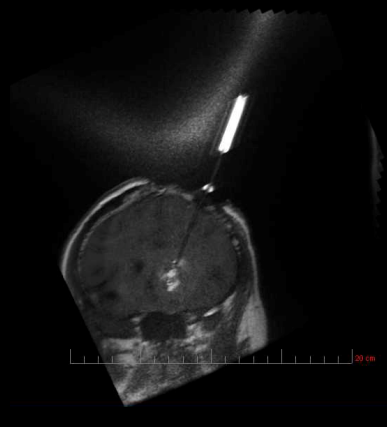
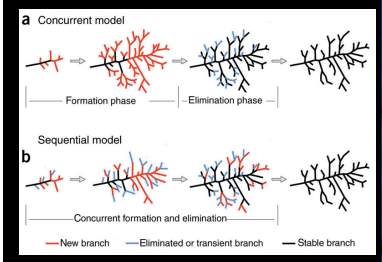
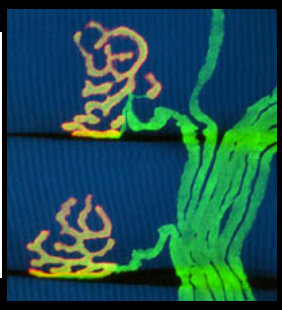
Gene therapy for the Nervous System



I fattori neurotrofici





La sopravvivenza dei motoneuroni delle corna anteriori del midollo spinale dipende strettamente dalla produzione di fattori neurotrofici da parte del muscolo innervato: solo i neuroni che raggiungono il muscolo bersaglio durante lo sviluppo sopravvivono, mentre gli altri vanno in contro ad un processo di apoptosi.
I fattori neurotrofici nell'adulto controllano la sintesi proteica e la sintesi di neurotrasmettitori.

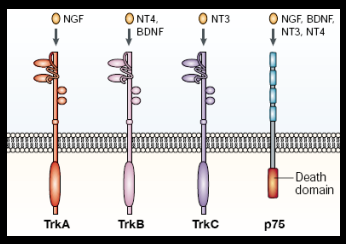
Families of neurotrophic factors

Box 1 | **Haploinsufficiency of neurotrophins**

NGF$^{+/-}$ mice
- Decreased cholinergic innervation of the hippocampus[15]
- Deficiency in memory acquisition and retention[15]
- Loss of neurons of the peripheral nervous system[121]

BDNF$^{+/-}$ mice
- Hyperphagia, obesity[16–18]
- Impairment of long-term potentiation[19,20,122]
- Elevated striatal dopamine levels[123]
- Loss of mechanosensitivity[124]
- Loss of neurons of the peripheral nervous system[125,126]

NT3$^{+/-}$ mice
- Deficient amygdala KINDLING activity[127]
- Cardiovascular defects[128]
- Reduced mechanoreceptors[129]
- Loss of neurons of the peripheral nervous system[130]

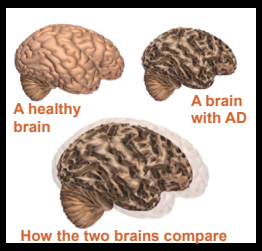


R. Levi Montalcini

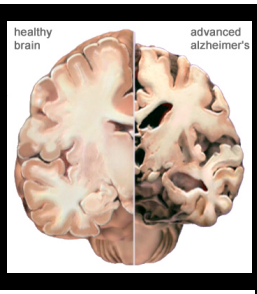
1. neurotrophins: NGF, BDNF, NT-3, NT-4
2. GDNF: GDNF, neurturin (NTN), artemin (ART), persephin (PSP)
3. CNTF/LIF

Alzheimer's Disease

7% of people over 65, 40% over 80



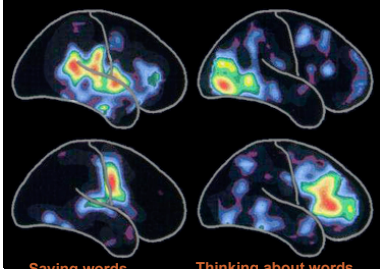
Dramatic loss of cholinergic neurons!



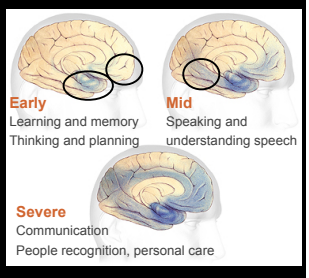
- The **cortex shrivels up**, damaging areas involved in thinking, planning and remembering.
- Shrinkage is especially severe in the **hippocampus**, an area of the cortex that plays a key role in formation of new memories.
- **Ventricles** (fluid-filled spaces within the brain) grow larger.

AD progression through the brain

Hearing words **Reading words**



Saying words **Thinking about words**

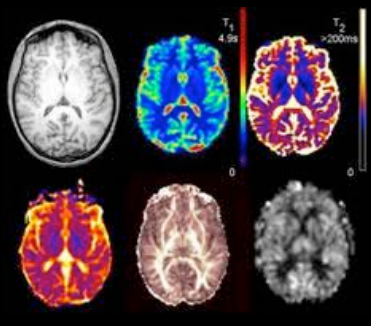


- **Earliest Alzheimer's** – changes may begin 20 years or more before diagnosis.
- **Mild to moderate Alzheimer stages** – generally last from 2 - 10 years.
- **Severe Alzheimer's** – may last from 1 - 5 years.

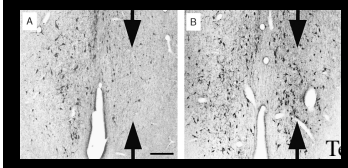
No effective therapy available able to modify disease progression

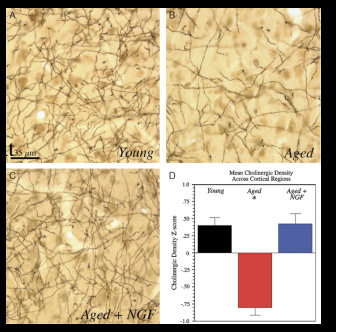
Dementia and normal ageing what makes the difference?

The boundaries between non-pathological brain ageing and the dementias are unclear and contentious...



NGF prevents cholinergic neuronal death in rodents and primates and restores cortical cholinergic terminal density in aged primates (dogma revolution: growth factor biology era!!)



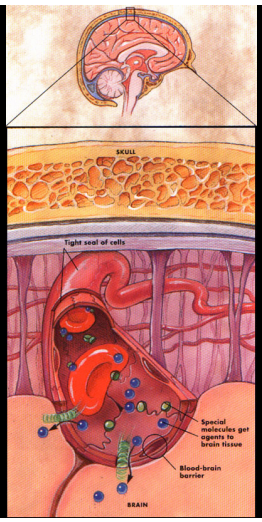


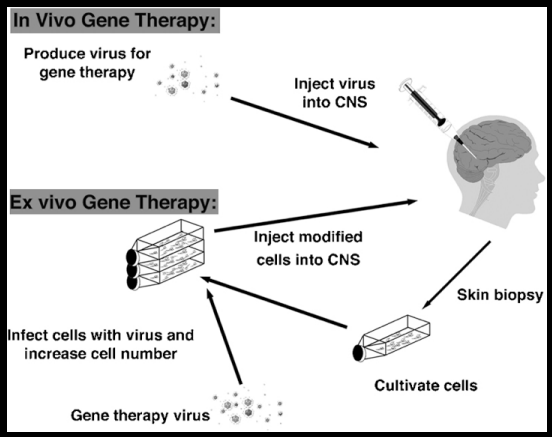
- protection of basal forebrain cholinergic neurons after axotomy
- reversion of age-related atrophy
- improved learning and memory

→ **INITIATION OF CLINICAL TRIALS**

- NGF is relatively large and polar molecule compared with most drugs
- NGF does not cross the BBB: it requires CNS administration
- Adverse effects arising from cells (other than cholinergic neurons) expressing NGF receptors: pain (stimulation of dorsal root ganglion nociceptive neurons), weight loss, sympathetic axon sprouting in the cerebral vasculature, Schwann cell proliferation.

