Table 2.

Safety data was reviewed after each dose in the first cohort of 8 patients and a data safety monitoring board reviewed all data prior to initiation of the second cohort

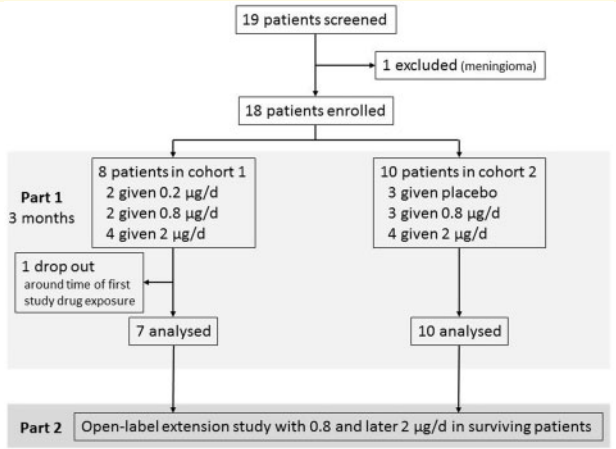


Figure 1 Trial design.

of 10 patients (see Fig. 1) and regularly during the continuation trial.

The first cohort of 8 patients was enrolled in an open-label study of 3 months (sNN0029-001); the first 2 patients received 0.2 µg/day, the next 2 patients 0.8 µg/day and the following 4 patients 2 µg VEGF/day. The inclusion of patients was staggered with at least 4 weeks for the first 6 patients to make sure that no acute toxicity was apparent before the VEGF was administered to the next patient.

The second cohort of 10 patients was treated in a placebo-controlled randomized study (ratio 3 placebo: 7 study drug). The duration of the study was also 3 months.

After the 3-month treatment period, all patients could participate in an open-label continuation study (sNN0029-002) with 0.8 µg/day or 2 µg/day (as soon as the 2 µg/day dose was considered safe by the data safety monitoring board, all patients could receive this dose).

Surgical procedures and initiation of treatment

All surgeries were performed under general anesthesia. MRI-guided stereotactic implantation (Radionics® CRW™ stereotactic system, Integra) of a ICV catheter (Model 8770-4 Investigational Use ICV Catheter, Medtronic, Minneapolis, MN) was performed via a burr hole on the coronal suture, 25–30 mm lateral to the midline on the right side, with the tip of the catheter in the lateral ventricle just anterior to the foramen of Monroe. The ICV catheter was fixed to the skull bone with a custom anchor (Medtronic Inc.) at the burr hole. The position of the catheter was verified by intraoperative fluorography, and by return of cerebrospinal fluid from the catheter. A Medtronic SynchroMed® II Programmable Pump (Model 8637-20) was implanted subcutaneously in the right lower abdominal quadrant after programming and filling the pump reservoir with saline. The pump was connected to the ICV catheter via a subcutaneously tunneled catheter. A post-operative cranial computed tomography scan was carried out to ensure correct position of the catheter.

During the surgery the pump was filled with sterile, preservative-free normal saline and set to run at 150 µl/day. Postoperatively, a computed tomography of the brain was performed to evaluate if complications had occurred. MRI of the brain was performed at regular intervals to monitor the position of the catheter. At least 14 days after the implantation procedure, saline was removed from the pump and the pump was rinsed and filled with 20 ml of investigational product. Depending on the dose cohort, the pump was programmed to deliver the appropriate amount at a continuous flow rate.

Safety, tolerability and efficacy assessments

Safety assessments included: vital signs and physical examination, electrocardiograms, clinical laboratory tests, fundus photography, lumbar puncture, magnetic resonance imaging of the brain, magnetic resonance angiography, formation of anti-VEGF antibodies [using an enzyme-linked immunosorbent assay develop by the Sponsor with a sensitivity of 10–32 ng/ml of anti-hVEGF165 in human plasma using Avastin (Genentech) as control], ALS functional rating scale-revised score, slow vital capacity and EuroQol five-dimension scale.