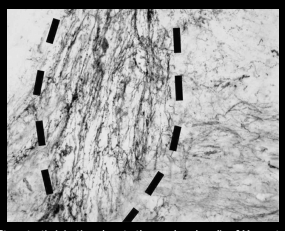
GENE DELIVERY





2001: A Phase 1 Human Trial of Ex Vivo NGF Gene Therapy for AD

- Skin biopsy to generate primary fibroblast cultures
- MLV-NGF vector to secrete hNGF within a range of 50-75 ng/10^6 cells/d (3 dose cohorts enrolled)
- Initially subjects were treated in a sedated but wakeful state but 2 subjects moved during injection resulting in intraparenchymal hemorrhage. Subsequently, all subjects underwent general anesthesia.

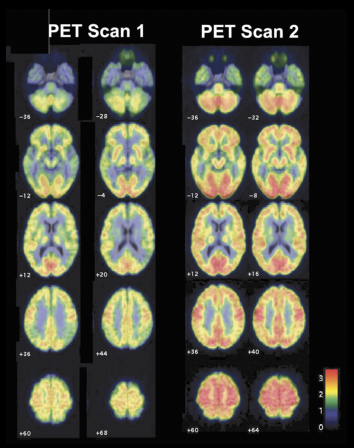


Stereotactic injection close to the nucleus basalis of Meynert, from where cholinergic neurons project toward the whole cortex

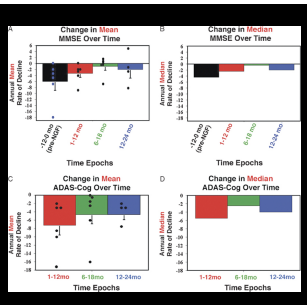
- One individual died 5 weeks post NGF delivery: cholinergic axons robustly extended toward the site of NGF gene transfer

Nat Med. 2005;11:551–555

- Mean follow-up: 3.5 years
- No long-term adverse effects
- Serial MRI and PET scans: statistically significant increase in cortical glucose uptake after 6-8 months (AD normally results in a steady decline)



- Cognitive tests not conclusive but suggestive of possible reduction in rate of decline (Mini-Mental Status Examination and AD Assessment Scale-Cognitive sub-component)



CONCLUSIONS

1. NGF can be delivered safely to the brain over an extended period using gene delivery but needs general anesthesia or deep sedation
2. Degenerating cholinergic neurons of the human brain exhibit trophic response to NGF
3. Broad cortical regions demonstrate enhance glucose metabolism
4. Larger, controlled, blinded clinical trials of NGF delivery are warranted

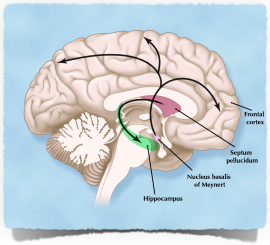
A phase1 study of stereotactic gene delivery of AAV2-NGF for Alzheimer's disease

Michael S. Rafii‡, Tiffany L. Baumann‡, Roy A.E. Bakay†, Jeffrey M. Ostrove, Joao Siffert, Adam S. Fleisher, Christopher D. Herzog, David Barba, Mary Pay, David P. Salmon, Yaping Chu, Jeffrey H. Kordower, Kathie Bishop, David Keator, Steven Potkin, Raymond T. Bartus‡

Alzheimers and Dementia, September 2014

MAY 15, 2014

RICHMOND, Calif., -- Sangamo BioSciences, Inc. announced positive data from the Phase 1 clinical trial of CERE-110 (AAV-NGF), a gene therapy approach designed to deliver nerve growth factor **(NGF)** for the treatment of Alzheimer's disease **(AD)**. This novel product was developed by Ceregene, Inc., which was recently acquired by Sangamo. The data were presented at the Sixth Clinical Trials on Alzheimer's Disease (CTAD) Meeting.



Results

AAV2-NGF was safe and well-tolerated for 2 years. Positron emission tomographic imaging and neuropsychological testing showed no evidence of accelerated decline. Brain autopsy tissue confirmed long-term, targeted, gene-mediated NGF expression and bioactivity.

Conclusions

This trial provides important evidence that bilateral stereotactic administration of AAV2-NGF to the nucleus basalis of Meynert is feasible, well-tolerated, and able to produce long-term, biologically active NGF expression, supporting the initiation of an ongoing multicenter, double-blind, sham-surgery-controlled trial.

JAMA Neurology | Original Investigation

Adeno-Associated Viral Vector (Serotype 2)–Nerve Growth Factor for Patients With Alzheimer Disease

A Randomized Clinical Trial

Michael S. Rafii, MD, PhD; Mark H. Tuszynski, MD, PhD; Ronald G. Thomas, PhD; David Barba, MD; James B. Brewer, MD, PhD; Robert A. Rissman, PhD; Joao Siffert, MD; Paul S. Aisen, MD; for The AAV2-NGF Study Team

IMPORTANCE Nerve growth factor (NGF) is an endogenous neurotrophic factor that prevents the death and augments the functional state of cholinergic neurons of the basal forebrain, a cell population that undergoes extensive degeneration in Alzheimer disease (AD).

OBJECTIVE To determine whether stereotactically guided intracerebral injections of adeno-associated viral vector (serotype 2)–nerve growth factor (AAV2-NGF) are well tolerated and exhibit preliminary evidence of impact on cognitive decline in mild to moderate AD-associated dementia.

DESIGN, SETTING, AND PARTICIPANTS In a multicenter phase 2 trial, 49 participants with mild to moderate AD were randomly assigned in a 1:1 ratio to receive stereotactically guided intracerebral injections of AAV2-NGF or sham surgery. Participants were enrolled between November 2009 and December 2012. Analyses began in February 2015. The study was conducted at 10 US academic medical centers. Eligibility required a diagnosis of mild to moderate dementia due to AD and individuals aged 55 to 80 years. A total of 39 participants did not pass screening; the most common reason was Mini-Mental State Examination scores below cutoff. Analyses were intention-to-treat.

INTERVENTIONS Stereotactically guided intracerebral injections of AAV2-NGF into the nucleus basalis of Meynert of each hemisphere or sham surgery.

MAIN OUTCOMES AND MEASURES Change from baseline on the Alzheimer Disease Assessment Scale–cognitive subscale at month 24.

RESULTS Among 49 participants, 21 (43%) were women, 42 (86%) self-identified as white, and the mean (SD) age was 68 (6.4) years. AAV2-NGF was safe and well-tolerated through 24 months. No significant difference was noted between the treatment group and placebo on the primary outcome measure, the Alzheimer Disease Assessment Scale–cognitive subscale (mean [SD] score, 14.52 [4.66] vs 9.11 [4.65], $P = .17$).

CONCLUSIONS AND RELEVANCE This multicenter randomized clinical trial demonstrated the feasibility of sham-surgery–controlled stereotactic gene delivery studies in patients with AD. AAV2-NGF delivery was well-tolerated but did not affect clinical outcomes or selected AD biomarkers. Pathological confirmation of accurate gene targeting is needed.

TRIAL REGISTRATION clinicaltrials.gov Identifier NCT00876863

Table 3. Change on Outcome Measures at 24 mo (N = 49)

Outcome Measure[a]	Mean Change (95% CI) Placebo Group (n = 23)	Treatment Group (n = 26)	*P* Value
ADAS-Cog 11[b]	9.11 (4.46 to 13.57)	14.52 (9.86 to 19.18)	.17
CDR-SOB	2.81 (1.34 to 4.28)	4.75 (3.20 to 6.30)	.09
mCGIC	5.33 (5.06 to 5.60)	5.59 (5.26 to 5.92)	.21
MMSE	−4.17 (−6.84 to 1.50)	−6.18 (−8.36 to 4.00)	.16
NPI	9.18 (−0.71 to 19.07)	6.61 (1.85 to 11.37)	.95
ADCS-ADL	−12.94 (−22.13 to 3.75)	−17.65 (−24.49 to 10.81)	.61

Abbreviations: ADAS-Cog 11, Alzheimer's Disease Assessment Scale–cognitive subscale; ADCS-ADL, Alzheimer Disease Cooperative Study–Activities of Daily Living; CDR-SOB, Clinical Dementia Rating-sum of boxes; mCGIC, modified Clinical Global Impression of Change; MMSE, Mini-Mental State Examination; NPI, Neuropsychiatric Inventory.

[a] In almost all outcome measures, there was a trend toward worsening in the treatment arm.

[b] There was no statistical difference in ADAS-Cog 11 at 24 months between treatment and placebo arms. Mean (SD) change was 14.52 (4.66) for treatment vs 9.11 (4.65) for the placebo group ($P = .17$).

JAMA Neurol. doi:10.1001/jamaneurol.2018.0233
Published online March 26, 2018.

EDITORIAL

Gene Therapy in Alzheimer Disease—It May Be Feasible, but Will It Be Beneficial?

Lawrence S. Honig, MD, PhD

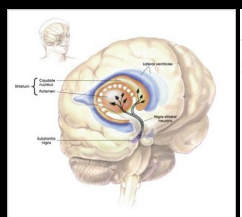
This study provides a lesson on historical controls because it was performed after an open-label phase 1 trial on 10 individuals seemed to show stability and decreased cognitive and functional decline compared with historical controls.

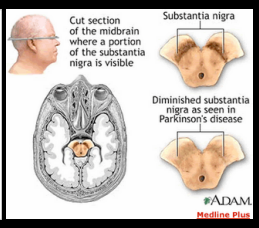
The fact that no benefit was evident in this randomized, double-blind phase 2 study emphasizes the lack of scientific validity for open-label comparisons with historical controls in clinical trials. The reasons why treatments so often appear beneficial in comparisons with untreated historical controls are well-known:

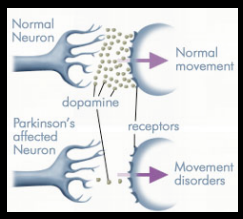
(1) individuals in a treatment trial are from a different population sample than those in observational studies, are often highly motivated, and receive better symptomatic and general treatments

(2) historical controls are from an earlier period, and given a secular trend toward earlier diagnosis and ascertainment earlier in the disease course, current trial participants usually appear to have more stable disease status than historical controls did. This may be relevant to other recent restorative therapy trials with controversial analyses in which historical controls were used as evidence of possible efficacy.

Gene therapy for Parkinson Disease



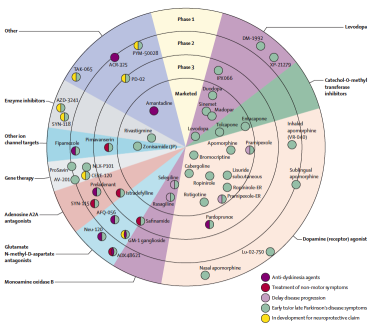




- **Rigidity** — Arms and legs become stiff and hard to move
- **Tremors** — Rapid shaking of the hands, arms or legs
- **Slowed Movements** — Difficulty starting or completing movements, called bradykinesia
- **Impaired Balance** — Lack of balance or difficulty adjusting to sudden changes in position

Current therapies for PD

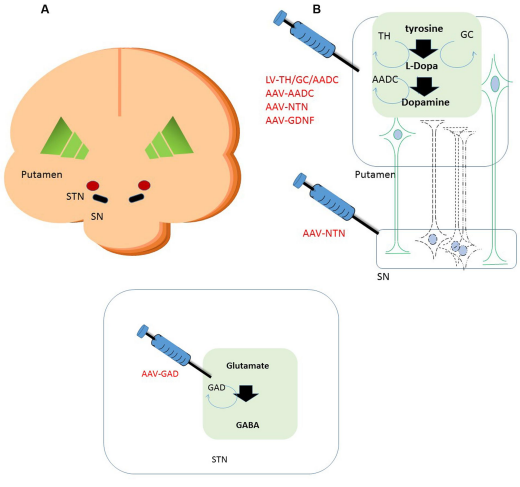
- Replacement therapy levo-dopa + carbidopa: long-term complications limiting the dose
- Deep brain stimulation: technically complex
- Human fetal mesencephalic cell transplantation: double-blind controlled trials disappointing



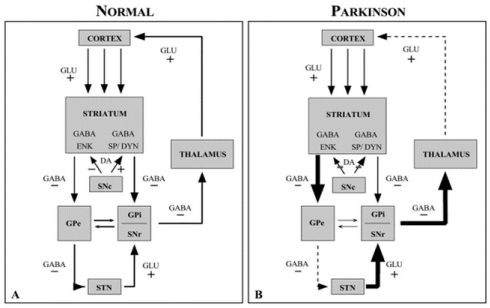
Gene therapy

- Enhancement of DA synthesis
- Delivery of neurotrophic factors (neurturin)
- Interference with aberrant protein aggregation
- AAV-GAD: conversion of the subthalamic nucleus in an inhibitory rather than an excitatory structure

Brain targets in gene therapy for PD



AAV-GAD Background & Rationale



In PD, loss of DA projections from the SN to the striatum results in overactivity of the subthalamic nucleus.

The subthalamic nucleus sends excitatory projections to the internal part of globus pallidus and the pars reticulata of the SN, which in turn inhibits motor output.

AAV-GAD Background & Rationale

- Adeno-associated virus (AAV) vectors can yield safe, stable gene transfer in the adult brain (Kaplitt, et. al. Nat. Gen. 8:148-154,1994)
- GAD is the rate-limiting enzyme in synthesis of GABA
- GABA infusion in STN reduces firing and improves symptoms transiently (Levy, et. al., Brain 124:2105-2118, 2001)
- AAV-GAD improves motor function and normalizes motor circuits in rodent and primate PD models (Luo, et. al., Science 298:425-429,2002; Emborg, et. al., J Cereb Blood Flow Metab 27:501-509, 2007)

Safety and tolerability of gene therapy with an adeno-associated virus (AAV) borne *GAD* gene for Parkinson's disease: an open label, phase I trial

Michael G Kaplitt, Andrew Feigin, Chengke Tang, Helen L Fitzsimons, Paul Mattis, Patricia A Lawlor, Ross J Bland, Deborah Young, Kristin Strybing, David E Eidelberg, Matthew J During

Lancet 2007; 369: 2097–105

- 12 patients
- 5x10^9-5x10^10 AAV2-GAD particles infused unilaterally

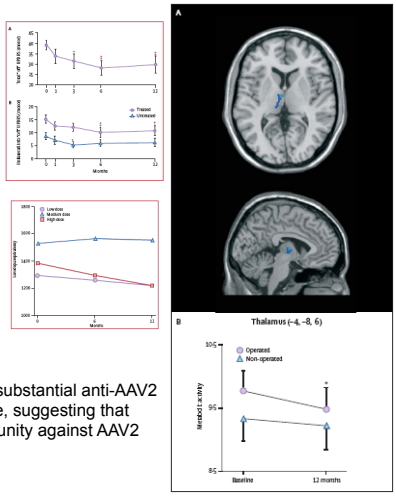
Results

No adverse events related to the gene therapy

Clinical improvement in motor rating

Changes of daily dose of dopaminergic medication

Reduction in glucose metabolism of the thalamus in the operated hemisphere



Two patients showed evidence of substantial anti-AAV2 immunity but no changes over time, suggesting that vector infusion did not induce immunity against AAV2

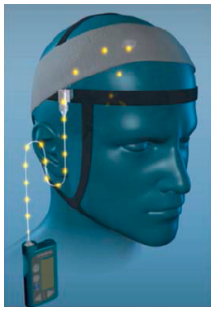
Surprising findings:
- bilateral improvement after unilateral therapy
- improvement in best on-medication function

Concerns and caveats:
- absence of sham-operated control group
- the excitatory role of the subthalamic nucleus suggests its role in learning: what might be the long-term effect of converting this nucleus from an excitatory to an inhibitory structure?