An autopsy was performed in 6 of the 17 patients who died during the course of the sNN0029-001 or sNN0029-002 continuation study.

CSF sampling and analysis

CSF samples were taken by lumbar puncture on day 11 (2 patients), day 39 and 85 (all patients). Routine CSF testing included measurements of cells, protein, glucose, bilirubin. For additional measurements, the cerebrospinal fluid samples were collected in pre-labeled, non-adhesive polypropylene tubes, immediately put on ice and centrifuged within 30 min for 10 min at 1300 rpm at 4°C. Aliquots of CSF were dispensed in pre-labeled, non-adhesive 1 ml cryo-vials were stored at −80°C until use. VEGF levels in CSF were measured using a validated enzyme-linked immunosorbent assay (Quantikine Human VEGF, R&D Systems, validated by the Sponsor for quantification of VEGF in CSF).

Statistical analysis

Data are shown as mean ± standard deviation. To compare cerebrospinal VEGF levels between different patient cohorts, a two-way ANOVA (repeated measures) was used. For an exploratory analysis of efficacy a combined analysis of survival and function was performed (Cudkowicz *et al.*, 2011). In addition, the decline in ALS

FRS-R (ALS functional rating scale revised), SVC and quality of life was studied using a one-way ANOVA or Kruskal–Wallis in the case of non-normality.

Study approval

The studies were approved by the Ethical Committee of the University Hospital Leuven and registered with Clinicaltrials.gov identifiers NCT00800501 and NCT01384162.

Data availability

All data are available on request.

Results

Patient characteristics and enrollment of patients

Between December 2008 and January 2011 19 ALS patients were screened and 18 patients were enrolled into two separate cohorts. The one screening failure was due to the coincidental finding on MRI of a brain meningioma. A scheme of the enrollment of patients is given in Fig. 1. As stipulated by the protocol, the first 8 patients received increasing doses of VEGF (0.2 µg/day in patient 1 and 2, 0.8 µg/day in patient 3 and 4, and 2 µg/day in patients 5–8). The second cohort of 10 patients was randomized to receive placebo, 0.8 or 2 µg/day. The patient characteristics are summarized in Table 1. We included 12/18 male patients (66.6%). The average age at onset was 47.9 ± 8.4 years, the average diagnostic delay was 10.4 ± 6.4 months, the average disease duration at the time of screening 33.0 ± 28.2 months. The proportion of patients with a bulbar onset was 1/18 (5.6%). Out of the 18 patients, 5 (27.8%) had a familial form of ALS. Genetic testing revealed a mutation in *SOD1* (2 patients), *C9orf72* (2 patients) or *FUS* (1 patient). There was one drop out due to pulmonary embolus early (see below), 2 patients (1 in the 0.8 µg/day group and 1 in the 2 µg/day group) had a very fast progressing form of ALS and died before the end of the 3-month period. Hence, 17/18 patients were analysed of which 15 patients completed the first part of the study.

The surgery for implantation of the ICV catheter in the right frontal horn of the lateral ventricle connected to a Medtronic SynchroMed® II Programmable Pump was well tolerated in all patients. In none of the patients a problem of delayed weaning from the ventilator was encountered. No respiratory complications occurred in the immediate postoperative period. Two patients experienced mild transient headache after the surgery. One patient had 2 epileptic insults with gaze deviation to the left and a tonic phase in the limbs with fast and full recovery of the consciousness on days 1 and 2 after the surgery. The computed tomography scan of the brain immediately after the surgery was unremarkable, but on follow-up imaging studies an ischemic lesion in the right frontal area surrounding the catheter tract was visible. Treatment with levetiracetam was initiated and no further seizures occurred. The patient could complete the study.