NEUROLOGIX

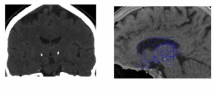
- The primary objective of the Phase 2 study is to evaluate the clinical antiparkinsonian efficacy of rAAV-GAD, administered bilaterally into the subthalamic nucleus of 20 subjects with advanced PD, for comparison to 20 sham-operated PD controls at 6 months after the procedure
- The secondary objectives are
  - To evaluate the safety of rAAV-GAD administered to bilateral subthalamic nuclei through 12 months after the procedure
  - To assess the outcomes of rAAV-GAD administration on PD disability, activities of daily living, motor fluctuations, dyskinesias, and quality of life assessments through 12 months after the procedure
  - To evaluate metabolic activity related to PD measured by FDG-PET through 12 months after the procedure

- With the patient under local anesthesia, the neurosurgeon will drill burr holes on both sides of the skull
- A stereotactic frame will be used to place small catheters in the subthalamic nucleus, after targeting based on presurgical CT scan or MRI; the planning procedure is comparable to DBS
- Once the catheters are in place, the burr holes will be covered with a special capping system and the patient will be transferred to the recovery room for infusion of the study agent or saline



- The infusion system was codeveloped by Neurologix and Medtronic and is approved for use in this procedure
  - It should be noted that this system is investigational and is not approved for other uses
- Infusion takes place in the recovery room for 150 minutes
- Imaging is used to verify placement of the catheter
- CT and MRI scans are used for safety measurements at 24 and 48 hours, respectively, before the patient is released from the hospital

Blinded Catheter Tip Localization



Target Area Relative to Mid-Commissural Point:
X=9-14mm lateral
Y=2mm anterior-5mm posterior
Z=1mm dorsal-7mm ventral

(Standard DBS tip coordinates in postero-ventral STN:
X=12mm lateral, Y=3.5mm posterior, Z=4mm ventral)

Medtronic
When Life Depends on Medical Technology

7 participating centers in the United States

Phase 2 Trial Sites

Site	Principal Investigator	Neurosurgeon
Henry Ford	Peter Lewitt, MD	Jason Schwalb, MD
Massachusetts General Hospital	Alice Flaherty, MD	Emad Eskandar, MD
The Ohio State University	Sandra Kostyk, MD, PhD	Atom Sarkar, MD, PhD Ali Rezai, MD
Stanford University	Kathleen Poston, MD, PhD	Jaimie Henderson, MD
University of Colorado	Maureen Leehey, MD	Steven Ojemann, MD
University of Rochester	Roger Kurlan, MD	Jason Schwalb, MD
Wake Forest University	Mustafa Siddiqui, MD	Stephen Tatter, MD, PhD
The Feinstein Institute (Centralized PET imaging)	Andrew Feigin, MD, PhD	-------

Each surgeon completed a minimum of 3 surgeries

Study Summary To Date

- Statistically significant improvements from baseline UPDRS III score seen in AAV-GAD compared to sham over six month blinded phase
  - Sham methodology effective, small sham effect, both treatment and sham effect stable over time
- Significantly greater responder rate in AAV-GAD compared to sham
  - Shulman, et. al. (2010) Ann Neurol 67:64-70
- Significant differences in certain secondary measures
- Correlation between AP and DV catheter tip location and outcome
- GAD therapy continued to be very safe and well-tolerated in this group
- Twelve month follow-up ongoing
  - Crossover of shams currently being planned
- Functional imaging analysis supports efficacy and safety clinical outcomes

Table I: Gene therapies under development for Parkinson's disease (from Prous Science Integrity®).

Gene therapy	Description	Source	Phase
AV-201	Adeno-associated virus (AAV) vector containing the gene that encodes the enzyme L-amino acid decarboxylase (AADC)	Genzyme/Avigen	I/II
CERE-120	AAV type 2 vector encoding a modified human neurturin (NRTN) gene that carries the preprodomain of human nerve growth factor β (NGF)	Ceregene	I
NLX-P101	Recombinant AAV vector comprising the human glutamate decarboxylase 65 (GAD65) coding sequence and the neuron-specific enolase (NSE) promoter	Neurologix	I
ProSavin®	Tricistonic lentiviral vector encoding human tyrosine hydroxylase, aromatic AADC and GTP cyclohydrolase 1	Oxford BioMedica	Preclinical
TREAT-HGF	Naked plasmid encoding human HGF	AnGes	Preclinical

Neurturin is a member of the GDNF family of ligands that has been shown to exert neuroprotective and restorative effects on nigrostriatal dopaminergic neurons in animal models and in humans. CERE-120 (AAV-hNGF-hNTN) is a novel gene therapy product that shows particular promise. CERE-120 delivers a modified human neurturin (*NRTN* or *NTN*) gene in which the prepro sequence of human neurturin cDNA is replaced with the prepro domain of human nerve growth factor β (NGFβ) via an adeno-associated virus type 2 (AAV2) vector under the control of the CAG promoter. The result is a gene product that is efficiently secreted from human cells with potent biological activity. CERE-120 was chosen for further development for the treatment of Parkinson's disease.

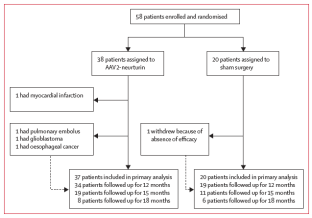
An recent phase I trial in 12 patients with Parkinson's disease (Hoehn/Yahr stage 3 or greater) and motor fluctuations has examined the safety, tolerability and efficacy of CERE-120 (2 x 10^{11} or 8 x 10^{11} vector genomes injected intraputaminally along 4 trajectories/ hemisphere). No surgical complications or serious adverse events have been reported and no treatment-related adverse events have been seen at 2-17 weeks of follow-up.

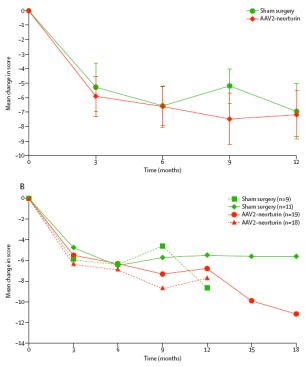
Based on promising preliminary results, a phase II study is currently ongoing.

www.thelancet.com/neurology Vol 9 December 2010

Gene delivery of AAV2-neurturin for Parkinson's disease: a double-blind, randomised, controlled trial

William J Marks Jr*, Raymond T Bartus*, Joao Siffert, Charles S Davis, Andres Lozano, Nicholas Boulis, Jerrold Vitek, Mark Stacy, Dennis Turner, Leonard Verhagen, Roy Bakay, Raymond Watts, Barton Guthrie, Joseph Jankovic, Richard Simpson, Michele Tagliati, Ron Alterman, Matthew Stern, Gordon Baltuch, Philip A Starr, Paul S Larson, Jill L Ostrem, John Nutt, Karl Kieburtz, Jeffrey H Kordower, C Warren Olanow*





Interpretation
Positive results with GDNF were reported in the open-label studies[10,11] but these results were not confirmed in the two double-blind studies.[12,13] The open-label study of gene delivery of neurturin as a treatment for Parkinson's disease met its primary endpoint of safety.[14] The present study did not meet its primary endpoint of efficacy but did report positive results in the subgroup of patients who were assessed for up to 18 months, suggesting that there might be a delay in transport of the trophic factor to the substantia nigra because of the extent of degeneration of the nigrostriatal tract. This study emphasises the importance of double-blind trials in assessment of novel surgical therapies for Parkinson's disease and suggests that future gene delivery studies should be done with direct injections into the substantia nigra as well as the putamen.

A Dose-Ranging Study of AAV-hAADC Therapy in Parkinsonian Monkeys

John R. Forsayeth,[1] Jamie L. Eberling,[1,2] Laura M. Sanftner,[3] Zhu Zhen,[3] Philip Pivirotto,[1] John Bringas,[1] Janet Cunningham,[1] and Krystof S. Bankiewicz[1,*]

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Available online 16 June 2006

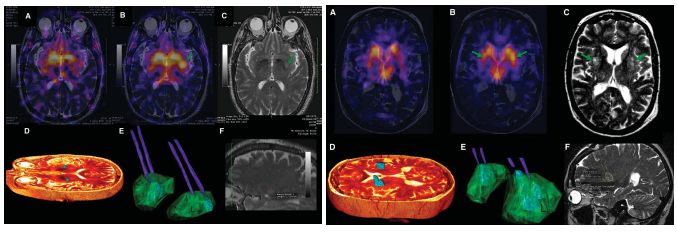
The main medication for idiopathic Parkinson disease is L-Dopa. Drug efficacy declines steadily in part because the converting enzyme, aromatic L-amino acid decarboxylase (AADC), is lost concomitant with substantia nigra atrophy. Over the past decade, we have developed a gene therapy approach in which AADC activity is restored to the brain by infusion into the striatum of a recombinant adeno-associated virus carrying human AADC cDNA. We report here the results of an investigation of the relationship between vector dose and a series of efficacy markers, such as PET, L-Dopa response, and AADC enzymatic activity. At low doses of vector, no effect of vector was seen on PET or behavioral response. At higher doses, a sharp improvement in both parameters was observed, resulting in an approximate 50% improvement in L-Dopa responsiveness. The relationship between vector dose and AADC enzymatic activity in tissue extracts was linear. We conclude that little behavioral improvement can be seen until AADC activity reaches a level that is no longer rate limiting for conversion of clinical doses of L-Dopa into dopamine or for trapping of the PET tracer FMT. These findings have implications for the design and interpretation of clinical studies of AAV-hAADC gene therapy.

Key Words: Parkinson disease, convection-enhanced delivery, adeno-associated virus, aromatic L-amino acid decarboxylase, AAV-hAADC, FMT-PET

Therapeutic effect maintained up to 6 years!

Qualitative Imaging of Adeno-Associated Virus Serotype 2–Human Aromatic L-Amino Acid Decarboxylase Gene Therapy in a Phase I Study for the Treatment of Parkinson Disease

Patient 102 **Patient 103**



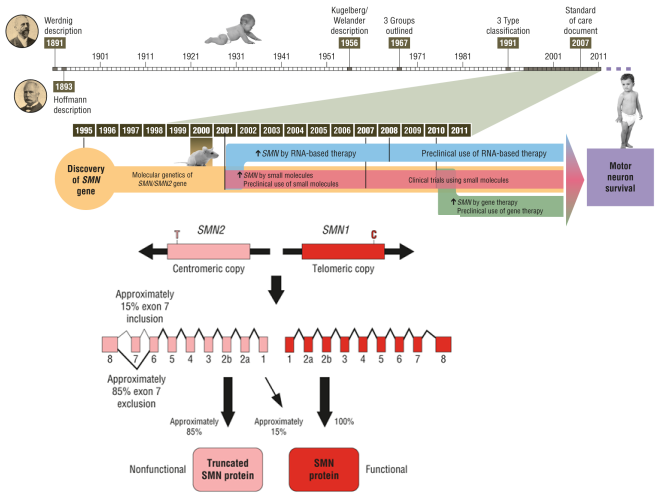
A through C, axial views of the T2 magnetic resonance imaging (MRI) after convection-enhanced delivery with [18F]-6-fluoro-meta-tyrosine (FMT) positron emission tomography coregistered before (A) and 1 month after (B) adeno-associated virus serotype 2–human aromatic L-amino acid decarboxylase gene transfer. T2 hyperintensity in C is indicated by the green arrows, and correlates well with the FMT uptake indicated by green arrows in B. **D**, T2 MRI 3-dimensional (3D) reconstruction of the bilateral infusions. Blue domains indicate the 3D reconstructions of the infusions. **E**, T2 MRI 3D reconstruction in the axial-oblique view. Green domains indicate putamen reconstruction; blue domains with the putamen indicate infusion reconstructions; purple lines indicate cannula tracks. Infusions appear to be confined primarily within the putamen. **F**, sagittal view on T2 MRI illustrating infusion hyperintensity on right side. The yellow line outlines the infusion region. The diameter of the infusion appears to be at least 9 mm, as indicated by the red line drawn across the widest region of T2 hyperintensity. Dorsoventral aspect of the infusion, indicated by the blue line, measures 13 mm. This is the same region indicated by the green arrow on the right side in the C.

The protocol for this trial on 15 moderately advanced subjects was publicly discussed at the recombinant DNA advisory committee in late 2003, but subjects were not treated until 1 year later, during which time Genzyme, Boston, MA acquired the program from Avigen (Alameda, CA).

This trial was successful in terms of safety. However, despite a reasonable scientific rationale and several animal studies demonstrating reasonably robust enhancement in nigrostriatal dopamine function with AAV2-AADC, the phase 1 trial found only very modest efficacy, and was even described as neither “clear cut” nor what “we needed” by a Genzyme spokesperson.

A second phase 1 study was performed in Japan using the identical vector (provided by Genzyme) and dosing paradigm, as well as a similar clinical protocol. Not surprisingly, the open-label efficacy results were not markedly different from the trial conducted in the USA.

Recently, Genzyme agreed to allow the program's academic originators, in collaboration with Michael J. Fox Foundation for Parkinson's Research (MJFF), to resurrect a modified version of the clinical program, admitting that without the MJFF financial support, the program would not likely have moved forward.



SMA Types: A Devastating Disease

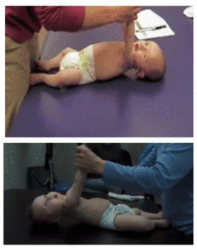
	TYPE 1	TYPE 2	TYPE 3	TYPE 4
SMN2 Copy Number	Two	Three or Four	Three or Four	Four to Eight
Onset	Before 6 Months	6-18 Months	Early childhood to early adulthood (juvenile)	Adulthood (20s-30s) usually after 30
Incidence per Live Birth	Approximately 60%	Approximately 27%	Approximately 13%	Uncommon; limited information available
Developmental Milestones	• Will never be able to sit without support • Difficulty breathing & swallowing • Can't crawl/will never walk	• Will never be able to walk or stand without support	• Stand alone and walk but may lose ability to walk in 30s-40s	• Stand alone and walk but may lose ability to walk in 30s-40s (Same as Type 3)
Survival	• <10% Event free* by two years of age	• 68% alive at age 25	• Normal	• Normal

*Event = Death or ≥16-hr/day ventilation continuously for ≥ 2 wks, in the absence of an acute reversible illness