Table 1 Patient characteristics

Patient number	Sex	Age at screening (year)	Age at onset (year)	Site of onset	ALS FRS-R at screening	SVC at screening (in %)	Familial form of ALS?	Disease duration at screening (months)	ALS FRS-R decline at screening (pints/month)	Survival after disease onset (months)	Dose of VEGF during first 3 months	Dose of VEGF during extension phase
1	M	48	45	L	30	79	No	39.6	0.45	56	0.2	0.8
2	M	34	32	L	18	92	No	17.9	1.68	43	0.2	0.8, 2
3	M	46	40	L	19	51	No	69.7	0.42	171	0.8	0.8, 2
4	F	63	59	L	33	108	No	42.6	0.35	67	0.8	0.8, 2
5	F	61	51	L	31	97	Yes (*SOD1*)	117.8	0.14	254	2	n.a.
6	M	58	53	L	37	130	No	56.3	0.20	124	2	2
7	M	63	60	L	38	109	Yes (*C9orf72*)	30.2	0.33	51	2	2
8	F	51	50	B	30	65	No	6.5	2.77	9	2	n.a.
9	M	55	53	L	36	90	Yes (*C9orf72*)	17.0	0.71	54	0.8	0.8, 2
10	F	41	40	L	35	82	No	8.4	1.55	29	2	2
11	M	47	46	L	25	45	No	11.9	1.93	14	2	n.a.
12	M	69	65	L	37	59	No	41.7	0.26	48	0.8	0.8, 2
13	F	59	54	L	33	83	Yes (*SOD1*)	55.5	0.27	97	2	2
14	M	47	45	L	41	77	No	12.7	0.55	27	0	2
15	M	44	43	L	39	117	No	13.9	0.65	40	0	2
16	F	40	38	L	38	143	Yes (*FUS*)	22.2	0.45	32	2	2
17	M	45	44	L	30	61	No	14.5	1.24	46	2	2
18	M	46	45	L	35	68	No	16.3	0.80	94	0	2

B, bulbar; F, female; L, limb; M, male; n.a., not applicable.

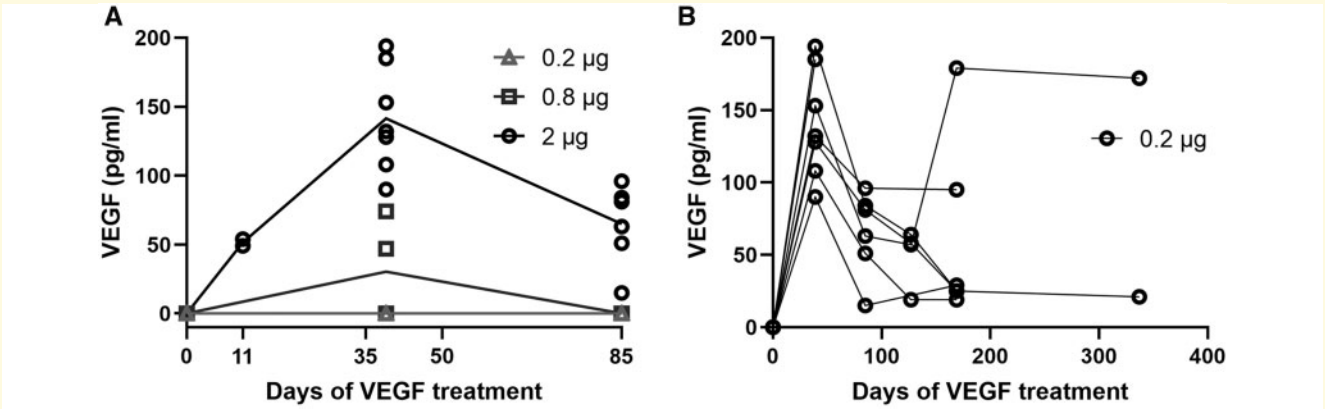


Figure 2 CSF concentrations of VEGF. (**A**) CSF levels over time during the first 3-month period of the study in patients treated with 0.2 µg/day, 0.8 µg/day or 2.0 µg/day measured by enzyme-linked immunosorbent assay. Results are shown as mean ± standard deviation. (**B**) CSF levels in patients on 2.0 µg/day surviving beyond 3 months after initiation of therapy ($n \geq 4$).

Pharmacokinetics of continuous ICV VEGF administration

To estimate the levels of VEGF in CSF after continuous ICV delivery, a lumbar puncture was performed at days 39 and 85 after the initiation of therapy. Patients were also sampled at later time points during the open-label extension study. A dose-dependent increase in CSF levels was noted (Fig. 2A). The baseline CSF levels and the levels in patients treated with placebo or 0.2 µg/day were below detection limit. In the 0.8 µg/day dose group, VEGF was measurable at day 39 (65.3 ± 9.9 pg/ml), but was below detection limit at later time points in 3 out of the 4 patients. In the 2 µg/day dose group, VEGF was always above detection limit (Fig. 2A and B). The average concentration on day 39 in this group was 125.4 ± 15.1 pg/ml. To rule out higher CSF concentrations before day 39, an additional measurement was performed on day 11 in 2 patients (51.5 ± 2.5 pg/ml). At the end of the 3-month period, the average VEGF concentration in patients treated with 2 µg VEGF/day ($n = 7$) was 76.9 ± 17.5 pg/ml (range: 19–204 pg/ml).

Safety, tolerability and feasibility of intracerebroventricular delivery of VEGF

The total duration of exposure to VEGF was 18.4, 70.5 and 300.7 person months for the 0.2, 0.8 and 2 µg/day, respectively. During the 3-month study and the open-label extension study, no technical problems occurred with the pump or catheter. The monthly filling of the abdominally implanted pump was well tolerated in all patients.

ICV VEGF administration was well tolerated in most patients. All adverse events reported during the studies are listed in Supplementary Table 1. Three patients had a pulmonary embolus. One patient had a saddle embolus on day 25 after surgery (1 week after initiation of VEGF at a dose of 2 µg/day), requiring cardiac surgery followed by anticoagulation. The patient fully recovered, but was withdrawn from the study. One patient had dyspnea due to pulmonary emboli 3.5 months after the initiation of treatment (2 µg/day) and was treated with low molecular heparin. The open-label treatment was also stopped in this patient, because of the use of anticoagulation was not allowed per protocol. A third patient collapsed, when getting up after a car drive of 8 h. The patient died shortly thereafter and turned out to have a saddle embolus at autopsy. This patient had received 2 µg/day for 8 months.

One patient developed episodes of dizziness, nausea and slight confusion after 34 months of treatment (2 µg/day). The study drug was stopped, but the episodes continued to occur. Other adverse events reported by different patients included nasofaryngitis (common cold), episodes of headache and anxiety. These adverse events were generally mild and did not require a change in management or compromise study participation.

As part of the safety assessment, ALS FRS-R and SVC were monitored. No unexpected decline in performance was observed. For the patients in whom data could be collected for the full 3-month period, the average decline in ALS FRS-R and SVC was 0.73 ± 0.16 points per month and 2.8 ± 5.5% per month, respectively.

Repeated MRI scans of the brain did not reveal signs of edema, bleeding, tumor or other abnormalities. There were also no concerns in follow-up of the fundus photographs. There were no signs of intracranial hypertension and no signs of capillary proliferation, edema or other retinal abnormalities.

Clinical chemistry, hematological measurements, coagulation tests and ECG's were unrevealing. No VEGF antibodies were identified in patients before or after treatment with ICV VEGF.