5

Children with SMA Type 1 Never Sit Unassisted

The Natural History of SMA Type 1 is marked by the inability to achieve or maintain developmental milestones



Disease Characteristics

- Disease onset <6 months
- Hypotonia and weakness
- Bulbar muscle weakness
- Difficulty breathing and swallowing
- Inexorable progression to nutritional failure
- Inexorable progression to respiratory failure

Developmental Milestone Prognosis

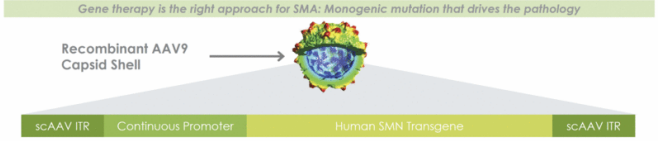
- Progressive decline in motor function soon after birth
- Rapid loss of any early milestones (e.g. head control, hands to mouth)
- Will never be able to sit unassisted
- Will never be able to roll
- Will never be able to crawl, stand, or walk

15

Our Solution: AVXS-101

An Innovative Treatment Approach for SMA

Gene therapy is the right approach for SMA: Monogenic mutation that drives the pathology



KEY COMPONENTS	PURPOSE
Recombinant AAV9 Capsid Shell	• Ability to deliver across the blood brain barrier (BBB) and into the spinal cord – Avoids the need for intrathecal delivery when treating infants • Non-replicating virus does not modify the existing DNA of the patient.
scAAV ITR (Self-complementary DNA technology)	• Enables rapid onset of effect which is key in a quickly deteriorating population
Continuous Promoter	• Activates the transgene to allow for continuous and sustained SMN expression
Human SMN Transgene	• Full copy of a stable, functioning SMN gene that is introduced into the cell's nucleus

Rendering adapted from DiMattia et al. Structural Insight into the Unique Properties of Adeno-Associated Virus Serotype 9. J. Virol. June 2012.

8

The NEW ENGLAND JOURNAL of MEDICINE

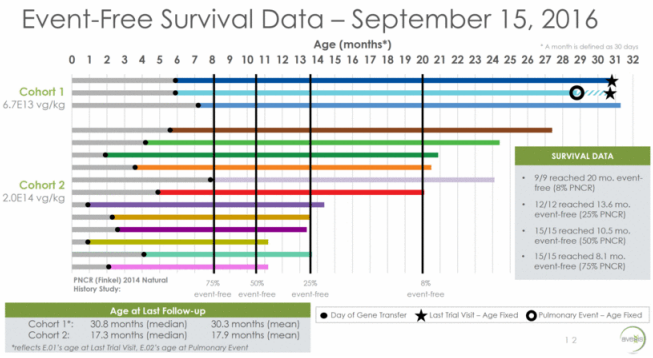
Single-Dose Gene-Replacement Therapy for Spinal Muscular Atrophy

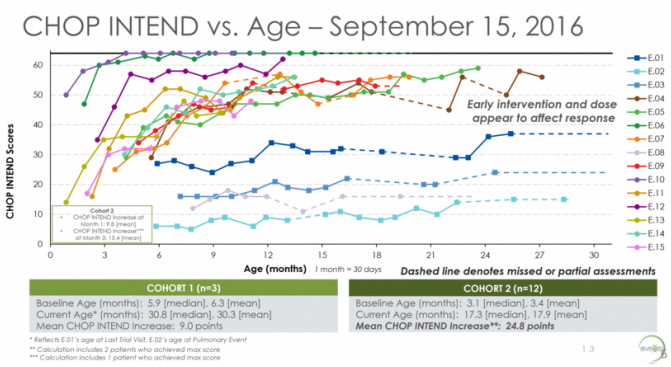
Jerry R. Mendell, M.D., Samiah Al-Zaidy, M.D., Richard Shell, M.D., W. Dave Arnold, M.D., Louise R Rodino-Klapac, Ph.D., Thomas W. Prior, Ph.D., Linda Lowes, P.T., Ph.D., Lindsay Alfano, D.P.T., Katherine Berry, P.T., Kathleen Church, M.S.W., John T. Kissel, M.D., Sukumar Nagendran, M.D., James L'Italien, Ph.D., Douglas M. Sproule, M.D., Courtney Wells, B.S., Jessica A. Cardenas, Ph.D., Marjet D. Heitzer, Ph.D., Allan Kaspar, Ph.D., Sarah Corcoran, B.S., Lyndsey Braun, B.S., Shibi Likhite, Ph.D., Carlos Miranda, Ph.D., Kathrin Meyer, Ph.D., K.D. Foust, Ph.D., Arthur H.M. Burghes, Ph.D., and Brian K. Kaspar, Ph.D. et al.

Feedback

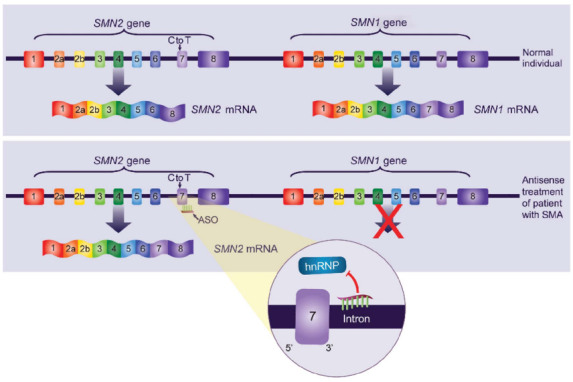
RESULTS As of the data cutoff on August 7, 2017, all 15 patients were alive and event-free at 20 months of age, as compared with a rate of survival of 8% in a historical cohort. In the high-dose cohort, a rapid increase from baseline in the score on the CHOP INTEND scale followed gene delivery, with an increase of 9.8 points at 1 month and 15.4 points at 3 months, as compared with a decline in this score in a historical cohort. Of the 12 patients who had received the high dose, 11 sat unassisted, 9 rolled over, 11 fed orally and could speak, and 2 walked independently. Elevated serum aminotransferase levels occurred in 4 patients and were attenuated by prednisolone.

Phase I, sc-AAV9





SPINRAZA® (nusinersen) injection 12 mg/5 mL



Phase III, repeated intrathecal administration

Nusinersen versus Sham Control in Infantile-Onset Spinal Muscular Atrophy

Richard S. Finkel, M.D., Eugenio Mercuri, M.D., Ph.D., Basil T. Darras, M.D., Anne M. Connolly, M.D., Nancy L. Kuntz, M.D., Janbernd Kirschner, M.D., Claudia A. Chiriboga, M.D., M.P.H., Kayoko Saito, M.D., Ph.D., Laurent Servais, M.D., Ph.D., Eduardo Tizzano, M.D., Ph.D., Haluk Topaloglu, M.D., Már Tulinius, M.D., Ph.D., Jacqueline Montes, P.T., Ed.D., N.C.S., Allan M. Glanzman, P.T., D.P.T., P.C.S., Kathie Bishop, Ph.D., Z. John Zhong, Ph.D., Sarah Gheuens, M.D., Ph.D., C. Frank Bennett, Ph.D., Eugene Schneider, M.D., Wildon Farwell, M.D., M.P.H., and Darryl C. De Vivo, M.D. et al., for the ENDEAR Study Group*

Feedback

The NEW ENGLAND JOURNAL of MEDICINE

RESULTS In the interim analysis, a significantly higher percentage of infants in the nusinersen group than in the control group had a motor-milestone response (21 of 51 infants [41%] vs. 0 of 27 [0%], P<0.001), and this result prompted early termination of the trial. In the final analysis, a significantly higher percentage of infants in the nusinersen group than in the control group had a motor-milestone response (37 of 73 infants [51%] vs. 0 of 37 [0%]), and the likelihood of event-free survival was higher in the nusinersen group than in the control group (hazard ratio for death or the use of permanent assisted ventilation, 0.53; P=0.005). The likelihood of overall survival was higher in the nusinersen group than in the control group (hazard ratio for death, 0.37; P=0.004), and infants with a shorter disease duration at screening were more likely than those with a longer disease duration to benefit from nusinersen. The incidence and severity of adverse events were similar in the two groups.