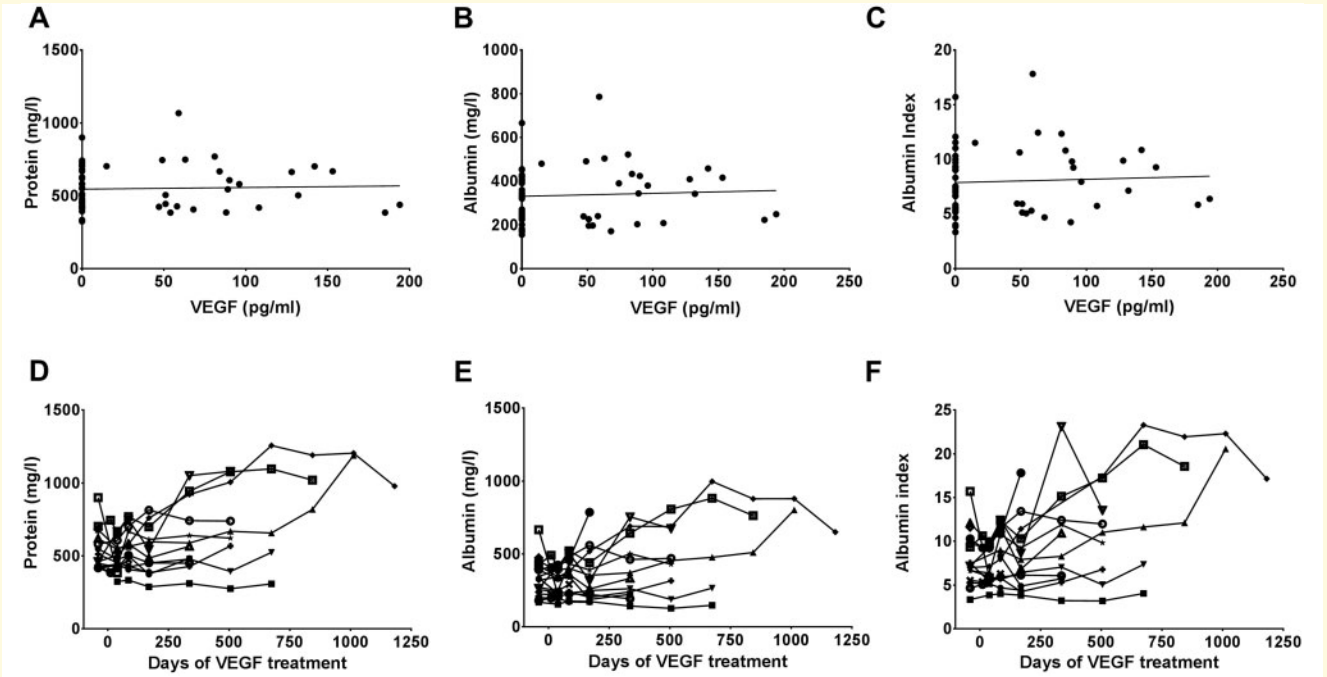


Figure 3 Correlation between CSF VEGF and protein levels. (**A**–**C**) Correlation between CSF VEGF levels and levels of protein (**A**), albumin (**B**) and albumin index (**C**) during part 1 of the study. (**D**, **E**). Evolution over time of CSF protein (**D**), albumin (**E**) levels and albumin index (**F**) of all participants.

Most patients developed a slight increase in the CSF protein levels, in 5 patients the protein levels remained normal. In 3 patients a slight lymphocytic pleocytosis, which was transient in 2/3, was noted (up to 16 cells/µl). During the 3-month study period, no correlation between CSF VEGF levels and CSF protein, albumin or albumin index was noted (Fig. 3A–C). In some of the patients treated for longer time periods an asymptomatic increase in CSF protein, albumin and albumin index was observed (Fig. 3D–F).

An autopsy was performed in 6/17 patients. None of these had mutations in *C9orf72*, *SOD1*, TAR DNA-binding protein or *FUS*. The presence of TPD-43 positive inclusions in remaining motor neurons confirmed the diagnosis of ALS in all 6 patients. No signs of toxicity, such as capillary proliferation, edema or tumor formation were observed. Only minor inflammatory changes along the trajectory of the catheter were seen. An estimation of the capillary density by counting the capillary intersections on CD31 stained slides from the frontal cortex revealed no changes in comparison with untreated control ALS brains (Supplementary Fig. 1).

ALS outcome measures in patients receiving ICV VEGF

This study was not designed to measure efficacy, but several outcome measures were collected for safety reasons. In 3 patients no data could be obtained for the full duration of the first part of the study. The analysis of ALS FRS-R, SVC, visual analogue scale quality of life and combined comparison of survival and ALS FRS-R data (combined analysis of function and survival) did not hint at accelerated disease progression in VEGF-treated patients (Fig. 4A–D). The average decline in ALS FRS-R was 0.82, 1.06, 0.79 and 0.47 points per month in the placebo, the 0.2, the 0.8 µg and the 2 µg/day group, respectively ($P = 0.81$). The decline in SVC (as % of control) was 2.6, 7.5, 2.9 and 1.3 ($P = 0.53$), for the visual analogue scale of the quality of life/EuroQol five-dimension scale, it was 3.6, 9.9, 4.3 and 0.4 points for the same groups ($P = 0.12$), respectively. The combined analysis of function and survival rank score after 3 months was 8.5, 8.5, 8.3 and 9.8 for the placebo, the 0.2, the 0.8 and the 2 µg/day group, respectively ($P = 0.95$).

Discussion

VEGF has been implicated in the pathogenesis of ALS and VEGF treatments have shown beneficial effect in rodent models of ALS. Animal data shows that VEGF can be neuroprotective without stimulating angiogenesis or increasing vascular permeability (Schanzer *et al.*, 2006; Storkebaum *et al.*, 2005). To deliver VEGF beyond the blood-brain barrier in patients with ALS, targeting a tissue concentration to elicit neuroprotection, we used an ICV administration method in this first-in-human phase I study. The