November 2, 2017

Nusinersen versus Sham Control in Later-Onset Spinal Muscular Atrophy

Eugenio Mercuri, M.D., Ph.D., Basil T. Darras, M.D., Claudia A. Chiriboga, M.D., M.P.H., John W. Day, M.D., Ph.D., Craig Campbell, M.D., Anne M. Connolly, M.D., Susan T. Iannaccone, M.D., Janbernd Kirschner, M.D., Nancy L. Kuntz, M.D., Kayoko Saito, M.D., Ph.D., Perry B. Shieh, M.D., Ph.D., Már Tulinius, M.D., Ph.D., Elena S. Mazzone, D.P.T., Jacqueline Montes, P.T., Ed.D., Kathie M. Bishop, Ph.D., Qingqing Yang, M.S., Richard Foster, M.Sc., Sarah Gheuens, M.D., Ph.D., C. Frank Bennett, Ph.D., Wildon Farwell, M.D., M.P.H., Eugene Schneider, M.D., Darryl C. De Vivo, M.D., and Richard S. Finkel, M.D. et al., for the CHERISH Study Group*

Feedback

RESULTS In the prespecified interim analysis, there was a least-squares mean increase from baseline to month 15 in the HFMSE score in the nusinersen group (by 4.0 points) and a least-squares mean decrease in the control group (by −1.9 points), with a significant between-group difference favoring nusinersen (least-squares mean difference in change, 5.9 points; 95% confidence interval, 3.7 to 8.1; P<0.001). This result prompted early termination of the trial. Results of the final analysis were consistent with results of the interim analysis. In the final analysis, 57% of the children in the nusinersen group as compared with 26% in the control group had an increase from baseline to month 15 in the HFMSE score of at least 3 points (P<0.001), and the overall incidence of adverse events was similar in the nusinersen group and the control group (93% and 100%, respectively).

CONCLUSIONS Among children with later-onset SMA, those who received nusinersen had significant and clinically meaningful improvement in motor function as compared with those in the control group. (Funded by Biogen and Ionis Pharmaceuticals; CHERISH ClinicalTrials.gov number, NCT02292537.)

EDITORIAL

The Dilemma of Two Innovative Therapies for Spinal Muscular Atrophy

Ans T. van der Ploeg, M.D., Ph.D.

Different study designs, hard to compare the results of these studies

scAAV9 gene therapy may require only a single intra-venous infusion (but difficult to repeat) , whereas nusinersen probably requires lifelong repetitive intrathecal treatment

As the children grow, the phenotype may expand to affect other organs and tissues (do scAAV9 and antisense oligonucleotides target other cell types?).

Neither therapy currently provides a cure. One option may be to start treatment earlier; the NURTURE study (ClinicalTrials.gov number, NCT02386553) is currently investigating the ef- fect of nusinersen in presymptomatic patients. Another option is to combine the two treatments.

An important constraint is the high anticipated cost of $750,000 for a course of nusinersen during the first year of therapy

The study of scAAV9 gene therapy enrolled 15 patients (3 low dose 12 high dose)

In the high-dose group 9 patients were able to sit without support for at least 30 seconds, and 2 were able to crawl, pull to stand, and walk independently and 7 patients did not require ventilatory support.

The trial of nusinersen enrolled 122 infants with onset of symptoms at 6 months of age or younger.

Of the infants who achieved motor milestones (51%), only 8% could sit independently and 1% could stand. 39% of the infants in the nusinersen group and 68% in the control group had died or received permanent assisted ventilation.

Best results in patients who started treatment within 13 weeks after disease onset.

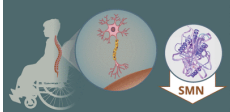
Nusinersen in later-onset spinal muscular atrophy

Long-term results from the phase 1/2 studies

Neurology® 2019;92:e2492-e2506. doi:10.1212/WNL.0000000000007527

Is long-term nusinersen effective for later-onset SMA?

Spinal muscular atrophy (SMA) is characterized by progressive muscular atrophy and weakness.



Analysis of the long-term efficacy of nusinersen on SMA is lacking.

Homozygous deletions or mutations in the **survival motor neuron 1 (*SMN1*)** gene cause decreased functional **SMN protein**.

Nusinersen has been shown to effectively increase full-length SMN protein levels.

Study Question
What is the long-term effectiveness and safety of nusinersen in children with later-onset SMA?

Integrated analysis

28 children (Aged: 2-15 years at enrollment)

SMA Type II: n = 11; SMA Type III: n = 17

Nusinersen (mg)

Phase 1b/2a study (253 days): 3, 6, 9, 12

Extension study (715 days): 12

Mean change from baseline over ~3 years of treatment

	SMA Type II	SMA Type III
Motor assesment		
Hammersmith Functional Motor Scale-Expanded score	+10.8 points	+1.8 points
Upper Limb Module score	+4.0 points	NR*
Six-Minute Walk Test distance	NR*	+92.0 points

*Not reported

No children discontinued treatment due to adverse events.

Long-term nusinersen treatment improved motor function and stabilized disease activity in children with later-onset SMA.

doi:10.1212/WNL.0000000000007527

Neurology®

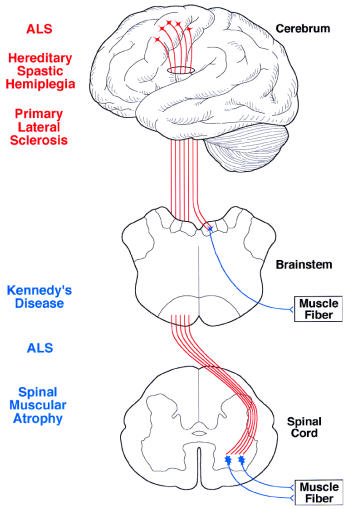
The SMA landscape – selected clinical-stage projects

			Annual sales ($m)			
Product	**Company**	**Mechanism**	**2018**	**2019**	**2022**	**2024**
Spinraza	Biogen	SMN 2 antisense	1,692	1,931	2,098	2,060
AVXS-101	Novartis	SMN gene therapy	-	189	1,159	1,339
Reldesemtiv*	Astellas	Troponin activator	-	-	171	189
Risdiplam	Roche	SMN 2 gene splicing modifier	-	-	33	82
LMI070	Novartis	SMN 2 gene splicing modifier	-	-	-	-
ALG-801	Biogen	Myostatin inhibitor	-	-	-	-

**Also in trials for ALS and COPD. Source: EvaluatePharma.*

Motor neuron diseases

involve lesions in one or both components of a two-neuron pathway



Amyotrophic lateral sclerosis
(Lou Gehrig's disease)

- Lower and upper motor degeneration
- Onset at 40-50 years
- Respiratory failure within 2-5 years
- Deterioration can be slowed by riluzole (glutamate-blocking drug) and antioxidant vitamins - but modest/no improvement
- 10% genetic forms: earlier onset, Lewy body inclusions and spinocerebellar degeneration

Superoxide dismutase (SOD)

SOD catalyzes the reaction of the superoxide free radical O_2' into H_2O_2

SOD1, cytosolic, requires copper and zinc
SOD2 in mitochondria
SOD3 extracellular

Mutations in SOD1 are an important cause of ALS - more then 60 mutations identified so far

Transgenic mice overexpressing mutant human SOD1 showed degeneration of spinal motor neurons similar to human ALS due to gain of function (survival inversely related to SOD1 activity)
The mutant SOD1 produces a toxic metabolite, probably peroxynitrite or nitrosamine peroxide

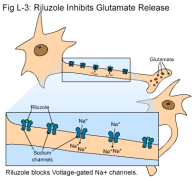
Mice with a knock-out mutation for SOD1 do not develop ALS-like disease

Gain of function is consistent with dominant inheritance seen in clinics

Therapeutic genes proposed/used so far:

- Calbindin
- Neurofilaments
- Bcl-2
- IL-1 converting enzyme inhibitors
- BDNF, CNTF, GDNF, IGF-1
- Neurotrophin 3
- Glutamate transporter

Fig L-3: Riluzole Inhibits Glutamate Release



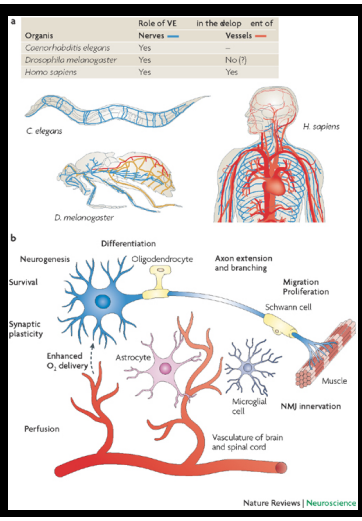
Abnormal motor performance in VEGF$^{\partial/\partial}$ mice

VEGF$^{+/+}$

VEGF$^{\partial/\partial}$

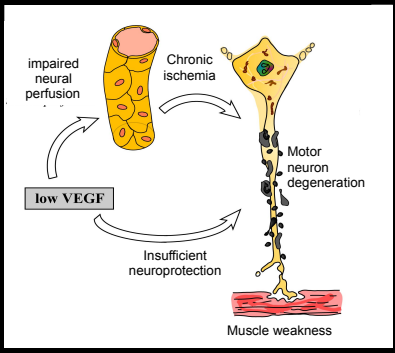
Oosthuyse et al. Nat. Genet., 2001

Evolution of VEGF as a multi-tasking neuronal factor

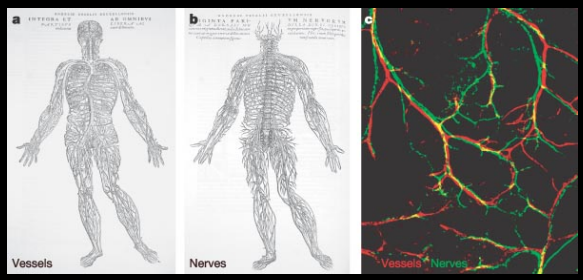


Zacchigna, Nature Review Neuroscience 2008

Low VEGF levels in VEGF$^{\partial/\partial}$ mice cause motoneuron degeneration



Parallels in vessel and nerve patterning



Peter Carmeliet and Marc Tessier-Lavigne

Nature 436, 193-200 (14 July 2005)

Axonal growth cones and endothelial tip cells share growth/ chemotactic factors and receptors

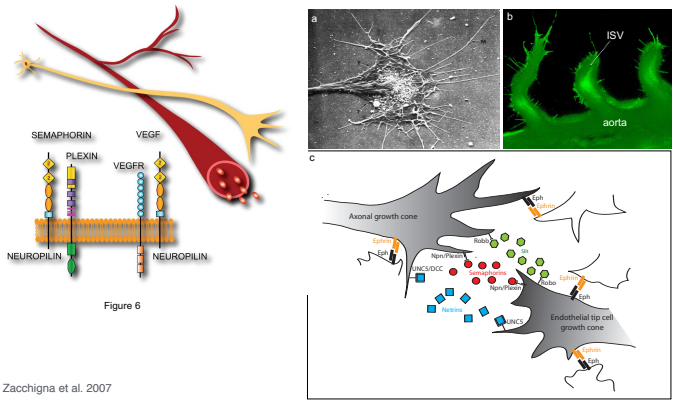
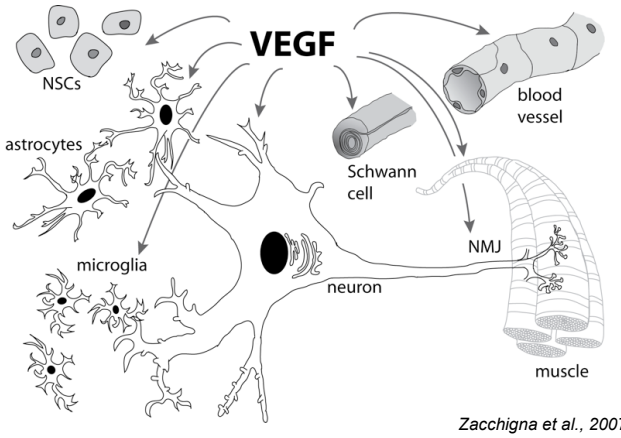


Figure 6

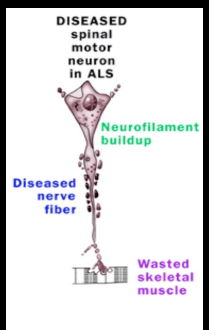
Zacchigna et al. 2007

...the advantage of being multi-tasking...



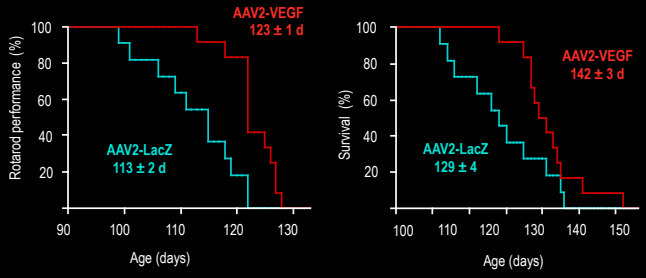
Zacchigna et al., 2007

The axon: an overlooked therapeutic candidate in ALS?

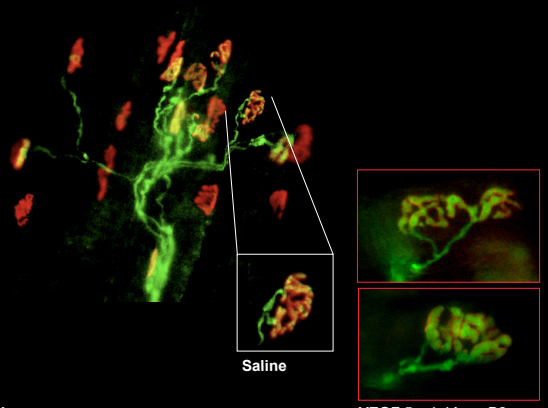


Do trophic factors (IGF-1, VEGF, etc.) affect the axon?

Intramuscular AAV2-VEGF prolongs survival of SOD1^{G93A}



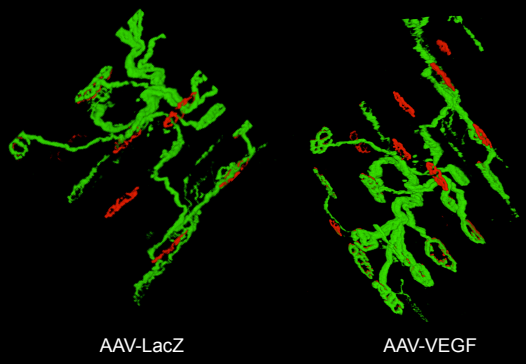
VEGF increases terminal axon sprouting



Thy1-YFP mice

VEGF 5μg/g/day - P8

VEGF effect on SOD mice: preserved or reinnervated NMJ?



AAV-LacZ

AAV-VEGF

Thy1-YFP/SOD mice

VEGF effect on SOD mice: stabilization of synaptic contacts?