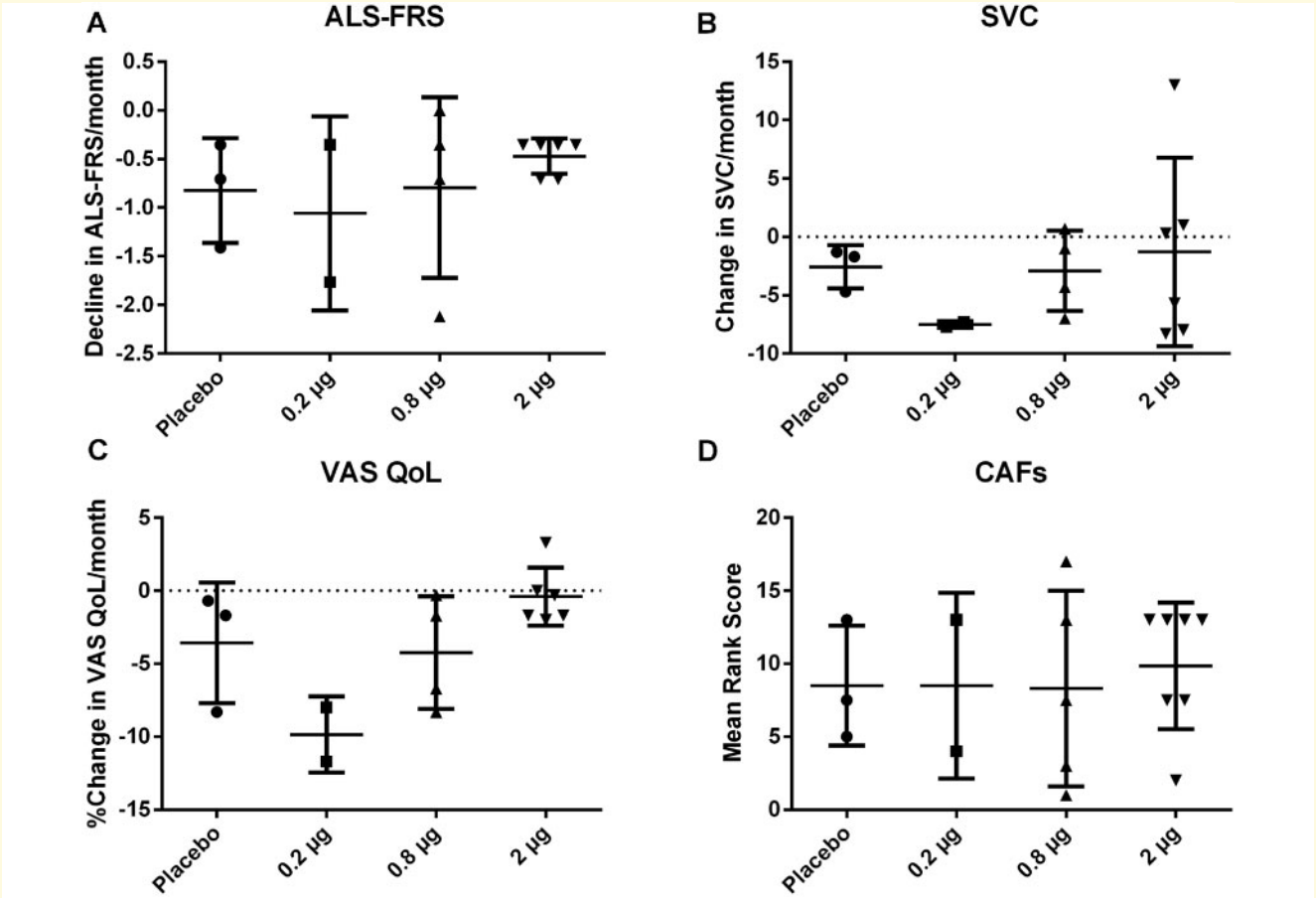


Figure 4 Outcome parameters from part 1 of the study. (**A**–**C**). Decline per month of the ALS FRS revised (in points per month, **B**), of the SVC (**C**) and of the visual analogue scale quality of life (D), for patients completing the first 3-month study period (n = 15). Results are shown as mean ± standard deviation. (D) Mean rank score of the combined analysis of function and survival analysis performed at the end of the 3-month study period ($n = 17$).

implantation of subcutaneous pumps connected to an implanted cerebroventricular catheter and the continuous delivery of VEGF via this method appeared to be feasible. The general anesthesia and surgery were well tolerated in most patients and no technical problems with the infusion system occurred. The distribution of VEGF occurred throughout the cerebrospinal fluid, as VEGF levels in patients receiving 2 µg per day were measurable in CSF taken by a lumbar spinal tap over several months of exposure. To our knowledge this represents a first example of a dose-exposure correlation after administration of a growth or neurotrophic factor into the brain or CSF, which is important in support of future direct-to brain trials for ALS (Miller *et al.*, 2013; Van Damme and Robberecht, 2014) or other neurodegenerative disorders. It is unclear why CSF VEGF levels at day 85 were lower than those at day 39, although similar drug level reductions have been observed after intrathecal administration of antisense oligonucleotides (Miller *et al.*, 2020).

Serious adverse events occurred in four patients. One patient developed postoperative seizures and had an ischemic lesion along the catheter tract. Although the patient fully recovered, this type of complications has to be taken into account in invasive studies requiring neurosurgery. A pulmonary embolus occurred in 3 patients receiving 2 µg/day of VEGF at different time points in the study. This suggests that it is not solely related to the increased postoperative risk for thromboembolic events. A possible association between the study drug and a pulmonary embolus can therefore not be excluded. However, deep venous thrombosis and pulmonary embolus are increasingly recognized in patients with ALS (Elman *et al.*, 2005; Qureshi *et al.*, 2007; Gladman *et al.*, 2014) suggesting that the prevalence is much higher than previously thought. The occurrence of deep venous thrombosis in this study is in accordance with the reported annual incidence rates (of 11% for all ALS patients and 35% of for patients with significant leg weakness) from a recent prospective study (Gladman *et al.*, 2014). The 3 patients with pulmonary embolus in this study all had severe leg weakness. Treatment with low molecular heparins to reduce the thrombosis risk

was not allowed in the study protocol, also not in the perioperative period before the initiation of VEGF therapy, because the risk for bleeding was considered to be too high in this first-in-human study. Measures to prevent deep venous thrombosis should be reconsidered in future trials with ICV delivery of VEGF in ALS patients.

Increased levels of albumin in CSF were seen at non-tolerable doses in toxicological studies and regarded as an expected dose dependent effect following VEGF infusion due to its ability to increase vascular permeability. Therefore we followed albumin levels in CSF and albumin index (CSF/plasma) as indicators of a potentially disrupted blood brain barrier. There was an increase in albumin levels and albumin index over time in some patients, but this was not associated to clear safety or tolerability problems. No CSF samples from placebo treated patients after the initial 3-month study period were available and therefore it is difficult to judge whether the CSF changes observed are related to the treatment. A mild pleocytosis was seen in 3 patients receiving 2 µg VEGF per day and may be associated to the infusion of study drug. It was transient in 2 patients and always asymptomatic.

A limitation of the study is the high variability in patient characteristics, most likely due to selection bias in this small cohort of ALS patients. Given the invasiveness of the delivery method a tendency towards younger age at onset was noted and some patients with a rapid disease progression participated as well. The survival after disease onset varied considerably, ranging from 9 months to 171 months. Unfortunately, 2 patients did not survive the first 3-month period of the trial due to very rapid disease progression.

Direct infusion of drugs in the central nervous system may become a novel approach in trials for ALS (Miller *et al.*, 2013; Van Damme and Robberecht, 2014; Miller *et al.*, 2020) or other neurodegenerative disorders. With more than 300 person months of exposure to the highest dose of ICV VEGF, the feasibility of ICV VEGF administration was shown. The possible association with pulmonary embolus requires further study. These results justify further trials in larger groups of ALS patients, possibly even with higher doses. As the CSF VEGF levels varied considerably between the different study participants, dose titrations based on CSF measurements should be considered in future dose-finding studies. The current study did not allow to assess the effect on disease progression, but the incorporation of biomarkers, such as neurofilaments (Poesen and Van Damme, 2019), could help to get a first sense of efficacy in a phase II study.

Acknowledgements

We are indebted to the patients who participated in this trial and to their families. We thank Lisa Shafer and Robert Coffey, former employees of Medtronic, Inc, for advice and technical support with the implantable catheters and programmable pumps. We thank Jimmy Beckers for the help with the graphical abstract (created using BioRender.com). P.V.D. holds a senior clinical investigatorship of the FWO-Vlaanderen. W.R., P.V.D. and K.C. are supported through the E. von Behring Chair for Neuromuscular Disorders. P.V.D. and D.R.T. received KU Leuven internal funding (C1—C14-17-107). D.R.T. received funds from Fonds Wetenschappelijk Onderzoek Vlaanderen (FWO—G0F8516N Odysseus) and Vlaamse Impulsfinanciering voor Netwerken voor Dementie-onderzoek (IWT 135043). P.V.D. is supported by the ALS Liga België and the KU Leuven funds 'Een Hart voor ALS', 'Laeversfonds voor ALS Onderzoek' and 'the Valéry Perrier Race against ALS fund'.

Funding

Newron Sweden AB (formerly NeuroNova AB) financed the studies and acted as study Sponsor.

Supplementary material

Supplementary material is available at *Brain Communications* online.

Competing interests

K.J.M., P.A., M.J., N.H., O.Z., F.B. and A.H. are, or have been, employees of Newron Sweden AB, a fully owned subsidiary of Newron S.P.A., a listed company. Medtronic, Inc. donated travel and research grants and a Chair of Neurosurgery for Psychiatric Disorders to B.N. D.R.T. received travel support and/or a speaker honorarium from GE-Healthcare (UK) and Novartis Pharma Basel (Switzerland) and collaborated with GE-Healthcare (UK), Novartis Pharma Basel (Switzerland), Probiodrug (Germany) and Janssen Pharmaceutical Companies (Belgium).

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